DRAFT FINAL REPORT

PLACENTAL AROMATASE VALIDATION STUDY: Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories (Volume I- Overall Summary, Appendices A and B)

EPA Contract Number 68-W-01-023
Work Assignment 4-16, Task 7

June 2006

Prepared for

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PLACENTAL AROMATASE ASSAY VALIDATION STUDY: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES

WA 4-16, Task 7

OVERALL TASK DRAFT FINAL REPORT

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Study Initiation Date:

September 8, 2004 (Work Plan Submission)

Task Initiation Date:

June 7, 2005 (QAPP Approved)

Experimental Performance Dates:

July 14, 2005 to October 10, 2005

Overall Draft Final Report Date:

April 14, 2006

ERRATA

Draft Final Report
For WA 4-16, Task 7

In the individual laboratory reports found in the appendices, the aminoglutethimide and ketoconazole IC_{50} values are incorrect by a factor of 10, i.e., the IC_{50} for aminoglutethimide is 10-fold high and for ketoconazole is 10-fold low. This error occurred due to a miscommunication by the Chemical Repository as to the identity of these two reference chemicals and their respective stock concentrations. The individual laboratory reports will be corrected by their respective study directors when the draft final reports are finalized.

In this overall report, the errors were corrected for each of the laboratories in order for an evaluation of the intralaboratory and interlaboratory results to be made without having to be concerned about this discrepancy.

OVERALL DRAFT FINAL REPORT

Title: Placental Aromatase Assay Validation Study: Conduct Multiple Chemical Studies With Microsomes Prepared in Participating Laboratories

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Quality Assurance Statement

Study Number: G608316

This study was inspected by the Quality Assurance Unit and reports were submitted to the Work Assignment Leader (WAL) and Management as follows:

Phase Inspected	Inspection Date	Date Reported to WAL/Management
Audit Overall Interlaboratory draft final report	5/02/2006	05/02/2006

Quality Assurance Unit **Date**

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EXECUTIVE SUMMARY

In this task, the aromatase assay was conducted by staff from a lead laboratory (RTI International) and three participating laboratories (Battelle, In Vitro Technologies, and WIL Research Laboratories). Battelle and In Vitro each obtained a human placenta, which was used to prepare and distribute microsomes (WA 4-16, Task 6). Each laboratory conducted at least three independent replicates of the placental aromatase assay using the following reference chemicals: aminoglutethimide, atrazine, chrysin, dibenz [a,h] anthracene, dicofol, econazole, fenarimol, ketoconazole, 4-nonylphenol, and prochloraz, as well as 4-hydroxyandrostenedione (positive control) and lindane (negative control). The study design involved, for each reference chemical, conducting three repetitions at each of eight concentrations, and for the positive and negative controls, conducting four repetitions at a single concentration, for each of at least three independent replicates. Reference chemical stock solutions were supplied by a central laboratory (Battelle's Endocrine Disruptor Screening Program Chemical Repository) and tested blind.

The objectives of this task were to evaluate the responsiveness of human placental microsomes (prepared by two participating laboratories) for conducting the aromatase assay using ten reference chemicals and a positive and negative control, as well as to obtain intralaboratory and interlaboratory values for the aromatase enzyme activity and aromatase inhibition (IC_{50}) for each of the reference chemicals.

For the Battelle-supplied microsomes (protein concentration determined to be 21 mg/mL by Battelle), the laboratory group mean (± SEM) protein concentration values were 26.8 ± 0.4, 25.0 ± 0.4, 18.6 ± 0.7, and 23.9 ± 0.8 mg/mL for RTI, Battelle, In Vitro, and WIL, respectively (% CV values ranged from 5.3 to 11.5 percent). The overall task mean ± SEM protein concentration was 23.6 ± 1.8 mg/mL with a percent CV of 15.0 percent. For the In Vitro-supplied microsomes (protein concentration determined to be 8 mg/mL by In Vitro), the laboratory group mean (± SEM) protein concentration values were 10.9 ± 0.4, 9.2 ± 0.1, 6.3 ± 0.6, and 8.5 ± 0.4 mg/mL for RTI, Battelle, In Vitro, and WIL, respectively (% CV values ranged from 5.7 to 13.8 percent). The overall task mean ± SEM protein concentration was 8.7 ± 1.0 mg/mL with a percent CV of 21.8 percent.

Protein QC samples (target concentrations of 10 and 100 µg/mL), had acceptable accuracy (±15 % RE) for three of four laboratories for the low QC (range was -54.7 to 13.0 percent) and all laboratories for the high QC (range was 0.4 to 13.9 percent). Precision was acceptable (±15 % CV) for one of four laboratories for the low QC (range was 10.9 to 83.5 percent) and all laboratories for the high QC (range was 2.4 to 8.7 percent).

For the full enzyme activity controls, the Battelle-prepared microsomes had laboratory overall average ± SEM (% CV) values of 0.048 ± 0.002 (6.3 %), 0.055 ± 0.005 (21.4 %), 0.058 ± 0.007 (22.0 %) and 0.059 ± 0.001 (3.4 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM full enzyme activity control value for the Battelle-prepared microsomes was 0.055 ± 0.002 nmol/mg protein/min with a percent CV of 8.6 percent. Using the In-Vitro-prepared microsomes, the laboratory overall average ± SEM (% CV) values were 0.036 ± 0.003 (16.1 %), 0.046 ± 0.001 (6.8 %), 0.075 ± 0.018 (42.4 %) and 0.050 ± 0.006 (20.9 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM full enzyme activity control value for the In Vitro-prepared

microsomes was 0.052 ± 0.008 nmol/mg protein/min with a percent CV of 31.7 percent. For the full enzyme activity control (irrespective of microsomal source), the laboratory overall average ± SEM (% CV) values were 0.043 ± 0.003 (17.7 %), 0.051 ± 0.003 (19.0 %), 0.066 ± 0.010 (35.4 %) and 0.055 ± 0.003 (15.3 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM full enzyme activity control value was 0.054 ± 0.005 nmol/mg protein/min with a percent CV of 17.9 percent.

Background enzyme activity controls indicated that there was no background activity that interfered with the interpretation of the results.

For the positive control (4-hydroxyandrostenedione, 4-OH ASDN, at a concentration of 5 x 10^{-8} M), the laboratory overall average ± SEM (% CV) enzyme activity values in the presence of 4-OH ASDN were 0.021 ± 0.002 (19.5 %), 0.025 ± 0.001 (15.0 %), 0.024 ± 0.003 (33.1 %), and 0.023 ± 0.001 (13.5 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM positive control value was 0.023 ± 0.001 nmol/mg protein/min with a percent CV of 7.5 percent. In regard to percent inhibition, the laboratory overall group mean ± SEM (% CV) values for 4-OH ASDN (as a percent of control) were 48.0 ± 0.4 (2.3 %), 48.7 ± 1.0 (6.8 %), 40.5 ± 7.4 (44.5 %), and 42.7 ± 3.1 (17.5 %) percent for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM percent of control value was 45.0 ± 2.0 percent with a percent CV of 9.0.

For the negative control (lindane, at a concentration of 10^{-6} M), the laboratory overall average ± SEM (% CV) enzyme activity values in the presence of lindane were 0.042 ± 0.003 (16.3 %), 0.049 ± 0.003 (18.7 %), 0.046 ± 0.007 (36.1 %), and 0.053 ± 0.003 (14.3 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM negative control value was 0.048 ± 0.002 nmol/mg protein/min with a percent CV of 9.8 percent. In regard to percent inhibition, the laboratory overall group mean ± SEM (% CV) inhibition values for lindane (as a percent of control) were 97.9 ± 0.8 (2.1 %), 97.3 ± 0.9 (2.8 %), 74.8 ± 13.0 (42.4 %), and 99.6 ± 8.8 (21.6 %) percent for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM percent of control value was 92.4 ± 5.9 percent with a percent CV of 12.8.

For aminoglutethimide, increasing concentrations decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent. At an aminoglutethimide concentration of 10^{-3} M, percent of control values were less than 1 percent. In contrast, at an aminoglutethimide concentration of approximately 10^{-7} M, there was little to no aromatase inhibition (percent of control values were greater than or equal to 94 percent for all labs). The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-7} M were 0.17 ± 0.10 and 95.16 ± 0.78 percent, respectively. The overall task assay precision ranged from 2 to 10 percent for those concentrations that were tested by 3 or more labs, except at a concentration of 10^{-3} M where the % CV value was 120 percent. The IC_{50} values for aminoglutethimide (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 4.02 ± 0.15 (6.5), 4.98 ± 0.51 (17.6), 4.09 ± 0.40 (17.1), and 4.11 ± 0.27 (11.5) μM, respectively. The overall task group mean ± SEM IC_{50} value was 4.30 ± 0.23 μM and the percent CV was 10.6 percent.

For atrazine, increasing concentrations did not decrease the activity of the placental microsomal aromatase activity. At an atrazine concentration of 10^{-3} M, aromatase activity was 80 to 90 percent of control, which was similar to the values obtained at the lower concentrations, e.g at approximately 10^{-10} M, the laboratory percent of control values were greater than 90 percent. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-10} M were 81.31 ± 1.46 and 93.62 ± 1.57 percent, respectively. The overall task assay precision ranged from 2 to 5 percent for those concentrations that were tested by 3 or more labs. The absence of any inhibition, precluded reporting an IC_{50} value for atrazine.

For chrysin, increasing concentrations decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent. At a chrysin concentration of 10^{-4} M, aromatase inhibition percent of control values ranged from 14 to 23 percent. In contrast, at chrysin concentrations $\leq 10^{-7}$ M, most laboratories reported percent of control values greater than 90 percent. The overall task mean ± SEM percent of control values at 10^{-4} and 10^{-7} M were 18.74 ± 1.88 and 89.51 ± 1.69 percent, respectively. The overall task assay precision ranged from 1 to 9 percent for those concentrations that were tested by 3 or more labs, except at a concentration of 10^{-4} M where the % CV value was 20 percent. IC_{50} values (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 1.72 ± 0.13 (13.4), 2.21 ± 0.13 (10.0), 3.02 ± 0.48 (27.4), and 3.06 ± 0.33 (18.4) µM, respectively. The overall task group mean ± SEM IC_{50} value was 2.50 ± 0.33 µM and the percent CV was 26.1 percent.

For dicofol, increasing concentrations decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent. At a dicofol concentration of 10^{-3} M, three of four laboratories reported percent of control values of 3 percent. The remaining laboratory reported a percent of control value at its lowest tested concentration (10^{-4} M) of 45 percent. In contrast, at a dicofol concentration of approximately 10^{-6} M, percent of control values were approximately 90 percent or higher. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-6} M were 2.86 ± 0.15 and 91.01 ± 0.80 percent, respectively. The overall task assay precision ranged from 2 to 9 percent for those concentrations that were tested by 3 or more labs, except at a concentration of 10^{-4} M where the % CV value was 30 percent. IC_{50} values (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 62.91 ± 35.86 (98.7), 24.14 ± 3.72 (26.7), 501 ± 489 (169.0), and 53.13 ± 16.56 (54.0) µM, respectively. The overall task group mean ± SEM IC_{50} value was 29.13 ± 8.62 µM and the percent CV was 59.2 percent.

For dibenz[a,h]anthracene, increasing concentrations did not decrease the activity of the placental microsomal aromatase activity. At a dibenz[a,h]anthracene concentration of 10^{-4} M (or 3.3×10^{-5} M for In Vitro) to 10^{-10} M, there was no indication of any aromatase inhibition. The overall task mean ± SEM percent of control values at 10^{-4} and 10^{-10} M were 100.77 ± 2.23 and 97.56 ± 3.96 percent, respectively. The overall task assay precision ranged from 4 to 11 percent for those concentrations that were tested by 3 or more labs. The absence of any inhibition precluded reporting an IC_{50} value for dibenz[a,h]anthracene.

For econazole, increasing concentrations decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent. At an econazole concentration of 10^{-7} M, percent of control values ranged from 1 to 2 percent. In contrast, at an econazole concentration of approximately 10^{-10} M, there was little to no aromatase inhibition

(percent of control values were approximately 90 percent or higher for three labs and 80 percent for one lab). The overall task mean ± SEM percent of control values at 10^{-7} and 10^{-10} M were 1.52 ± 0.07 and 89.94 ± 3.34 percent, respectively. The overall task assay precision ranged from 7 to 14 percent for those concentrations that were tested by 3 or more labs, except at concentrations $\geq 10^{-6}$ M where the % CV values were very large. The IC_{50} values for econazole (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 1.50 ± 0.06 (6.8), 2.43 ± 0.27 (19.5), 0.818 ± 0.127 (27.0), and 1.69 ± 0.04 (4.2) nM, respectively. The overall task group mean ± SEM IC_{50} value was 1.61 ± 0.33 nM and the percent CV was 41.3 percent.

For fenarimol, increasing concentrations decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent. At a fenarimol concentration of 10^{-3} M, percent of control values ranged from 1 to -6 percent for three of the four labs (the highest concentration tested by the remaining lab, 3.3 x 10^{-5} M, resulted in a value of 15 percent). In contrast, at a fenarimol concentration of approximately 10^{-7} M, there was little to no aromatase inhibition (percent of control values were greater than 90 percent for three of the labs and 86 for the fourth lab). The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-7} M were -1.11 ± 2.40 and 91.88 ± 2.27 percent, respectively. The overall task assay precision ranged from 3 to 9 percent for those concentrations that were tested by 3 or more labs, except at a concentration of 10^{-3} and 10^{-4} M where the % CV values were 375 and 113 percent, respectively. The IC_{50} values for fenarimol (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 6.01 ± 0.31 (9.0), 6.05 ± 0.04 (1.2), 4.25 ± 0.65 (26.5), and 7.05 ± 0.40 (9.7) µM, respectively. The overall task group mean ± SEM IC_{50} value was 5.84 ± 0.58 µM and the percent CV was 20.0 percent.

For ketoconozole, increasing concentrations decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent. At a ketoconazole concentration of 10^{-4} M, percent of control values ranged from 5 to 6 percent for three of the four labs (one lab reported a value of 29 percent). In contrast, at a concentration of approximately 10^{-7} M, there was little to no aromatase inhibition (percent of control values were greater than 95 percent for all labs). The overall task mean ± SEM percent of control values at 10^{-4} and 10^{-7} M were 11.17 ± 5.99 and 100.51 ± 2.35 percent, respectively. The overall task assay precision ranged from 3 to 5 percent for those concentrations that were tested by 3 or more labs, except at a concentration of 10^{-4} M where the % CV value was 107 percent. The IC_{50} values for ketoconazole (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 6.72 ± 0.11 (2.8), 8.10 ± 1.26 (26.9), 4.58 ± 1.77 (67.0), and 6.48 ± 0.15 (4.1) µM, respectively. The overall task group mean ± SEM IC_{50} value was 6.47 ± 0.72 µM and the percent CV was 22.4 percent.

For 4-nonylphenol, increasing concentrations decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent. At a 4-nonylphenol concentration of 10^{-3} M, percent of control values were less than 1 percent for three of the four labs (the highest concentration tested for the remaining lab, 10^{-4} M, resulted in a value of 16 percent). In contrast, at a 4-nonylphenol concentration of approximately 10^{-6} M, there was little to no aromatase inhibition (percent of control values were greater than or equal to 91 percent for all labs). The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-6} M were 0.26 ± 0.10 and 97.16 ± 2.24 percent, respectively. The overall task assay precision ranged from 3 to 17 percent for those concentrations that were tested by 3 or more labs, except at

concentrations of 10^{-3} and 10^{-4} M where the % CV values were 65 and 40 percent respectively. The IC_{50} values for 4-nonylphenol (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 25.37 ± 0.65 (4.4), 37.98 ± 10.57 (48.2), 26.7 ± 0.2 (1.5), and 26.31 ± 0.71 (4.7) μM, respectively. The overall task group mean ± SEM IC_{50} value was 29.10 ± 2.97 μM and the percent CV was 20.4 percent.

For prochloraz, increasing concentrations decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent. At a procloraz concentration of 10^{-6} M, percent of control values ranged from 2 to 3 percent for three of the four labs (one lab reported a negative value at this concentration). In contrast, at a prochloraz concentration of approximately 10^{-9} M, there was little to no aromatase inhibition (percent of control values were approximately 95 percent or greater for three of four labs and, for the remaining lab, was 88 percent). The overall task mean ± SEM percent of control values at 10^{-6} and 10^{-9} M were 0.76 ± 1.64 and 94.46 ± 2.44 percent, respectively. The overall task assay precision ranged from 3 to 5 percent for those concentrations $\leq 10^{-8}$ M that were tested by 3 or more labs, whereas at higher concentrations, the overall task assay precision ranged from 16 to 434 percent. The IC_{50} values for prochloraz (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 0.0202 ± 0.0018 (15.8), 0.0269 ± 0.0031 (20.3), 0.0408 ± 0.0220 (93.2), and 0.0258 ± 0.0013 (8.9) μM, respectively. The overall task group mean ± SEM IC_{50} value was 0.0284 ± 0.0044 μM and the percent CV was 30.8 percent.

Inhibition curves were characterized as "complete" by all laboratories for all reference chemicals, except for atrazine and dibenz[a,h]anthracene, which were non-inhibitors.

The principal results of the interlaboratory analysis are summarized below.

A. Concentration Response Relations

Concentration response relations were fitted to eight of the ten test substances. Two test substances, dibenz[a,h]anthracene and atrazine, were noninhibitors and concentration response relation fits were not carried out.

1. Differences between the Battelle and In Vitro microsomes. For the most part there were little or no significant differences between the Battelle and In Vitro microsomes. For the $\log_{10}IC_{50}$ there was a slight difference for 4-nonylphenol, with the Battelle microsomes having a slightly (but significantly) lower $\log_{10}IC_{50}$ than the In Vitro microsomes (i.e. the Battelle microsomes were slightly more active on average). There were no other significant differences. For slope there were no significant differences. For the top threshold, for ketoconazole the Battelle microsomes were on average lower than the In Vitro microsomes. Otherwise there were no significant differences between microsome sources. For the bottom threshold, there were no significant differences between microsome sources.

2. Differences among laboratories for $\log_{10}IC_{50}$. The estimates for the $\log_{10}IC_{50}$ were similar among the four laboratories for most chemicals except for econazole. For econazole, the estimated $\log_{10}IC_{50}$ for In Vitro was more negative than the other three laboratories, and the associated 95 percent confidence interval for In Vitro did not overlap with those for the other three laboratories. The CVs for the IC_{50} (note that CVs were calculated for IC_{50} rather than

$\log_{10}IC_{50}$) were 22.5 percent for econazole, 22.6 percent for chrysin, 47.4 percent for dicofol, and less than 15 percent for the other five chemicals. The ratios of the among laboratory variances to the pooled within laboratory variance ratios were 6.8 for econazole, 4.6 for chrysin, 2.3 for prochloraz, and less than 0.05 for the remaining five chemicals. The relatively high CVs for econazole and for chrysin might be explained by their relatively large among laboratory variances. However the relatively large CV for dicofol was due to the relatively large within laboratory variances, which were for the most part an order of magnitude larger than those for the other test substances. The pooled within laboratory variances were 0.045 for dicofol and less than or equal to 0.002 for the other test substances.

3. Differences among laboratories for slope. The estimates for the slope were similar among the four laboratories for most chemicals except for econazole and 4-nonylphenol. For econazole the estimated slope for In Vitro was less negative than those for the other three laboratories. The 95 percent confidence interval for In Vitro did not overlap with those for the other three laboratories. For 4-nonylphenol the estimated slope for In Vitro was more negative than those for the other three laboratories. The CVs for the slope were 10.2 percent for chrysin, 15.2 percent for dicofol, and were less than nine percent for the other six test substances. The among laboratory variances to the pooled within laboratory variance ratios were 9.0 for econazole and zero for the other seven test substances.

4. Differences among laboratories for top and bottom thresholds. The estimates for top were similar across all the laboratories. The CVs were less than 2.8 percent. For the bottom estimates In Vitro differed somewhat from the other laboratories for 4-nonylphenol, chrysin, and dicofol. For 4-nonylphenol and dicofol the bottom threshold at In Vitro was higher than those of the other laboratories. For chrysin the bottom threshold at In Vitro was lower. For the other five test substances the bottom threshold estimates were in agreement among laboratories.

B. Control Activity

The control responses were analyzed combined across chemicals. Comparisons between the ends and the beginnings of replicates within laboratories were carried out for the full enzyme activity, background activity, positive, and negative controls. Comparisons between the Battelle microsomes and the In Vitro microsomes were carried out for positive and negative controls.

1. Comparisons between the ends and the beginnings of replicates.

   a. For the background activity controls there were no significant differences between the beginnings and the ends of replicates, for each laboratory and combined across laboratories.
   b. For the full enzyme activity controls the ends of replicates had lower activity than the beginnings, for each laboratory and combined across laboratories.
   c. For the negative controls the ends of replicates had lower activity than the beginnings for RTI, Battelle, and WIL. The ends had greater activity than the beginnings for In Vitro. The confidence interval at In Vitro did not overlap with those from any of the other laboratories. The results at In Vitro were inconsistent with those at the other laboratories. Combined across laboratories, there was not a significant difference

between beginnings and ends of replicates. This was due to the very large among laboratories variance.

d. The results for the positive controls were similar to those for the negative controls, but not as extreme. For the positive controls the ends of replicates had lower activity than the beginnings for RTI, Battelle, and WIL. The ends had nearly significantly greater activity than the beginnings for In Vitro. The confidence interval at In Vitro did not overlap with those from any of the other laboratories. The results at In Vitro were inconsistent with those at the other laboratories. Combined across laboratories, there was not a significant difference between beginnings and ends of replicates. This was due to the relatively large among laboratories variance.

2. Comparisons between the Battelle and the In Vitro microsomes.

Comparisons were not carried out for the background activity and the full enzyme activity controls, since the sum of their percent of control within each replicate of each test was necessarily constrained to 0 percent or 100 percent by definition, thereby masking any differences across laboratories.

a. For the positive controls there were no significant differences between the Battelle microsomes and the In Vitro microsomes for each laboratory and combined across laboratories.

b. For the negative controls there were no significant differences between the Battelle microsomes and the In Vitro microsomes for Battelle and for WIL Laboratories. The Battelle microsomes had significantly less activity than the In Vitro microsomes for RTI, for In Vitro, and combined across laboratories. The difference between microsomes sources was much more pronounced for In Vitro than for RTI or combined across laboratories.

C. Interlaboratory Statistical Analysis Summary and Conclusions

In Vitro differed from the other laboratories with respect to $\log_{10}IC_{50}$ and slope for econazole, for slope for 4-nonylphenol, for differences between end and beginning portions for negative and positive controls, and for the difference between Battelle and In Vitro for negative controls. It is not understood why the activity for the negative and positive controls at In Vitro increased between the beginning and end portions of replicates.

In future analyses comparisons between sources of microsomes should be based on unnormalized aromatase activity endpoints rather than percent of control. Normalizing by the sum of the full enzyme activity control values within each replicate completely masks differences between sources in aromatase activity of full enzyme activity controls and likely at least partially masks differences between sources in aromatase activity of positive and negative controls.

1.0 INTRODUCTION

1.1 BACKGROUND

The Food Quality Protection Act of 1996 was enacted by Congress to authorize the Environmental Protection Agency (EPA) to implement a screening program on pesticides and other chemicals found in food or water sources for endocrine effects in humans. Thus, the U.S. EPA is implementing an Endocrine Disruptor Screening Program (EDSP). In this program, comprehensive toxicological and ecotoxicological screens and tests are being developed for identifying and characterizing the endocrine effects of various environmental contaminants, industrial chemicals, and pesticides. The program's aim is to develop a two-tiered approach, e.g., a combination of *in vitro* and *in vivo* mammalian and ecotoxicological screens (Tier 1) and a set of *in vivo* tests (Tier 2) for identifying and characterizing endocrine effects of pesticides, industrial chemicals, and environmental contaminants. Validation of the individual screens and tests is required, and the Endocrine Disruptor Methods Validation Advisory Committee (EDMVAC) will provide advice and counsel on the validation assays.

Estrogens are sex steroid hormones that are necessary for female reproduction and affect the development of secondary sex characteristics of females. Estrogens are biosynthesized from cholesterol by a series of enzymatic steps, with the last step involving the conversion of androgens into estrogens by the enzyme aromatase. Estrogen biosynthesis occurs primarily in the ovary in mature, premenopausal women. During pregnancy, the placenta is the main source of estrogen biosynthesis and pathways for production change. Small amounts of these hormones are also synthesized by the testes in the male and by the adrenal cortex, the hypothalamus, and the anterior pituitary in both sexes. The major source of estrogens in both postmenopausal women and men occurs in extraglandular sites, particularly in adipose tissue. One potential endocrine target for environmental chemicals is the enzyme aromatase, which catalyzes the biosynthesis of estrogens. An aromatase assay is proposed as one of the Tier 1 Screening Battery Alternate Methods. A detailed literature review on aromatase was performed and encompassed (1) searching the literature databases, (2) contacting individuals to obtain information on unpublished research, and (3) evaluating the literature and personal communications.

Aromatase is a cytochrome $P450_{arom}$ enzyme complex responsible for estrogen biosynthesis and converts androgens, such as testosterone and androstenedione, into the estrogens estradiol and estrone. Aromatase is present in the ovary, placenta, uterus, testis, brain, and extraglandular adipose tissues. Two proteins, cytochrome $P450_{arom}$ and NADPH-cytochrome P450 reductase, are necessary for enzymatic activity, and the enzyme complex is localized in the smooth endoplasmic reticulum. The aromatase gene, designated CYP19, encodes the cytochrome $P450_{arom}$ and consists of ten exons, with the exact size of the gene exceeding 70 kilobases. Aromatase is found in breast tissue, and the importance of intratumoral aromatase and local estrogen production is being unraveled. Effective aromatase inhibitors have been developed as therapeutic agents for estrogen-dependent breast cancer to reduce the growth stimulatory effects of estrogens in breast cancer. Investigations on the development of aromatase inhibitors began in the 1970's and have expanded greatly in the past three decades.

An *in vitro* aromatase assay could easily be utilized as an alternative screening method in the Tier 1 Screening Battery to assess the potential effects of various environmental toxicants on

aromatase activity. Both *in vitro* subcellular (microsomal) assays and cell-based assays are available for measuring aromatase activity. The *in vitro* subcellular assay using human placental microsomes is commonly used to evaluate the ability of pharmaceuticals and environmental chemicals to inhibit aromatase activity. In addition, human JEG-3 and JAR choriocarcinoma cell culture lines, originally isolated from cytotrophoblasts of malignant placental tissues, have been used as *in vitro* systems for measuring the effects of compounds on aromatase activity. These cell lines are also utilized for investigations on the effects of agents in placental toxicology.

Numerous flavonoids and related phytoestrogen derivatives have been extensively evaluated for their ability to inhibit aromatase activity for two primary reasons: (1) these natural plant products can serve as possible leads for the development of new nonsteroidal aromatase inhibitors; and (2) humans and other animals are exposed to these agents through the diet. In general, the flavonoids and related analogs demonstrate aromatase inhibition with IC_{50} values in the micromolar range; however, these compounds lack both the potency and specificity of aromatase inhibitors developed for breast cancer therapy. Several pesticides have also demonstrated inhibition of aromatase activity in the human placental microsomal assay system, with IC_{50} values for aromatase inhibition ranging from 0.04 μM to greater than 50 μM.

The human placental microsomal aromatase assay was recommended as the *in vitro* aromatase screening assay to be included in the Tier 1 Screening Battery. This assay will detect environmental toxicants that possess the ability to inhibit aromatase activity. Prevalidation studies on recombinant aromatase (WA 2-24) were conducted to optimize the microsomal aromatase assay protocol for human placenta, demonstrate the utility of the microsomal assay to detect known aromatase inhibitors, and compare the performance of a recombinant assay system and the placental microsomal assays.

1.2 TASK DESCRIPTION AND OBJECTIVES

In this task, the aromatase assay was conducted by staff from a lead laboratory (RTI International) and three participating laboratories (Battelle, In Vitro Technologies, and WIL Research Laboratories). Battelle and In Vitro each obtained a human placenta, which was used to prepare microsomes (see report for WA 4-16, Task 6). The task was organized so that across the ten chemicals the allocation of microsomes to reference chemicals was approximately balanced. Each laboratory analyzed five chemicals with the microsomes prepared by Battelle and five chemicals with the microsomes prepared by In Vitro. Across the 10 reference chemicals the laboratories were approximately balanced for comparison of the microsomes from the two laboratories. Each laboratory conducted at least three independent replicates of the placental aromatase assay using the following reference chemicals: aminoglutethimide, atrazine, chrysin, dibenz [a,h] anthracene, dicofol, econazole, fenarimol, ketoconazole, 4-nonylphenol, and prochloraz, as well as 4- hydroxyandrostenedione (positive control) and lindane (negative control). The study design involved, for each reference chemical, conducting three repetitions at each of eight concentrations, and for the positive and negative controls, conducting four repetitions at a single concentration, for each of at least three independent replicates. Reagents and assay solutions were made fresh for each replicate so that the replicates were truly independent. The reference chemicals and positive and negative control stock solutions used for testing were prepared and analyzed at a central laboratory (Chemical Repository at Battelle) before they were distributed to the laboratories.

The objectives of this task were to evaluate the responsiveness of human placental microsomes (prepared by two participating laboratories) for conducting the aromatase assay using ten reference chemicals and a positive and negative control when the assay was performed by a lead and three participating laboratories, as well as to obtain intralaboratory and interlaboratory values for the aromatase enzyme activity and aromatase inhibition for each of the reference chemicals and the positive and negative controls.

1.3 OVERALL REPORT CONTENT AND FORMAT

The overall report body includes salient information about the methods used and results obtained by the individual laboratories, summary and conclusions of the intralaboratory statistical analysis, the interlaboratory statistical analysis, discussion and conclusions. Detailed information about the methods used and results obtained by the individual laboratories can be found in their reports, which are included in the appendices of the overall report. In addition, there are a few important supplemental documents that were the same for all laboratories, i.e. chemistry reports and quality assurance project plan (QAPP), and these documents are also included in the appendices. Other laboratory specific documents, i.e. protocol, spreadsheets, intralaboratory statistical analysis reports, etc., were included in the appendices of the individual laboratory reports.

2.0 MATERIALS AND METHODS

2.1 CHEMISTRY

2.1.1 Substrate – Androstenedione (ASDN)

The substrate for the assay was androstenedione (ASDN). Non-radiolabeled and radiolabeled ASDN were obtained by Battelle's Chemical Repository and then distributed to the lead and participating laboratories so that all laboratories used the same lot of the substrate. The non-radiolabeled ASDN had a reported purity of 100%. The radiolabeled androstenedione ([1β-^{3}H]-androstenedione, [^{3}H]ASDN had a reported specific activity of 25.3 Ci/mmol. Radiochemical purity was reported by the supplier to be > 97%. Radiochemical purity was assessed by high performance liquid chromatography (HPLC) by the lead laboratory and was determined to be 97% (RTI's report for radiochemical purity is included in the individual laboratory report appendices).

ASDN substrate solution preparation is described in detail in the laboratory reports. Briefly, the substrate solution was prepared fresh each day by combining solutions of [^{3}H]ASDN and non-radiolabeled ASDN in order to prepare a solution that contained 2 µM ASDN with approximately 1 µCi/mL. The addition of 100 µL of the substrate solution to each 2 mL assay volume yielded a final [^{3}H]ASDN concentration of 100 nM with approximately 0.1 µCi/tube.

2.1.2 Reference Chemicals and Control Substances

The Chemical Repository at Battelle was responsible for the chemistry activities performed on the ten reference chemicals and two control substances. The chemistry activities included chemical procurement, solubility, formulation stability assessment, formulation preparation, formulation analysis and shipment of the stock formulations to the lead and participating laboratories. These chemistry activities and results of the analysis and stability

determinations are described in the Chemical Repository chemistry reports (see appendices). Table 1 summarizes the salient information for the reference chemicals and control substances.

Table 1. Chemistry Information for the Reference Chemicals and Control Substances

Reference Chemical (Code Number)[a]	Mfr. Purity	CAS No.	Molecular Formula	Molecular Weight (g/mol)	Basis for Selection
Reference Chemicals					
Aminoglutethimide (1)	>99	125-84-8	$C_{13}H_{16}N_2O_2$	232.3	Non-steroidal aromatase inhibitor
Atrazine (10)	98	1912-24-9	$C_8H_{14}ClN_5$	215.7	Affects aromatase gene; no aromatase inhibition
Chrysin (8)	98.2	480-40-0	$C_{15}H_{10}O_4$	254.2	Potent flavenoid aromatase inhibitor
Dibenz [a,h] anthracene (5)	97	53-70-3	$C_{22}H_{14}$	278.4	Non-aromatase inhibitor; Ah receptor agonist
Dicofol (9)	96.5	115-32-2	$C_{14}H_9Cl_5O$	370.5	Organochlorine
Econazole (7)	98	24169-02-6	$C_{18}H_{15}Cl_3N_2O \cdot HNO_3$	444.7	Potent imidazole anti-fungal aromatase inhibitor
Fenarimol (6)	99	60168-88-9	$C_{17}H_{12}Cl_2N_2O$	331.2	Pyrimidine fungicide
Ketoconazole (2)	>99	65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	531.4	Weak imidazole anti-fungal aromatase inhibitor
4-Nonylphenol (4)	>98.5	84852-15-3	$C_{15}H_{24}O$	220.4	Affects AR/ER; no known aromatase inhibition
Prochloraz (3)	99.5	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	Conazole fungicide
Controls					
4-hydroxyandrostenedione (Positive Control)	99	566-48-3	$C_{19}H_{26}O_3$	302.4	Known aromatase inhibitor
Lindane (Negative Control)	99.6	58-89-9	$C_6H_6Cl_6$	290.8	Known not to inhibit aromatase

a. Reference chemicals were tested blind using the assigned code number.

The reference chemical and control chemical stock formulations were prepared by the Chemical Repository (CR) and shipped to the laboratories. The laboratories instituted appropriate procedures so that the reference chemical stock formulations were tested blind. The reference chemical stock formulation concentration was 0.1 M for all reference chemicals, except for ketoconazole, dibenz[a,h]anthracene, and chrysin, which had a stock concentration of 0.01 M. Due to a miscommunication by the CR, RC-1 and RC-2 were erroneously logged in at

the laboratories as ketoconazole and aminoglutethimide, respectively, and the stock concentrations were switched. For this reason, the actual dilutions tested for aminoglutethimide were 10^{-3} to 10^{-9} M rather than 10^{-3} to 10^{-10} M as planned and, for ketoconazole were 10^{-4} to 10^{-11} M rather than 10^{-4} to 10^{-10} as planned.

The dilution schemes used by the laboratories to prepare the appropriate concentrations of the reference chemicals and control substances for testing are presented in the individual laboratory reports. Fresh dilutions were prepared on the day of testing. The same vehicle used to prepare the stock solution was used in the preparation of the dilutions. For the first replicate, the reference chemicals were tested at eight target concentrations ranging from 10^{-3} to 10^{-10} M. For the second and third replicates, the results for replicate 1 were reviewed and adjustments were made as necessary to the concentrations tested in replicates 2 and 3 in order to obtain data that better described the curve. The control substances were each tested at a single concentration of 5 x 10^{-8} M for 4-OH ASDN and 10^{-6} M for lindane. The vehicle for the ten reference chemicals and lindane was dimethylsulfoxide (DMSO), whereas for 4-OH ASDN it was ethanol.

2.2 HUMAN PLACENTAL MICROSOMES

Human placental microsomes were provided to each of the laboratories by Battelle (Lot # 6-041305, 21 mg protein/mL, ~200 µL/vial) and In Vitro (Lot # BAA, 8 mg protein/mL, ~ 150 µL/vial). The microsomes were stored at approximately -70°C until the time of the assay. On the day of use, the microsomes were thawed rapidly in a 37 ± 1°C water bath, rehomogenized using a Potter-Elvejhem homogenizer and then kept on ice until used. For use in the assay, the microsomes were diluted in the assay buffer (see below for the various dilution schemes used). The time between thawing of the microsomes and their use in the assay was limited to less than 2 hours and, in most cases, was about 30 to 60 minutes. The final target protein concentration in the incubation mixture was approximately 0.0125 mg/mL. None of the microsome vials used were thawed, re-frozen, and thawed again for use; rather, the microsomes used for a given experiment came from a single use vial.

RTI's dilution scheme for the Battelle-prepared microsomes involved a serial dilution of 1:80 and then 1:10 for a total dilution of 1:800. For the In Vitro-prepared microsomes, RTI combined two separate tubes and diluted in a single step to a final dilution of 1:320. Battelle's dilution scheme for the Battelle-prepared microsomes involved a serial dilution of 1:50 and then 1:19 for a total dilution scheme of 1:950. For the In Vitro-prepared microsomes, Battelle performed a single dilution of 1:350. In Vitro's dilution scheme for the Battelle-prepared microsomes involved a single direct dilution of 1:800 and, for their own microsomes (In Vitro-prepared), a single direct dilution of 1:320 was used. WIL's dilution scheme for the Battelle-prepared microsomes involved a single direct dilution of 1:843 and, for the In Vitro-prepared microsomes, involved a single direct dilution of 1:321 or 1:401, depending on the replicate.

The source of microsomes used by each laboratory in the testing of each reference chemical was defined in the protocol and is presented in Table 2. The microsomes from Battelle are shown in the table as "BATTELLE", whereas the microsomes from In Vitro are shown in the table as "*IN VITRO*".

Table 2. Source of Microsomes by Reference Chemical and Laboratory[a]

Reference Chemical (RC Blind Code)	RTI	Battelle	In Vitro	WIL
Aminoglutethimide (1)	BATTELLE	BATTELLE	*IN VITRO*	*IN VITRO*
Ketoconazole (2)	BATTELLE	BATTELLE	*IN VITRO*	*IN VITRO*
Prochloraz (3)	*IN VITRO*	*IN VITRO*	BATTELLE	BATTELLE
4-Nonylphenol (4)	BATTELLE	*IN VITRO*	*IN VITRO*	BATTELLE
Dibenz[a,h]anthracene (5)	*IN VITRO*	BATTELLE	BATTELLE	*IN VITRO*
Fenarimol (6)	*IN VITRO*	*IN VITRO*	BATTELLE	BATTELLE
Econazole (7)	BATTELLE	*IN VITRO*	BATTELLE	*IN VITRO*
Chrysin (8)	BATTELLE	*IN VITRO*	BATTELLE	*IN VITRO*
Dicofol (9)	*IN VITRO*	BATTELLE	*IN VITRO*	BATTELLE
Atrazine (10)	*IN VITRO*	BATTELLE	*IN VITRO*	BATTELLE

a. BATTELLE – denotes Battelle was the source of the microsomes. *IN VITRO* - denotes In Vitro was the source of the microsomes.

2.3 OTHER ASSAY COMPONENTS

In addition to substrate, reference or control chemical or vehicle, and microsomes, the aromatase assay contained NADPH (β-Nicotinamide Adenine Dinucleotide Phosphate, reduced form), propylene glycol, and phosphate buffer. Information about these other assay components is provided in Table 3. The Chemical Repository (CR) obtained the NADPH, DMSO, and ethanol and distributed it to the participating laboratories. The laboratories purchased their own propylene glycol and buffer materials.

Table 3. Source Information about the Other Assay Components

Chemical	RTI	Battelle	In Vitro	WIL
NADPH (co-factor)[a]	Sigma	Sigma	Sigma	Sigma-Aldrich
Propylene glycol	J. T. Baker	Spectrum Chemical	J. T. Baker	J. T. Baker
Sodium phosphate dibasic (buffer)	J. T. Baker	Sigma	J. T. Baker	J. T. Baker
Sodium phosphate monobasic (buffer)	J. T. Baker	Sigma	J. T. Baker	J. T. Baker
DMSO[b]	Battelle CR	Battelle CR	Battelle CR	Battelle CR
95% Ethanol[c]	Battelle CR	Battelle CR	Battelle CR	Battelle CR

a. Two different lots of NADPH were used.
b. Five different lots of DMSO were used.
c. Three different lots of ethanol were used.

NADPH was prepared fresh each day (6 mM, 5 mg/mL) in 0.1 M sodium phosphate buffer (pH of 7.4) and kept on ice until used.

Sodium phosphate monobasic and sodium phosphate dibasic solutions (0.1 M of each) were combined to prepare a final 0.1 M solution at a pH of 7.4. The assay buffer was stored refrigerated for up to one month.

Further details about preparation and storage are provided in the laboratory reports (see appendices).

2.4 PROTEIN DETERMINATION

Protein concentration of the microsomal preparation was determined on each day of use of the microsomes in the aromatase assay. The microsomal protein concentration was determined using a DC Protein Assay kit from Bio-Rad (Hercules, CA). The 6-point standard curve was prepared using bovine serum albumin (BSA) reconstituted in Milli-Q water. The standard curve range was 5 to 250 µg protein/mL. For all laboratories, except In Vitro, an unacceptable level of non-linearity occurred when the 250 µg/mL standard was included, which resulted in the three of the four labs using a 5-point standard curve ranging from 5 to 125 µg/mL. In Vitro chose to retain the 250 µg/mL standard and used a 6-point standard curve in the analysis. The absorbance at a wavelength of 750 nm was measured using a spectrophotometer. The protein concentration of the microsomal sample was determined from the absorbance value using linear regression to the absorbance of the protein standards.

During the conduct of the work assignment, quality control (QC) samples were included in the assay. QC samples were supplied by RTI. The target concentrations of the QC samples were 10 and 100 µg/mL and the reported concentrations were 12 and 110 µg/mL (RTI report). The QC samples were analyzed in duplicate using the protein determination method described in the preceding paragraph. Additional information about the unknowns and QC sample protein determinations can be found in the laboratory reports.

2.5 AROMATASE ASSAY PROCEDURE

Details of how the assay was actually performed by each participating laboratory are presented in the individual laboratory reports. The general procedure followed by the laboratories is described below.

Three independent replicates were conducted (on separate days) for each reference chemical with all replicates for a given reference chemical conducted by the same technician. In some instances, a laboratory tested two reference chemicals on the same day (making sure to pair the reference chemicals with the appropriate source of microsomes), thereby sharing a set of controls. Each reference chemical was tested at eight concentrations and there were three (triplicate) repetitions for each concentration of a given replicate. A single replicate study of an example reference chemical is shown in Table 4.

Table 4. Reference Chemical Study Design

Sample Type	Repetitions (Test Tubes)	Description	Final Reference Chemical or Control Substance Concentration (M)[a]
Full Enzyme Activity Control	4	Complete assay[b] with inhibitor vehicle control	N/A
Background Activity Control	4	Complete assay with inhibitor vehicle control omitting NADPH	N/A
Positive Control	4	Complete assay with positive control chemical (4-OH ASDN) added	5×10^{-8}
Negative Control	4	Complete assay with negative control chemical (lindane) added	1×10^{-6}
Reference Chemical Concentration 1	3	Complete assay with Reference Chemical added	1×10^{-3}
Reference Chemical Concentration 2	3	Complete assay with Reference Chemical added	1×10^{-4}
Reference Chemical Concentration 3	3	Complete assay with Reference Chemical added	1×10^{-5}
Reference Chemical Concentration 4	3	Complete assay with Reference Chemical added	1×10^{-6}
Reference Chemical Concentration 5	3	Complete assay with Reference Chemical added	1×10^{-7}
Reference Chemical Concentration 6	3	Complete assay with Reference Chemical added	1×10^{-8}
Reference Chemical Concentration 7	3	Complete assay with Reference Chemical added	1×10^{-9}
Reference Chemical Concentration 8	3	Complete assay with Reference Chemical added	1×10^{-10}

a. Reference chemical concentrations for first replicate are shown (assuming the stock solution concentration was 0.1 M) . The concentrations tested for replicates 2 and 3 varied depending on the results of the first replicate.
b. The complete assay contained buffer, propylene glycol, microsomal protein, [^{3}H]ASDN and NADPH.

Four types of control samples were included with each replicate. These included:

- Full enzyme (aromatase) activity controls (substrate, NADPH, propylene glycol, buffer, vehicle [used for preparation of reference chemical solutions] and microsomes).
- Background activity controls (all components found in the full aromatase activity control, except NADPH).
- Positive controls (all components found in the full aromatase activity controls, except vehicle, and with the addition of 4-OH ASDN at a concentration of 5×10^{-8} M).
- Negative controls (all components found in full aromatase activity controls, except vehicle, and with the addition of lindane at a concentration of 1×10^{-6} M).

Four test tubes of each type of control were included with each replicate and were treated the same as the other samples. The controls sets were split so that two tubes (of each control type) were run at the beginning and two at the end of each replicate set.

The aromatase assay was performed in test tubes maintained at 37 ± 1°C in a shaking water bath. Propylene glycol, [^{3}H]ASDN, NADPH, and reference chemical (or control or vehicle), and assay buffer were combined in the test tubes to a total volume of 1.0 mL. The volumes and final concentrations for the assay major components are presented in Table 5. The tubes and the microsomal suspension were placed at 37 ± 1°C in the water bath for approximately 5 minutes prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension.

Table 5. Aromatase Assay Components (Volumes and Final Concentrations)

Assay Components	Component Volume Added to the Assay	Final Concentration in the Assay
Microsomal Protein	1.0 mL	0.0125 mg/mL
NADPH	100 µL	0.3 mM
[^{3}H]ASDN	100 µL	100 nM
Propylene glycol	100 µL	5% (v/v)
Reference Chemical, Control Substance, or Vehicle	20 µL	Varied
Incubation Time	15 min	Not Applicable

The total assay volume was 2.0 mL and the tubes were incubated for 15 minutes at 37 ± 1°C. The incubations were stopped by the addition of methylene chloride (2 mL); the tubes were vortex-mixed for ca. 5 seconds and placed on ice. The tubes were then vortex-mixed an additional 20-25 seconds to extract unreacted ASDN, then centrifuged for 10 minutes to facilitate separation of the organic and aqueous layers. The methylene chloride layer was removed and discarded; the aqueous layers were extracted two more times, each time with 2 mL of methylene chloride. The aqueous layers were transferred to vials and duplicate aliquots (0.5 mL) were transferred to 20 mL liquid scintillation counting vials. Liquid scintillation cocktail was added to each counting vial and the vials shaken to mix.

Analysis of the samples was performed using liquid scintillation spectrometry (LSS). Radioactivity found in the aqueous fractions represented ^3H_2O formed from the hydrolysis of [^{3}H]-ASDN. One H_2O molecule was released per molecule of ASDN converted to estrogen in a stereospecific reaction. Thus, the amount of estrogen product formed was determined by dividing the total amount of ^3H_2O formed by the specific activity of the [^{3}H]ASDN substrate (expressed in dpm/nmol). Results are presented as the activity (velocity) of the enzyme reaction and expressed in nmol (mg protein)$^{-1}$min^{-1}.

2.6 DATA ANALYSIS - AROMATASE ACTIVITY AND PERCENT OF CONTROL CALCULATIONS

Each laboratory entered data into an Excel spreadsheet (provided by RTI and Battelle) for calculation of aromatase activity and percent of control. For each repeat tube (full enzyme activity control, background activity control, positive and negative controls and each reference chemical concentration), the Excel spreadsheet included total observed (uncorrected) disintegration per minute (dpm) per tube and total aromatase activity per tube. The dpm and aromatase activity values were corrected for the background dpm's, as measured by the average of the background activity control tubes. The aromatase activity was calculated as the corrected dpm, normalized by the specific activity of the [^{3}H]ASDN, the mg of protein of the aromatase, and the incubation time. The average (corrected) dpm and aromatase activity across the four background activity control repeat tubes must necessarily be equal to 0 (zero) within each replicate.

For each tube, percent of control was determined by dividing the background corrected aromatase activity for that tube by the average background corrected aromatase activity for the four full enzyme activity control tubes and multiplying by 100. It was expected that for an inhibitor the percent of control activity values could vary between approximately 0% near the high inhibition concentrations and approximately 100% near the low inhibitions concentrations. However, due to experimental variation individual observed percent of control values sometimes extended below 0% or above 100%.

The spreadsheet calculated dpm/mL for each aliquot of extracted aqueous incubation mixture and average dpm/mL and total dpm for each aqueous portion (after extraction). Multiplication of the volume (mL) of substrate solution added to the incubation by the substrate solution radiochemical content (dpm/mL) yielded the total dpm present in the assay tube at initiation. The total dpm remaining in the aqueous portion after extraction divided by the total dpm present in the assay tube at initiation times 100 yielded the percent of the substrate that was converted to product. The total dpm remaining in the aqueous portion after extraction was corrected for background by subtracting the average dpm present in the aqueous portion of the background activity control tubes (for that day/assay). This corrected dpm was then converted to nmol product formed by dividing by the substrate specific activity (dpm/nmol). The activity of the enzyme was expressed in nmol (mg protein)$^{-1}$min^{-1} and was calculated by dividing the amount of estrogen formed (nmol) by the amount of microsomal protein used (in mg) times the incubation time (in min). Average activity in the full activity control samples for a given study was calculated. Percent of activity remaining in the presence of various inhibitor concentrations was calculated by dividing the aromatase activity at a given inhibitor concentration by the average positive full activity control and multiplying by 100.

2.7 STATISTICAL ANALYSIS

The intralaboratory statistical analysis for RTI, Battelle, and WIL were performed by the laboratories statistical analysis services. For In Vitro, Battelle provided the intralaboratory statistical analysis. This analysis was performed according to the plan in the protocol.

The interlaboratory statistical analysis was conducted by Battelle (EDSP Data Coordination Center).

2.7.1 Intralaboratory Statistical Analysis

2.7.1.1 Concentration Response Trend Curves

For each test substance and replicate within test substance a concentration response curve was fitted to the percent of control activity values at the three repetitions at each of the eight graded test substance inhibitor concentrations.

For purposes of response curve fitting, concentration was expressed on the log scale. In agreement with past convention, common logarithms (i.e. base 10) were used. Let X denote the logarithm of the concentration of inhibitor compound (e.g. if concentration = 10^{-5} then X = -5). Let

Y ≡ (background corrected) percent of control in the inhibitor tube
X ≡ logarithm (base 10) of the concentration
DAVG ≡ average (not corrected for background) DPMs across the repeat tubes with the same inhibitor concentration
T ≡ top of plateau (maximum response)
B ≡ bottom of plateau (baseline response)
β ≡ slope of the concentration response curve (β is negative)
$\mu \equiv \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to the percent of control halfway between baseline and maximum responses).

The following four parameter concentration response curve was fitted to relate percent of control activity to logarithm of concentration within each replicate

$$Y = \mathbf{B} + (\mathbf{T}-\mathbf{B})/[1 + 10^{(\mu - X)\beta}] + \epsilon$$

where ϵ is the variation among repetitions, distributed with mean 0 and variance proportional to DAVG (based on Poisson distribution theory for radiation counts) and also approximately proportional to the response Y.

The response curve was fitted by non-weighted least squares nonlinear regression analysis. Model fits were carried out using PRISM software (Version 4).

The slope "β" is an alternative and equivalent notation to what is referred to in amendment 2 to the protocol as the hillslope "H". The random variation term "ϵ" is not explicitly included in the concentration response curve equation stated in Amendment 2 to the protocol but is tacitly assumed to be included in the model specified there.

For each replicate, the estimated baseline response (**B**) and its associated standard error, the estimated maximum response (**T**) and its associated standard error, the estimated $\log_{10}IC_{50}$ (μ) and its associated standard error, the IC_{50} and its associated geometric standard error, the slope (β) and its associated standard error, and the "Status" of each response curve are reported.

The "Status" of each response curve is indicated as "C", complete, if the concentration response curve inhibition ranges from essentially 0 percent to at least 80 percent of control. It is indicated as "I", incomplete, if the concentration response curve inhibition ranges from essentially 100% inhibition to less than 50% inhibition. It is also indicated as "I", incomplete, if the concentration response curve decreases to between 80% and 50% of control. If the concentration response curve inhibition does not go below 80% of control, the test substance is a non-inhibitor, parameter estimates are not calculated.

For each of the test substances a one-way random effects analysis of variance model with heterogeneous variances among the replicates was fitted to the parameter estimates top (**T**), bottom (**B**), $\log_{10}IC_{50}$ (μ), and slope (β), from the concentration response curve fits within each replicates. The random effect was replicate within test substance. The within replicate variances were estimated as the squares of the standard errors for each replicate. The analysis of variance fits provide estimated averages effects (mean) across the replicates within test substances and their associated standard errors. These standard errors include both within replicate and between replicate components of variation. Degrees of freedom associated with the mean effects were calculated based on Satterthwaite's approximation.

The estimated IC_{50} for the test substance was estimated as 10 to the power mean $\log_{10}IC_{50}$. The geometric standard error associated with the estimated IC_{50} was calculated as 10 to the power standard error associated with mean $\log_{10}IC_{50}$.

Top (**T**), bottom (**B**), slope (β), and $\log_{10}IC_{50}$ (μ) were each compared across replicates within test substances based on the one-way random effects analysis of variance model fits. For each of **T**, **B**, β, and μ, plots were prepared that display the parameters within each replicate with associated 95% confidence intervals based on the within replicate standard error and the average across replicates with associated 95% confidence interval incorporating replicate-to-replicate variation.

Concentration response curves were fitted to the averages of the three repetitions within each replicate. Estimates and associated standard errors (or geometric standard error) for top (**T**), bottom (**B**), $\log_{10}IC_{50}$ (μ), IC_{50}, and slope (β) were displayed. The averages of the three repetitions for each replicate were plotted in the same plot with plotting symbols distinguishing among replicates. The concentration response curves for each replicate, fitted to the average data, were superimposed on the same plot to compare the percent of control activity values across replicates.

On a separate plot the average percent of control values for each replicate were plotted versus logarithm of inhibitor concentrations. The average concentration response curve across replicates was superimposed on the same plot. The average response curve was calculated as

$$Y_{avg} = \mathbf{B}_{avg} + (\mathbf{T}_{avg} - \mathbf{B}_{avg})/[1 + 10^{\beta_{avg}(\mu_{avg} - X)}]$$

where $\mathbf{B}_{avg}$, $\mathbf{T}_{avg}$, β_{avg}, and μ_{avg} were estimated across the three replicates, based on the random effects one-way analysis of variance model discussed above.

All concentration response curves were fitted to the data using the non-linear regression analysis features in the PRISM statistical analysis package, Version 4. Supplemental statistical analyses and displays such as summary tables, graphical displays, analysis of variance, and multiple comparisons were carried out using PRISM and the SAS statistical analysis system-Version 9.

2.7.1.2 Analysis of Full Enzyme Activity, Background Activity, Positive, and Negative Controls Across Test substances and Replicates Within Test Substances

Within each test substance and replicate within test substance, quadruplicate repetitions were made of the full enzyme activity, background activity, positive, and negative control responses. Half the repetitions were carried out at the beginning of the replicate and half at the end. If the test conditions were consistent throughout the replicate, the control tube responses at the beginning should be equivalent to those at the end.

The control response analyses were carried out with the control data combined across 30 replicates within the ten test substances. The control responses were expressed as percent of control. The full enzyme activity, background activity, positive, and negative controls percent of control responses were plotted across replicates, with plotting symbol distinguishing between beginning and end, and with reference line at 0% (background activity controls), at 100% (full enzyme activity controls), or at 100% (negative controls). These plots indicate the extent of consistency across replicates with respect to average value and variability, and provide comparisons of beginning versus end of each replicate. Two separate plots (with the same vertical scale) were generated for chemicals with microsomal preparation made in the two laboratories. Therefore, chemicals RC-1, RC-2, RC-5, RC-9, and RC-10 were included in one plot (microsomal preparation made at Battelle); chemicals RC-3, RC-4, RC-6, RC-7, and RC-8 were included in the other plot (microsomal preparation made at In Vitro). Additional plots were prepared displaying the difference of the average of the first two percent of control values (i.e. those based on the "beginning" tubes) and the average of the last two percent of control values (i.e. those based on the "end" tubes) across replicates (end minus beginning). Each plot has a reference line at 0. Also, two separate plots (with the same vertical scale) were generated for chemicals with microsomal preparation made in the two laboratories.

Mixed effects analysis of variance models were fitted to the full enzyme activity, background activity, positive, and negative controls percent of control responses. The fixed effect factors in the analysis of variance were source of microsomes, test substance, portion (beginning or end), and portion by test substance interaction. The random effects were replicate nested within test substance and portion by replicate within test substance interaction. The residual error variation was based on the variation among repetitions within replicate and portion. The response was percent of control. For the background activity and full enzyme activity controls the average of the repetitions within a replicate are constrained to be 0 and 100 respectively, which implies that the variation associated with the replication effect is necessarily constrained to be 0.

2.7.1.3 Round Off

Some derived numbers in the results tables may differ from those in the computer printouts or from those obtained using hand calculations by several units in the least significant

digit due to round off in intermediate numbers and in intermediate calculations.

2.7.2 Interlaboratory Statistical Analysis

Statistical analyses were carried out for each of the ten concentration response relation and control endpoints discussed above: $\log_{10}IC_{50}$, slope, top, bottom, portion effects (end minus beginning) for background activity control, full enzyme activity control, negative, and positive controls, and microsome source effects (Battelle microsomes minus In Vitro microsomes) for negative and positive controls. The analyses for the $\log_{10}IC_{50}$, the slope, the top and the bottom were performed separately for each chemical, while the analyses for the control endpoints were performed combined across chemicals.

For each of the $\log_{10}IC_{50}$, the slope, the top and the bottom parameters, a mixed effects analysis of variance model with heterogeneous variances among the participating laboratories was fitted to the summary responses within laboratories. Miscrosome source was treated as a fixed effect, and laboratory was treated as a random effect. For the control endpoints, a random effects analysis of variance model with heterogeneous variances among the participating laboratories was fitted to the summary responses within laboratories. Laboratory was treated as a random effect. For each endpoint, the within laboratory variances were based on the squares of the standard errors associated with the endpoint estimates obtained in each of the intralaboratory analyses. The analysis of variance provided an estimated weighted average across all the laboratories and its associated standard error as well as an estimate of the laboratory-to-laboratory component of variation. The weights included in the weighted averages incorporated both laboratory-to-laboratory variation and within laboratory variation. The degrees of freedom associated with the overall weighted average were calculated as

$$2*[((1/K)*\sum(S_L^2 + S_i^2))^2]/[(var(S_L^2)+(2/K^2)*\sum(S_i^4/df_i))]$$

where S_L^2 is the random laboratory to laboratory variance, S_i^2 and df_i are the reported within laboratory variance and degrees of freedom for the i^{th} laboratory, $var(S_L^2)$ is the variance of S_L^2, and K is the number of laboratories (Hartung and Makambi, 2001).

For each endpoint, the estimated overall average and its associated standard error (incorporating both within laboratory and among laboratory components of variation) and associated degrees of freedom were used to construct a 95 percent confidence interval. For each laboratory the individual effect and associated 95 percent confidence interval (based on the within laboratory standard error) were also determined. These were plotted side-by-side to provide a graphical comparison among the laboratories.

When calculating the within laboratory mean $\log_{10}IC_{50}$, slope, top and bottom threshold parameters across replicates, all four laboratories incorporated the replicate-to-replicate component of variation into the standard errors of the averages and reported summary results in the same manner. However the four laboratories reported summary results in different manners for the background activity, the full enzyme activity, the negative and the positive controls. The differences in activity between the beginning and the end portions between the two sources of microsome were therefore necessarily determined differently for each of the laboratories.

The differences in activity between the beginning and the end portions (end minus beginning) were determined for the control endpoints as follows:

1. For RTI, the differences between the end and beginning were first calculated for each chemical. The associated standard errors for these differences were calculated as |LS Mean|/(F-value)$^{1/2}$, where F-values (and p-values) were test results for portion effect for that chemical, as provided in the intralaboratory report. Then the differences between the beginning and the end were averaged across all chemicals. The associated standard errors were calculated as $(1/10)(\sum s_i^2)^{1/2}$, where s_i is the standard error associated with the difference for the chemical.

2. For WIL, the differences between the beginning and the end portion values (averaged across chemicals) were calculated. The associated standard errors for the differences were calculated as |LS Mean|/(F-value)$^{1/2}$, where F-values were corresponding to the p-values for the portion main effect, as reported in the WIL Laboratories intralaboratory analysis report.

3. Results for Battelle and In Vitro were used as reported in the intralaboratory analysis reports (averaged across chemicals with associated standard errors).

Within each of the laboratories the intralaboratory analyses of the control responses combined across chemicals were based on mixed effects analysis of variance models that assumed random replicate effects and independent responses within replicates. The models did not account for the constraints on the full enzyme activity control responses (sum to 100 percent) or the background activity control responses (sum to 0 percent) within each replicate. This affects the standard errors that are presented in the tables in Appendix E. As an example, for the RTI aminoglutethimide background activity control the replicate-to-replicate variability is zero and so the standard error of the end minus beginning portions is given as $0.305 \equiv 0.216\ \sqrt{2}$. These standard errors given in the Statistical Analysis Report Appendix do not account for the constraints on the sums of the background activity control and the full enzyme activity control values within replicates.

For each chemical, the sum of the four repetitions within a replicate is necessarily zero for the background controls, and 100 for the full enzyme activity controls, by the definitions of the responses. Therefore the differences between the Battelle and In Vitro were always necessarily zero for the background controls and for the full enzyme activity controls and so they provide no information concerning differences between the two sources of microsomes.

For the positive controls and the negative controls the differences in activity between the two sources of microsomes (Battelle minus In Vitro) were determined as follows:

1. For RTI, for each chemical averages were calculated averages across the beginning and the end portions. For each chemical, the associated standard errors for the averages were calculated as $(1/2)(2(S_e^2+S_b^2)-S_d^2)^{1/2}$, where S_e, S_b, and S_d were the standard errors associated with the end, the beginning, and the difference between the end and the beginning respectively. S_e and S_b were reported in the intralaboratory analysis, and S_d was calculated as discussed above (see above item 1). This expression accounts for possible correlation between the beginning and the end portions within each replicate due

to replicate-to-replicate variation. The difference between the two microsome sources was then calculated as the difference between the averages over the five chemicals per source, $(1/5)(\sum M_{1i}-\sum M_{2i})$, with associated standard errors $[(\sum s_{1i}^2+\sum s_{2i}^2)/5]^{1/2}$, where M_{1i} and s_{1i} are the average and associated standard error for the i^{th} chemical within the Battelle microsomes and M_{2i} and s_{2i} are the average and associated standard error for the i^{th} chemical within the In Vitro microsomes.

2. For WIL, the ten chemical runs were combined into six groups, resulting in a total of six combinations, three for each microsome source. One set of controls were run for each grouping. The averages and the associated standard errors across the two portions for each chemical combination were reported. The differences between the two microsome sources were calculated as $(1/3)(\sum M_{1i}-\sum M_{2i})$ and the associated standard errors as $[((\sum s_{1i}^2+\sum s_{2i}^2)/3]^{1/2}$, where M_{1i} and s_{1i} are the average and associated standard error for the i^{th} chemical tested with Battelle microsomes and M_{2i} and s_{2i} are the average and associated standard error for the i^{th} chemical tested with In Vitro microsomes.

3. Results for Battelle and In Vitro were as reported in the intralaboratory analysis reports.

For the $\log_{10}IC_{50}$, slope, top, and bottom parameters, the overall mean values and associated standard errors across laboratories were calculated for each chemical. For each chemical two laboratories tested with microsomes prepared by Battelle and two laboratories tested with microsomes prepared by In Vitro. The microsome source was therefore included into the mixed effect models as a fixed effect. The differences in response between the two microsome sources were based on the mixed model fits.

To describe the variability among the individual laboratory values relative to the overall average value, coefficients of variation (CV) and their associated 95 percent confidence intervals (CI) were calculated for the IC_{50}, the slope, and the top parameters. (Note that the CV was calculated for IC_{50} in the original domain, rather than $\log_{10}IC_{50}$.) The coefficient of variation is defined as the standard deviation of the response divided by its mean. The method for calculating the CV and the associated 95 percent CI was different depending on the underlying assumption about the distribution of the endpoint parameter.

The within laboratory IC_{50} estimates were assumed to be approximately log normally distributed. The CV for IC_{50} is

$$CV = (10^{S^2(\ln 10)} - 1)^{1/2}$$

where S^2 is the total logarithmic variance among the four laboratories. S^2 is approximated by 4 (se) 2 where se is the standard error of the pooled mean ($\log_{10}IC_{50}$) estimates. This would be exact if the within laboratory components of variance were equal across laboratories. The 95 percent confidence interval associated with the CV is based on the chi square distribution and is calculated as

$$[(10^{(df*S^2(\ln 10)/(\chi_{df,0.975}))} - 1)^{1/2}, (10^{(df*S^2(\ln 10)/(\chi_{df,0.025}))} - 1)^{1/2}]$$

where df is the estimated degree of freedom among the four laboratories, shown above.

For the slope (β) and the top (T), the measurements are assumed to be approximately normal. The CV is expressed as

$$CV=|S/M|$$

where M is the mean and S is the associated standard deviation. S is approximated by $\sqrt{4}(se)$ where se is the standard error of the pooled mean estimate. The endpoints of CI for the CV are related to the solutions to the two nonlinear equations:

$$t_{df}(NC)^{-1}(0.025) = M/(S/\sqrt{4}) \text{ and } t_{df}(NC)^{-1}(0.975) = M/(S/\sqrt{4})$$

where $t_{df}(NC)$ is noncentral t distribution with noncentrality parameter NC ($= \mu/(\sigma/\sqrt{4})$) and degrees of freedom df is the estimated degree of freedom among the four laboratories, shown above. The solution to the first equation gives the lower bound of the CI, and the solution for the second equation gives the upper bound of the CI. A Newton-type algorithm is used to solve for the noncentrality parameters in these equations (Lehmann, 1986).

CV was not calculated for the bottom threshold parameter, for the differences between the end and the beginning for the background activity, the full enzyme activity, the negative and the positive controls, and for the differences between the two microsome sources for the negative control and the positive controls. The range of the mean values for these endpoints included zero and so the estimates fluctuate between positive and negative. CV is therefore not stable for these parameters and could be very large, or even infinite.

To describe the variability among laboratories relative to variability within laboratories the ratio of the variance among laboratories to the average variance within laboratories was calculated as

$$R=S^2_{lab} /[(1/4)(s_1^2 + s_2^2 + s_3^2 + s_4^2)]$$

where S^2_{lab} is the variance among the four laboratories and (s_1^2, s_2^2, s_3^2, s_4^2) are the squares of the within laboratory standard errors for the four laboratories. A confidence interval for this ratio is based on the F-distribution with (ν_{lab}, ν_{wi}) degree of freedom

$$[R/F^{-1}(0.975), R/F^{-1}(0.025)]$$

$\nu_{lab}=3$ and ν_{wi} is based on Satterthwaite's approximation

$$\nu_{wi} = [(s_1^2 + s_2^2 + s_3^2+ s_4^2)^2]/[s_1^4/ \nu_1 + s_2^4/ \nu_2 + s_3^4/ \nu_3 + s_4^4/ \nu_4]$$

where (ν_1, ν_2, ν_3, ν_4) are the degrees of freedom associated with the within laboratory standard errors at the four laboratories. This ratio is calculated for each of the ten endpoint parameters.

In several places entries in the interlaboratory analysis report tables differ from corresponding entries in the intralaboratory analysis report tables by one or a small number of trailing digits in the last decimal place. This is due to rounding differences in intermediate calculations between the intralaboratory analyses and the interlaboratory analysis.

The report includes a number of figures, to be discussed in greater detail in the results section. Within each graph the symbol farthest to the right (corresponding to "all labs" on the horizontal axis) is a circle. The legend states that the circle is to be filled, but intermittently in some graphs the circle is empty. This is due to an issue in the graphing program however the interpretation is unambiguous since there is just one circle plotted in each graph. For purposes of interpretation of each graph, whether the circle is filled or is empty should be disregarded.

2.8 GOOD LABORATORY PRACTICES

The toxicology laboratories at RTI, Battelle (and Battelle's chemistry laboratories), and WIL Research Laboratories are operated in compliance with the U.S. EPA FIFRA Good Laboratory Practices (GLP) Standards. Thus, these studies were conducted in compliance with EPA FIFRA Regulations for GLPs. In Vitro Technologies operates in compliance with the U.S. FDA GLP standards and conducted these studies according to 21 CFR Part 58.

2.9 PERSONNEL

The personnel involved in the conduct of this task are listed in their respective laboratory reports that are included in the appendix. The study directors and the lead technician at each of the laboratories were:

- James M. Mathews, Ph.D., D.A.B.T. and Sherry Black, B.S. – RTI International
- Bozena D. Lusiak, Ph.D. and Thomas Deck, B.S. – Battelle
- Aruna Koganti, Ph.D. and Tim Moeller, M.Sc. – In Vitro Technologies
- Jennifer A. Thomas-Wohlever, Ph.D. and Justin Godsey, B.S. – WIL Research Laboratories

3.0 RESULTS

3.1 [^{3}H]-ASDN RADIOCHEMICAL PURITY

The radiochemical purity for the substrate was 97 percent. The radiochemical purity report is included as an appendix of the individual laboratory reports.

3.2 REFERENCE CHEMICAL AND CONTROL SUBSTANCES ANALYSES

The actual reference chemical and control substance stock formulation concentrations were within less than approximately 8 percent of their respective target concentrations (Table 6). The formulations were determined to be stable (within 10 percent of target) when stored refrigerated for at least the period of time reported in the table. All laboratories used the reference or control chemicals within their denoted stability days, except Battelle and WIL used the dibenz[a,h]anthracene one day beyond the 20 day stability and In Vitro used the 4-nonylphenol stock formulation seven days beyond the 20 day stability. The short period beyond

the stability and absence of any degradation during the 20-day stability period suggested that these formulations were acceptable for use. The full chemistry reports from the Chemical Repository are included in the appendices.

Table 6. Reference and Control Chemical Stock Formulation Analysis and Stability

Reference Chemical Code	Reference or Control Chemical	Target Conc. (mg/mL) [M]	Measured Conc. (mg/mL)	% of Target[a]	Stability[b] (Days)
1	Aminoglutethimide	23.2 [0.1]	22.3	-3.9	59
2	Ketoconazole	5.31 [0.01]	5.47	3.0	60
3	Prochloraz	38.0 [0.1]	37.5	-1.3	63
4	4-Nonylphenol	24.1 [0.1]	24.7	2.5	20
5	Dibenz[a,h]anthracene	2.78 [0.01]	2.79	0.4	20
6	Fenarimol	33.2 [0.1]	31.2	-6.0	30
7	Econazole	44.5 [0.1]	44.4	-0.2	56
8	Chrysin	2.54 [0.01]	2.50	-1.6	100
9	Dicofol	36.8 [0.1]	33.7	-8.4	29
10	Atrazine	21.3 [0.1]	20.3	-4.7	28
NA	4-OH ASDN	3.02 [0.01]	2.95	-2.3	173
NA	Lindane	29.1 [0.1]	29.4	1.0	168

a. % of Target = ((Measured Concentration – Target Concentration)/Target Concentration) x 100

b. Denotes end of stability testing period conducted for this work assignment (stability of reference or control chemical may be longer than reported in table).

3.3 MICROSOMAL PROTEIN ANALYSIS

The microsomal protein concentration was determined on the day that the microsomes were used in the assay. The number of protein concentrations differed somewhat because some laboratories performed two independent replicates of the assay but used the same protein analysis results for both replicates, thereby reducing the total number of protein determinations for a given laboratory. Results are presented separately for the Battelle-supplied vs the In Vitro-supplied microsomes due to the difference in concentrations between the two preparations.

For the Battelle-supplied microsomes, the original protein concentration provided by Battelle at the time of distribution was 21 mg/mL. The laboratory group mean (± Standard Error of the Mean, SEM) protein concentration values were 26.8 ± 0.4, 25.0 ± 0.4, 18.6 ± 0.7, and 23.9 ± 0.8 mg/mL for RTI, Battelle, In Vitro, and WIL, respectively (Table 7). The measured protein concentration within a laboratory showed low variability, i.e. % CV values ranged from 5.3 to 11.5 percent. A comparison of the original reported protein concentration to the values determined by the laboratories resulted in a percent relative error (%RE) of 27.7, 19.1, -11.5, and 14.0 percent for RTI, Battelle, In Vitro, and WIL, respectively. In general, the laboratories obtained a similar protein concentration to that reported by the supplier. If a comparison is made to the results obtained by the lead laboratory (RTI), then the %RE was -6.7, -30.7, and -10.7 percent for Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM protein concentration was 23.6 ± 1.8 mg/mL with a percent CV of 15.0 percent.

For the In Vitro-supplied microsomes, the original protein concentration provided by In Vitro at the time of distribution was 8 mg/mL. The laboratory group mean (± Standard Error of

the Mean, SEM) protein concentration values were 10.9 ± 0.4, 9.2 ± 0.1, 6.3 ± 0.6, and 8.5 ± 0.4 mg/mL for RTI, Battelle, In Vitro, and WIL, respectively (Table 7). The measured protein concentration within a laboratory showed low variability for three of four labs, i.e. % CV values ranged from 5.7 to 13.8 percent, whereas In Vitro had a larger measure of variability compared to its original reported value, i.e. 28.5 percent. A comparison of the original reported protein concentration to the values determined by the laboratories resulted in a percent relative error (%RE) of 36.1, 15.3, -21.4, and 6.5 percent for RTI, Battelle, In Vitro, and WIL, respectively. In general, the laboratories obtained a similar protein concentration to that reported by the supplier. If a comparison is made to the results obtained by the lead laboratory (RTI), then the %RE was -15.3, -42.2, and -21.7 percent for Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM protein concentration was 8.7 ± 1.0 mg/mL with a percent CV of 21.8 percent.

Table 7. Human Placental Microsomal Protein Concentration[a]

Parameter	RTI	Battelle	In Vitro	WIL
Battelle-Supplied Microsomes				
Average (mg/mL)	26.8	25.0	18.6	23.9
sd	1.4	1.6	2.1	2.5
SEM	0.4	0.4	0.7	0.8
Minimum (mg/mL)	25.3	20.9	14.8	19.4
Maximum (mg/mL)	29.8	27.3	22.4	27.9
% CV	5.3	6.5	11.5	10.6
In Vitro-Supplied Microsomes				
Average (mg/mL)	10.9	9.2	6.3	8.5
sd	1.2	0.5	1.8	1.2
SEM	0.4	0.1	0.6	0.4
Minimum (mg/mL)	9.7	8.2	4.0	6.6
Maximum (mg/mL)	13.2	10.1	8.8	10.5
% CV	10.7	5.7	28.5	13.8

a. Table values were based on all protein concentrations reported by labs for all replicates (regardless of whether the replicates were used or not for reporting enzyme activity and IC_{50} value).

Protein QCs were analyzed with the unknowns during the study (Table 8). The target QC concentrations were 10 and 100 µg/mL (RTI's reported concentrations were 12 and 110 µg/mL). Accuracy (% RE) ranged from -3.3 to 13.0 percent for the low QC, except for In Vitro that had a % RE value of -54.7 percent, whereas accuracy ranged from 0.4 to 13.9 percent for the high QC, thereby indicating that an acceptable level of accuracy could be achieved for both QC standards (based on acceptance criteria of 15 percent). Precision (% CV) ranged from 10.9 to 40.2 percent for the low QC, except for In Vitro that reported a value of 83.5 percent, whereas the precision ranged from 2.4 to 8.7 for the high QC, thereby indicating that precision was poor for the low QC but within an acceptable level for the high QC (based on acceptance criterion of 15 percent). In Vitro's poorer accuracy and precision results were attributed to their use of a six-point standard curve (included the 250 µg/mL standard) rather than the 5-point standard curve (excluded the 250 µg/mL standard), which was used by the other laboratories when they found increased variability and poorer fit when the 250 µg/mL standard was included.

Table 8. Protein QC Standards[a]

Parameter	RTI	Battelle	In Vitro	WIL
Low Standard (10 µg/mL)				
Overall Average	11	11	5	10
Overall sd	4	1	4	4
% CV	36.8	10.9	83.5	40.2
% RE[b]	13.0	6.3	-54.7	-3.3
High Standard (100 µg/mL)				
Overall Average	114	107	106	100
Overall sd	5	3	9	5
% CV	4.4	2.4	8.7	4.6
% RE[b]	13.9	6.8	5.8	0.4

a. Protein concentrations were rounded to the nearest whole µg/mL.
b. Based on target values.

3.4 FULL ENZYME AROMATASE ACTIVITY

Full enzyme activity controls were conducted in duplicate repetitions at the beginning and end of each replicate of the assay (a total of four tubes/replicate). There were at least three replicates conducted for each of ten reference chemicals by each of the four laboratories. In those instances where a laboratory assayed two reference chemicals on the same day and at the same time, there was only one set (four tubes) assayed, which resulted in one average enzyme activity for both reference chemicals. Thus, even though such controls were used for two reference chemicals, the control value was only used once in the calculation of any overall control value. Finally, since the microsomes were supplied by two sources, the enzyme activity control results are presented separately – first, by source and second, together, since the full enzyme aromatase activities were similar regardless of the source of the microsomes.

Using the Battelle-prepared microsomes, the laboratory overall average ± SEM (% CV) full enzyme activity control values were 0.048 ± 0.002 (6.3 %), 0.055 ± 0.005 (21.4 %), 0.058 ± 0.007 (22.0 %) and 0.059 ± 0.001 (3.4 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM full enzyme activity control value for the Battelle-prepared microsomes was 0.055 ± 0.002 nmol/mg protein/min with a percent CV of 8.6 percent. These results indicate that the laboratories obtained similar enzyme activity values for the Battelle-prepared microsomes.

Using the In-Vitro-prepared microsomes, the laboratory overall average ± SEM (% CV) full enzyme activity control values were 0.036 ± 0.003 (16.1 %), 0.046 ± 0.001 (6.8 %), 0.075 ± 0.018 (42.4 %) and 0.050 ± 0.006 (20.9 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM full enzyme activity control value for the In Vitro-prepared microsomes was 0.052 ± 0.008 nmol/mg protein/min with a percent CV of 31.7 percent. The In Vitro determined activity was approximately twice that of the lead laboratory, which was attributed to four replicates where the beginning and ending activity values were approximately twice as high as was measured in the other replicates. In Vitro had no explanation for the replicates with this higher level of enzyme activity.

A comparison of the enzyme activity values obtained for a given laboratory when it used the Battelle- or In Vitro-supplied microsomes indicated that the activity was similar (taking into consideration the variability). For this reason, it was believed reasonable to determine an overall enzyme activity value using the data generated by all laboratories and the microsomes from both suppliers (Table 9). As such, the laboratory overall average ± SEM (% CV) full enzyme activity control values were 0.043 ± 0.003 (17.7 %), 0.051 ± 0.003 (19.0 %), 0.066 ± 0.010 (35.4 %) and 0.055 ± 0.003 (15.3 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM full enzyme activity control value for both sources of microsomes was 0.054 ± 0.005 nmol/mg protein/min with a percent CV of 17.9 percent. Comparison of the lead laboratory's results relative to the individual laboratory results produced % RE values of 17.2, 53.3, and 26.1 percent for Battelle, In Vitro, and WIL, respectively. These results indicated that microsomes prepared by different laboratories and analyzed by different laboratories can result in microsomes with similar aromatase activity.

Table 9. Human Placental Full Enzyme Activity Control Determinations[a]

Parameter	Aromatase Activity (nmol/mg protein/min)			
	RTI	Battelle	In Vitro	WIL
Overall Average	0.043	0.051	0.066	0.055
Overall sd	0.008	0.010	0.023	0.008
Overall SEM	0.003	0.003	0.010	0.003
% CV	17.7	19.0	35.4	15.3

a. The overall average value for the laboratory was calculated using the mean values for the ten reference chemicals, regardless of the microsome source (mean values from paired reference chemicals were only used once).

For most labs, the variability (% CV) of the full enzyme activity control values for a given replicate was generally less than 10 percent. There was a time or two when a laboratory had a much higher % CV for a given replicate, e.g. > 25 percent, but these instances were few. Although the lead laboratory mostly had % CV values less than 10 percent, the other laboratories had % CV values that tended to range between 10 and 20 percent (one laboratory had values of approximately 40 percent).

3.5 BACKGROUND ACTIVITY

Background enzyme activity controls were conducted in duplicate repetitions at the beginning and end of each replicate of the assay (a total of four tubes/replicate). There were at least three replicates conducted for each of ten reference chemicals by each of the four laboratories. For the most part, the aromatase activity in these samples for all laboratories and reference chemicals was negligible, indicating that there was no background activity that interfered with the interpretation of the results.

3.6 POSITIVE CONTROL ACTIVITY

4-OH ASDN, at a final concentration of 5×10^{-8} M, was used as the positive control with each replicate of the assay because it is a known aromatase inhibitor and this concentration has been shown to produce an approximately 50 percent inhibition of the enzyme. Since the full

enzyme activity values were similar for both sources of microsomes, the results using microsomes from the two sources were combined to report the results by laboratory and task.

The laboratory overall average ± SEM (% CV) enzyme activity values in the presence of 4-OH ASDN were 0.021 ± 0.002 (19.5 %), 0.025 ± 0.001 (15.0 %), 0.024 ± 0.003 (33.1 %), and 0.023 ± 0.001 (13.5 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively (Table 10). Comparison of the lead laboratory to the individual laboratories resulted in % RE values of 17.8, 17.5, and 9.9 percent for Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM full enzyme activity control value was 0.023 ± 0.001 nmol/mg protein/min with a percent CV of 7.5 percent.

Table 10. Human Placental Enzyme Activity in the Presence of 4-OH ASDN (Positive-Control)[a]

Parameter	Aromatase Activity (nmol/mg protein/min)			
	RTI	Battelle	In Vitro	WIL
Overall Average	0.021	0.025	0.024	0.023
Overall sd	0.004	0.004	0.008	0.003
Overall SEM	0.002	0.001	0.003	0.001
% CV	19.5	15.0	33.1	13.5

a. The overall average value for the laboratory was calculated using the mean values for the ten reference chemicals, regardless of the microsome source (mean values from paired reference chemicals were only used once).

The laboratory overall group mean ± SEM (% CV) inhibition values for 4-OH ASDN (as a percent of control) were 48.0 ± 0.4 (2.3 %), 48.7 ± 1.0 (6.8 %), 40.5 ± 7.4 (44.5 %), and 42.7 ± 3.1 (17.5 %) percent for RTI, Battelle, In Vitro, and WIL, respectively (Table 11). Comparison of the lead laboratory to the individual laboratories resulted in % RE values of 1.4, -15.7, and -11.1 percent for Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM percent of control value was 45.0 ± 2.0 percent with a percent CV of 9.0.

Table 11. Human Placental Enzyme Activity Inhibition by 4-OH ASDN (Positive Control)[a]

Parameter	Percent of Control			
	RTI	Battelle	In Vitro	WIL
Overall Average	48.0	48.7	40.5	42.7
Overall sd	1.1	3.3	18.0	7.5
Overall SEM	0.4	1.0	7.4	3.1
% CV	2.3	6.8	44.5	17.5

a. The overall average value for the laboratory was calculated using the mean values for the ten reference chemicals, regardless of the microsome source (mean values from paired reference chemicals were only used once).

3.7 NEGATIVE CONTROL ACTIVITY

Lindane, at a final concentration of 10^{-6} M, was used as the negative control because it is known not to inhibit aromatase at this concentration. The negative control was analyzed with each replicate of the assay. Since the full enzyme activity values were similar for both sources of microsomes, the results using microsomes from the two sources were combined to report the results by laboratory and task.

The laboratory overall average ± SEM (% CV) enzyme activity values in the presence of lindane were 0.042 ± 0.003 (16.3 %), 0.049 ± 0.003 (18.7 %), 0.046 ± 0.007 (36.1 %), and 0.053 ± 0.003 (14.3 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively (Table 12). Comparison of the lead laboratory to the individual laboratories resulted in % RE values of 16.8, 9.1, and 26.0 percent for Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM full enzyme activity control value was 0.048 ± 0.002 nmol/mg protein/min with a percent CV of 9.8 percent. This enzyme activity value is similar to that measured in the absence of any inhibitor (full enzyme activity control value).

Table 12. Human Placental Enzyme Activity in the Presence of Lindane (Negative Control)[a]

Parameter	Aromatase Activity (nmol/mg protein/min)			
	RTI	Battelle	In Vitro	WIL
Overall Average	0.042	0.049	0.046	0.053
Overall sd	0.007	0.009	0.017	0.008
Overall SEM	0.003	0.003	0.007	0.003
% CV	16.3	18.7	36.1	14.3

a. The overall average value for the laboratory was calculated using the mean values for the ten reference chemicals, regardless of the microsome source (mean values from paired reference chemicals were only used once).

The laboratory overall group mean ± SEM (% CV) inhibition values for lindane (as a percent of control) were 97.9 ± 0.8 (2.1 %), 97.3 ± 0.9 (2.8 %), 74.8 ± 13.0 (42.4 %), and 99.6 ± 8.8 (21.6 %) percent for RTI, Battelle, In Vitro, and WIL, respectively (Table 13). It is interesting to note that In Vitro reported a decrease in enzyme activity of approximately 25 percent. The laboratory reviewed its dilution scheme, procedures, and results for a possible explanation for the lindane-related decrease in enzyme activity but was not able to find any. Comparison of the lead laboratory to the individual laboratories resulted in % RE values of -0.7, -23.6, and 1.8 percent for Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM percent of control value was 92.4 ± 5.9 percent with a percent CV of 12.8.

Table 13. Human Placental Enzyme Activity Inhibition by Lindane (Negative Control)[a]

Parameter	Percent of Control			
	RTI	Battelle	In Vitro	WIL
Overall Average	97.9	97.3	74.8	99.6
Overall sd	2.1	2.8	31.7	21.5
Overall SEM	0.8	0.9	13.0	8.8
% CV	2.1	2.8	42.4	21.6

a. The overall average value for the laboratory was calculated using the mean values for the ten reference chemicals, regardless of the microsome source (mean values from paired reference chemicals were only used once).

3.8 INHIBITION OF AROMATASE ACTIVITY (PERCENT OF CONTROL)

The effect of increasing concentrations of the reference chemicals on aromatase activity was determined and the results were expressed as a percent of the control aromatase activity. A summary of these results by laboratory and overall task are provided in the following subsections for each reference chemical. The individual replicate percent of control results for each laboratory can be found in the appendices.

Within a given subsection, the percent of control results were evaluated with respect to: 1) the modifications made by the study directors in reference chemical concentrations tested in replicates 2 and 3 after reviewing the results from replicate 1, 2) the effect of the reference chemical over the concentration ranges tested on aromatase activity, and 3) the assay precision.

3.8.1 Aminoglutethimide

The effect of increasing concentrations of aminoglutethimide on aromatase activity is reported by laboratory in Table 14 and by overall task in Table 15. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 1 and 2.

After the first replicate was completed, only the Battelle study director modified the aminoglutethimide concentrations selected for testing in replicates 2 and 3 by adding a single intermediate concentration of 5×10^{-6} M, which was used in place of the 10^{-9} M (Table 14). For the other three labs, since the highest concentration that could be tested was 10^{-3} M, one additional concentration had to be selected, which was 5×10^{-6} M for RTI, 10^{-10} M for In Vitro, and 5×10^{-5} M for WIL. The laboratories tested a dilution higher than the lowest planned concentration, i.e. 10^{-9} M rather than 10^{-10} M, due to a miscommunication by the CR regarding the correct RC blind number and its corresponding concentration.

Table 14. Effect of Aminoglutethimide on Aromatase Activity (Percent of Control) by Laboratory

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
RTI	-3.00	3	-0.12	0.88	0.51	736.32
	-4.00	3	3.60	1.21	0.70	33.52
	-5.00	3	28.43	0.70	0.40	2.45
	-5.30	3	43.07	2.25	1.30	5.23
	-6.00	3	74.82	3.51	2.03	4.69
	-7.00	3	93.74	4.85	2.80	5.17
	-8.00	3	96.28	3.79	2.19	3.93
	-9.00	3	95.82	3.76	2.17	3.92
Battelle	-3.00	3	0.36	0.26	0.15	72.68
	-4.00	3	4.48	0.68	0.39	15.11
	-5.00	3	32.53	2.68	1.55	8.24
	-5.30	2	49.12	NC[a]	NC	NC
	-5.60	3	64.88	1.74	1.00	2.68
	-6.00	3	80.48	2.28	1.31	2.83
	-7.00	3	94.72	3.32	1.92	3.51
	-8.00	3	98.68	2.01	1.16	2.04
	-9.00	1	99.66	NC	NC	NC
In Vitro	-3.00	3	0.21	0.04	0.02	20.8
	-4.00	3	4.45	1.22	0.70	27.4
	-5.00	3	28.50	6.73	3.89	23.6
	-6.00	3	80.04	6.72	3.88	8.4
	-7.00	3	94.80	5.03	2.90	5.3
	-8.00	3	99.32	6.62	3.82	6.7
	-9.00	3	100.92	7.82	4.51	7.8
	-10.00[b]	3	98.75	10.11	5.84	10.2
WIL	-3.00	3	0.23	0.06	0.03	26.45
	-4.00	3	4.07	0.63	0.36	15.43
	-4.30	3	8.26	0.94	0.54	11.41
	-5.00	3	28.25	3.41	1.97	12.07
	-6.00	3	80.84	3.97	2.29	4.91
	-7.00	3	97.38	2.12	1.23	2.18
	-8.00	3	100.09	2.49	1.44	2.48
	-9.00	3	98.71	2.77	1.60	2.81

a. NC – value **N**ot **C**alculated when n ≤2.
b. See text for explanation for this dilution.

Table 15. Effect of Aminoglutethimide on Aromatase Activity (Percent of Control) by Overall Task

Reference Chemical Log Conc (M)	Number of Labs[a]	Overall Mean	Overall sd	Overall SEM	Overall %CV
-3.00	4	0.17	0.20	0.10	120.3
-4.00	4	4.15	0.41	0.21	9.9
-4.30	1	8.26	NC[b]	NC	NC
-5.00	4	29.43	2.07	1.04	7.0
-5.30	2	46.10	NC	NC	NC
-5.60	1	64.88	NC	NC	NC
-6.00	4	79.05	2.84	1.42	3.6
-7.00	4	95.16	1.56	0.78	1.6
-8.00	4	98.59	1.65	0.82	1.7
-9.00	4	98.78	2.17	1.08	2.2
-10.00	1	98.75	NC	NC	NC

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because each laboratory selected some concentrations for testing that differed, as well as Battelle revised the concentrations used for replicates 2 and 3 after reviewing their replicate 1 results.

b. NC – Not Calculated. If the overall mean was calculated using an n ≤2, then the sd, SEM, and %CV were not calculated.

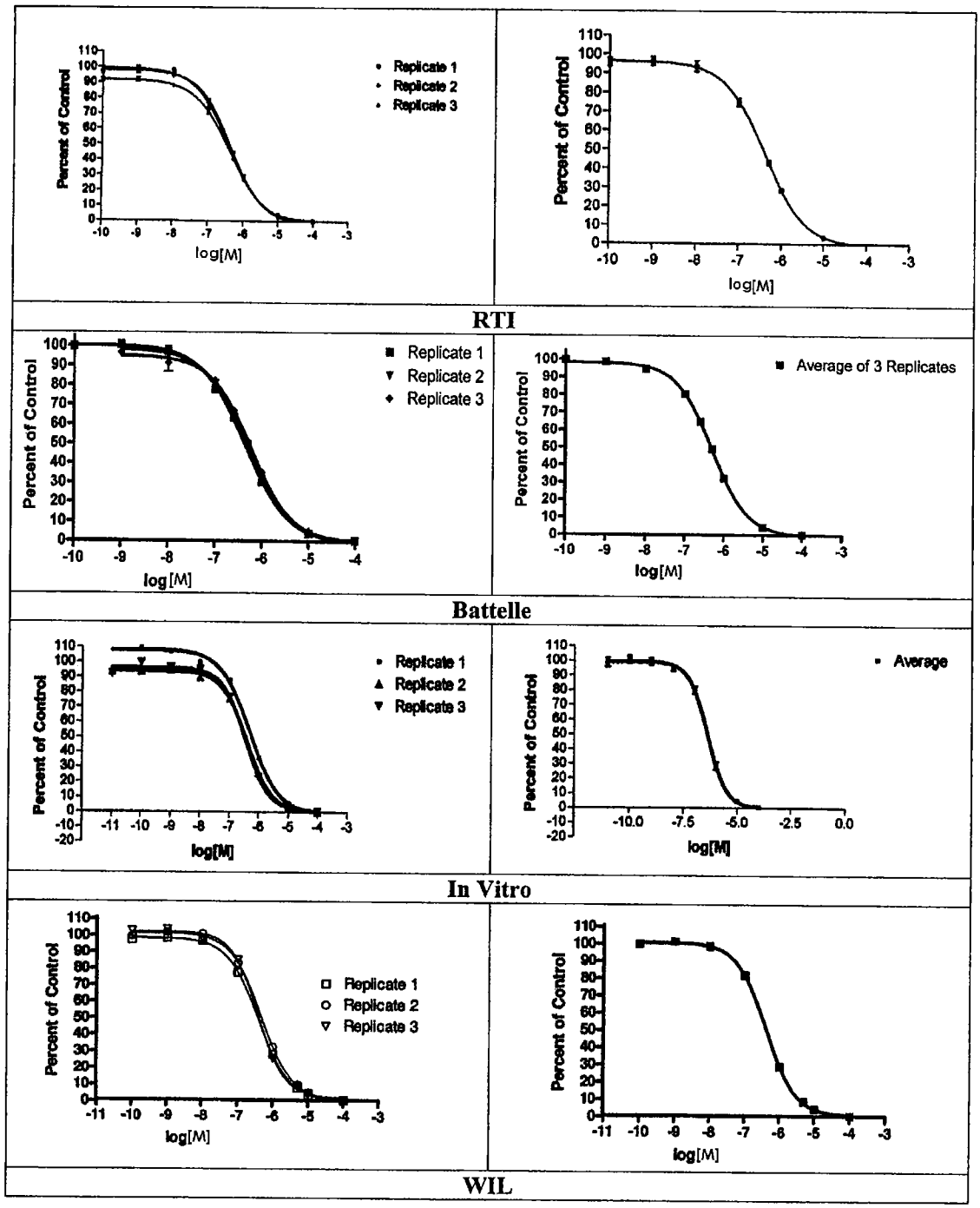

Figure 1. **Individual Replicate and Average Aminoglutethimide Inhibition Response Curves (percent of control) by Laboratory**

[note: X axis values are 10-fold low, e.g., 10^{-4} M should actually be 10^{-3} M.]

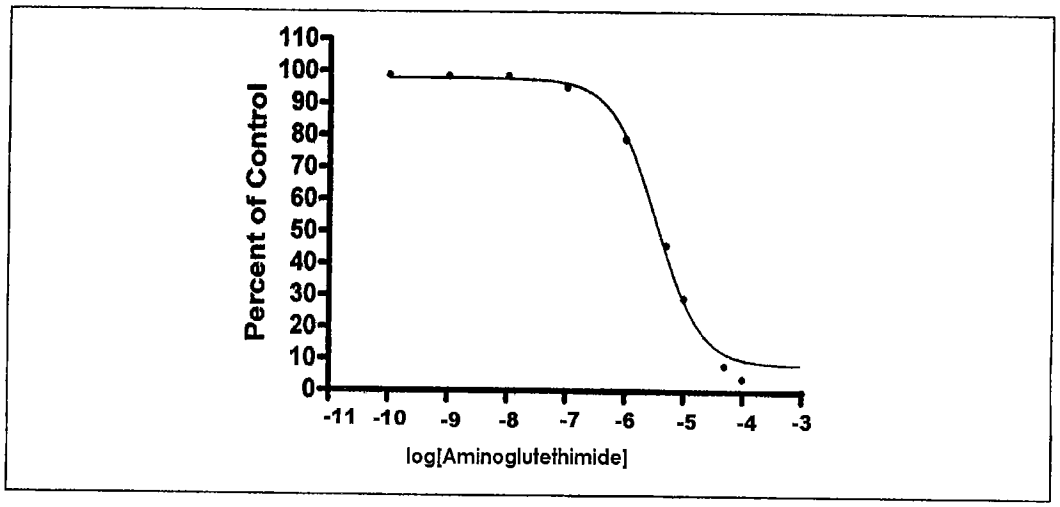

Figure 2. Overall Task Aminoglutethimide Inhibition Response Curve (percent of control)

For all four laboratories, increasing concentrations of aminoglutethimide decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent (Table 14). The shapes of the enzyme activity vs. aminoglutethimide curves were sigmoidal (Figure 1). At an aminoglutethimide concentration of 10^{-3} M, the aromatase inhibition was complete; the laboratory percent of control values were less than 1 percent. In contrast, at an aminoglutethimide concentration of approximately 10^{-7} M, there was little to no aromatase inhibition; the laboratory percent of control values were greater than or equal to 94 percent for all labs. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-7} M were 0.17 ± 0.10 and 95.16 ± 0.78 percent, respectively (Table 15).

All labs have assay precision values (% CV) that were less than or equal to 12 percent for those concentrations used in at least three replicates, except at a concentration of 10^{-3} and 10^{-4} M (and 10^{-5} M for In Vitro) where the precision varied widely, i.e. 15 to 736 percent (Table 14). The overall task assay precision ranged from 2 to 10 percent for those concentrations that were tested by 3 or more labs, except at a concentration of 10^{-3} M where the % CV value was 120 percent (Table 15).

3.8.2 Atrazine

The effect of increasing concentrations of atrazine on aromatase activity is reported by laboratory in Table 16 and by overall task in Table 17. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 3 and 4.

After the first replicate was completed, the study directors from two of the four laboratories modified the atrazine concentrations selected for testing in replicates 2 and 3 (Table 16). In Vitro and WIL used the same eight concentrations for their three replicates. In general, the modifications that were made in an attempt to characterize the shape of the curve included adding mid-level concentrations between 10^{-3} and 10^{-5} M. No modifications were made below 10^{-5} M because there was little, if any, inhibition observed at lower concentrations. Even with the modifications, there was no further characterization of the curve beyond what was originally determined by the first replicate.

For all four laboratories, increasing concentrations of atrazine did not decrease the activity of the placental microsomal aromatase activity, although there was a trend toward inhibition at the highest concentration (Table 16). The shapes of the enzyme activity vs atrazine curves approached a horizontal line (Figure 3). At an atrazine concentration of 10^{-3} M, aromatase activity approximated 80 to 90 percent of control. Similarly, at an atrazine concentration of approximately 10^{-10} M, there was little to no aromatase inhibition; the laboratory percent of control values were greater than 90 percent for all labs. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-10} M were 81.31 ± 1.46 and 93.62 ± 1.57 percent, respectively (Table 17).

All labs have assay precision values (% CV) that were less than 8 percent for those concentrations used in at least three replicates (Table 16). The overall task assay precision ranged from 2 to 5 percent for those concentrations that were tested by 3 or more labs (Table 17).

Table 16. Effect of Atrazine on Aromatase Activity (Percent of Control) by Laboratory

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
RTI	-3.00	3	78.41	3.73	2.15	4.75
	-3.30	2	78.54	NC[a]	NC	NC
	-3.60	2	85.75	NC	NC	NC
	-4.00	3	90.87	4.12	2.38	4.53
	-4.30	2	94.17	NC	NC	NC
	-5.00	3	95.71	1.75	1.01	1.83
	-6.00	3	95.44	1.68	0.97	1.76
	-7.00	3	96.64	2.01	1.16	2.08
	-8.00	1	94.14	NC	NC	NC
	-9.00	1	94.48	NC	NC	NC
	-10.00	1	93.12	NC	NC	NC
Battelle	-3.00	3	83.05	2.11	1.22	2.54
	-3.05	2	83.63	NC	NC	NC
	-3.10	2	81.99	NC	NC	NC
	-3.12	2	84.21	NC	NC	NC
	-3.30	2	84.41	NC	NC	NC
	-4.00	3	93.74	1.00	0.58	1.07
	-5.00	3	96.89	4.56	2.63	4.70
	-6.00	3	97.35	2.79	1.61	2.86
	-7.00	1	97.59	NC	NC	NC
	-8.00	1	97.89	NC	NC	NC
	-9.00	1	95.60	NC	NC	NC
	-10.00	1	93.71	NC	NC	NC
In Vitro	-4.00	3	89.31	5.83	3.37	6.5
	-4.48	3	89.83	3.47	2.00	3.9
	-5.00	3	95.04	3.68	2.12	3.9
	-6.00	3	92.80	5.16	2.98	5.6
	-7.00	3	89.21	6.33	3.65	7.1
	-8.00	3	89.24	5.73	3.31	6.4
	-9.00	3	90.74	6.98	4.03	7.7
	-10.00	3	89.98	6.60	3.81	7.3
WIL	-3.00	3	82.48	6.09	3.51	7.38
	-4.00	3	94.17	2.55	1.47	2.71
	-5.00	3	99.18	2.76	1.59	2.78
	-6.00	3	98.43	3.40	1.96	3.45
	-7.00	3	98.09	0.18	0.10	0.18
	-8.00	3	99.69	3.76	2.17	3.77
	-9.00	3	97.92	2.35	1.36	2.40
	-10.00	3	97.65	1.33	0.77	1.36

a. NC – value **N**ot **C**alculated when n ≤2.

Table 17. Effect of Atrazine on Aromatase Activity (Percent of Control) by Overall Task

Reference Chemical Log Conc (M)	Number of Labs[a]	Overall Mean	Overall sd	Overall SEM	Overall %CV
-3.00	3	81.31	2.53	1.46	3.1
-3.05	1	83.63	NC[b]	NC	NC
-3.10	1	81.99	NC	NC	NC
-3.12	1	84.21	NC	NC	NC
-3.30	2	81.48	NC	NC	NC
-3.60	1	85.75	NC	NC	NC
-4.00	4	92.02	2.33	1.16	2.5
-4.30	1	94.17	NC	NC	NC
-4.48	1	89.83	NC	NC	NC
-5.00	4	96.71	1.82	0.91	1.9
-6.00	4	96.01	2.47	1.23	2.6
-7.00	4	95.38	4.16	2.08	4.4
-8.00	4	95.24	4.62	2.31	4.9
-9.00	4	94.69	2.99	1.50	3.2
-10.00	4	93.62	3.15	1.57	3.4

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because each laboratory used the results from their first replicate to select concentrations to be used in replicates 2 and 3 that were believed would further (In Vitro and WIL) or better characterize the percent inhibition curve.

b. NC – Not Calculated. If the overall mean was calculated using an n $\leq$2, then the sd, SEM, and %CV were not calculated.

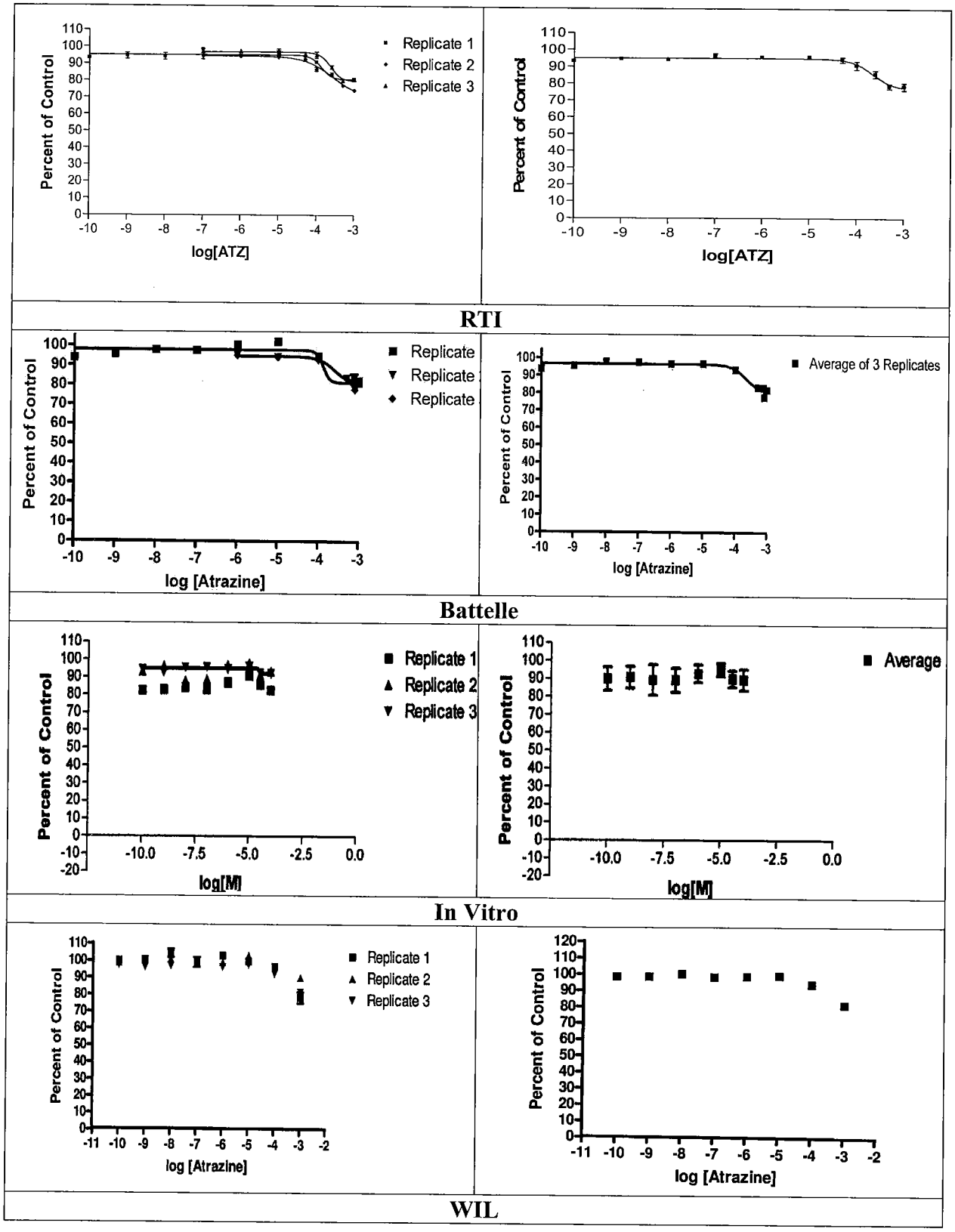

Figure 3. **Individual Replicate and Average Atrazine Inhibition Response Curves (percent of control) by Laboratory**

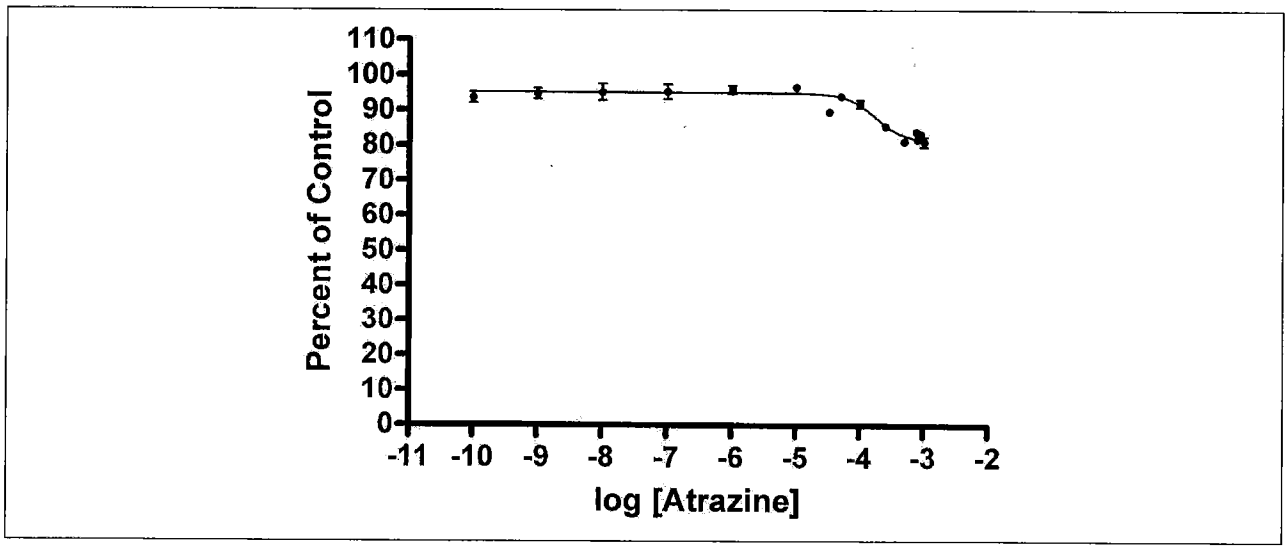

Figure 4. Overall Task Atrazine Inhibition Response Curve (percent of control)

3.8.3 Chrysin

The effect of increasing concentrations of chrysin on aromatase activity is reported by laboratory in Table 18 and by overall task in Table 19. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 5 and 6.

After the first replicate was completed, the study directors from all laboratories modified the chrysin concentrations selected for testing in replicates 2 and 3 (Table 18). In general, the modifications involved including additional mid-concentrations between 10^{-4} and 10^{-7} M and using them in place of concentrations at 10^{-9} to 10^{-10} M that were tested in replicate 1.

For all four laboratories, increasing concentrations of chrysin decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent (Table 18). The shapes of the enzyme activity vs chrysin curves were sigmoidal (Figure 5). At a chrysin concentration of 10^{-4} M (the highest achievable concentration), aromatase inhibition percent of control values ranged from 14 to 23 percent. In contrast, at chrysin concentrations $\leq 10^{-7}$ M there was little aromatase inhibition with most laboratories reporting percent of control values greater than 90 percent. The overall task mean ± SEM percent of control values at 10^{-4} and 10^{-7} M were 18.74 ± 1.88 and 89.51 ± 1.69 percent, respectively (Table 19).

Assay precision values (% CV) were less than 8 percent for those concentrations used in at least three replicates for RTI and Battelle, ranged from 8 to 33 percent for In Vitro, and ranged from 1 to 19 percent for WIL (Table 18). The overall task assay precision ranged from 1 to 9 percent for those concentrations that were tested by 3 or more labs, except at a concentration of 10^{-4} M where the % CV value was 20 percent (Table 19).

Table 18. Effect of Chrysin on Aromatase Activity (Percent of Control) by Laboratory

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
RTI	-4.00	3	22.63	0.51	0.29	2.24
	-5.00	3	22.79	0.04	0.03	0.19
	-5.30	2	35.45	NC[a]	NC	NC
	-5.60	2	50.48	NC	NC	NC
	-6.00	3	70.35	0.68	0.39	0.96
	-6.30	3	80.70	1.23	0.71	1.53
	-7.00	3	91.71	2.45	1.41	2.67
	-8.00	3	94.14	3.45	1.99	3.66
	-9.00	1	95.41	NC	NC	NC
	-10.00	1	93.30	NC	NC	NC
Battelle	-4.00	3	20.52	1.43	0.82	6.95
	-5.00	3	26.24	1.37	0.79	5.21
	-5.30	2	39.52	NC	NC	NC
	-5.60	2	53.73	NC	NC	NC
	-6.00	3	73.68	2.88	1.66	3.91
	-6.60	1	86.99	NC	NC	NC
	-7.00	3	87.18	7.00	4.04	8.03
	-8.00	3	92.31	3.04	1.75	3.29
	-9.00	3	93.43	1.08	0.63	1.16
	-10.00	1	90.23	NC	NC	NC
In Vitro	-4.00	1	17.90	NC	NC	NC
	-4.48	3	8.19	2.67	1.54	32.6
	-5.00	3	21.94	4.03	2.33	18.3
	-5.48	2	47.21	NC	NC	NC
	-6.00	3	71.99	7.00	4.04	9.7
	-7.00	3	86.10	6.92	4.00	8.0
	-8.00	3	91.05	12.36	7.14	13.6
	-9.00	3	88.36	15.37	8.87	17.4
	-10.00	3	93.90	9.50	5.48	10.1
WIL	-4.00	3	13.91	2.14	1.23	15.37
	-5.00	3	26.13	2.37	1.37	9.09
	-5.30	3	40.01	1.91	1.10	4.78
	-5.60	2	56.70	NC	NC	NC
	-6.00	3	75.70	0.65	0.38	0.86
	-7.00	3	93.05	5.65	3.26	6.07
	-8.00	3	91.74	9.41	5.43	10.26
	-9.00	1	102.08	NC	NC	NC
	-10.00	3	82.75	15.61	9.01	18.86

a. NC – value Not Calculated when n ≤2.

Table 19. Effect of Chrysin on Aromatase Activity (Percent of Control) by Overall Task

Reference Chemical Log Conc (M)	Number of Labs[a]	Overall Mean	Overall sd	Overall SEM	Overall %CV
-4.00	4	18.74	3.76	1.88	20.0
-4.48	1	8.19	NC[b]	NC	NC
-5.00	4	24.28	2.23	1.12	9.2
-5.30	3	38.33	2.50	1.45	6.5
-5.48	1	47.21	NC	NC	NC
-5.60	3	53.64	3.11	1.80	5.8
-6.00	4	72.93	2.29	1.15	3.1
-6.30	1	80.70	NC	NC	NC
-6.60	1	86.99	NC	NC	NC
-7.00	4	89.51	3.39	1.69	3.8
-8.00	4	92.31	1.32	0.66	1.4
-9.00	4	94.82	5.68	2.84	6.0
-10.00	4	90.05	5.12	2.56	5.7

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because each laboratory used the results from their first replicate to select concentrations to be used in replicates 2 and 3 that were believed would better characterize the percent inhibition curve.

b. NC – Not Calculated. If the overall mean was calculated using an n ≤2, then the sd, SEM, and %CV were not calculated.

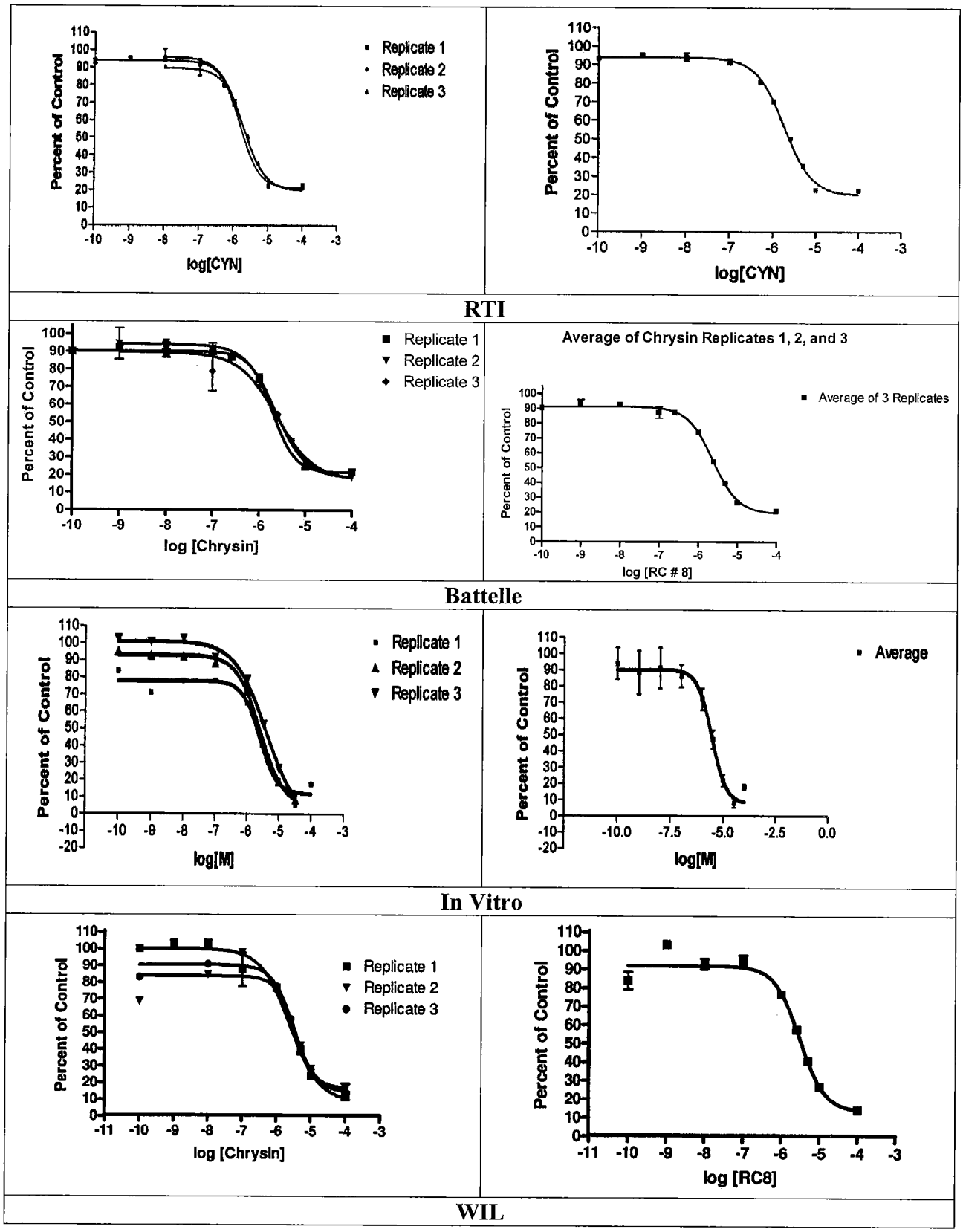

Figure 5. **Individual Replicate and Average Chrysin Inhibition Response Curves (percent of control) by Laboratory**

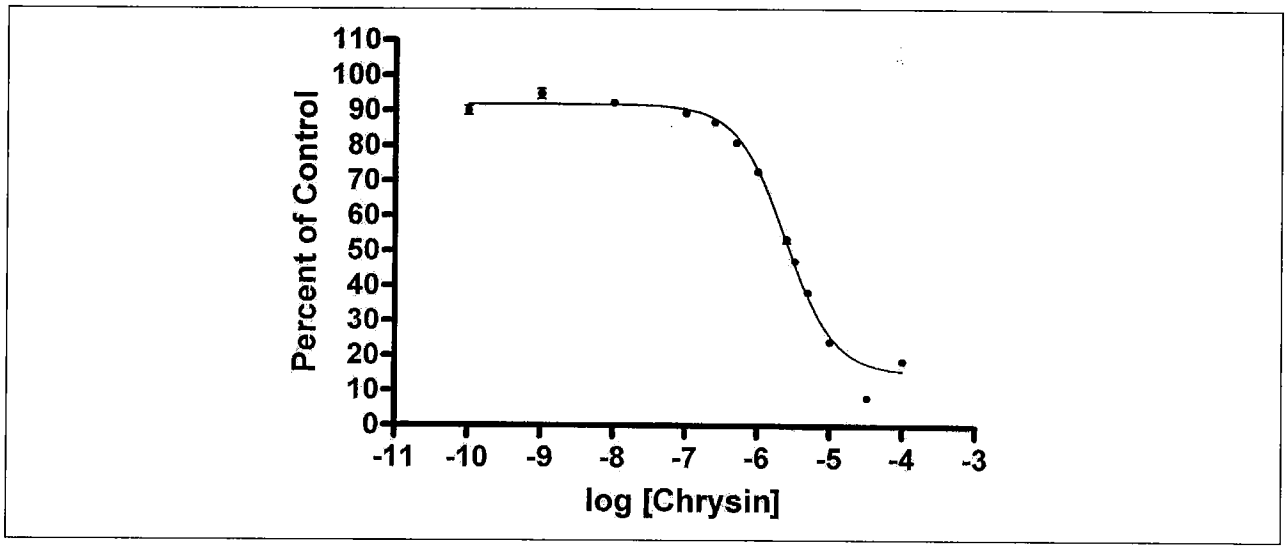

Figure 6. Overall Task Chrysin Inhibition Response Curve (percent of control)

3.8.4 Dicofol

The effect of increasing concentrations of dicofol on aromatase activity is reported by laboratory in Table 20 and by overall task in Table 21. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 7 and 8.

After the first replicate was completed, the study directors from three of four laboratories modified the dicofol concentrations selected for testing in replicates 2 and 3 (Table 20). In Vitro used the same dicofol concentrations for all three replicates. It is not clear why In Vitro did not test at a concentration of 10^{-3} M. In general, the modifications involved including additional mid-concentrations between 10^{-3} and 10^{-6} M, which replaced concentrations ranging from 10^{-7} to 10^{-10} M that were tested in replicate 1.

For all four laboratories, increasing concentrations of dicofol decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent (Table 20). The shapes of the enzyme activity vs dicofol curves were sigmoidal (Figure 7), although the curve for In Vitro was a partial sigmoidal curve since they did not test at the highest possible concentration. At a dicofol concentration of 10^{-3} M, aromatase inhibition was almost complete for the three of four laboratories that tested at this concentration. The laboratory percent of control value was 3 percent at 10^{-3} M. For In Vitro, the percent of control value at its highest tested concentration (10^{-4} M) was 45 percent. In contrast, at a dicofol concentration of approximately 10^{-6} M, there was little to no aromatase inhibition; the laboratory percent of control values were approximately 90 percent or higher for all labs. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-6} M were 2.86 ± 0.15 and 91.01 ± 0.80 percent, respectively (Table 21).

All labs have assay precision values (% CV) that were equal or less than 10 percent for concentrations at 10^{-5} M and lower (for those concentrations with at least three replicates), except for Battelle which had a precision value of 19 percent at this concentration. At concentrations higher than 10^{-5} M, the precision varied widely, i.e. 6 to 87 percent (Table 20). The overall task assay precision ranged from 2 to 9 percent for those concentrations that were tested by 3 or more labs, except at a concentration of 10^{-4} M where the % CV value was 30 percent (Table 21).

Table 20. Effect of Dicofol on Aromatase Activity (Percent of Control) by Laboratory

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
RTI	-3.00	3	2.78	1.06	0.61	37.97
	-3.30	2	5.59	NC[a]	NC	NC
	-3.60	2	12.05	NC	NC	NC
	-4.00	3	28.66	9.69	5.60	33.82
	-5.00	3	66.79	2.14	1.23	3.20
	-6.00	3	90.12	2.78	1.61	3.08
	-7.00	3	96.91	2.58	1.49	2.66
	-8.00	3	99.69	2.39	1.38	2.40
	-9.00	1	100.89	NC	NC	NC
	-10.00	1	100.13	NC	NC	NC
Battelle	-3.00	3	2.65	0.40	0.23	15.01
	-4.00	3	22.04	1.32	0.76	6.00
	-4.30	2	33.03	NC	NC	NC
	-5.00	3	67.69	12.77	7.38	18.87
	-6.00	3	91.41	1.45	0.83	1.58
	-7.00	3	97.52	1.11	0.64	1.14
	-8.00	3	97.74	0.58	0.33	0.59
	-9.00	3	96.25	0.52	0.30	0.54
	-10.00	1	98.05	NC	NC	NC
In Vitro	-4.00	3	44.60	10.27	5.93	23.0
	-4.48	3	61.52	11.31	6.53	18.4
	-5.00	3	68.81	4.05	2.34	5.9
	-6.00	3	89.42	2.31	1.33	2.6
	-7.00	3	95.67	6.32	3.65	6.6
	-8.00	3	91.54	6.27	3.62	6.9
	-9.00	3	94.49	1.71	0.99	1.8
	-10.00	3	96.35	5.70	3.29	5.9
WIL	-3.00	3	3.14	2.73	1.58	87.00
	-3.52	2	13.57	NC	NC	NC
	-4.00	3	31.83	4.96	2.87	15.60
	-4.52	2	60.61	NC	NC	NC
	-5.00	3	66.33	6.72	3.88	10.13
	-5.52	2	84.67	NC	NC	NC
	-6.00	3	93.07	3.26	1.88	3.50
	-7.00	1	99.34	NC	NC	NC
	-8.00	3	100.47	2.37	1.37	2.36
	-9.00	1	101.22	NC	NC	NC
	-10.00	1	98.88	NC	NC	NC

a. NC – value **N**ot **C**alculated when n ≤2.

Table 21. Effect of Dicofol on Aromatase Activity (Percent of Control) by Overall Task

Reference Chemical Log Conc (M)	Number of Labs[a]	Overall Mean	Overall sd	Overall SEM	Overall %CV
-3.00	3	2.86	0.25	0.15	8.9
-3.30	1	5.59	NC[b]	NC	NC
-3.52	1	13.57	NC	NC	NC
-3.60	1	12.05	NC	NC	NC
-4.00	4	31.78	9.47	4.73	29.8
-4.30	1	33.03	NC	NC	NC
-4.48	1	61.52	NC	NC	NC
-4.52	1	60.61	NC	NC	NC
-5.00	4	67.41	1.09	0.55	1.6
-5.52	1	84.67	NC	NC	NC
-6.00	4	91.01	1.60	0.80	1.8
-7.00	4	97.36	1.53	0.76	1.6
-8.00	4	97.36	4.05	2.02	4.2
-9.00	4	98.21	3.36	1.68	3.4
-10.00	4	98.35	1.59	0.79	1.6

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because each laboratory used the results from their first replicate to select concentrations to be used in replicates 2 and 3 that were believed would further (In Vitro) or better characterize the percent inhibition curve.

b. NC – Not Calculated. If the overall mean was calculated using an n $\leq$2, then the sd, SEM, and %CV were not calculated.

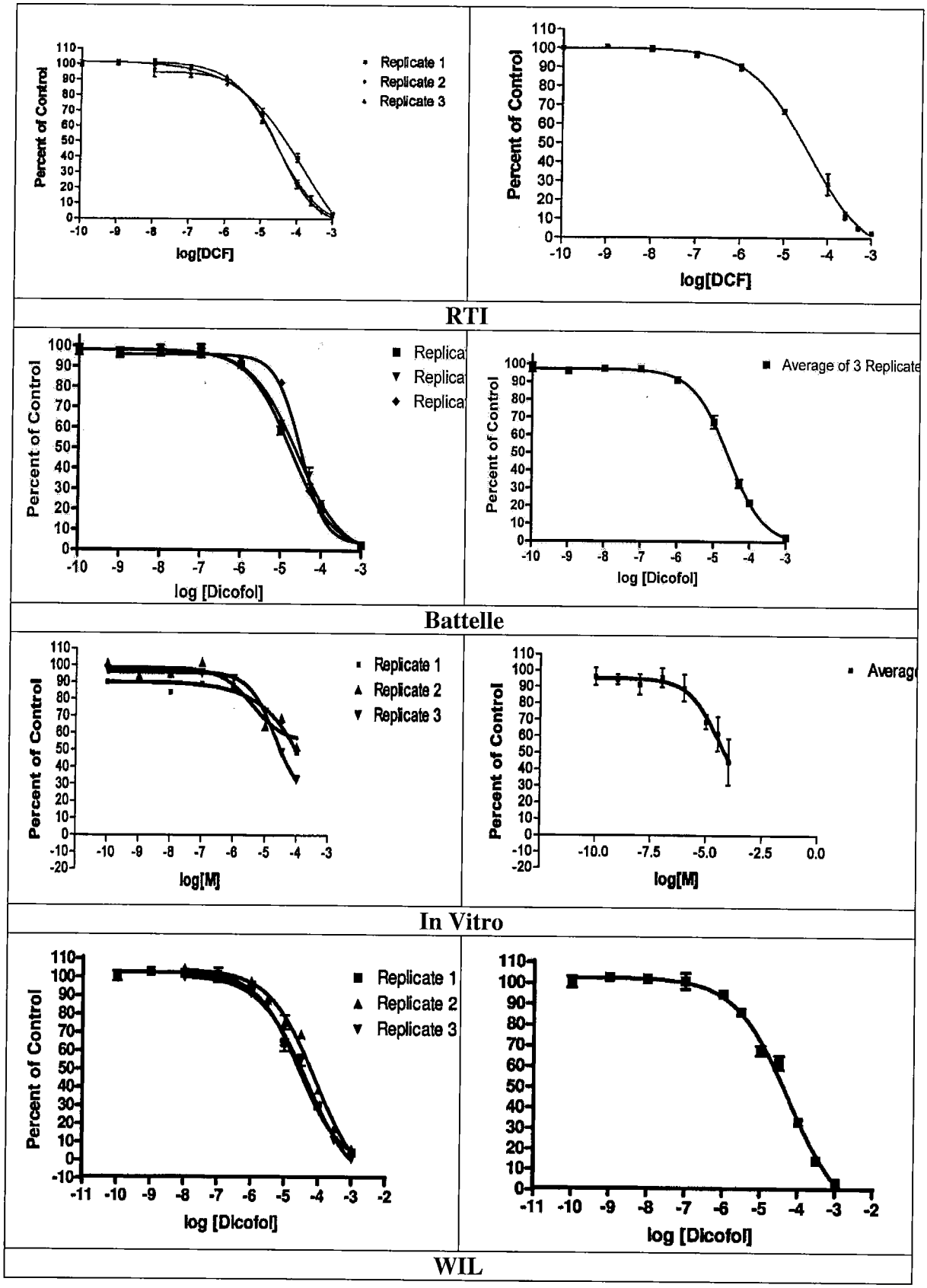

Figure 7. **Individual Replicate and Average Dicofol Inhibition Response Curves (percent of control) by Laboratory**

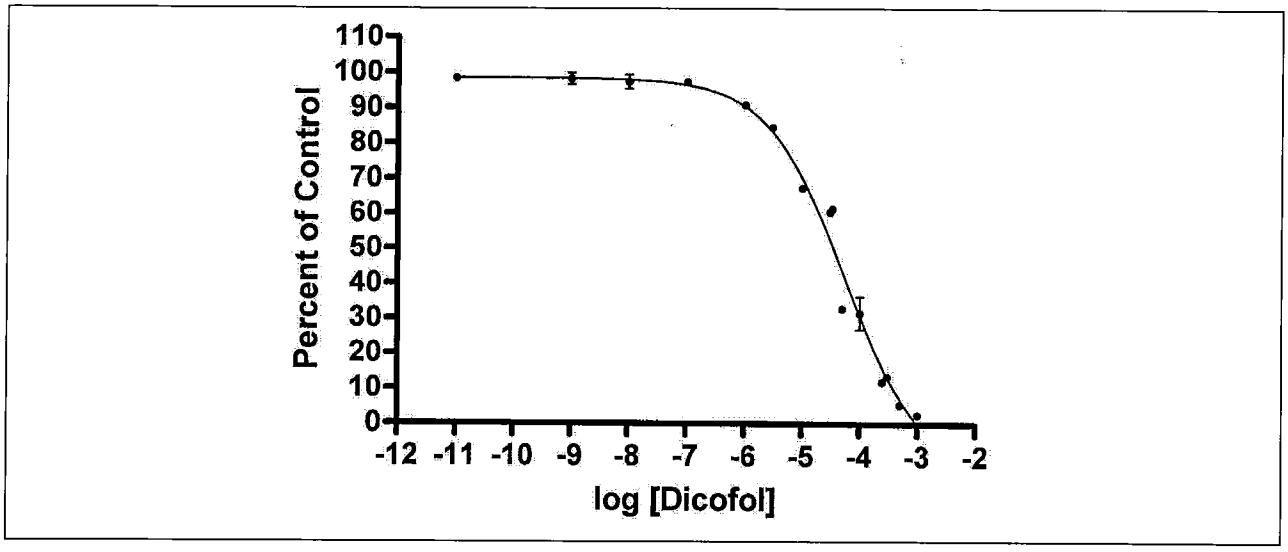

Figure 8. Overall Task Dicofol Inhibition Response Curve (percent of control)

3.8.5 Dibenz [a,h] anthracene

The effect of increasing concentrations of dibenz[a,h]anthracene on aromatase activity is reported by laboratory in Table 22 and by overall task in Table 23. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 9 and 10.

After the first replicate was completed, the study directors did not make any modifications of the dibenz[a,h]anthracene concentrations selected for testing in replicates 2 and 3 (Table 22). The absence of any inhibition precluded the need to do so.

For all four laboratories, increasing concentrations of dibenz[a,h]anthracene did not decrease the activity of the placental microsomal aromatase activity (Table 22). The shapes of the enzyme activity vs dibenz[a,h]anthracene curves approached a horizontal line (Figure 9). At a dibenz[a,h]anthracene concentration of 10^{-4} M (or 3.3 x 10^{-5} M for In Vitro) to 10^{-10} M, there was no indication of any aromatase inhibition. The overall task mean ± SEM percent of control values at 10^{-4} and 10^{-10} M were 100.77 ± 2.23 and 97.56 ± 3.96 percent, respectively (Table 23).

All labs have assay precision values (% CV) that were less than or equal to 12 percent for those concentrations used in at least three replicates (Table 22). The overall task assay precision ranged from 4 to 11 percent for those concentrations that were tested by 3 or more labs (Table 23).

Table 22. Effect of Dibenz[a,h]anthracene on Aromatase Activity (Percent of Control) by Laboratory

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
RTI	-4.00	3	96.42	7.74	4.47	8.03
	-5.00	3	102.08	1.98	1.14	1.94
	-6.00	3	102.64	1.39	0.80	1.36
	-6.30	3	101.70	2.60	1.50	2.55
	-7.00	3	103.11	2.01	1.16	1.95
	-8.00	3	103.57	2.42	1.40	2.34
	-9.00	3	102.74	1.61	0.93	1.57
	-10.00	3	100.57	3.67	2.12	3.65
Battelle	-4.00	3	102.10	3.63	2.10	3.56
	-5.00	3	101.95	4.40	2.54	4.31
	-6.00	3	104.78	4.23	2.44	4.04
	-6.60	3	104.17	4.04	2.33	3.88
	-7.00	3	101.95	2.49	1.44	2.44
	-8.00	3	100.82	1.80	1.04	1.78
	-9.00	3	101.38	3.11	1.80	3.07
	-10.00	3	96.68	6.30	3.64	6.51
In Vitro	-4.48	3	83.46	10.19	5.88	12.2
	-5.00	3	92.47	7.57	4.37	8.2
	-5.48	3	88.28	4.91	2.83	5.6
	-6.00	3	81.59	9.83	5.68	12.1
	-7.00	3	93.05	4.11	2.37	4.4
	-8.00	3	89.33	5.92	3.42	6.6
	-9.00	3	84.17	7.36	4.25	8.7
	-10.00	3	87.10	8.50	4.91	9.8
WIL	-4.00	3	103.78	5.47	3.16	5.27
	-5.00	3	106.46	7.62	4.40	7.15
	-5.30	3	105.04	6.07	3.50	5.78
	-6.00	3	103.54	5.28	3.05	5.10
	-7.00	3	106.22	6.28	3.63	5.92
	-8.00	3	103.75	4.30	2.48	4.15
	-9.00	3	108.87	5.27	3.05	4.85
	-10.00	3	105.87	5.01	2.89	4.73

Table 23. Effect of Dibenz[a,h]anthracene on Aromatase Activity (Percent of Control) by Overall Task

Reference Chemical Log Conc (M)	Number of Labs[a]	Overall Mean	Overall sd	Overall SEM	Overall %CV
-4.00	3	100.77	3.86	2.23	3.8
-4.48	1	83.46	NC[b]	NC	NC
-5.00	4	100.74	5.90	2.95	5.9
-5.30	1	105.04	NC	NC	NC
-5.48	1	88.28	NC	NC	NC
-6.00	4	98.14	11.07	5.53	11.3
-6.30	1	101.70	NC	NC	NC
-6.60	1	104.17	NC	NC	NC
-7.00	4	101.08	5.65	2.83	5.6
-8.00	4	99.37	6.82	3.41	6.9
-9.00	4	99.29	10.59	5.30	10.7
-10.00	4	97.56	7.92	3.96	8.1

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because the concentrations selected by the laboratories for testing in the replicates differed in some instances.

b. NC – Not Calculated. If the overall mean was calculated using an n $\leq$2, then the sd, SEM, and %CV were not calculated.

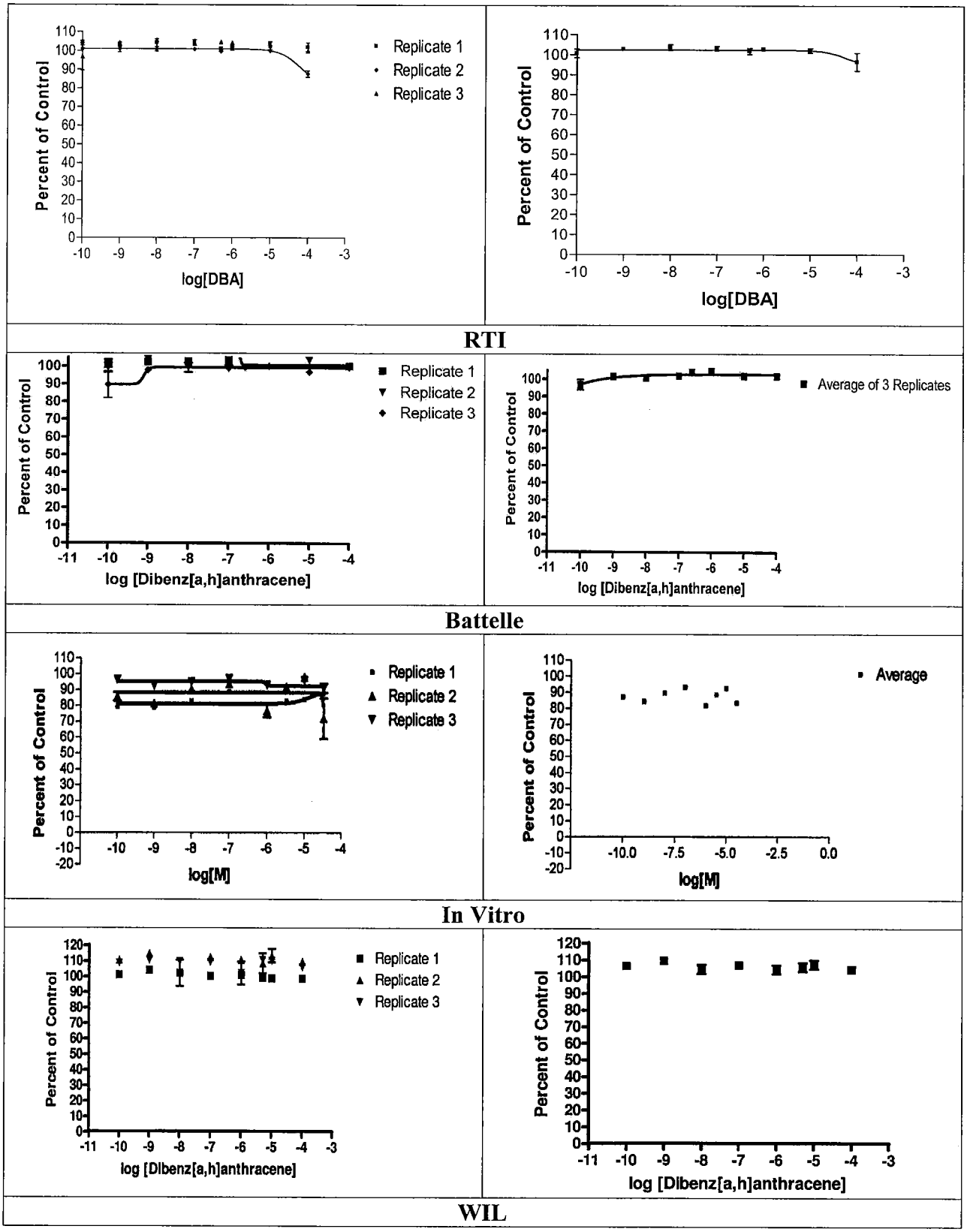

Figure 9. Individual Replicate and Average Dibenz[a,h]anthracene Inhibition Response Curves (percent of control) by Laboratory

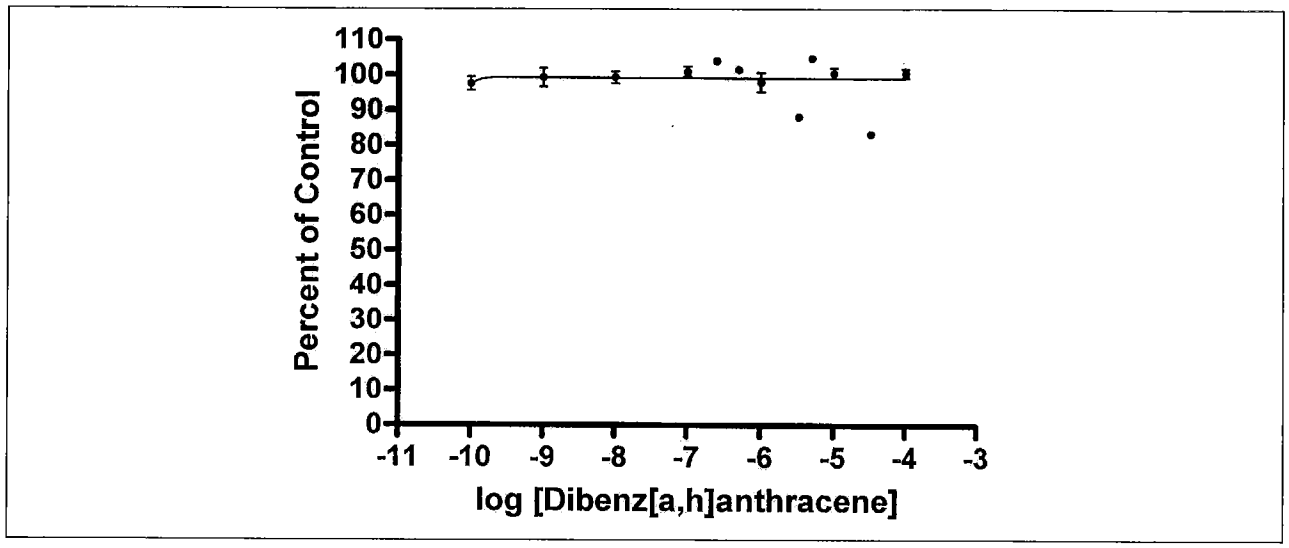

Figure 10. Overall Task Dibenz[a,h]anthracene Inhibition Response Curve (percent of control)

3.8.6 Econazole

The effect of increasing concentrations of econazole on aromatase activity is reported by laboratory in Table 24 and by overall task in Table 25. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 11 and 12.

After the first replicate was completed, the study directors from all four laboratories modified the econazole concentrations selected for testing in replicates 2 and 3 (Table 24). In general, the modifications involved including additional mid-concentrations between 10^{-8} and 10^{-10} M, which replaced the concentrations ranging from 10^{-3} and 10^{-4} M that were tested in replicate 1.

For all laboratories, increasing concentrations of econazole decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent (Table 24). The shapes of the enzyme activity vs. econazole curves were sigmoidal (Figure 11). However, the top portions of the curves (lowest inhibitor concentrations) were not clearly defined to the extent of a well characterized plateau. Lower concentrations would have provided further characterization. Even so, the curve was sufficiently defined to evaluate the effect of econazole on aromatase activity. Approximately 1- 2 percent of the enzyme activity remained at econazole concentrations of 10^{-7} M. In contrast, at an econazole concentration of approximately 10^{-10} M, there was little to no aromatase inhibition; the laboratory percent of control values were approximately 90 percent or higher for three of four labs and approximately 80 percent for Battelle. The overall task mean ± SEM percent of control values at 10^{-7} and 10^{-10} M were 1.52 ± 0.07 and 89.94 ± 3.34 percent, respectively (Table 25).

The lead laboratory (RTI) had assay precision values (% CV) that were less than or equal to approximately 8 percent at $\leq 10^{-7}$ M (for those concentrations with at least three replicates), but at a concentration of 10^{-6} M had a % CV value of 84 percent (Table 24). Battelle's % CV

values differed from the lead and other laboratories. This finding was attributed to the results of their first replicate, which showed that significant inhibition occurred at concentrations that did not cause inhibition to the same extent in the second and third replicates. Battelle's % CV values ranged from 15 to 58 percent. The remaining two laboratories reported % CV values that showed a similar trend as was observed for the lead laboratory. At a concentration of 10^{-6} M or higher, the % CV values were large, whereas lower concentrations had % CV values ranging from 8 to 30 percent for In Vitro and 3 to 13 percent for WIL. The overall task assay precision ranged from 7 to 14 percent for those concentrations that were tested by 3 or more labs, except at concentrations $\geq 10^{-6}$ M where the % CV values were very large (Table 25).

Table 24. Effect of Econazole on Aromatase Activity (Percent of Control) by Laboratory

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
RTI	-3.00	1	0.09	NC[a]	NC	NC
	-4.00	1	0.06	NC	NC	NC
	-5.00	1	0.01	NC	NC	NC
	-6.00	3	0.10	0.08	0.05	83.80
	-7.00	3	1.34	0.09	0.05	7.08
	-8.00	3	12.20	1.02	0.59	8.33
	-8.30	2	22.32	NC	NC	NC
	-9.00	3	59.60	1.42	0.82	2.39
	-9.30	2	76.05	NC	NC	NC
	-9.60	2	86.43	NC	NC	NC
	-10.00	3	93.28	1.12	0.65	1.21
Battelle	-3.00	1	-0.34	NC	NC	NC
	-4.00	3	-0.36	0.16	0.09	45.47
	-5.00	3	-0.23	0.13	0.08	58.41
	-6.00	3	-0.25	0.09	0.05	37.41
	-7.00	3	1.65	0.41	0.23	24.52
	-8.00	3	14.93	2.30	1.33	15.42
	-8.60	2	44.40	NC	NC	NC
	-9.00	3	58.89	14.21	8.20	24.13
	-10.00	3	80.39	22.31	12.88	27.75
In Vitro	-4.00	1	0.04	NC	NC	NC
	-4.48	1	-0.35	NC	NC	NC
	-5.00	3	-0.21	0.33	0.19	154.5
	-6.00	3	-0.15	0.14	0.08	95.0
	-7.00	3	1.48	0.45	0.26	30.3
	-8.00	3	10.77	0.29	0.17	2.7
	-8.48	2	29.15	NC	NC	NC
	-9.00	3	50.39	7.81	4.51	15.5
	-9.48	2	72.39	NC	NC	NC
	-10.00	3	90.62	6.76	3.90	7.5
WIL	-3.00	1	0.27	NC	NC	NC
	-4.00	1	-0.18	NC	NC	NC
	-5.00	3	0.36	0.62	0.36	171.69
	-6.00	3	0.19	0.14	0.08	74.56
	-7.00	3	1.59	0.04	0.02	2.62
	-8.00	3	13.54	1.72	0.99	12.68
	-8.52	2	35.32	NC	NC	NC
	-9.00	3	64.00	2.08	1.20	3.25
	-9.52	2	89.83	NC	NC	NC
	-10.00	3	95.48	3.28	1.89	3.44

a. NC – value **N**ot **C**alculated when n ≤2.

Table 25. Effect of Econazole on Aromatase Activity (Percent of Control) by Overall Task

Reference Chemical Log Conc (M)	Number of Labs[a]	Overall Mean	Overall sd	Overall SEM	Overall %CV
-3.00	3	0.01	0.31	0.18	4701.3
-4.00	4	-0.11	0.20	0.10	180.9
-4.48	1	-0.35	NC[b]	NC	NC
-5.00	4	-0.02	0.27	0.14	1566.6
-6.00	4	-0.03	0.21	0.10	751.3
-7.00	4	1.52	0.14	0.07	9.0
-8.00	4	12.86	1.78	0.89	13.9
-8.30	1	22.32	NC	NC	NC
-8.48	1	29.15	NC	NC	NC
-8.52	1	35.32	NC	NC	NC
-8.60	1	44.40	NC	NC	NC
-9.00	4	58.22	5.69	2.84	9.8
-9.30	1	76.05	NC	NC	NC
-9.48	1	72.39	NC	NC	NC
-9.52	1	89.83	NC	NC	NC
-9.60	1	86.43	NC	NC	NC
-10.00	4	89.94	6.67	3.34	7.4

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because each laboratory used the results from their first replicate to select concentrations to be used in replicates 2 and 3 that were believed would better characterize the percent inhibition curve.

b. NC – Not Calculated. If the overall mean was calculated using an n $\leq$2, then the sd, SEM, and %CV were not calculated.

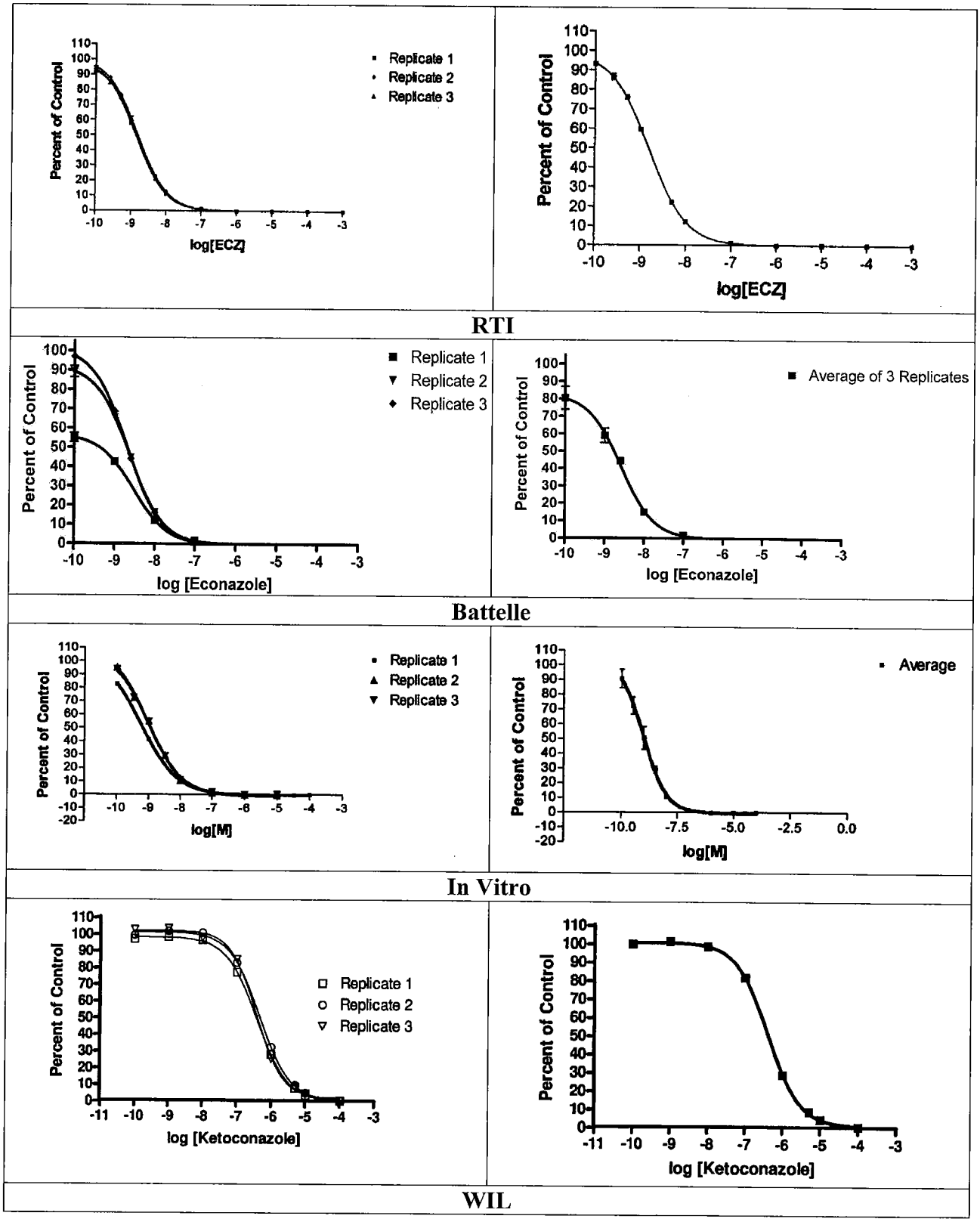

Figure 11. Individual Replicate and Average Econazole Inhibition Response Curves (percent of control) by Laboratory

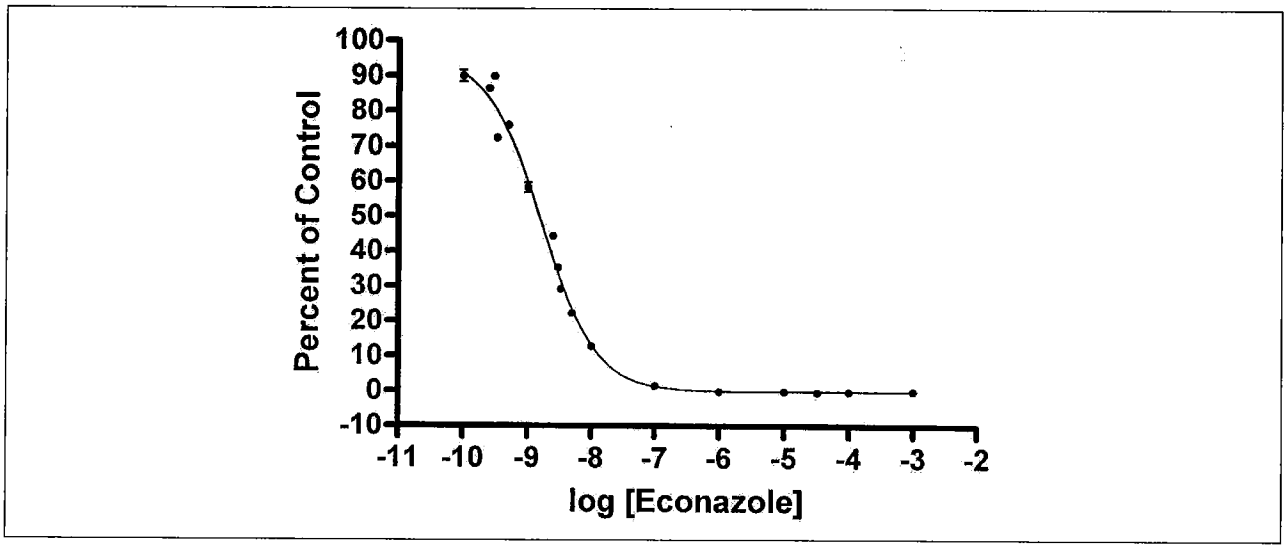

Figure 12. Overall Task Econazole Inhibition Response Curve (percent of control)

3.8.7 Fenarimol

The effect of increasing concentrations of fenarimol on aromatase activity is reported by laboratory in Table 26 and by overall task in Table 27. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 13 and 14.

After the first replicate was completed, the study directors from three of four laboratories modified the fenarimol concentrations selected for testing in replicates 2 and 3 (Table 26). In general, the modifications for these three laboratories involved including additional mid-concentrations between 10^{-4} and 10^{-6} M, which replaced one to two of the concentrations from 10^{-8} to 10^{-10} M that were tested in replicate 1. In Vitro tested the same concentrations for their three replicates, starting at a concentration of 3.3 x 10^{-5} M. This starting concentration was selected by In Vitro because they reported seeing visible precipitate at concentrations of 10^{-3} and 10^{-4} M.

For all four laboratories, increasing concentrations of fenarimol decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent (Table 26). The shapes of the enzyme activity vs fenarimol curves were sigmoidal (Figure 13). At a fenarimol concentration of 10^{-3} M, aromatase inhibition was almost complete; the laboratory percent of control values ranged from 1 to -6 percent for three of the labs and, for In Vitro at its highest concentration of 3.3 x 10^{-5} M, the percent of control value was 15 percent. In contrast, at a fenarimol concentration of approximately 10^{-7} M, there was little to no aromatase inhibition; the laboratory percent of control values were greater than 90 percent for all labs, except WIL with a value of 86 percent. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-7} M were -1.11 ± 2.40 and 91.88 ± 2.27 percent, respectively (Table 27).

Table 26. Effect of Fenarimol on Aromatase Activity (Percent of Control) by Laboratory

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
RTI	-3.00	3	1.23	0.49	0.28	40.17
	-4.00	3	6.09	0.40	0.23	6.61
	-4.60	2	20.26	NC[a]	NC	NC
	-5.00	3	35.61	2.52	1.45	7.08
	-5.30	2	52.63	NC	NC	NC
	-6.00	3	78.26	5.81	3.36	7.43
	-7.00	3	91.47	8.60	4.96	9.40
	-8.00	3	92.96	8.56	4.94	9.21
	-9.00	1	83.54	NC	NC	NC
	-10.00	1	81.48	NC	NC	NC
Battelle	-3.00	3	1.35	0.21	0.12	15.73
	-4.00	3	6.01	0.20	0.11	3.30
	-5.00	3	37.69	0.31	0.18	0.83
	-5.60	2	69.43	NC	NC	NC
	-6.00	3	84.28	3.29	1.90	3.91
	-7.00	3	97.43	2.62	1.51	2.69
	-8.00	3	97.82	0.57	0.33	0.59
	-9.00	3	98.72	1.10	0.64	1.12
	-10.00	1	98.91	NC	NC	NC
In Vitro	-4.48	3	14.85	3.20	1.85	21.6
	-5.00	3	31.51	1.05	0.61	3.3
	-5.48	3	55.23	4.98	2.88	9.0
	-6.00	3	80.46	4.95	2.86	6.2
	-7.00	3	92.25	4.65	2.68	5.0
	-8.00	3	95.88	5.60	3.23	5.8
	-9.00	3	96.89	3.75	2.17	3.9
	-10.00	3	95.60	3.76	2.17	3.9
WIL	-3.00	3	-5.91	12.06	6.96	204.0
	-4.00	3	-1.11	11.74	6.78	1060
	-4.52	2	9.44	NC	NC	NC
	-5.00	3	32.44	10.38	5.99	32.00
	-5.52	2	57.22	NC	NC	NC
	-6.00	3	79.91	7.85	4.53	9.82
	-7.00	3	86.36	12.12	7.00	14.03
	-8.00	1	105.45	NC	NC	NC
	-9.00	3	92.35	9.35	5.40	10.13
	-10.00	1	97.62	NC	NC	NC

a. NC – value **N**ot **C**alculated when n ≤2.

Table 27. Effect of Fenarimol on Aromatase Activity (Percent of Control) by Overall Task

Reference Chemical Log Conc (M)	Number of Labs[a]	Overall Mean	Overall sd	Overall SEM	Overall %CV
-3.00	3	-1.11	4.16	2.40	374.5
-4.00	3	3.66	4.13	2.39	112.8
-4.48	1	14.85	NC[b]	NC	NC
-4.52	1	9.44	NC	NC	NC
-4.60	1	20.26	NC	NC	NC
-5.00	4	34.31	2.85	1.43	8.3
-5.30	1	52.63	NC	NC	NC
-5.48	1	55.23	NC	NC	NC
-5.52	1	57.22	NC	NC	NC
-5.60	1	69.43	NC	NC	NC
-6.00	4	80.73	2.55	1.27	3.2
-7.00	4	91.88	4.53	2.27	4.9
-8.00	4	98.03	5.34	2.67	5.4
-9.00	4	92.88	6.78	3.39	7.3
-10.00	4	93.40	8.06	4.03	8.6

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because each laboratory used the results from their first replicate to select concentrations to be used in replicates 2 and 3 that were believed would further (In Vitro) or better characterize the percent inhibition curve.

b. NC – Not Calculated. If the overall mean was calculated using an n ≤2, then the sd, SEM, and %CV were not calculated.

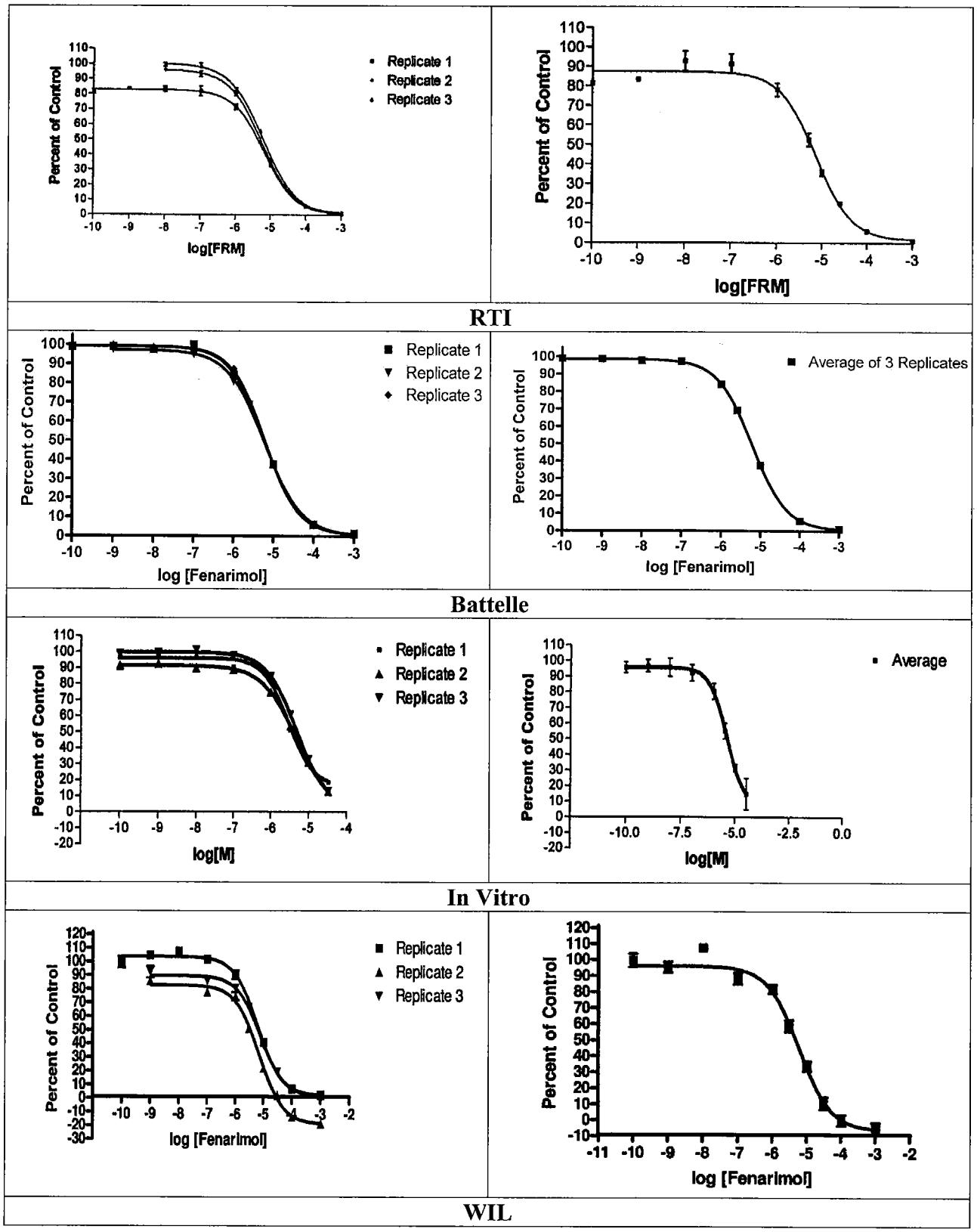

Figure 13. Individual Replicate and Average Fenarimol Inhibition Response Curves (percent of control) by Laboratory

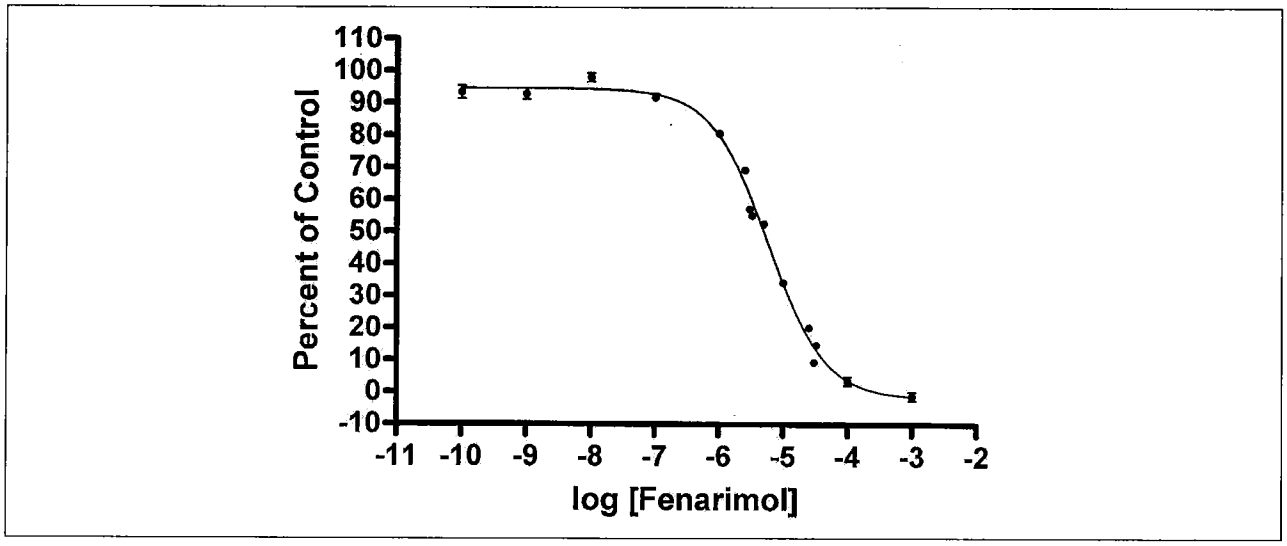

Figure 14. Overall Task Fenarimol Inhibition Response Curve (percent of control)

Three of four labs have assay precision values (% CV) that were less than or equal to 9 percent for those concentrations used in at least three replicates, except at a concentration of 10^{-3} M for RTI and Battelle where the precision varied widely, i.e. 16 to 40 percent, and at a concentration of 3.3 x 10^{-5} M for In Vitro, i.e. 22 percent (Table 26). For WIL, the assay precision values ranged from 10 to 32 percent, except at the highest concentrations tested (10^{-3} and 10^{-4} M) where the precision was estimated at 204 and 1060 percent. The overall task assay precision ranged from 3 to 9 percent for those concentrations that were tested by 3 or more labs, except at a concentration of 10^{-3} and 10^{-4} M where the % CV values were 375 and 113 percent (Table 27). Again, the large variability at the highest fenarimol concentrations tested were attributed to the small percentage of enzyme activity remaining, thereby allowing small differences in remaining enzyme activity to appear to vary widely when, in fact, the enzyme activity was virtually fully inhibited.

3.8.8 Ketoconazole

The effect of increasing concentrations of ketoconazole on aromatase activity is reported by laboratory in Table 28 and by overall task in Table 29. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 15 and 16.

After the first replicate was completed, the study directors from all laboratories modified the ketoconazole concentrations selected for testing in replicates 2 and 3 (Table 28). In general, the modifications involved including additional mid-concentrations between 10^{-4} and 10^{-7} M, which replaced the concentrations from 10^{-8} to 10^{-11} M (or 10^{-12} M for Battelle) that were tested in replicate 1. Battelle tested concentrations ranging from 10^{-5} M to 10^{-12} M rather than 10^{-4} M to 10^{-11} M due to a miscalculation. The error was identified and corrected prior to proceeding with replicates 2 and 3. The laboratories did not test over the planned concentration range, i.e. 10^{-4} to 10^{-10} M, due to a miscommunication with the CR regarding the correct RC blind number and its corresponding concentration.

For all four laboratories, increasing concentrations of ketoconazole decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent (Table 28). The shapes of the enzyme activity vs ketoconazole curves were sigmoidal (Figure 15). At a ketoconazole concentration of 10^{-4} M, aromatase inhibition was complete; the laboratory percent of control values ranged from 5 to 6 percent for three of the labs and was 29 percent of control for In Vitro. In contrast, at a ketoconazole concentration of approximately 10^{-7} M, there was little to no aromatase inhibition; the laboratory percent of control values were greater than 95 percent for all labs. The overall task mean ± SEM percent of control values at 10^{-4} and 10^{-7} M were 11.17 ± 5.99 and 100.51 ± 2.35 percent, respectively (Table 29).

All labs have assay precision values (% CV) that were less than 9 percent for those concentrations used in at least three replicates, except at a concentration of 10^{-4} M where the precision varied widely, i.e. 21 to 151 percent (Table 28). The overall task assay precision ranged from 3 to 5 percent for those concentrations that were tested by 3 or more labs, except at a concentration of 10^{-4} M where the % CV value was 107 percent (Table 29). The large variability at the highest ketoconazole concentration tested (10^{-4} M) was attributed to the small percentage of enzyme activity remaining, thereby allowing small differences in remaining enzyme activity to appear to vary widely when, in fact, the enzyme activity was virtually fully inhibited.

Table 28. Effect of Ketoconazole on Aromatase Activity (Percent of Control) by Laboratory

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
RTI	-4.00	3	4.70	0.97	0.56	20.69
	-4.30	2	11.08	NC[a]	NC	NC
	-4.60	2	21.02	NC	NC	NC
	-5.00	3	39.35	1.46	0.84	3.71
	-5.30	2	55.64	NC	NC	NC
	-6.00	3	85.46	3.73	2.15	4.36
	-7.00	3	98.20	2.35	1.36	2.39
	-8.00	3	98.98	1.45	0.84	1.46
	-9.00	1	96.15	NC	NC	NC
	-10.00	1	95.39	NC	NC	NC
	-11.00	1	94.48	NC	NC	NC
Battelle	-4.00	2	5.54	NC	NC	NC
	-4.60	2	19.79	NC	NC	NC
	-5.00	3	37.87	1.31	0.76	3.46
	-5.30	2	55.00	NC	NC	NC
	-5.60	2	70.19	NC	NC	NC
	-6.00	3	84.51	0.64	0.37	0.76
	-6.60	2	91.15	NC	NC	NC
	-7.00	3	95.87	1.84	1.06	1.92
	-8.00	1	97.50	NC	NC	NC
	-9.00	1	98.57	NC	NC	NC
	-10.00	1	98.90	NC	NC	NC
	-11.00	1	100.9	NC	NC	NC
	-12.00[b]	1	98.71	NC	NC	NC

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
In Vitro	-4.00	3	29.14	44.12	25.47	151.4
	-4.52	1	33.01	NC	NC	NC
	-5.00	3	39.85	1.86	1.07	4.7
	-5.48	2	63.05	NC	NC	NC
	-6.00	3	83.62	4.36	2.52	5.2
	-7.00	3	106.74	3.96	2.29	3.7
	-8.00	3	104.53	3.22	1.86	3.1
	-9.00	3	100.56	6.02	3.48	6.0
	-10.00	3	106.46	9.01	5.20	8.5
WIL	-4.00	3	5.31	1.82	1.05	34.20
	-4.30	2	12.59	NC	NC	NC
	-5.00	3	40.07	2.36	1.37	5.90
	-5.30	2	58.53	NC	NC	NC
	-6.00	3	89.05	2.74	1.58	3.08
	-7.00	3	101.21	3.28	1.89	3.24
	-8.00	3	103.31	2.68	1.55	2.59
	-9.00	1	105.95	NC	NC	NC
	-10.00	3	101.71	2.68	1.55	2.64
	-11.00	1	104.59	NC	NC	NC

a. NC – value **N**ot **C**alculated when n ≤2.
b. See text for an explanation for this dilution.

Table 29. Effect of Ketoconazole on Aromatase Activity (Percent of Control) by Overall Task

Reference Chemical Log Conc (M)	Number of Labs[a]	Overall Mean	Overall sd	Overall SEM	Overall %CV
-4.00	4	11.17	11.98	5.99	107.3
-4.30	2	11.84	NC[b]	NC	NC
-4.52	1	33.01	NC	NC	NC
-4.60	2	20.41	NC	NC	NC
-5.00	4	39.29	0.99	0.50	2.5
-5.30	3	56.39	1.88	1.09	3.3
-5.48	1	63.05	NC	NC	NC
-5.60	1	70.19	NC	NC	NC
-6.00	4	85.66	2.38	1.19	2.8
-6.60	1	91.15	NC	NC	NC
-7.00	4	100.51	4.70	2.35	4.7
-8.00	4	101.08	3.37	1.69	3.3
-9.00	4	100.31	4.17	2.09	4.2
-10.00	4	100.62	4.68	2.34	4.6
-11.00	3	99.99	5.12	2.95	5.1
-12.00	1	98.71	NC	NC	NC

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because each laboratory used the results from their first replicate to select concentrations to be used in replicates 2 and 3 that were believed would better characterize the percent inhibition curve.

b. NC – Not Calculated. If the overall mean was calculated using an n ≤2, then the sd, SEM, and %CV were not calculated.

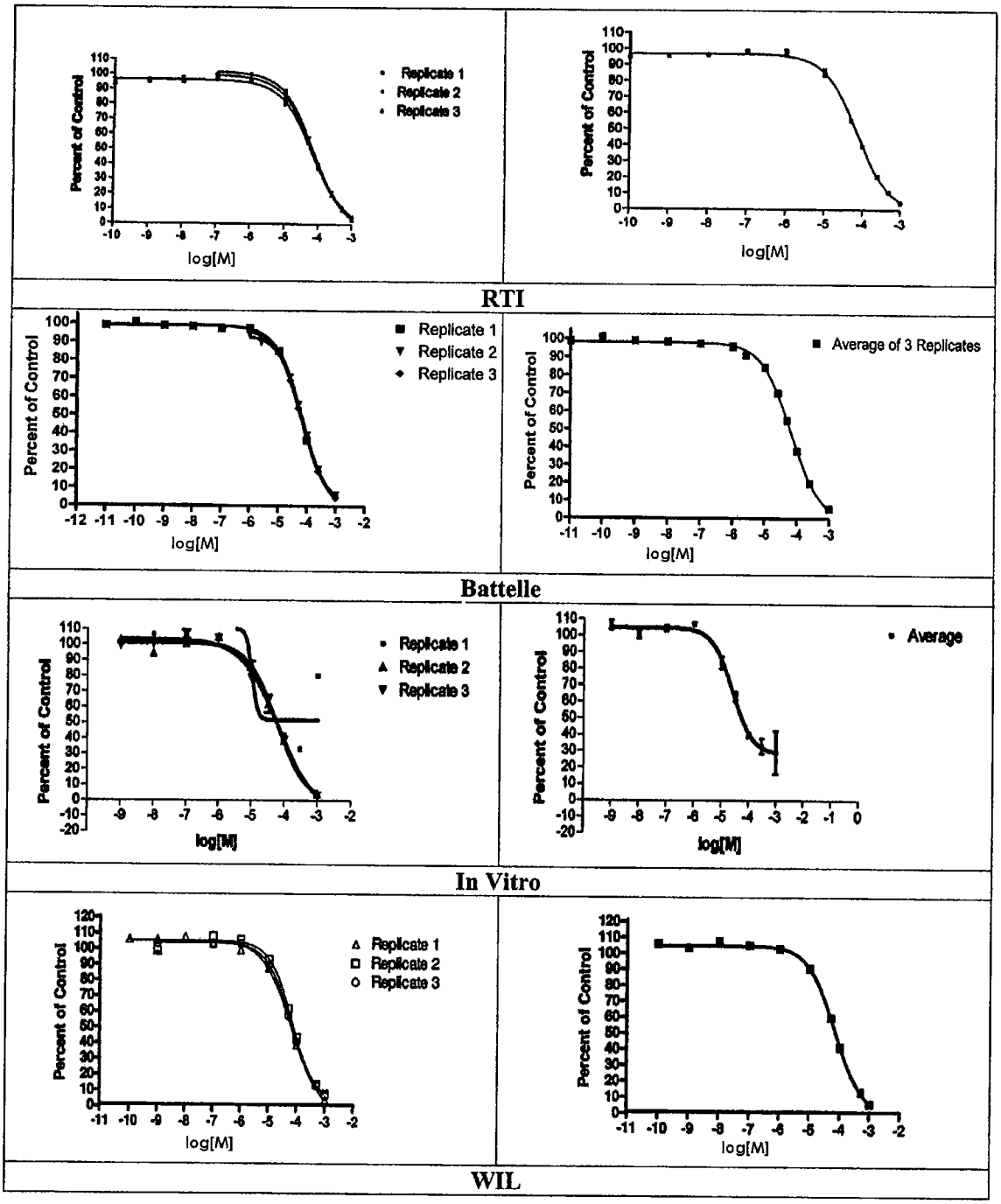

Figure 15. Individual Replicate and Average Ketoconazole Inhibition Response Curves (percent of control) by Laboratory

[Note: x-axis values are 10-fold high, e.g., 10^{-4} M should actually be 10^{-5} M.]

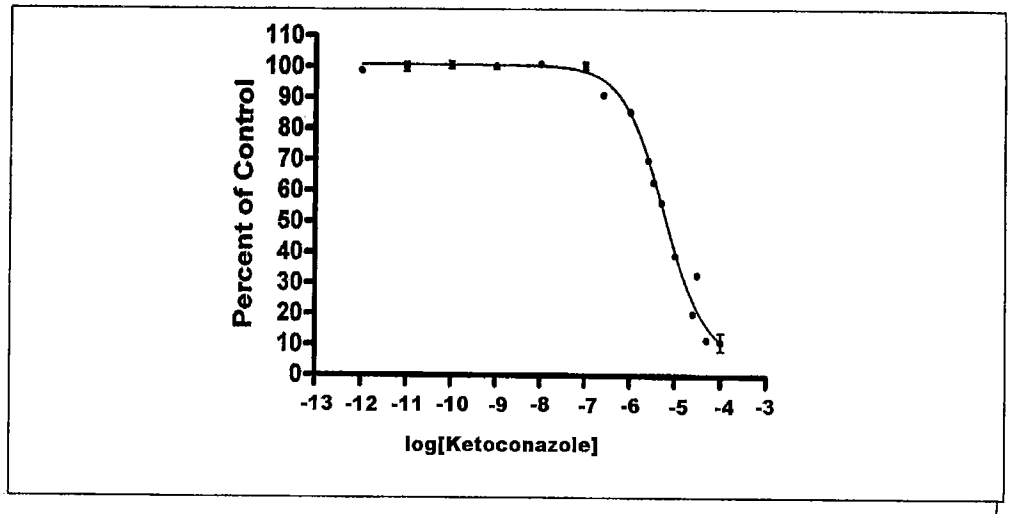

Figure 16. Overall Task Ketoconazole Inhibition Response Curve (percent of control)

3.8.9 4-Nonylphenol

The effect of increasing concentrations of 4-nonylphenol on aromatase activity is reported by laboratory in Table 30 and by overall task in Table 31. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 17 and 18.

Table 30. Effect of 4-Nonylphenol on Aromatase Activity (Percent of Control) by Laboratory

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
RTI	-3.00	3	0.07	0.06	0.03	79.51
	-4.00	3	6.78	0.31	0.18	4.52
	-4.30	2	22.87	NC[a]	NC	NC
	-4.60	2	46.63	NC	NC	NC
	-5.00	3	86.28	1.56	0.90	1.81
	-5.30	2	95.10	NC	NC	NC
	-6.00	3	98.18	1.15	0.66	1.17
	-7.00	3	98.30	2.20	1.27	2.24
	-8.00	1	100.10	NC	NC	NC
	-9.00	1	99.48	NC	NC	NC
	-10.00	1	99.58	NC	NC	NC
Battelle	-3.00	3	0.39	0.17	0.10	43.48
	-4.00	3	14.30	2.35	1.35	16.41
	-4.30	2	29.96	NC	NC	NC
	-4.60	2	47.26	NC	NC	NC
	-5.00	3	89.25	6.45	3.72	7.23
	-6.00	3	97.87	2.47	1.43	2.53
	-7.00	3	98.18	7.16	4.13	7.29
	-8.00	3	94.80	2.69	1.55	2.84
	-9.00	1	90.22	NC	NC	NC
	-10.00	1	94.73	NC	NC	NC
In Vitro	-4.00	3	15.62	1.24	0.72	7.9
	-4.48	3	40.85	1.32	0.76	3.2
	-5.00	3	83.72	4.13	2.38	4.9
	-6.00	3	90.94	4.95	2.86	5.4
	-7.00	3	90.62	0.60	0.35	0.7
	-8.00	3	90.10	5.94	3.43	6.6
	-9.00	3	88.18	4.28	2.47	4.8
	-10.00	3	86.12	1.71	0.99	2.0

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
WIL	-3.00	3	0.32	0.14	0.08	42.27
	-4.00	3	7.84	0.17	0.10	2.13
	-4.30	2	22.46	NC	NC	NC
	-5.00	3	87.97	0.45	0.26	0.52
	-6.00	3	101.63	3.19	1.84	3.14
	-7.00	3	100.58	2.97	1.71	2.95
	-8.00	3	101.38	2.99	1.73	2.95
	-9.00	3	101.02	3.47	2.00	3.44
	-10.00	1	104.55	NC	NC	NC

a. NC – value **N**ot **C**alculated when n ≤2.

Table 31. Effect of 4-Nonylphenol on Aromatase Activity (Percent of Control) by Overall Task

Reference Chemical Log Conc (M)	Number of Labs[a]	Overall Mean	Overall sd	Overall SEM	Overall %CV
-3.00	3	0.26	0.17	0.10	64.7
-4.00	4	11.14	4.47	2.24	40.1
-4.30	3	25.10	4.22	2.43	16.8
-4.48	1	40.85	NC[b]	NC	NC
-4.60	2	46.95	NC	NC	NC
-5.00	4	86.81	2.39	1.19	2.8
-5.30	1	95.10	NC	NC	NC
-6.00	4	97.16	4.48	2.24	4.6
-7.00	4	96.92	4.34	2.17	4.5
-8.00	4	96.60	5.18	2.59	5.4
-9.00	4	94.73	6.46	3.23	6.8
-10.0	4	96.25	7.85	3.93	8.2

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because each laboratory used the results from their first replicate to select concentrations to be used in replicates 2 and 3 that were believed would further (In Vitro) or better characterize the percent inhibition curve.

b. NC – Not Calculated. If the overall mean was calculated using an n ≤2, then the sd, SEM, and %CV were not calculated.

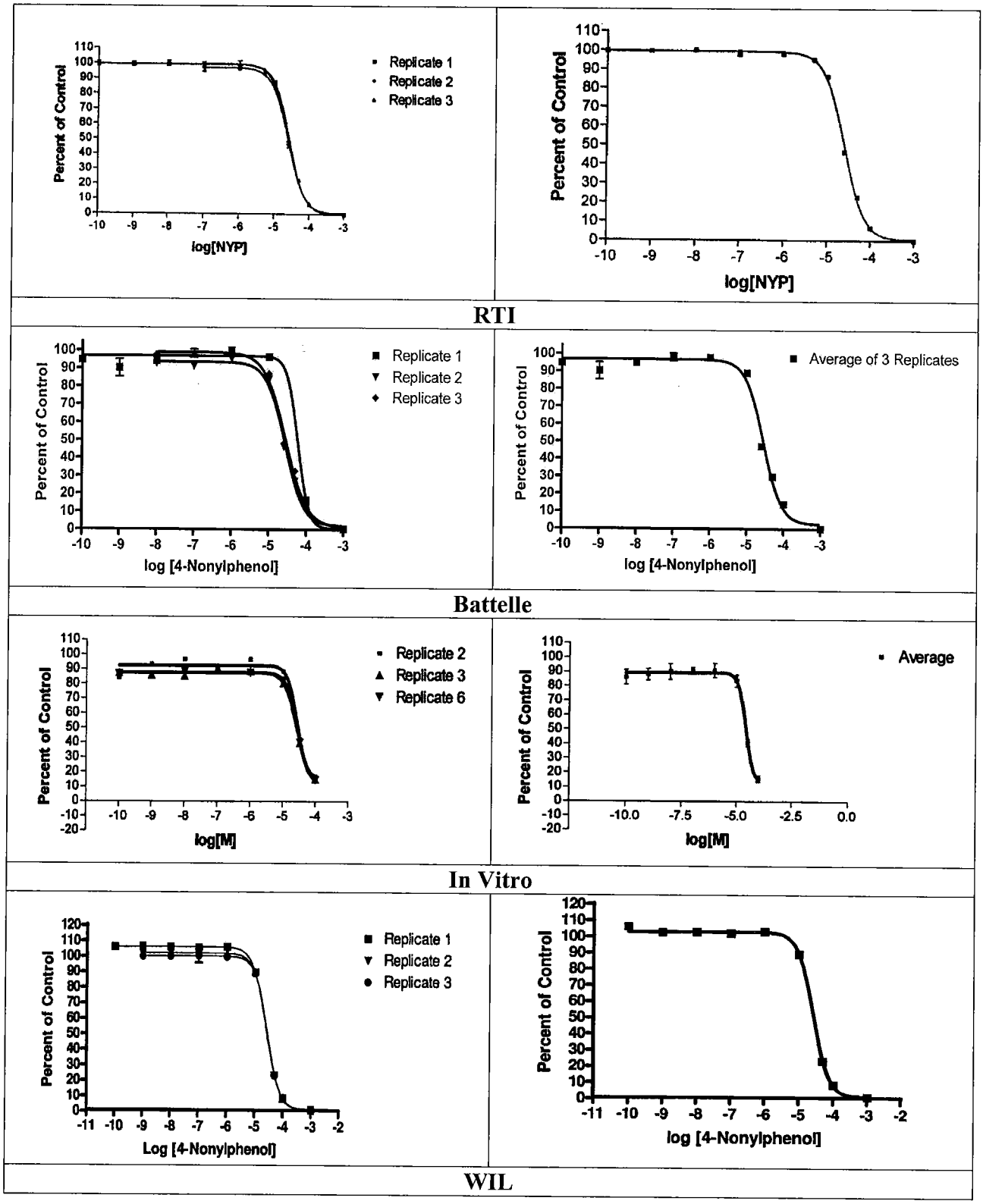

Figure 17. Individual Replicate and Average 4-Nonylphenol Inhibition Response Curves (percent of control) by Laboratory

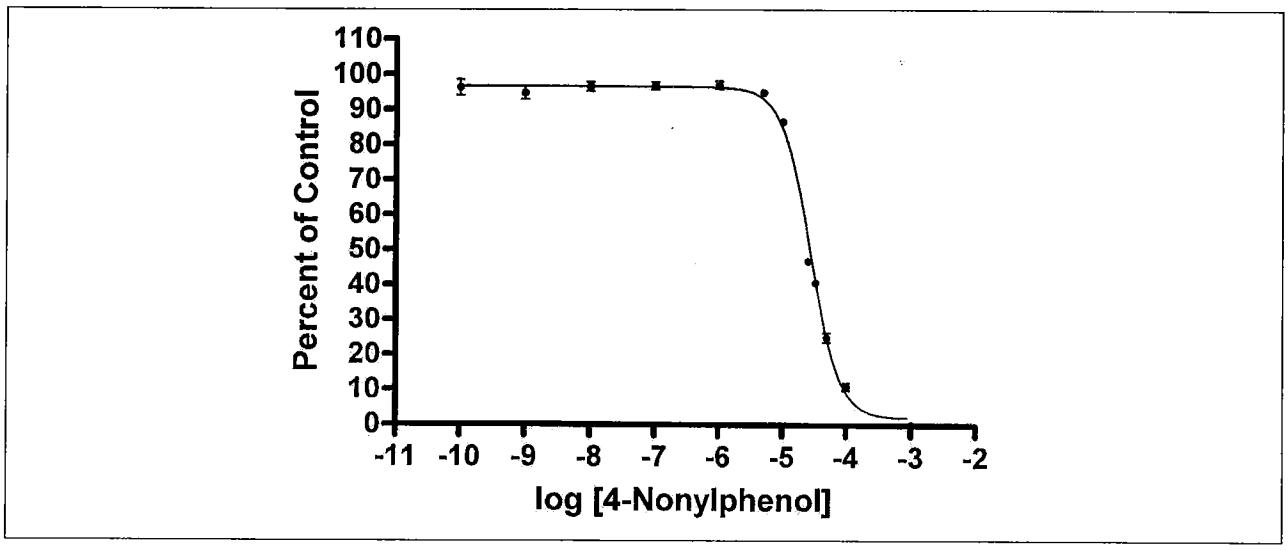

Figure 18. Overall Task 4-Nonylphenol Inhibition Response Curve (percent of control)

After the first replicate was completed, the study directors from three of four laboratories modified the 4-nonylphenol concentrations selected for testing in replicates 2 and 3 (Table 30). In Vitro used the same concentrations for all three replicates and mistakenly did not test at a concentration of 10^{-3} M. In general, the modifications involved including additional mid-concentrations between 10^{-4} and 10^{-6} M for RTI and 10^{-4} M and 10^{-5} M for Battelle and WIL. The concentration(s) that were replaced by these modifications differed for the three labs but ranged from 10^{-8} to 10^{-10} M.

For all four laboratories, increasing concentrations of 4-nonylphenol decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent (Table 30). The shapes of the enzyme activity vs 4-nonylphenol curves were sigmoidal (Figure 17). At a 4-nonylphenol concentration of 10^{-3} M, aromatase inhibition was complete; the laboratory percent of control values were less than 1 percent for the three labs that tested at this concentration. For In Vitro, the percent of control at the highest concentration tested (10^{-4} M) was 16 percent. In contrast, at a 4-nonylphenol concentration of approximately 10^{-6} M, there was little to no aromatase inhibition; the laboratory percent of control values were $\geq$98 percent, except for In Vitro that reported a percent of control value of approximately 91 percent at this concentration. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-6} M were 0.26 ± 0.10 and 97.16 ± 2.24 percent, respectively (Table 31).

All labs have assay precision values (% CV) that were less than 8 percent for those concentrations used in at least three replicates with the following exceptions: the precision varied widely at a concentration of 10^{-3} M for three of four labs, i.e. 42 to 80 percent (In Vitro did not test at this concentration, Table 30); and Battelle had a percent of control value of 16 percent at a concentration of 10^{-4} M. The overall task assay precision ranged from 3 to 17 percent for those concentrations that were tested by 3 or more labs, except at concentrations of 10^{-3} and 10^{-4} M where the % CV values were 65 and 40 percent, respectively (Table 31).

3.8.10 Prochloraz

The effect of increasing concentrations of prochloraz on aromatase activity is reported by laboratory in Table 32 and by overall task in Table 33. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 19 and 20.

After the first replicate was completed, the study directors from three of four laboratories modified the prochloraz concentrations selected for testing in replicates 2 and 3 (Table 32). In general, the modifications involved including additional mid-concentrations between 10^{-7} and 10^{-9} M, which replaced the concentrations from 10^{-3} and 10^{-4} M that were tested in replicate 1 for RTI and WIL, whereas Battelle replaced concentrations at 10^{-4} and 10^{-11} M. Battelle tested concentrations ranging from 10^{-4} M to 10^{-11} M rather than 10^{-3} M to 10^{-10} M due to a miscalculation. The error was identified and corrected prior to proceeding with replicates 2 and 3. In Vitro used the same concentrations for the first two replicates, but for its last replicate, used a concentration of 3.3×10^{-8} M rather than what it used in the previous two replicates, i.e. 3.3×10^{-9} M. Changing the concentration after the second rather than the first replicate was an unplanned change but was done so believing that it might result in a more well-defined IC_{50}.

For all four laboratories, increasing concentrations of prochloraz decreased the activity of the placental microsomal aromatase activity and the decrease was concentration-dependent (Table 32). The shapes of the enzyme activity vs prochloraz curves were sigmoidal (Figure 19). At a prochloraz concentration of 10^{-6} M, aromatase inhibition was almost complete; the laboratory percent of control values ranged from 2 to 3 percent for three of the labs; the exception was WIL, which reported a negative percent of control value at this concentration. In contrast, at a prochloraz concentration of approximately 10^{-9} M, there was little to no aromatase inhibition; the laboratory percent of control values were ≥95 percent for three of the four labs, and for In Vitro the percent of control value was 88 percent at this concentration. The overall task mean ± SEM percent of control values at 10^{-6} and 10^{-9} M were 0.76 ± 1.64 and 94.46 ± 2.44 percent, respectively (Table 33).

Assay precision values (% CV) varied widely for prochloraz concentrations ≥10^{-8} M, i.e. 4 to 281 percent. For prochloraz concentrations ≤10^{-8} M, the precision values ranged from less than 1 to 12 percent (Table 32). The overall task assay precision ranged from 3 to 5 percent for concentrations ≤10^{-8} M that were tested by 3 or more labs, whereas at higher concentrations, the overall task assay precision ranged from 16 to 434 percent (Table 33).

Table 32. Effect of Prochloraz on Aromatase Activity (Percent of Control) by Laboratory

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
RTI	-3.00	1	0.01	NC[a]	NC	NC
	-4.00	1	-0.07	NC	NC	NC
	-5.00	3	0.13	0.13	0.07	95.06
	-6.00	3	2.41	0.48	0.28	20.04
	-7.00	3	17.79	1.51	0.87	8.47
	-7.60	2	47.64	NC	NC	NC
	-8.00	3	66.59	4.11	2.37	6.17
	-8.30	2	84.11	NC	NC	NC
	-9.00	3	95.97	5.01	2.89	5.22
	-10.00	3	100.77	6.25	3.61	6.20

Laboratory	Reference Chemical Log Conc (M)	Number of Replicates	Percent of Control			
			Mean	sd	SEM	%CV
Battelle	-4.00	1	0.07	NC	NC	NC
	-5.00	3	0.14	0.11	0.07	79.54
	-6.00	3	2.68	0.47	0.27	17.70
	-7.00	3	21.52	3.05	1.76	14.20
	-7.60	2	52.31	NC	NC	NC
	-8.00	3	70.44	4.08	2.35	5.79
	-8.60	2	88.90	NC	NC	NC
	-9.00	3	94.70	0.45	0.26	0.47
	-10.00	3	97.38	1.34	0.77	1.37
	-11.00[b]	1	96.71	NC	NC	NC
In Vitro	-4.00	3	1.13	1.14	0.66	100.1
	-5.00	3	0.15	0.19	0.11	125.3
	-6.00	3	2.07	0.70	0.40	33.6
	-7.00	3	15.68	2.28	1.32	14.6
	-7.48	1	78.51	NC	NC	NC
	-8.00	3	65.02	3.42	1.97	5.3
	-8.48	2	82.65	NC	NC	NC
	-9.00	3	87.78	10.10	5.83	11.5
	-10.00	3	96.36	7.99	4.61	8.3
WIL	-3.00	1	0.06	NC	NC	NC
	-4.00	1	-0.21	NC	NC	NC
	-5.00	3	-6.80	11.97	6.91	176.1
	-6.00	3	-4.14	11.63	6.71	281.1
	-7.00	3	15.72	9.12	5.27	58.03
	-7.52	2	42.22	NC	NC	NC
	-8.00	3	72.91	2.87	1.66	3.94
	-8.52	2	90.14	NC	NC	NC
	-9.00	3	99.40	5.06	2.92	5.10
	-10.00	3	103.27	3.61	2.08	3.49

a. NC – value **N**ot **C**alculated when n ≤2.
b. See text for an explanation for this dilution.

Table 33. Effect of Prochloraz on Aromatase Activity (Percent of Control) by Overall Task

Reference Chemical Log Conc (M)	Number of Labs[a]	Overall Mean	Overall sd	Overall SEM	Overall %CV
-3.00	2	0.04	NC[b]	NC	NC
-4.00	4	0.23	0.61	0.31	265.6
-5.00	4	-1.60	3.47	1.74	217.6
-6.00	4	0.76	3.27	1.64	433.5
-7.00	4	17.68	2.74	1.37	15.5
-7.48	1	78.51	NC	NC	NC
-7.52	1	42.22	NC	NC	NC
-7.60	2	49.98	NC	NC	NC
-8.00	4	68.74	3.59	1.80	5.2
-8.30	1	84.11	NC	NC	NC
-8.48	1	82.65	NC	NC	NC
-8.52	1	90.14	NC	NC	NC
-8.60	1	88.90	NC	NC	NC
-9.00	4	94.46	4.88	2.44	5.2
-10.00	4	99.45	3.17	1.59	3.2
-11.00	1	96.71	NC	NC	NC

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because each laboratory used the results from their first replicate to select concentrations to be used in replicates 2 and 3 that were believed would better characterize the percent inhibition curve. (In Vitro modified their concentrations between replicates 3 and 4).

b. NC – Not Calculated. If the overall mean was calculated using an n ≤2, then the sd, SEM, and %CV were not calculated.

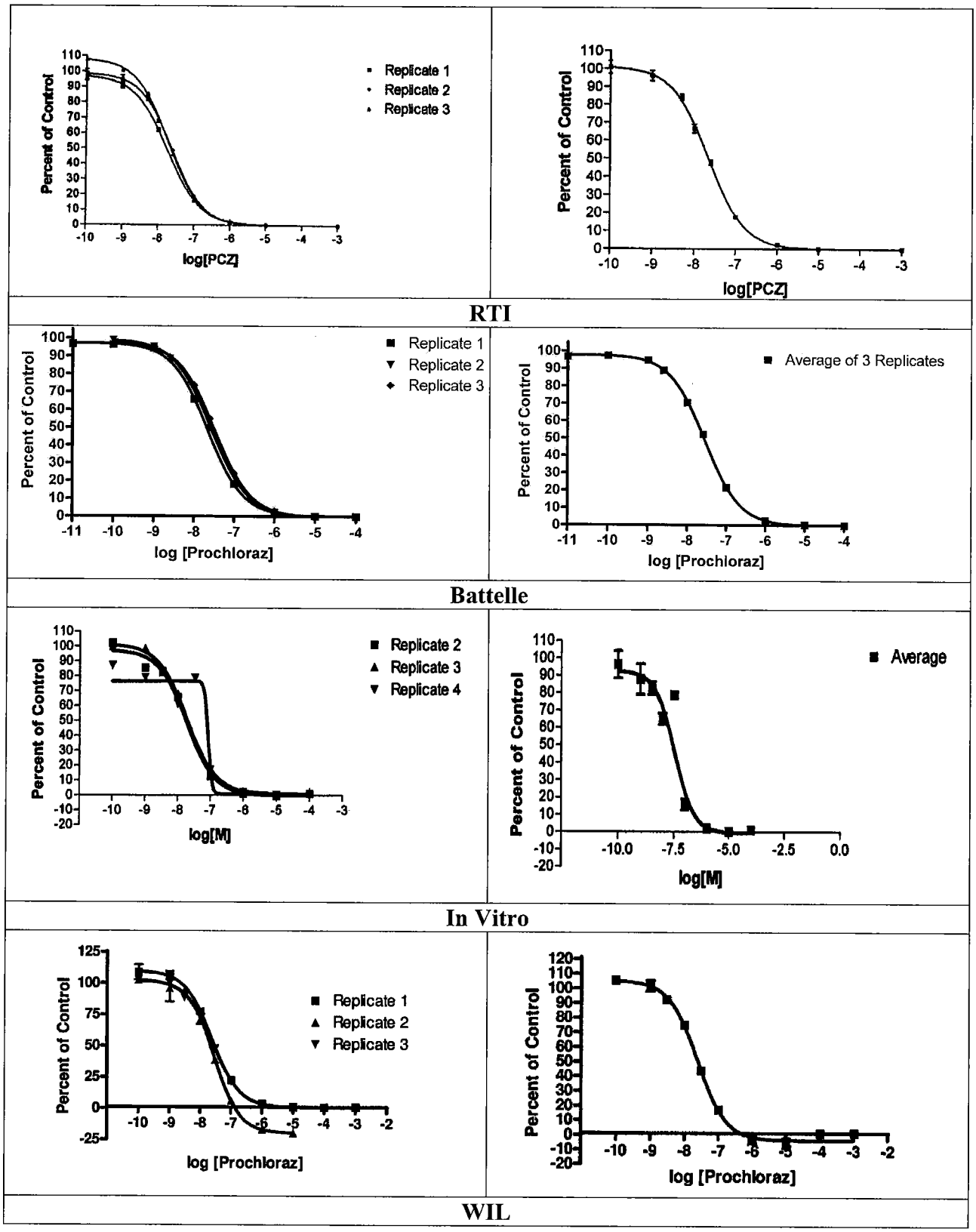

Figure 19. Individual Replicate and Average Prochloraz Inhibition Response Curves (percent of control) by Laboratory

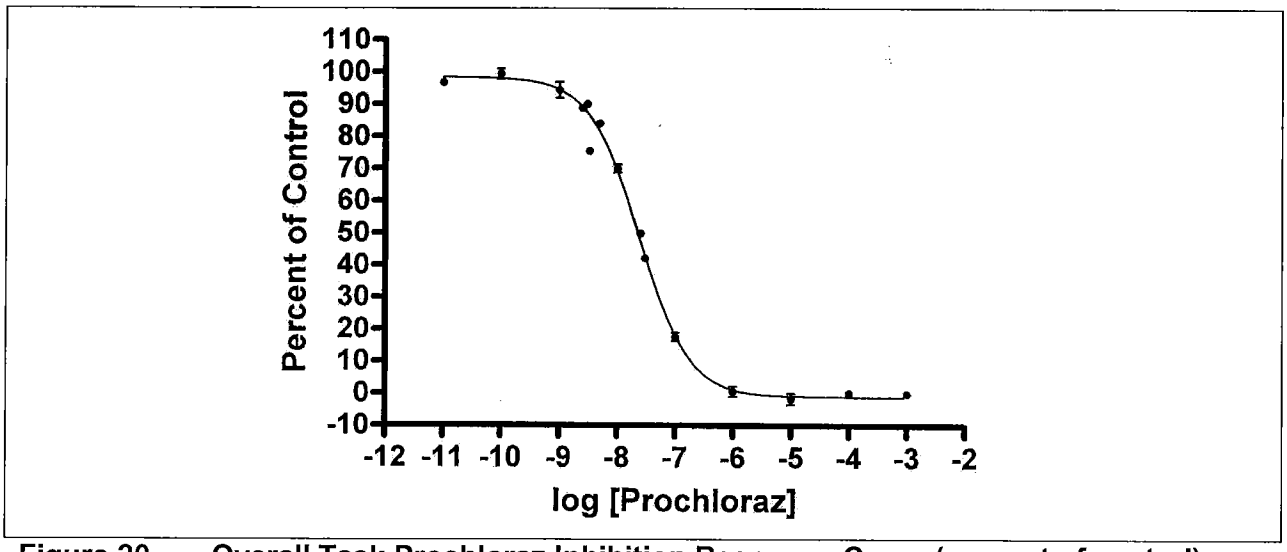

Figure 20. Overall Task Prochloraz Inhibition Response Curve (percent of control)

3.9 IC_{50} AND SLOPE DETERMINATION AND CURVE CLASSIFICATION

3.9.1 Laboratory IC_{50} Values

Based on the curve-fit of the percent of control aromatase activity values across the various number of concentrations for each reference chemical, the calculated IC_{50} values by replicate and laboratory are summarized in Table 34.

Table 34. Reference Chemical IC_{50} Values

Replicate	IC_{50} Values			
	RTI	Battelle	In Vitro	WIL
Aminoglutethimide (µM)				
1	4.16	4.26	4.85	3.89
2	4.19	4.73	3.95	4.65
3	3.72	5.96	3.47	3.78
Average	4.02	4.98	4.09	4.11
sd	0.26	0.88	0.70	0.47
SEM	0.15	0.51	0.40	0.27
% CV	6.5	17.6	17.1	11.5
Atrazine (µM)				
1	no inhibition[a]	no inhibition[a]	no inhibition	no inhibition
2	no inhibition[a]	no inhibition[a]	no inhibition	no inhibition
3	no inhibition[a]	no inhibition[a]	no inhibition[a]	no inhibition
Chrysin (µM)				
1	1.48	1.97	2.47	2.44
2	1.93	2.25	2.62	3.54
3	1.75	2.40	3.97	3.21
Average	1.72	2.21	3.02	3.06
sd	0.23	0.22	0.83	0.56
SEM	0.13	0.13	0.48	0.33
% CV	13.4	10.0	27.4	18.4
Dibenz[a,h]anthracene (µM)				
1	no inhibition	no inhibition	no inhibition[a]	no inhibition
2	no inhibition[a]	no inhibition[a]	no inhibition[a]	no inhibition
3	no inhibition	no inhibition[a]	no inhibition[a]	no inhibition

Replicate	IC_{50} Values			
	RTI	Battelle	In Vitro	WIL
Dicofol (µM)				
1	134.59	17.41	1478	26.56
2	29.24	24.75	3.966	83.54
3	24.89	30.27	20.42	49.28
Mean	62.91	24.14	501	53.13
sd	62.12	6.45	846	28.68
SEM	35.86	3.72	489	16.56
% CV	98.7	26.7	169.0	54.0
Econazole (µM)				
1	0.00138	0.00294	0.000566	0.00161
2	0.00154	0.00235	0.000914	0.00172
3	0.00157	0.00201	0.000975	0.00173
Mean	0.00150	0.00243	0.000818	0.00169
sd	0.00010	0.00047	0.000221	0.00007
SEM	0.00006	0.00027	0.000127	0.00004
% CV	6.8	19.5	27.0	4.2
Fenarimol (µM)				
1	6.49	5.97	2.95	6.35
2	5.42	6.06	4.93	7.10
3	6.12	6.11	4.86	7.72
Mean	6.01	6.05	4.25	7.05
sd	0.54	0.07	1.12	0.69
SEM	0.31	0.04	0.65	0.40
% CV	9.0	1.2	26.5	9.7
Ketoconazole (µM)				
1	6.58	10.6	1.07	6.37
2	6.64	7.11	5.91	6.78
3	6.93	6.59	6.76	6.28
Mean	6.72	8.10	4.58	6.48
sd	0.19	2.18	3.07	0.26
SEM	0.11	1.26	1.77	0.15
% CV	2.8	26.9	67.0	4.1
4-Nonylphenol (µM)				
1	26.67	59.11	26.82	25.03
2	24.66	26.84	26.31	26.43
3	24.77	27.98	27.10	27.48
Mean	25.37	37.98	26.74	26.31
sd	1.13	18.31	0.40	1.23
SEM	0.65	10.57	0.23	0.71
% CV	4.4	48.2	1.5	4.7
Prochloraz (µM)				
1	0.0180	0.0218	0.0186	0.0240
2	0.0239	0.0263	0.0191	0.0284
3	0.0188	0.0326	0.0848	0.0251
Mean	0.0202	0.0269	0.0408	0.0258
sd	0.0032	0.0054	0.0381	0.0023
SEM	0.0018	0.0031	0.0220	0.0013
% CV	15.8	20.3	93.2	8.9

a. Although a value was reported, the percent of control curve indicated that there was no inhibition.

For aminogluthetimide, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 4.02 ± 0.15, 4.98 ± 0.51, 4.09 ± 0.40, and 4.11 ± 0.27 μM, respectively. The percent CV values were 6.5, 17.6, 17.1, and 11.5 percent, respectively. The IC_{50} values were reasonably precise for all replicates and laboratories, which suggested that the IC_{50} could be precisely estimated using the replicate 1 starting concentrations. The overall task group mean ± SEM IC_{50} value was 4.30 ± 0.23 μM and the percent CV was 10.6 percent.

Atrazine did not inhibit aromatase. There were no valid IC_{50} values reported by the laboratories. An examination of the percent of control curves indicated that there was no inhibition.

For chrysin, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 1.72 ± 0.13, 2.21 ± 0.13, 3.02 ± 0.48, and 3.06 ± 0.33 μM, respectively. The percent CV values were 13.4, 10.0, 27.4, and 18.4 percent, respectively. The higher % CV values for In Vitro and WIL were attributed to the IC_{50} value obtained from one of the three replicates. For In Vitro, replicate 3 was relatively higher and, for WIL, replicate 1 was relatively lower. The overall task group mean ± SEM IC_{50} value was 2.50 ± 0.33 μM and the percent CV was 26.1 percent.

For dicofol, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 62.91 ± 35.86, 24.14 ± 3.72, 501 ± 489, and 53.13 ± 16.56 μM, respectively. The percent CV values were 98.7, 26.7, 169.0, and 54.0 percent, respectively. The higher % CV values for RTI and In Vitro were attributed to the IC_{50} value obtained from replicate 1. Modifications to the concentrations tested by RTI and In Vitro after replicate 1 resulted in IC_{50} values that were in better agreement with subsequent estimates, as well as estimates by the other labs. The results from Battelle and WIL indicate that the IC_{50} could be precisely estimated using the replicate 1 starting concentrations. However, three of four labs showed that modifications to the replicate 1 concentrations tested did improve the characterization of the steep portion of the inhibition curve and IC_{50} estimate. Due to the extremely high IC_{50} values reported by RTI and In Vitro from their replicate 1 results, these values were not included in the overall task estimate. The overall task group mean ± SEM IC_{50} value was 29.13 ± 8.62 μM and the percent CV was 59.2 percent.

Dibenz[a,h]anthracene did not inhibit aromatase. There were no valid IC_{50} values reported by the laboratories. An examination of the percent of control curves indicated that there was no inhibition.

For econazole, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 1.50 ± 0.06, 2.43 ± 0.27, 0.818 ± 0.127, and 1.69 ± 0.04 nM, respectively. The percent CV values were 6.8, 19.5, 27.0, and 4.2 percent, respectively. The higher % CV values for Battelle and In Vitro were attributed to the IC_{50} value obtained from replicate 1. In general, modifications to the concentrations tested after replicate 1 seemed to result in IC_{50} values that were in closer agreement for replicates 2 and 3. Although, the IC_{50} values determined were in reasonably good agreement regardless of the modifications. The overall task group mean ± SEM IC_{50} value was 1.61 ± 0.33 nM and the percent CV was 41.3 percent.

For fenarimol, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 6.01 ± 0.31, 6.05 ± 0.04, 4.25 ± 0.65, and 7.05 ± 0.40 μM, respectively. The percent CV values were 9.0, 1.2, 26.5, and 9.7 percent, respectively. The higher % CV value for In Vitro was attributed to the IC_{50} value obtained from replicate 1. However, no modifications to the

concentrations tested were made by In Vitro after replicate 1. The results from RTI, Battelle, and WIL indicate that the IC_{50} could be precisely estimated using the replicate 1 starting concentrations. However, three of four labs showed that modifications to the replicate 1 concentrations tested did improve the characterization of the steep portion of the inhibition curve and IC_{50} estimate. The overall task group mean ± SEM IC_{50} value was 5.84 ± 0.58 µM and the percent CV was 20.0 percent.

For ketoconazole, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 6.72 ± 0.11, 8.10 ± 1.26, 4.58 ± 1.77, and 6.48 ± 0.15 µM, respectively. The percent CV values were 2.8, 26.9, 67.0, and 4.1 percent, respectively. The higher % CV values for Battelle and In Vitro were attributed to the IC_{50} value obtained from replicate 1. Modifications to the concentrations tested by Battelle and In Vitro after replicate 1 resulted in IC_{50} values that were in better agreement with subsequent estimates, as well as estimates by the other labs. The results from RTI and WIL indicate that the IC_{50} could be precisely estimated using the replicate 1 starting concentrations. However, three of four labs showed that modifications to the replicate 1 concentrations tested did improve the characterization of the steep portion of the inhibition curve and IC_{50} estimate. The overall task group mean ± SEM IC_{50} value was 6.47 ± 0.72 µM and the percent CV was 22.4 percent.

For 4-nonylphenol, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 25.37 ± 0.65, 37.98 ± 10.57, 26.74 ± 0.23, and 26.31 ± 0.71 µM, respectively. The percent CV values were 4.4, 48.2, 1.5, and 4.7 percent, respectively. The higher % CV value for Battelle was attributed to the IC_{50} value obtained from replicate 1. Modifications to the concentrations tested by Battelle after replicate 1 resulted in IC_{50} values that were in better agreement with subsequent estimates, as well as estimates by the other labs. The results from RTI, In Vitro, and WIL indicate that the IC_{50} could be precisely estimated using the replicate 1 starting concentrations. However, three of four labs showed that modifications to the replicate 1 concentrations tested did improve the characterization of the steep portion of the inhibition curve and IC_{50} estimate. The overall task group mean ± SEM IC_{50} value was 29.10 ± 2.97 µM and the percent CV was 20.4 percent.

For prochloraz, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 0.0202 ± 0.0018, 0.0269 ± 0.0031, 0.0408 ± 0.0220, and 0.0258 ± 0.0013 µM, respectively. The percent CV values were 15.8, 20.3, 93.2, and 8.9 percent, respectively. The higher % CV value for In Vitro was attributed to the IC_{50} value obtained from replicate 3. The results from RTI, Battelle, and WIL indicate that the IC_{50} could be precisely estimated using the replicate 1 starting concentrations. However, three of four labs showed that modifications to the replicate 1 concentrations tested did improve the characterization of the steep portion of the inhibition curve and IC_{50} estimate. The overall task group mean ± SEM IC_{50} value was 0.0284 ± 0.0044 µM and the percent CV was 30.8 percent.

3.9.2 Laboratory Slope Values

The slope values by replicate and laboratory are summarized in Table 35 for each reference chemical.

Table 35. Reference Chemical Slope Values

Replicate	Slope Values			
	RTI	Battelle	In Vitro	WIL
Aminoglutethimide				
1	-0.9437	-0.8987	-5.155	-0.8613
2	-0.9842	-1.073	-0.9889	-1.0799
3	-0.9547	-0.9702	-0.7764	-0.9987
Average	-0.9609	-0.9806	-2.3068	-0.9800
sd	0.0209	0.0876	2.4689	0.1105
SEM	0.0121	0.0506	1.4254	0.0638
% CV	2.2	8.9	107.0	11.3
Atrazine				
1	no inhibition[a]	no inhibition[a]	no inhibition	no inhibition
2	no inhibition[a]	no inhibition[a]	no inhibition	no inhibition
3	no inhibition[a]	no inhibition[a]	no inhibition[a]	no inhibition
Chrysin				
1	-1.528	-1.785	-1.599	-1.0139
2	-1.432	-1.325	-1.167	-1.5485
3	-1.302	-1.075	-0.872	-1.3875
Average	-1.421	-1.395	-1.213	-1.317
sd	0.114	0.360	0.366	0.274
SEM	0.066	0.208	0.211	0.158
% CV	8.0	25.8	30.2	20.8
Dibenz[a,h]anthracene				
1	no inhibition	no inhibition	no inhibition[a]	no inhibition
2	no inhibition[a]	no inhibition[a]	no inhibition[a]	no inhibition
3	no inhibition	no inhibition[a]	no inhibition[a]	no inhibition
Dicofol				
1	-0.4643	-0.8235	-0.4795	-0.6920
2	-0.8303	-0.7637	-0.8759	-0.6235
3	-0.7067	-1.408	-1.038	-0.6028
Mean	-0.6671	-0.9984	-0.7978	-0.6394
sd	0.1862	0.3560	0.2873	0.0467
SEM	0.1075	0.2055	0.1659	0.0270
% CV	27.9	35.7	36.0	7.3
Econazole				
1	-1.048	-1.025	-0.7479	-1.0946
2	-1.030	-1.048	-0.8011	-1.0703
3	-1.073	-1.038	-0.8255	-1.1339
Mean	-1.0503	-1.0370	-0.7915	-1.0996
sd	0.0216	0.0115	0.0397	0.0321
SEM	0.0125	0.0067	0.0229	0.0185
% CV	2.1	1.1	5.0	2.9
Fenarimol				
1	-0.9924	-1.005	-1.292	-1.0253
2	-0.9430	-0.9361	-0.9339	-1.0224
3	-0.9089	-1.039	-1.072	-1.0465
Mean	-0.9481	-0.9934	-1.0993	-1.0314
sd	0.0420	0.0524	0.1806	0.0132
SEM	0.0242	0.0303	0.1043	0.0076
% CV	4.4	5.3	16.4	1.3

Replicate	Slope Values			
	RTI	Battelle	In Vitro	WIL
Ketoconazole				
1	-0.9772	-0.9384	-0.8716	-0.9736
2	-0.8726	-0.9801	-1.014	-0.9924
3	-0.9062	-1.009	-1.097	-1.1402
Mean	-0.9187	-0.9758	-0.9942	-1.0354
sd	0.0534	0.0355	0.1140	0.0912
SEM	0.0308	0.0205	0.0658	0.0527
% CV	5.8	3.6	11.5	8.8
4-Nonylphenol				
1	-2.017	-3.042	-2.939	-1.8580
2	-1.948	-1.766	-2.340	-1.9735
3	-1.908	-1.558	-2.601	-2.0307
Mean	-1.9577	-2.1220	-2.627	-1.9541
sd	0.0551	0.8035	0.3003	0.0880
SEM	0.0318	0.4639	0.1734	0.0508
% CV	2.8	37.9	11.4	4.5
Prochloraz				
1	-0.9392	-0.9853	-1.005	-0.9841
2	-0.9971	-0.9386	-0.9771	-1.0622
3	-0.9697	-0.9641	-7.924	-0.9521
Mean	-0.9687	-0.9627	-3.3020	-0.9995
sd	0.0290	0.0234	4.0028	0.0566
SEM	0.0167	0.0135	2.3110	0.0327
% CV	3.0	2.4	121.2	5.7

a. Although a value was reported, the percent of control curve indicated that there was no inhibition.

For aminoglutethimide, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -0.9609 ± 0.0121, -0.9806 ± 0.0506, -2.3068 ± 1.4254, and -0.9800 ± 0.0638, respectively. The percent CV value were 2.2, 8.9, 107.0, and 11.3 percent, respectively. The higher % CV values for In Vitro was attributed to a slope obtained from replicate 1. The results from RTI, Battelle, and WIL indicate that the slope could be precisely estimated using the replicate 1 starting concentrations. However, three of four labs showed that modifications to the replicate 1 concentrations tested did improve the characterization of the steep portion of the inhibition curve and slope estimate. The replicate 1 slope value reported by In Vitro was not included in the calculation used to determine the overall task values. The overall task group mean ± SEM slope value was -0.9510 ± 0.0232 and the percent CV was 4.9 percent.

Atrazine did not inhibit aromatase. There were no valid IC_{50} values reported by the laboratories. An examination of the percent of control curves indicated that there was no inhibition.

For chrysin, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -1.421 ± 0.066, -1.395 ± 0.208, -1.213 ± 0.211, and -1.317 ± 0.158, respectively. The percent CV values were 8.0, 25.8, 30.2, and 20.8 percent, respectively. The higher % CV values for Battelle, In Vitro, and WIL were attributed to a slope obtained from replicate 1. The results from RTI indicate that the slope could be precisely estimated using the replicate 1 starting concentrations. However, all four labs showed that modifications to the replicate 1 concentrations tested did improve the characterization of the steep portion of the inhibition curve and slope estimate.

Regardless, the slope estimates were in good agreement for the labs. The overall task group mean ± SEM slope value was -1.336 ± 0.047 and the percent CV was 7.0 percent.

For dicofol, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -0.6671 ± 0.1075, -0.9984 ± 0.2055, -0.7978 ± 0.1659, and -0.6394 ± 0.0270, respectively. The percent CV values were 27.9, 35.7, 36.0, and 7.3 percent, respectively. The higher % CV values for RTI and In Vitro were attributed to a slope obtained from replicate 1, whereas for Battelle it was associated with the slope determined from replicate 3. The results from WIL indicate that the slope could be precisely estimated using the replicate 1 starting concentrations. However, three of four labs showed that modifications to the replicate 1 concentrations tested did improve the characterization of the steep portion of the inhibition curve and slope estimate. The overall task group mean ± SEM slope value was -0.7757 ± 0.0819 and the percent CV was 21.1 percent.

Dibenz[a,h]anthracene did not inhibit aromatase. There were no valid IC_{50} values reported by the laboratories. An examination of the percent of control curves indicated that there was no inhibition.

For econazole, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -1.0503 ± 0.0125, -1.0370 ± 0.0067, -0.7915 ± 0.0229, and -1.0996 ± 0.0185, respectively. The percent CV values were 2.1, 1.1, 5.0, and 2.9 percent, respectively. The replicates for a given lab and between labs were in close agreement. Modifications to concentrations or lack thereof did not seem to have any overt effect on slope values. The overall task group mean ± SEM slope value was -0.9946 ± 0.0690 and the percent CV was 13.9 percent.

For fenarimol, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -0.9481 ± 0.0242, -0.9934 ± 0.0303, -1.0993 ± 0.1043, and -1.0314 ± 0.0076, respectively. The percent CV values were 4.4, 5.3, 16.4, and 1.3 percent, respectively. The replicates for a given lab and between labs were in close agreement. Modifications to concentrations or lack thereof did not seem to have any overt effect on slope values. The overall task group mean ± SEM slope value was -1.0180 ± 0.0320 and the percent CV was 6.3 percent.

For ketoconazole, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -0.9187 ± 0.0308, -0.9758 ± 0.0205, -0.9942 ± 0.0658, and -1.0354 ± 0.0527, respectively. The percent CV values were 5.8, 3.6, 11.5, and 8.8 percent, respectively. The replicates for a given lab and between labs were in close agreement. Modifications to concentrations or lack thereof did not seem to have any overt effect on slope values. The overall task group mean ± SEM slope value was -0.9810 ± 0.0242 and the percent CV was 4.9 percent.

For 4-nonylphenol, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -1.9577 ± 0.0318, -2.1220 ± 0.4639, -2.627 ± 1.734, and -1.9541 ± 0.0508, respectively. The percent CV values were 2.8, 37.9, 11.4, and 4.5 percent, respectively. The replicates for a given lab and between labs were in close agreement. Modifications to concentrations or lack thereof did not seem to have any overt effect on slope values. The overall task group mean ± SEM slope value was -2.1651 ± 0.1588 and the percent CV was 14.7 percent.

For prochloraz, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -0.9687 ± 0.0167, -0.9627 ± 0.0135, -3.3020± 2.3110 and -0.9995 ± 0.0327, respectively. The percent CV values were 3.0, 2.4, 121.2, and 5.7 percent, respectively. The higher % CV

value for In Vitro was attributed to a slope obtained from replicate 3. The results from RTI, Battelle, and WIL indicate that the slope could be precisely estimated using the replicate 1 starting concentrations. However, three of four labs showed that modifications to the replicate 1 concentrations tested did improve the characterization of the steep portion of the inhibition curve and slope estimate. The replicate 3 slope values reported by In Vitro was not included in the calculation used to determine the overall task values. The overall task group mean ± SEM slope value was -0.9805 ± 0.0088 and the percent CV was 1.8 percent.

3.9.3 Reference Chemical Response Classifications

All four laboratories characterized the reference chemicals as "Complete (C)" inhibitors, except for atrazine and dibenz[a,h]anthracene, which were classified as non-inhibitors.

3.10 INTRALABORATORY STATISTICAL ANALYSIS

The intralaboratory analyses from each laboratory for background activity, full enzyme activity, and negative and positive controls, IC_{50}s, and slopes are provided in the appendices of the individual laboratory reports, which can be found in their entirety in the appendices of this overall report. Due to the integrated manner in which the intralaboratory statistical analysis reports were presented, as well as the interrelationships among the endpoints, it was decided to present the salient information from the intralaboratory statistical analysis reports in a single section rather than attempt to extract information about individual endpoints and incorporate them into their previously presented respective sections.

3.10.1 RTI Intralaboratory Statistical Analysis

The full statistical analysis report and corresponding tables and graphs can be found in the appendices of the RTI report. Since the RTI report did not include a summary of the intralaboratory statistical analysis results, Battelle prepared the following summary from the information that was presented by RTI:

> Dose response model curve fits were not achievable for dibenz[a,h]anthracene (DBA) and atrazine (ATZ) due to the low aromatase inhibition and thus these two chemical were categorized as non-inhibitors. Among the remaining eight chemicals, the estimated values for the $\log_{10}IC_{50}$ were -8.823 for ECZ and -7.698 for PCZ, and ranged from -6.395 to -4.171 for the rest. Thus ECZ and PCZ were relatively strong inhibitors. The steepest slope was observed in NYP (-1.949) and the shallowest slope was observed in DCF (-0.658). The slopes for the rest of the chemicals were around -1.0. The bottom thresholds were around zero for seven of the eight chemicals, with the exception of CYN (20.631). The top thresholds were around 100 for all eight chemicals. There was very little variance in the IC_{50}, slope, top and bottom estimates over the three replicates for any of the eight chemicals. Significantly decreasing control activities between the beginnings and the ends of replicates were observed for the full enzyme activity control, the negative control, and the positive control.

3.10.2 Battelle Intralaboratory Statistical Analysis

The full statistical analysis report and corresponding tables and graphs can be found in the appendices of the Battelle report. The following information was taken from the Summary and Conclusion section of the statistical analysis report.

Reference chemicals dibenz[a,h]anthracene and atrazine are non-inhibitors. The majority of concentration responses were above 80% of control.

For chrysin, the low threshold of responses was in the range of 17 % and 25 %. Bottom parameter estimates ranged from 17.370 to 21.550 for the three replicates.

Among the four parameters of the concentration response curves (the baseline response [bottom], the maximum response [top], the concentration that provokes a response halfway between baseline and maximum [IC_{50}], and the slope), the top parameter had the most replicate-to-replicate variation. The other three parameters all had zero, close to zero, or minimal replicate-to-replicate variations.

Except for 4-nonylphenol (Replicate 1), the within-replicate variations were all close to zero for $\log_{10}IC_{50}$ and slope.

Except for background controls, the differences between the beginning and the end portions for the other three control types (full enzyme activity and positive and negative controls), when averaged across replicates, were significant. The end portion was significantly lower than the beginning portion, which implied a reduction in aromatase activity between the beginning and the end of a replicate.

For all the control types, chemical main effect and chemical by portion interaction effect were not significant.

Source of microsomes did not have a significant effect on the activities of the four controls (full enzyme activity, background activity, and positive and negative controls) .

For econazole, the variation in replicate 1 for the full enzyme activity controls, the positive controls, and the negative controls was substantially larger and out of line with that for any replicate for all the chemicals.

For positive controls, the majority of variation was from replicate-to-replicate within chemicals.

3.10.3 In Vitro Intralaboratory Statistical Analysis

The full statistical analysis report and corresponding tables and graphs can be found in the appendices of the In Vitro report. The following information was taken from the Summary and Conclusion section of the statistical analysis report.

The reference chemicals dibenz[a,h]anthracene and atrazine are non-inhibitors. With only a few exceptions, the majority of concentration responses were close to or above 80% of control.

Most of the reference chemicals exhibited replicate-to-replicate variation in one or more characteristics of the concentration response curve fits. These included:

- Aminoglutethimide: The lower end of the concentration response curve for replicate 1 did not exhibit a threshold. This resulted in inflation in the estimate and variability of the average bottom parameter.
- Chrysin: The top, bottom, and slope parameters varied somewhat across replicates.
- Dicofol: The bottom threshold was particularly variable among replicates and to a lesser extent the top threshold. This resulted in variation of the IC_{50} estimates across replicates since they estimated different percentiles [(B+T)/2] of the response distributions and introduced bias into their comparisons.
- Econazole: The replicate 1 concentration response curve was out of line with those from the other replicates.
- Ketoconazole: The top threshold from replicate 1 differed somewhat from those of the other replicates. This inflated the variability of the average top threshold.
- 4-Nonylphenol: The top threshold for replicate 2 was out of line with and higher than those from the other two replicates. This inflated the variability of the overall average estimate.
- Prochloraz: The concentration response curve for replicate 4 was well out of line with the response curves for the other replicates. The top threshold and the slope were particularly different.

Econazole is a strong aromatase inhibitor. The bottom thresholds of the concentration response distributions extended below a concentration 10^{-7} M. The concentration response distributions exhibit inhibition and do not attain their top thresholds even at concentration 10^{-10} M.

Dicofol is a weak inhibitor. The concentration response curves maintain their top thresholds to approximately a concentration of 10^{-6} M and do not approach their bottom threshold even at a concentration of 10^{-4} M. The average percent of control at a concentration of 10^{-4} M varies from 33 percent to 52 percent across replicates.

The behavior of the full enzyme activity controls and the background activity controls was for the most part (except for a couple of isolated replicates) consistent across chemicals and replicates. The activity at the beginning of the replicate was greater than the activity at the end of the replicate. The full enzyme activity control portion effect (end minus beginning) averaged across replicates was significant.

The behavior of the positive controls was for the most part (except for an isolated replicate, fenarimol replicate 1) consistent across chemicals and replicates. For the microsomes prepared at Battelle the activity at the end of the replicate was greater than the activity at the beginning of the replicate. For the microsomes prepared at In Vitro the activity at the end and at the beginning of the replicate was more similar.

The behavior of the negative controls was for the most part (except for an isolated replicate, fenarimol replicate 1) consistent across chemicals and replicates. For the microsomes prepared at Battelle the activity at the end of the replicate was considerably greater than the activity at the beginning of the replicate. For the microsomes prepared at In Vitro the activity at the end of the replicate was greater than the activity at the beginning of the replicate but the average difference was less than for the microsomes prepared at Battelle.

The source of microsomes fixed effect and the chemical by portion interaction fixed effect were not significant for full enzyme activity, background activity, and positive controls. However, the source of microsomes fixed effect and the chemical by portion interaction fixed effect were significant for the negative controls. Test substances whose microsomes were prepared at In Vitro had significantly higher average negative control values than test substances whose microsomes were prepared at Battelle. This is due to the considerably lower activity at the beginning of each replicate for the microsomes prepared at Battelle, as compared to the microsomes prepared at In Vitro.

3.10.4 WIL Intralaboratory Statistical Analysis

The full statistical analysis report and corresponding tables and graphs can be found in the appendices of the WIL report. Since the WIL report did not include a summary of the intralaboratory statistical analysis results, Battelle prepared the following summary from the information that was presented by WIL:

> The results for WIL were similar to those for RTI in most part. The dose response model curve fits were not achievable for, chemicals 5 and 10, dibenz[a,h]anthracene (DBA) and atrazine (ATZ) due to their low aromatase inhibition. ECZ and PCZ (chemicals 7 and 3) were relatively strong inhibitors. The estimated values for the $\log_{10}$IC50 were -8.78 for ECZ and -7.60 for PCZ, and ranged from -6.390 to -4.193 for the six remaining chemicals. The deepest slopes were observed in NYP (-1.952) and CYN (-1.258), and the slowest slope was observed in DCF (-0.639). The slopes for the rest of the chemicals were around -1.0. Chrysin (chemical 8) had a relatively high bottom threshold. The bottoms were -10.38, -6.70 and -6.47 for DCF, PCZ and FRM respectively, 13.30 for CYN, and around zero (between -1.11 to 0.44) for the other four chemicals. The top were all around 100 (ranging from 90.23 to 103.82). There was very little variance in the IC_{50}, slope, top and bottom estimates over the three replicates for any of the eight reference chemicals. Significantly decreasing control activity between the beginnings and the ends of replicates were observed for the full enzyme activity controls (P=0.002), the negative controls (P=0.001) and the positive controls (P=0.012).

3.11 INTERLABORATORY STATISTICAL ANALYSIS

3.11.1 Concentration Response Relations: $\log_{10}IC_{50}$, Slope, Top and Bottom

Convergence problems prevented successful concentration response curve fits for the two non-inhibitor chemicals dibenz[a,h]anthracene (DBA) and atrazine (ATZ) in all four intralaboratory analyses of the individual laboratory data. Therefore the interlaboratory analyses of the concentration response curve parameters were performed only for the other eight

chemicals. The mixed model analysis of variance comparisons among the laboratories accounted for potential systematic differences between the two sources of microsomes.

Table 36 displays the estimated $\log_{10}IC_{50}$ and slope within each laboratory and the associated 95 percent confidence intervals about the $\log_{10}IC_{50}$ and slope for each chemical[1]. Table 36 also displays the mean difference between the two sources of microsomes and the overall mean values across laboratories and their associated 95 percent confidence intervals, incorporating among laboratory variation based on the random effects analysis of variance and heterogeneous within laboratory variation. These means and confidence intervals are displayed in Figures 21 through 36. Each figure includes a reference line corresponding to the overall average. The estimated CVs and their associated 95 percent confidence intervals for the overall means for the $\log_{10}IC_{50}$ and the slope parameters are also presented in Table 36.

Table 37 displays the within laboratory variances and their associated degrees of freedom for each laboratory for the $\log_{10}IC_{50}$ and the slope parameters[2]. These are the squares of the within laboratory standard errors associated with the estimated parameter values. Table 37 also displays the among laboratory component of random variation, the p-values associated with the tests that the among laboratory variation is zero, and the squares of the standard errors of the overall mean values, as well as their associated degrees of freedom. The ratios of the among laboratory variances to the unweighted average within laboratory variances are also displayed, with their associated 95 percent confidence intervals.

Similar statistics for the top and the bottom parameters are displayed in the Interlaboratory Statistical Analysis Report Appendix (Tables A-1 and A-2 and Figures A-1 through A-16). Note that the CV was not calculated for the bottom parameter since its distribution straddles zero.

The estimated $\log_{10}IC_{50}$ with the Battelle microsomes was significantly lower than that with In Vitro microsome for 4-nonylphenol (NYP), but this difference was small (-0.028) compared to the mean estimated $\log_{10}IC_{50}$ (-4.583). No other significant differences between the two sources of microsomes were observed for either the $\log_{10}IC_{50}$ or the slope (Table 36). The estimated top threshold for ketoconazole was significantly lower for the Battelle microsomes that for the In Vitro microsomes. No other significant differences between the two sources of microsomes were observed for either the top or bottom thresholds (Table A-1).

The estimates for $\log_{10}IC_{50}$ (Table 36) were similar among the four laboratories for most chemicals except for econazole (ECZ). For econazole, the estimated $\log_{10}IC_{50}$ for In Vitro was

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[1] The confidence intervals in Table 36 for the individual laboratory means were based on the least squares means, standard errors, and degrees of freedom reported in the intralaboratory analyses within each laboratory. The confidence intervals in Table 36 may differ in the low significant digits from those displayed in the intralaboratory analysis reports due to round off in intermediate calculations.

[2] Degrees of freedom (Table 37) for Battelle and In Vitro were based on those specified in the intralaboratory analysis reports. Degrees of freedom for WIL Laboratories were based on the number of replicates (3) minus 1. Two degrees of freedom were assigned. The degrees of freedom for RTI were based on an analysis of variance model for eight chemicals combined. There were 24 observations (8 chemicals × 3 replicates per chemical) and 8 effects estimated, leaving 16 degrees of freedom for residual.

smaller than for any of the other three laboratories and the associated 95 percent confidence interval for In Vitro did not overlap with those for the other three laboratories (Figure 31). The CVs for IC_{50} exceeded 22.5 percent for econazole and for chrysin and 47.3 percent for dicofol. They were less than 14.7 percent for the IC_{50}s for the other chemicals (Table 36). The ratios of the among laboratory variance to the average within laboratory variance were 6.8 for econazole, 4.6 for chrysin, 2.3 for prochloraz, and less than 0.05 for the remaining five chemicals (Table 37). The large CV for dicofol (47.4 percent, Table 36) was caused by the relatively large within laboratory variance. The pooled within laboratory variance was 0.045 for dicofol, compared to less than or equal to 0.002 for the other seven chemicals. For chrysin, the estimated $\log_{10}IC_{50}$ for RTI was smaller than those for the other three laboratories (Figure 33), which was the principal reason for the relatively large CV and among laboratory variance for this chemical.

The estimates for slope (Table 36) were similar among the four laboratories for most chemicals except for econazole (ECZ). For econazole, the estimated slope for In Vitro was less negative than the other three laboratories and the associated 95 percent confidence interval did not overlap with those for the other three laboratories (Figure 32). For 4-nonylphenol the In Vitro slope was more negative than those at the other laboratories (Figure 28). The ratios of the among laboratory variances to the pooled within laboratory variance were 9.0 for econazole and zero for the seven other chemicals (Table 37). The CVs for the slope were 10.2 percent for chrysin, 15.2 percent for dicofol, and less than or equal to 8.8 percent for the other chemicals (Table 36). The relatively large CVs for chrysin and for dicofol were due to the relatively large within laboratory variances for these chemicals. The pooled within laboratory variances were 0.024 for dicofol, 0.021 for chrysin, 0.013 for 4-nonylphenol, and less than or equal to 0.004 for the other chemicals. Note that for 4-nonylphenol (NYP), the estimated slope for In Vitro was more negative than for the other three laboratories (-2.48 compared to the closest one at -1.95) (Table 36 and Figure 28). However for 4-nonylphenol (NYP), the within-laboratory variance for In Vitro was at least 3 times of the other three laboratories, and therefore less weight was given to In Vitro results when combining the intralaboratory results (Table 37).

The estimates for the top were similar for the four laboratories. The CVs for the top were less than 3 percent for each of the chemicals. The among laboratory variances to the pooled within laboratory variance ratios were 2.3 for 4-nonylphenol, 0.7 for dicofol, and zero for the rest. Some laboratories had very large within laboratory variances relatively to the others for some chemicals, however there were no consistent patterns for any laboratory.

The estimated bottom thresholds for In Vitro were higher than those for the other three laboratories for 4-nonylphenol and for dicofol. The associated 95 percent 4-nonylphenol confidence interval for In Vitro did not overlap with those for the other three laboratories. The ratios of the among laboratory variances to the pooled within laboratory variance ratios were 12.6 for 4-nonylphenol and 5.6 for chrysin, and zero for the other chemicals. Some laboratories had very large within laboratory variances relatively to the others for some chemicals, however there were no consistent patterns for any laboratory.

3.11.2 Control Activity: Differences Between the Beginning and the End Portions and Differences Between the Two Sources of Microsomes

Table 38 displays the estimated parameter values and the associated within laboratory 95 percent confidence intervals for the differences between the beginning and the end, and for the differences between the two sources of microsomes[3]. It also displays the overall mean differences across laboratories and their associated 95 percent confidence intervals, incorporating among laboratory variation based on the random effects analysis of variance. The mean differences and confidence intervals are displayed in Figures 37 to 42. Each figure includes a reference line corresponding to the overall average. Figures 37 through 40 display the results for the differences between the beginning and the end, one for each control endpoint. Figures 41 and 42 display the results for the differences between the two microsome sources for the negative and positive controls respectively.

Table 39 displays the within laboratory variances and their associated degrees of freedom for each laboratory. These are the squares of the within laboratory standard errors associated with the estimated parameter values. Table 39 also displays the laboratory to laboratory random variation and the p-values, and the squares of the standard errors of the overall mean values, as well as their associated degrees of freedom. The ratios of the among laboratory variances to the unweighted average within laboratory variances are also displayed, with their associated 95 percent confidence intervals.

The following results were observed:

For the background activity controls, there were no significant differences between the beginnings and the ends of replicates.

For the full enzyme activity control, the end portion was statistically significantly lower than the beginning portion for each individual laboratory and on average across laboratories. The estimated variance among the laboratories was zero.

For the negative controls, the result for In Vitro was different than those from any of the other three laboratories. The end portion was significantly higher than the beginning portion for In Vitro, but significantly lower than the beginning for each of the other three laboratories. As a result of this inconsistency, the estimated variance among the laboratories was more than 16 times higher than the average within-laboratory variance. The two portions on average across laboratories did not differ significantly.

For the positive controls, the result for In Vitro was different from the other three laboratories. The end portion was (nearly significantly) higher than the beginning portion for In Vitro, but significantly lower than the beginning for each of the other three laboratories. As a result of this disagreement, the estimated variance among the laboratories was more than six times higher than the average within laboratory variance. The two portions did not differ significantly on average across laboratories.

[3] The confidence intervals are based on the least squares means, standard errors, and degrees of freedom shown in Appendix E, which in turn are based on those reported in the intralaboratory analyses.

Comparisons between the microsomes prepared at Battelle and those prepared at In Vitro were carried out for the negative and the positive controls. For the negative controls the microsomes prepared at Battelle were significantly lower than the microsomes prepared at In Vitro for RTI and In Vitro and on average across laboratories. For the positive controls the microsomes prepared at Battelle were not significantly different than the microsomes prepared at In Vitro for any of the individual laboratories or on average across all the laboratories. The estimated variance among the laboratories was zero for the negative and positive controls. Note that the difference between the two sources of microsomes at In Vitro was larger in absolute value for both the negative and the positive controls.

Table 36. Parameter Estimates and the 95 percent Confidence Intervals for $Log_{10}IC_{50}$ and Slope Parameter of Concentration Response Curves for Placental Assay. Estimated by Chemical

Chemical[5]	Param	Estimate and 95 percent Confidence Interval						CV(percent) and 95 percent CI[4]
		RTI[1]	Battelle[1]	WIL[1]	In Vitro[1]	Difference Between Two Microsome Sources[3] (Battelle – In Vitro)	Overall[2]	
AG	Log_IC50	-5.395(-5.429,-5.361)	-5.310(-5.501,-5.119)	-5.390(-5.506,-5.274)	-5.407(-5.651,-5.163)	0.014(-0.050,0.078)	-5.391(-5.422,-5.359)	5.765(3.621,13.865)
AG	Slope	-0.913(-0.977,-0.850)	-0.971(-1.006,-0.936)	-1.026(-1.237,-0.815)	-1.007(-1.650,-0.364)	0.059(-0.042,0.160)	-0.987(-1.037,-0.936)	3.751(2.268,10.388)
KCZ	Log_IC50	-5.171(-5.200,-5.141)	-5.166(-5.199,-5.133)	-5.193(-5.270,-5.116)	-5.205(-5.316,-5.094)	0.026(-0.015,0.067)	-5.181(-5.202,-5.161)	4.616(3.667,6.230)
KCZ	Slope	-0.966(-1.029,-0.904)	-1.011(-1.113,-0.909)	-0.963(-1.221,-0.705)	-0.858(-1.073,-0.643)	-0.042(-0.161,0.077)	-0.957(-1.017,-0.898)	6.017(4.680,8.439)
PCZ	Log_IC50	-7.698(-7.776,-7.621)	-7.577(-7.794,-7.360)	-7.597(-7.683,-7.511)	-7.723(-7.779,-7.667)	-0.012(-0.183,0.159)	-7.651(-7.736,-7.566)	14.682(8.903,41.008)
PCZ	Slope	-0.966(-1.006,-0.926)	-0.961(-1.013,-0.909)	-0.971(-1.139,-0.803)	-0.983(-1.079,-0.887)	-0.013(-0.083,0.057)	-0.970(-1.004,-0.935)	3.317(2.398,5.381)
NYP	Log_IC50	-4.604(-4.621,-4.586)	-4.562(-4.595,-4.529)	-4.579(-4.639,-4.519)	-4.574(-4.600,-4.548)	-0.028(-0.054,-0.002)	-4.583(-4.596,-4.570)	2.845(2.181,4.092)
NYP	Slope	-1.949(-2.064,-1.833)	-1.655(-1.872,-1.438)	-1.952(-2.219,-1.685)	-2.479(-2.855,-2.103)	-0.096(-0.295,0.104)	-1.902(-2.002,-1.803)	5.225(4.357,6.534)
FRM	Log_IC50	-5.227(-5.275,-5.180)	-5.218(-5.240,-5.196)	-5.145(-5.244,-5.046)	-5.373(-5.716,-5.030)	0.051(-0.031,0.133)	-5.194(-5.235,-5.153)	5.504(3.038,23.906)
FRM	Slope	-0.931(-0.986,-0.877)	-0.990(-1.112,-0.868)	-1.034(-1.241,-0.827)	-1.021(-1.158,-0.884)	-0.076(-0.168,0.016)	-0.992(-1.038,-0.946)	4.434(3.363,6.516)
ECZ	Log_IC50	-8.823(-8.859,-8.788)	-8.624(-8.835,-8.413)	-8.776(-8.823,-8.729)	-9.055(-9.150,-8.960)	-0.219(-0.490,0.052)	-8.818(-8.953,-8.682)	22.545(13.342,72.698)
ECZ	Slope	-1.054(-1.090,-1.018)	-1.039(-1.091,-0.987)	-1.094(-1.150,-1.038)	-0.797(-0.887,-0.707)	0.128(-0.127,0.384)	-1.003(-1.131,-0.875)	8.824(5.181,27.943)
CYN	Log_IC50	-5.767(-5.839,-5.695)	-5.670(-5.739,-5.601)	-5.503(-5.645,-5.361)	-5.543(-5.724,-5.362)	-0.081(-0.354,0.191)	-5.627(-5.763,-5.490)	22.583(13.335,73.684)
CYN	Slope	-1.394(-1.533,-1.255)	-1.386(-2.369,-0.403)	-1.258(-1.934,-0.582)	-1.082(-1.430,-0.734)	-0.028(-0.356,0.299)	-1.324(-1.487,-1.160)	10.199(6.580,22.526)
DCF	Log_IC50	-4.379(-4.824,-3.934)	-4.626(-4.927,-4.325)	-4.334(-4.954,-3.714)	-4.935(-5.626,-4.244)	-0.015(-0.477,0.447)	-4.549(-4.780,-4.318)	47.367(30.420,114.548)
DCF	Slope	-0.658(-0.882,-0.434)	-0.983(-1.904,-0.062)	-0.639(-0.820,-0.458)	-0.830(-1.404,-0.256)	0.039(-0.203,0.282)	-0.673(-0.795,-0.552)	15.242(9.950,32.692)

1. The estimates and 95 percent confidence intervals are based on the intralaboratory analyses for the four participating laboratories. The intralaboratory analyses were performed by individual chemical.
2. The overall estimates and confidence intervals in this table were estimated using a mixed effects analysis of variance, with microsome source as a fixed effect and laboratory as a random effect, and with heterogeneous variances among the four laboratories. The variances for each laboratory were specified as the squares of the within laboratory standard errors. The degrees of freedom were reported in Table 37.
3. Microsomes were prepared by Battelle and In Vitro laboratories and distributed to the four laboratories. The differences in average responses between the two sources of microsomes were estimated based on the model fit, accounting for among laboratory variation and heterogeneous within laboratory variation. The degrees of freedom are same as those for the overall means.
4. CV was calculated for IC_{50} and the slope parameters based on the average results. CVs associated with $log_{10}IC_{50}$ are actually CVs on the IC_{50}s.
5. Concentration response relations were not fitted for dibenz [a,h]anthracene and for atrazine, since they resulted in no aromatase inhibition.

Table 37. Variance Components and Ratio of Between and Within Laboratories Variances for $Log_{10}IC_{50}$ and Slope Parameter of Concentration Response Curves for Placental Assay. Estimated by Chemical

Chemical[7]	Param	Within Laboratory Variance[1]					Among Laboratory Variance[3] and (p-value) (df=3)	Mean Variance[4,5]	Ratio and 95 percent CI[6]
		RTI	Battelle	WIL	In Vitro	Pooled Results[2]			
AG	Log_IC50	0.000/df=16.0	0.002/df=1.8	0.001/df=2.0	0.001/df=1.2	0.001/df=5.2	0.000 (1)	0.000/df=5.2	0.000 (0.000, 0.000)
AG	Slope	0.001/df=16.0	0.000/df=32.4	0.002/df=2.0	0.002/df=1.0	0.001/df=4.2	0.000 (1)	0.000/df=4.2	0.000 (0.000, 0.000)
KCZ	Log_IC50	0.000/df=16.0	0.000/df=20.1	0.000/df=2.0	0.003/df=20.0	0.001/df=28.4	0.000 (1)	0.000/df=28.4	0.000 (0.000, 0.000)
KCZ	Slope	0.001/df=16.0	0.002/df=14.7	0.004/df=2.0	0.011/df=20.0	0.004/df=24.1	0.000 (1)	0.001/df=24.1	0.000 (0.000, 0.000)
PCZ	Log_IC50	0.001/df=16.0	0.003/df=2.0	0.000/df=2.0	0.001/df=20.0	0.001/df=7.3	0.003 (0.14617)	0.001/df=4.3	2.272 (0.397, 33.164)
PCZ	Slope	0.000/df=16.0	0.000/df=2.9	0.002/df=2.0	0.002/df=20.0	0.001/df=12.8	0.000 (1)	0.000/df=12.8	0.000 (0.000, 0.000)
NYP	Log_IC50	0.000/df=16.0	0.000/df=20.6	0.000/df=2.0	0.000/df=44.3	0.000/df=20.4	0.000 (1)	0.000/df=20.4	0.000 (0.000, 0.000)
NYP	Slope	0.003/df=16.0	0.011/df=20.2	0.004/df=2.0	0.034/df=30.1	0.013/df=51.2	0.000 (1)	0.002/df=51.2	0.000 (0.000, 0.000)
FRM	Log_IC50	0.000/df=16.0	0.000/df=56.9	0.001/df=2.0	0.004/df=1.7	0.001/df=2.6	0.000 (1)	0.000/df=2.6	0.000 (0.000, 0.000)
FRM	Slope	0.001/df=16.0	0.001/df=2.4	0.002/df=2.0	0.004/df=29.5	0.002/df=18.9	0.000 (1)	0.000/df=18.9	0.000 (0.000, 0.000)
ECZ	Log_IC50	0.000/df=16.0	0.002/df=1.9	0.000/df=2.0	0.002/df=43.5	0.001/df=8.7	0.008 (0.11037)	0.002/df=3.9	6.843 (1.324, 99.158)
ECZ	Slope	0.000/df=16.0	0.001/df=49.9	0.000/df=2.0	0.002/df=56.8	0.001/df=99.1	0.007 (0.11215)	0.002/df=3.6	8.965 (2.758, 125.120)
CYN	Log_IC50	0.001/df=16.0	0.001/df=42.9	0.001/df=2.0	0.003/df=3.1	0.002/df=10.6	0.008 (0.12552)	0.002/df=3.9	4.576 (0.972, 65.867)
CYN	Slope	0.004/df=16.0	0.036/df=1.7	0.025/df=2.0	0.020/df=5.6	0.021/df=6.2	0.000 (1)	0.005/df=6.2	0.000 (0.000, 0.000)
DCF	Log_IC50	0.044/df=16.0	0.005/df=2.0	0.021/df=2.0	0.110/df=20.8	0.045/df=34.8	0.002 (0.46330)	0.010/df=7.0	0.047 (0.013, 0.654)
DCF	Slope	0.011/df=16.0	0.039/df=1.9	0.002/df=2.0	0.043/df=4.1	0.024/df=7.0	0.000 (1)	0.003/df=7.0	0.000 (0.000, 0.000)

1. The within laboratory variance for each laboratory is the square of the standard error associated with parameter estimate, which was reported in the intralaboratory analyses for each of the four participating laboratories.
2. Pooled average for the within laboratory variances is the unweighted average of the within laboratory variances among the four laboratories. Associated degrees of freedom were based on Satterthwaite's approximation
3. Variance among laboratories is based on a mixed effects analysis of variance model with heterogeneous variances among the four laboratories, equal to the squares of the within laboratory standard errors.
4. Mean variance is the square of the standard error of the pooled weighted mean value. It includes both within and among laboratory variation.
5. Degrees of freedom for the (mean) overall effect variance were estimated as $2*((1/K)*\sum(S_L^2 + S_i^2))^2/(var(S_L^2)+(2/K^2)*\sum(S_i^4/df_i))$, where S_L^2 is the among laboratory variance, S_i^2 and df_i are the reported variance and degrees of freedom for laboratory i, $var(S_L^2)$ is the variance of S_L^2, and K is the number of laboratories (Hartung and Makambi, 2001).
6. Ratio of the among-laboratory variance and the pooled average within laboratory variance.
7. Concentration response relations were not fitted for dibenz [a,h]anthracene and for atrazine, since they resulted in no aromatase inhibition.

Table 38. Parameter Estimate and the 95 percent Confidence Interval for Differences Between Beginning and End (End Minus Beginning) and Between the Two Sources of Microsomes (Battelle minus In Vitro) for the Percent of Control Responses in the Placental Aromatase Assay.

Parameter	Estimate and 95 percent Confidence Interval				
	RTI[1,2]	Battelle[1,2]	WIL[1,2]	In Vitro[1,2]	Mean[3]
End – Beginning					
Background Activity Control	0.172 (-0.034, 0.378)	0.062 (-0.118, 0.242)	0.056 (-0.918, 1.030)	-0.407 (-0.921, 0.107)	0.071 (-0.061, 0.203)
Full Enzyme Activity Control	-9.873 (-11.092, -8.654)	-10.444 (-14.975, -5.913)	-6.062 (-9.399, -2.725)	-10.016 (-12.854, -7.178)	-9.543 (-10.552, -8.535)
Negative Control	-11.212 (-12.541, -9.883)	-8.294 (-13.226, -3.362)	-9.995 (-15.102, -4.888)	17.384 (7.898, 26.870)	-3.611 (-19.896, 12.674)
Positive Control	-4.913 (-6.122, -3.704)	-2.233 (-4.100, -0.366)	-3.740 (-6.484, -0.996)	3.581 (-0.269, 7.431)	-2.086 (-6.538, 2.366)
Battelle Microsomes – In Vitro Microsomes[4]					
Negative Control	-1.446 (-2.775, -0.117)	-0.782 (-6.031, 4.467)	-2.216 (-8.358, 3.926)	-12.063 (-21.609, -2.517)	-1.636 (-2.858, -0.413)
Positive Control	1.210 (-0.979, 3.399)	1.109 (-1.949, 4.168)	-2.318 (-6.793, 2.158)	-4.905 (-13.509, 3.699)	0.343 (-1.311, 1.996)

1. The estimates and 95 percent confidence intervals are based on the intralaboratory analyses for the four participating laboratories.
2. The results from the four laboratories are listed in Appendix E.
3. The overall (mean) effects and confidence intervals in this table were estimated using a one-way random effects analysis of variance, with laboratory as a random effect, and with heterogeneous variances among the four laboratories. The variances for each laboratory were specified as the squares of the within laboratory standard errors.
4. Two microsomes were prepared by Battelle and In Vitro laboratories and distributed to the four laboratories. Results for the difference between the two sources of microsomes within each laboratory were listed in Appendix E.

Table 39. Variance Components and Ratio of among and Within Laboratories Variances for Differences between Beginning and End and between the Two Sources of Microsomes by Battelle and In Vitro for the Percent of Control Responses for Placental Aromatase Assay

Parameter	Within Laboratory Variance[1]					Among Laboratory Variance[3] and (p-value) (df=3)	Mean Variance[4,5]	Ratio and 95 percent CI[6]
	RTI	Battelle	WIL	In Vitro	Pooled Results[2]			
End - Beginning								
Background Activity Control	0.010/df=20.0	0.008/df=61.8	0.200/df=12.0	0.052/df=9.3	0.067/df=20.1	0.000 (1)	0.004/df=20.1	0.000 (0.000, 0.000)
Full Enzyme Activity Control	0.341/df=20.0	3.375/df=5.8	2.346/df=12.0	1.880/df=22.7	1.985/df=24.4	0.000 (1)	0.239/df=24.4	0.000 (0.000, 0.000)
Negative Control	0.406/df=20.0	4.360/df=7.0	5.495/df=12.0	18.173/df=10.1	7.108/df=21.3	117.867 (0.10411)	31.159/df=3.6	16.581 (4.356, 234.648)
Positive Control	0.336/df=20.0	0.585/df=6.1	1.586/df=12.0	2.676/df=7.2	1.296/df=21.1	7.813 (0.12999)	2.255/df=3.4	6.029 (1.581, 85.332)
Battelle Microsome – In Vitro Microsome[7]								
Negative Control	0.406/df=20.0	5.328/df=8.7	7.946/df=12.0	18.770/df=10.9	8.112/df=25.8	0.000 (1)	0.353/df=25.8	0.000 (0.000, 0.000)
Positive Control	1.101/df=20.0	1.981/df=12.3	4.219/df=12.0	9.641/df=4.0	4.235/df=11.5	0.000 (1)	0.570/df=11.5	0.000 (0.000, 0.000)

1. The within laboratory variance for each laboratory is the square of the standard error associated with the parameter estimate, as reported in the intralaboratory analyses for the four participating laboratories (Appendix E).
2. Pooled average for the within laboratory variances is the unweighted average of the within laboratory variances among the four laboratories. Associated degrees of freedom were based on Satterthwaite's approximation
3. Among laboratories variance is based on a one-way random effects analysis of variance model with heterogeneous within laboratory variances, equal to the squares of the within laboratory standard errors.
4. Mean variance is the square of the standard error of the pooled weighted mean value. It includes both within and among laboratory variation.
5. Degrees of freedom for the (mean) overall effect variance were estimated as $2*((1/K)*\sum(S_L^2 + S_i^2))^2/(var(S_L^2)+(2/K^2)*\sum(S_i^4/df_i))$, where S_L^2 is the among laboratory variance, S_i^2 and df_i are the reported variance and degrees of freedom for laboratory i, $var(S_L^2)$ is the variance of S_L^2, and K is the number of laboratories (Hartung and Makambi, 2001).
6. Ratio of the among-laboratory variance and the pooled average within laboratory variance.
7. Microsomes were prepared by Battelle and In Vitro laboratories and distributed to the four laboratories. Results for the difference between the two sources of microsomes within each laboratory were listed in Appendix E.

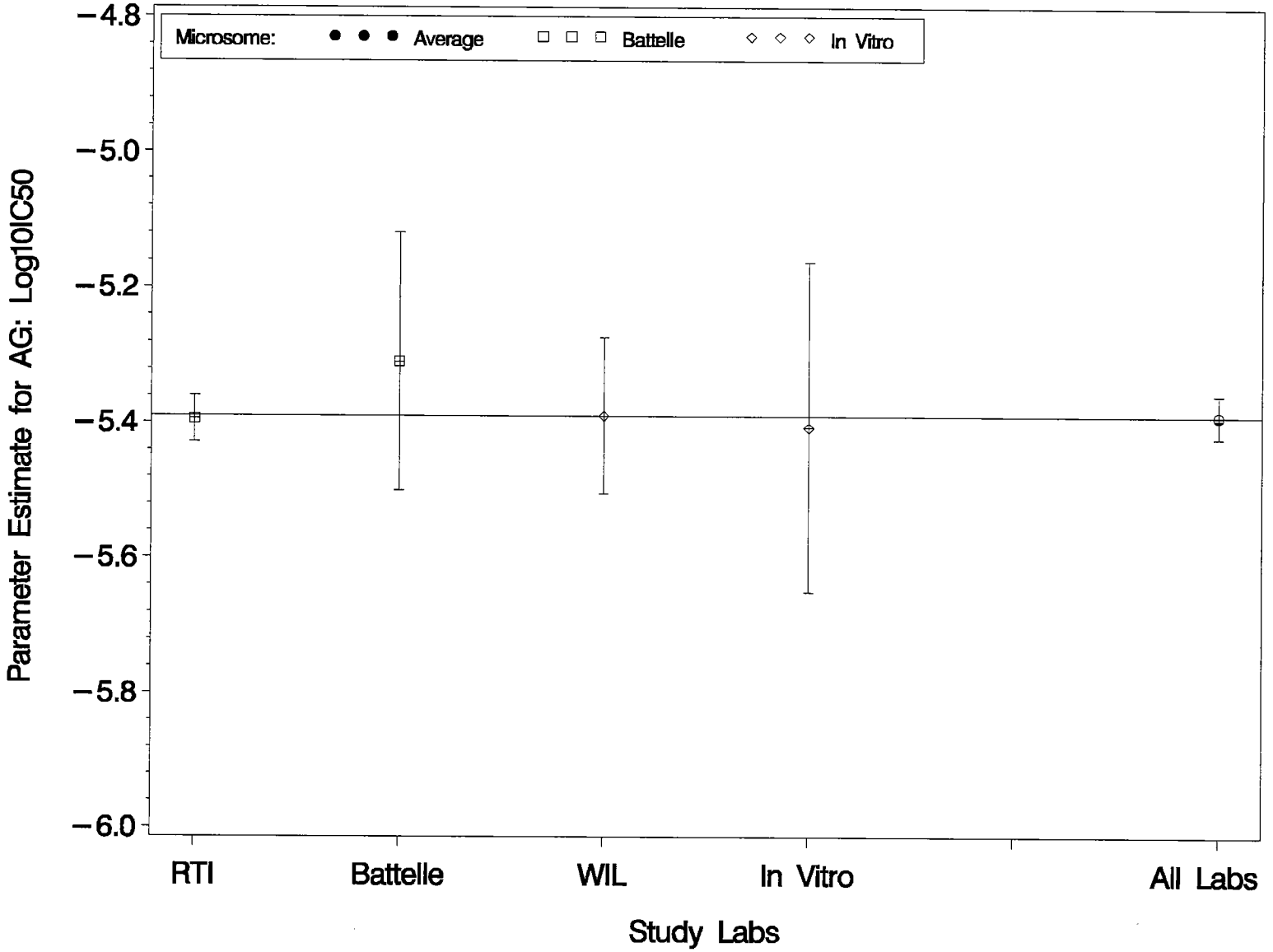

Figure 21. **Aminoglutethimide: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

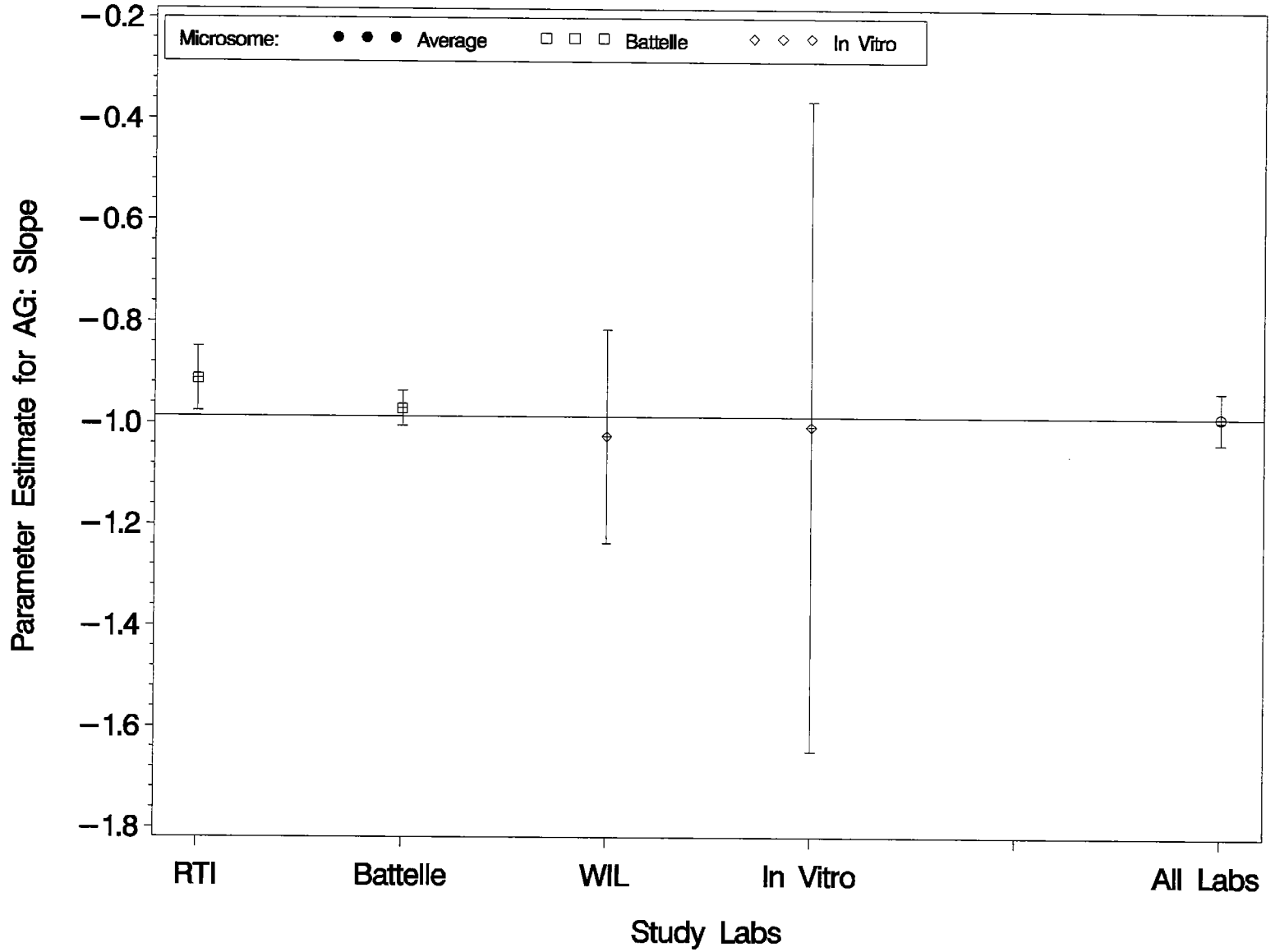

Figure 22. **Aminoglutethimide: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

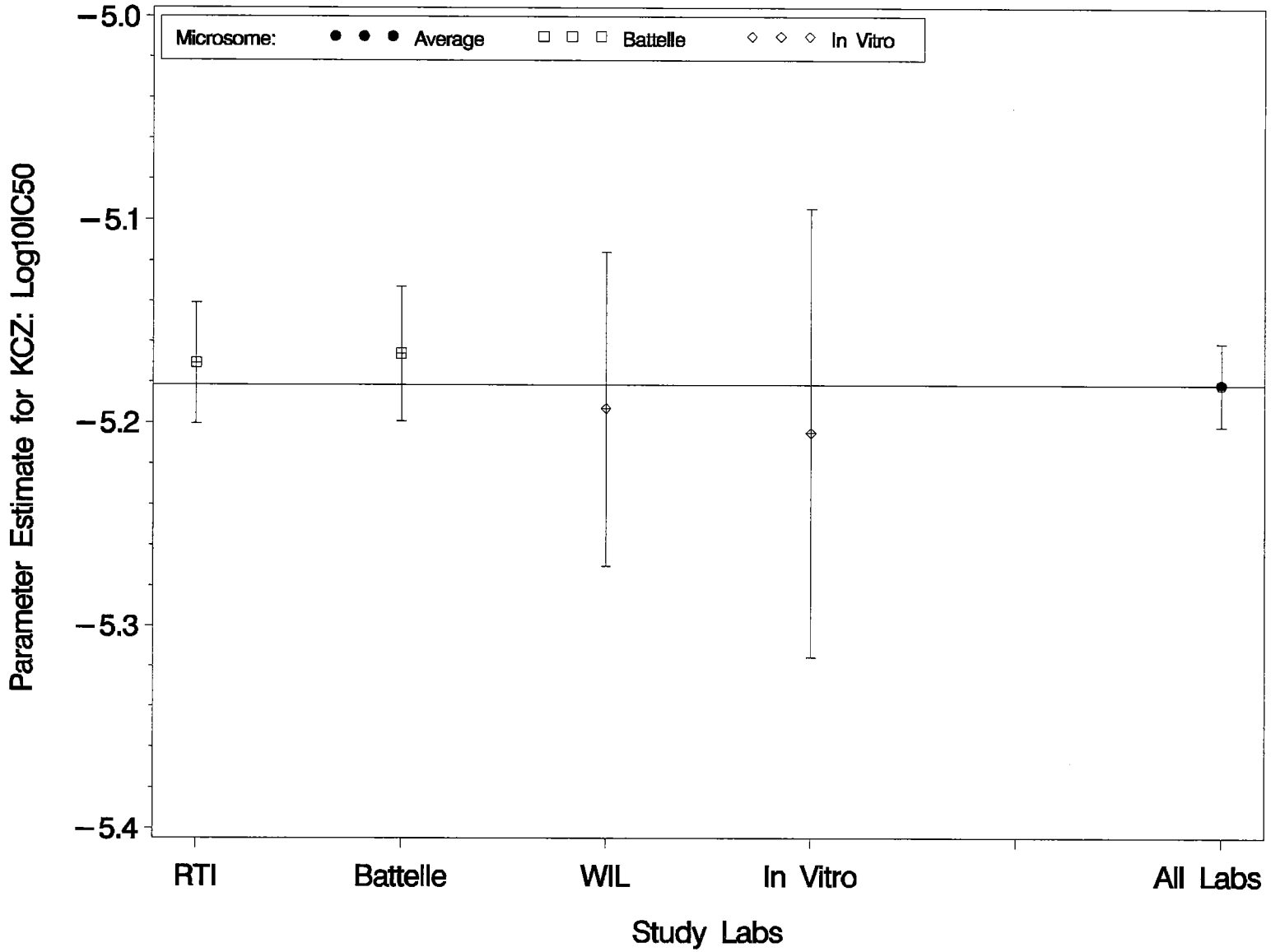

Figure 23. **Ketoconazole: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

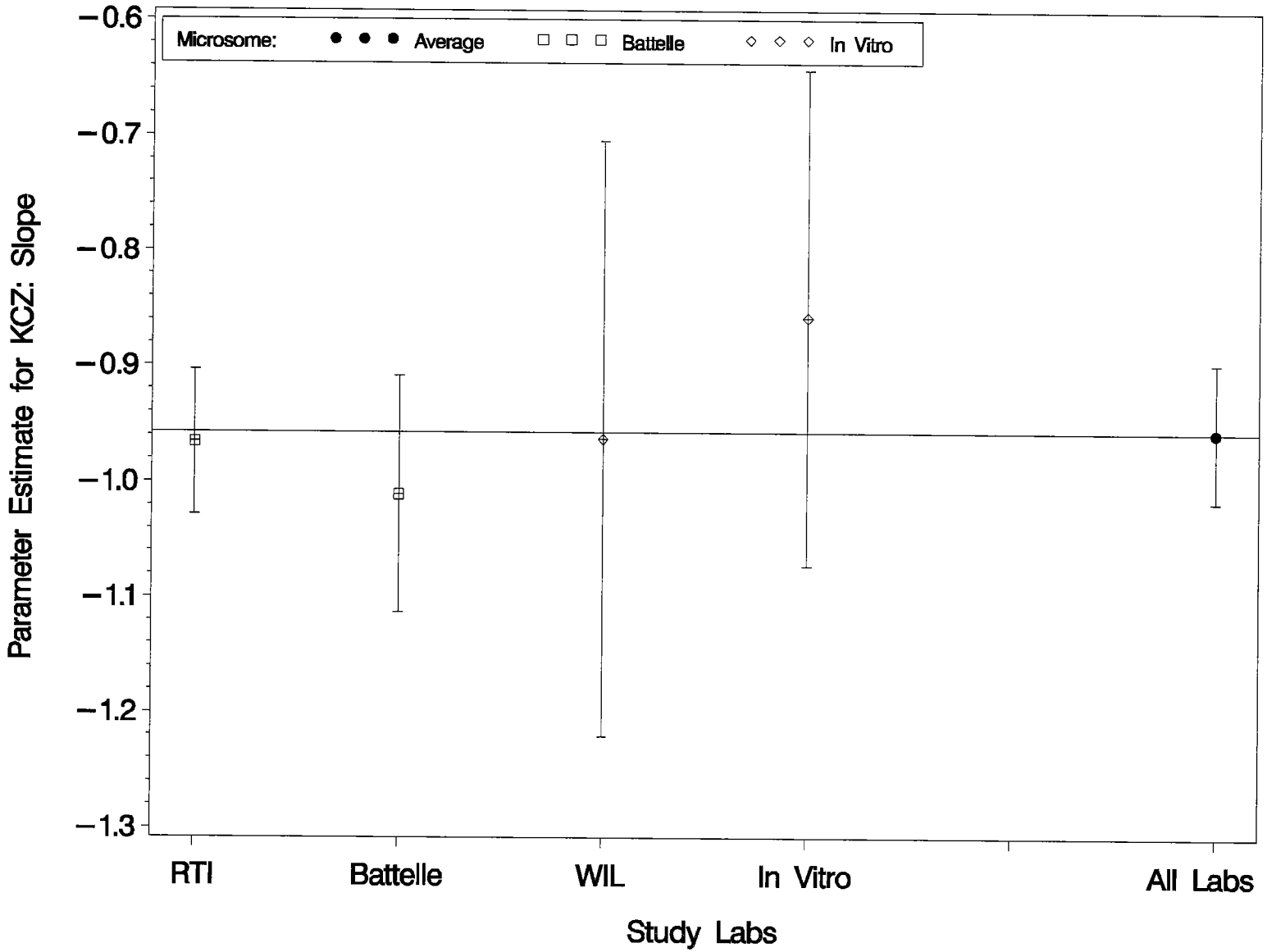

Figure 24. **<u>Ketoconazole</u>: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

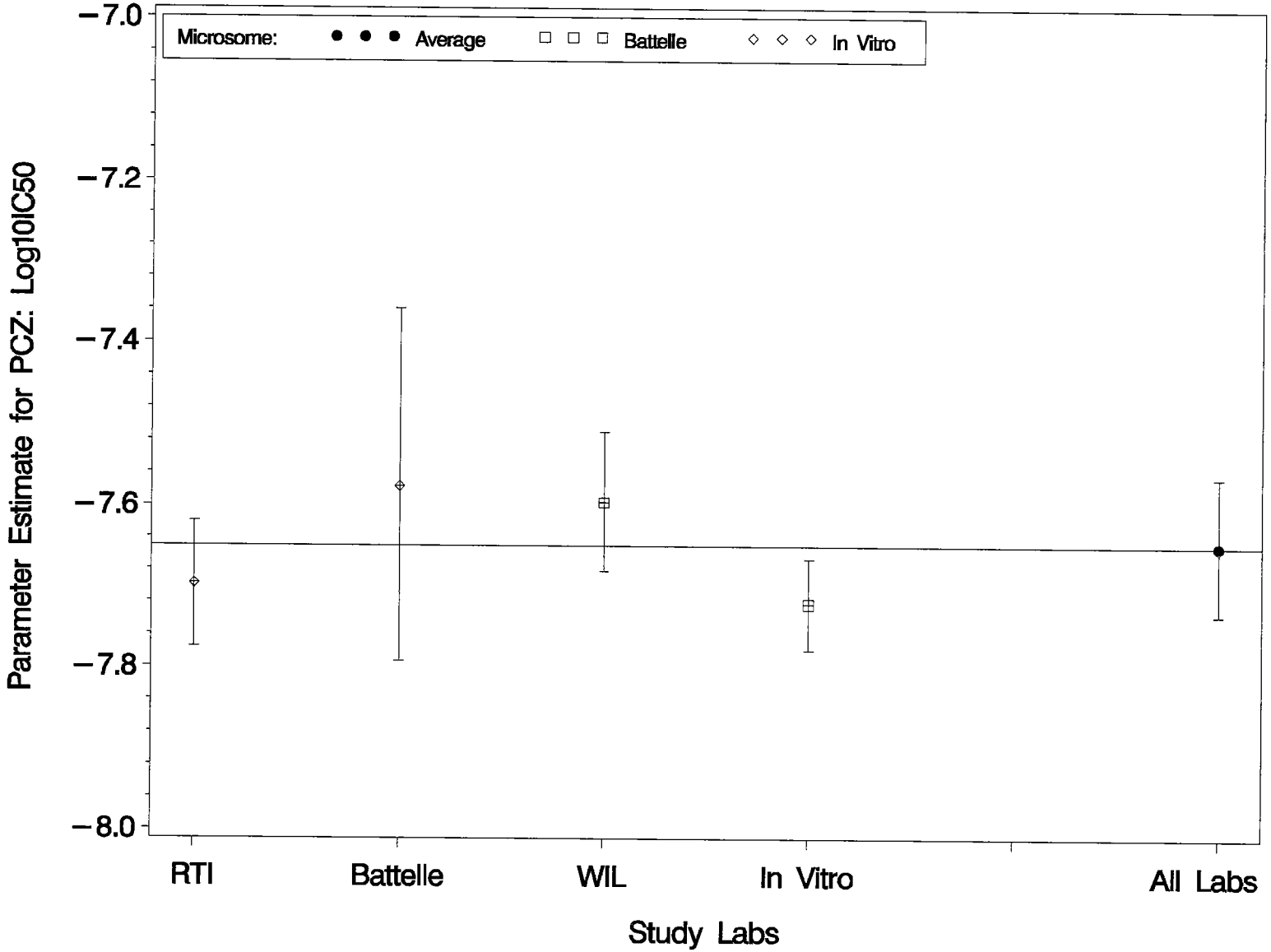

Figure 25. **Prochloraz: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

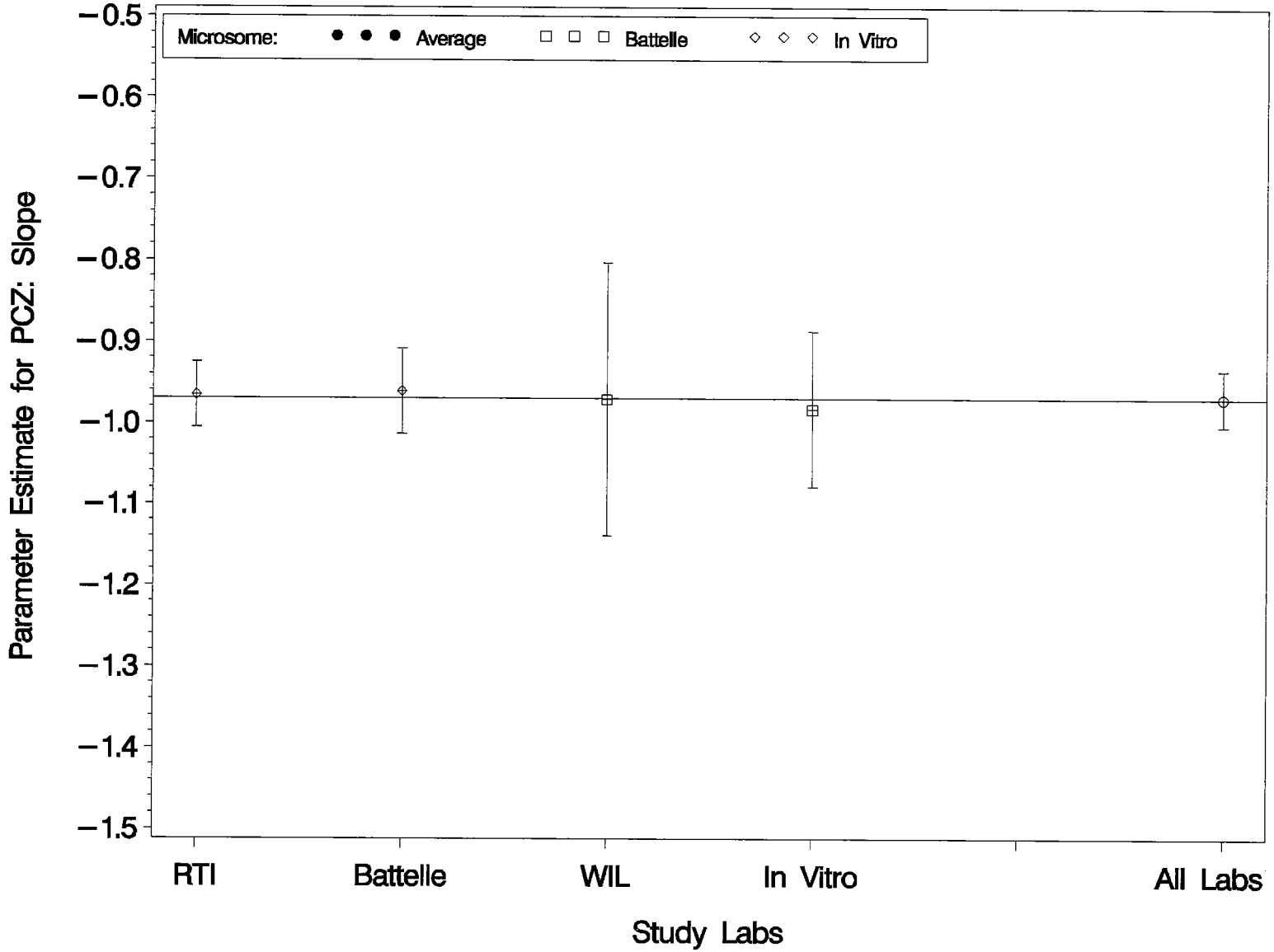

Figure 26. **<u>Prochloraz:</u> Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

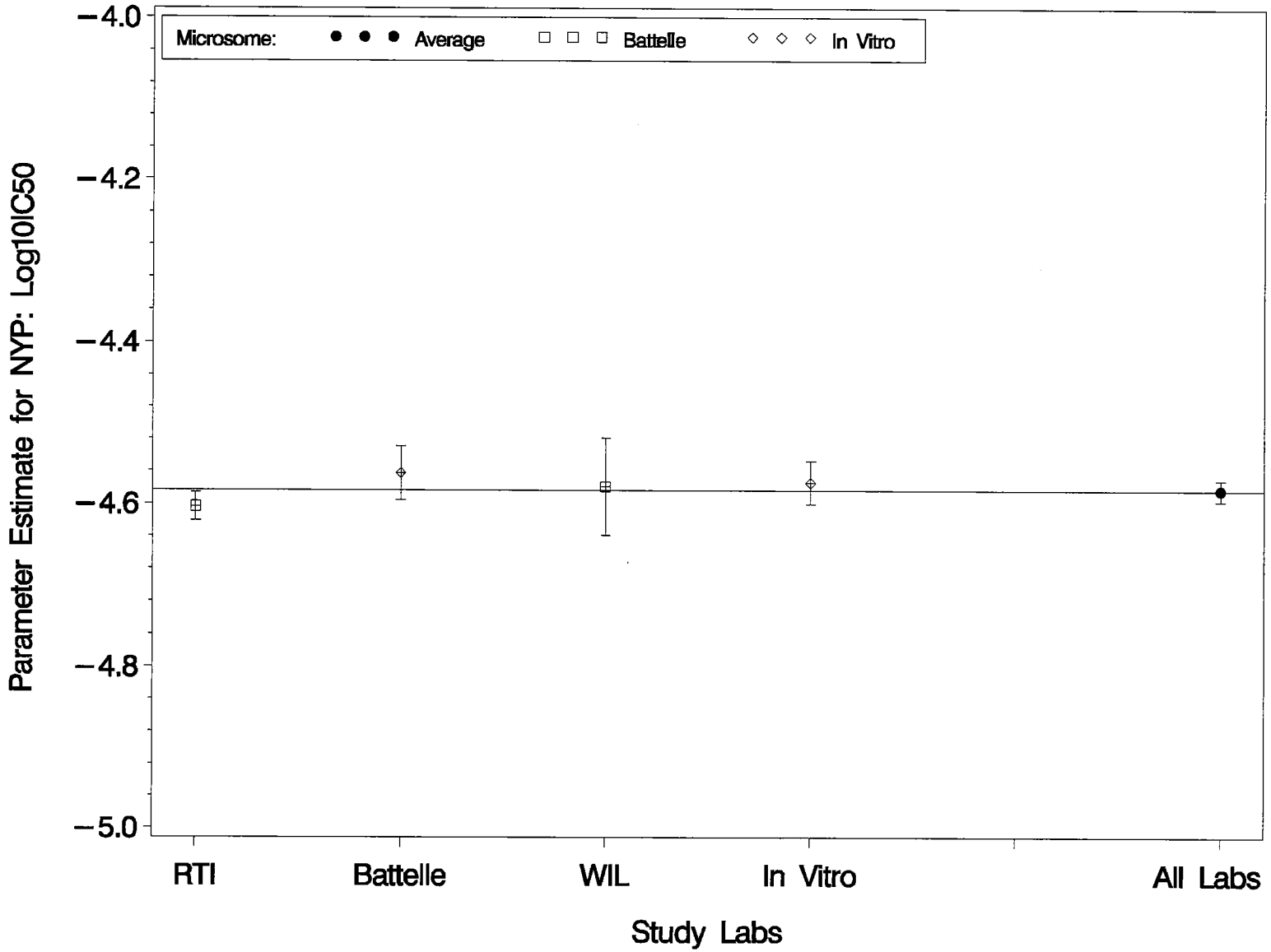

Figure 27. **<u>4-Nonylphenol</u>: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

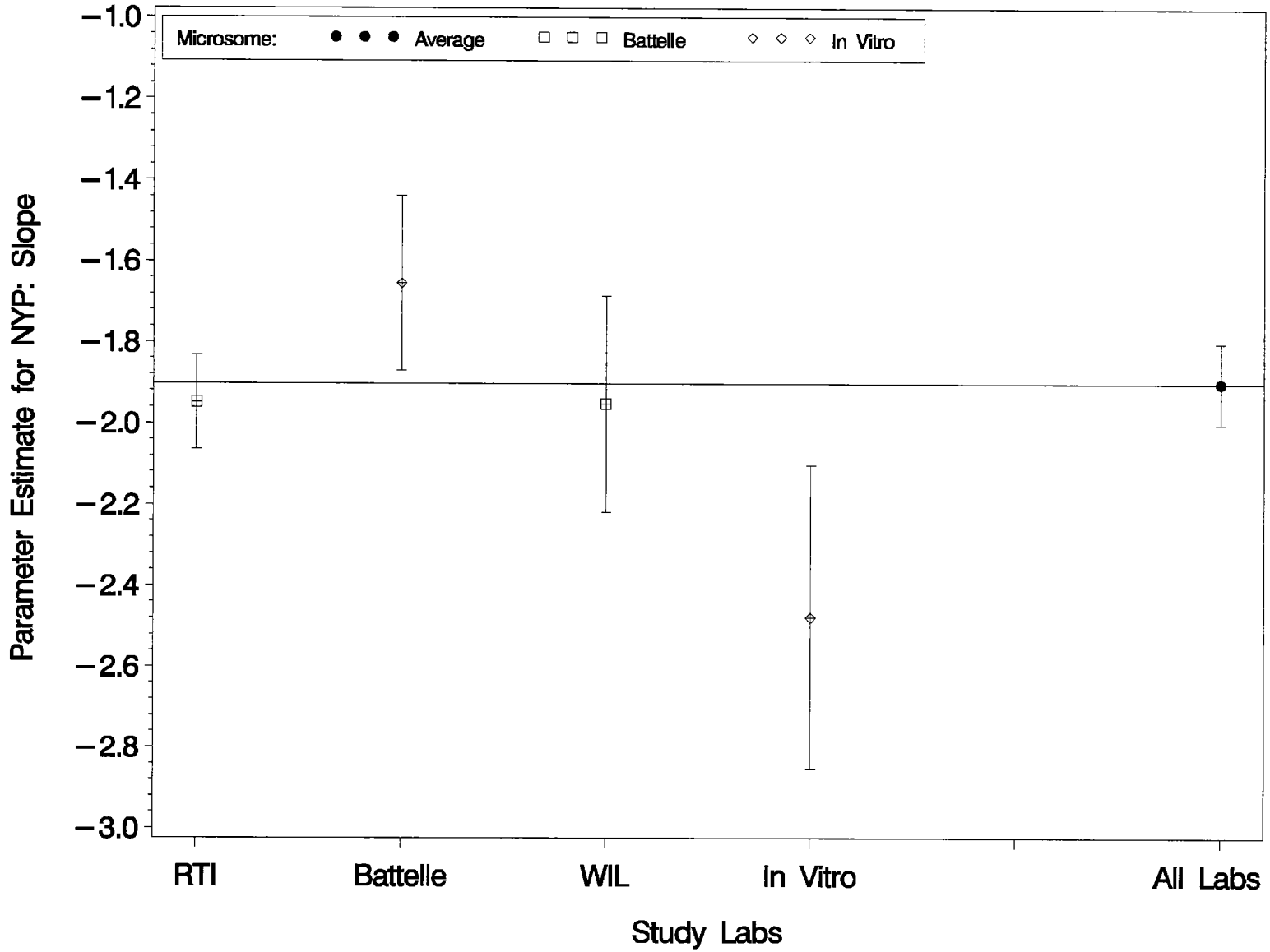

Figure 28. **4-Nonylphenol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

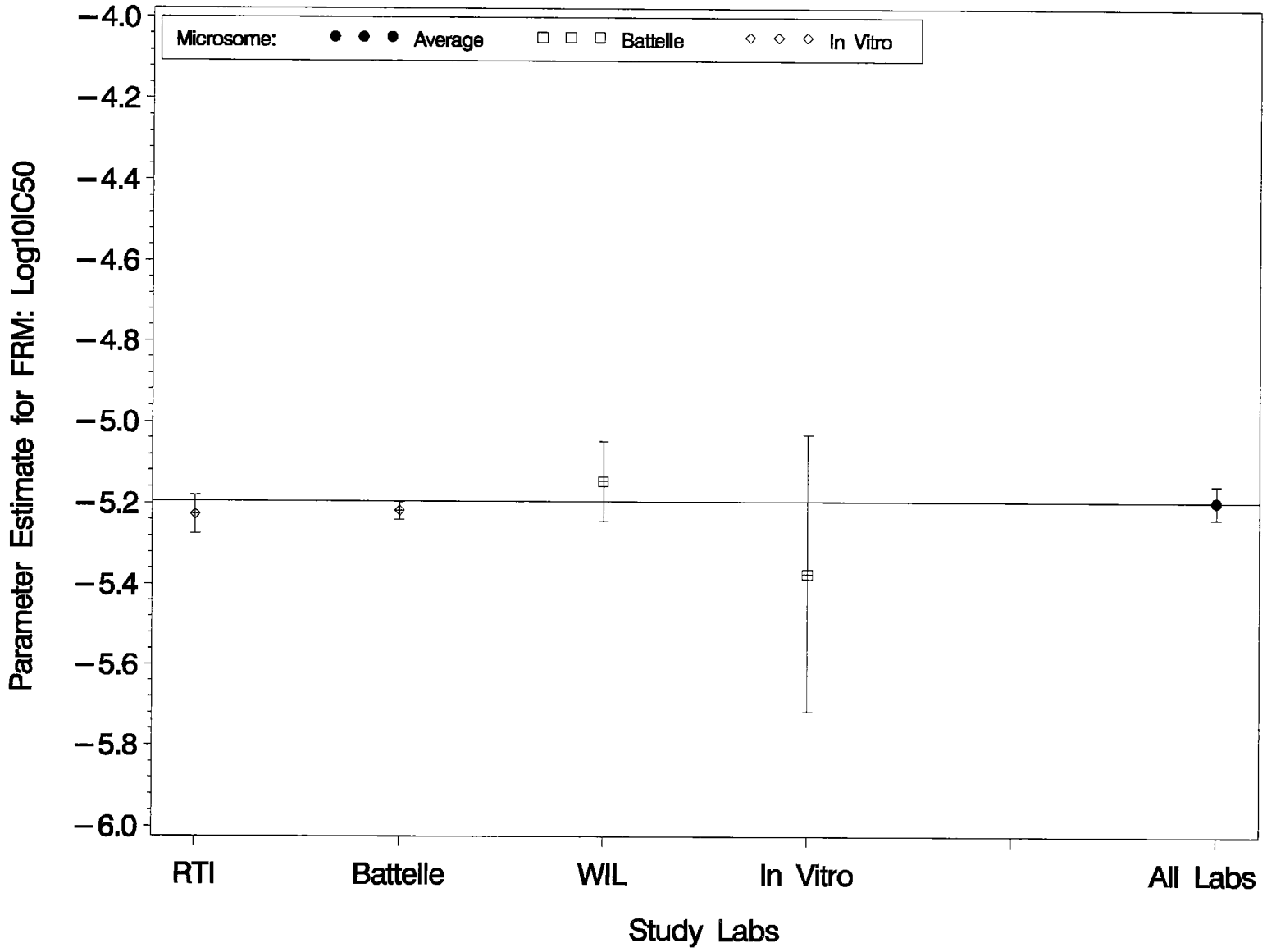

Figure 29. **Fenarimol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

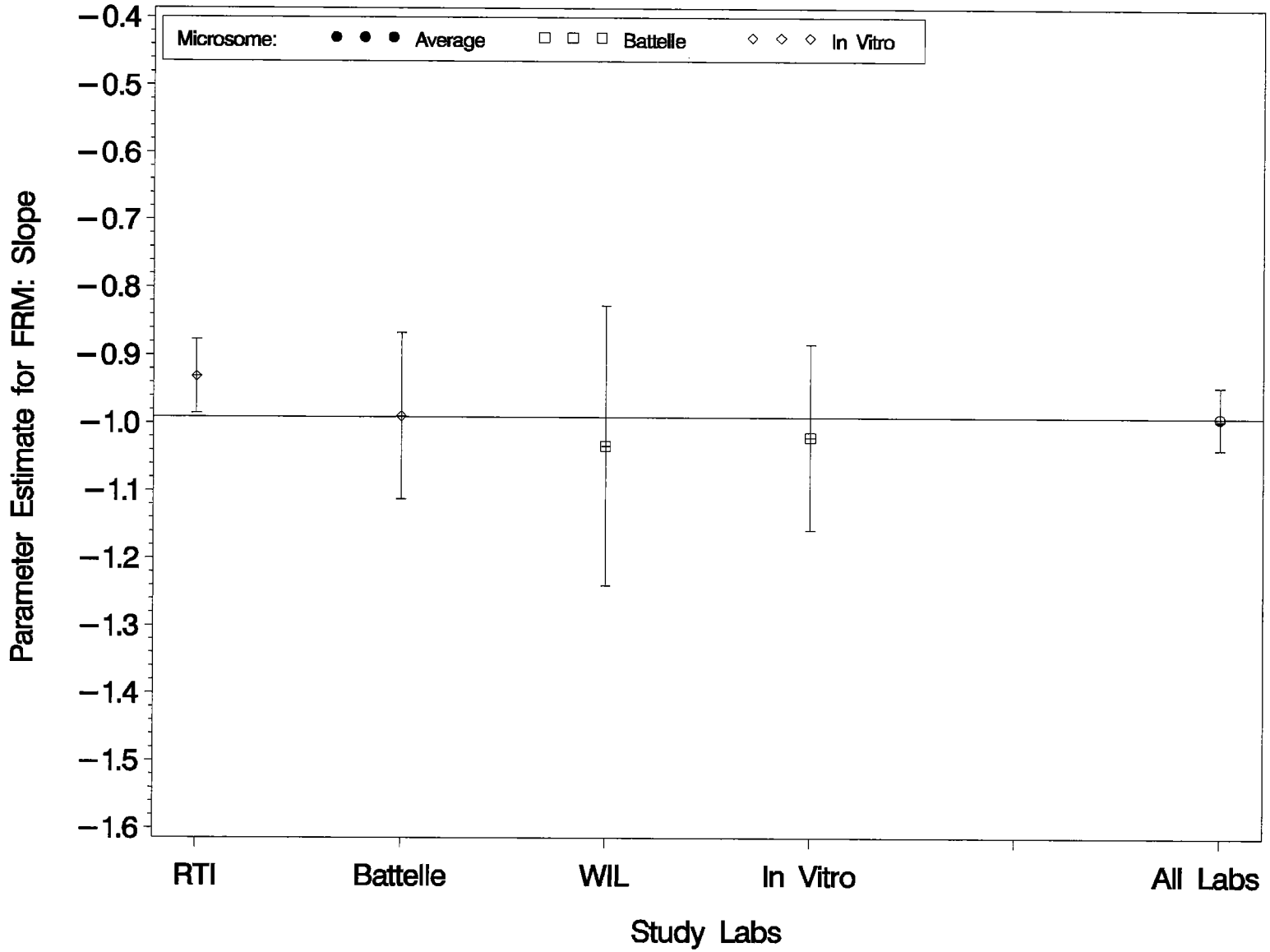

Figure 30. **Fenarimol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

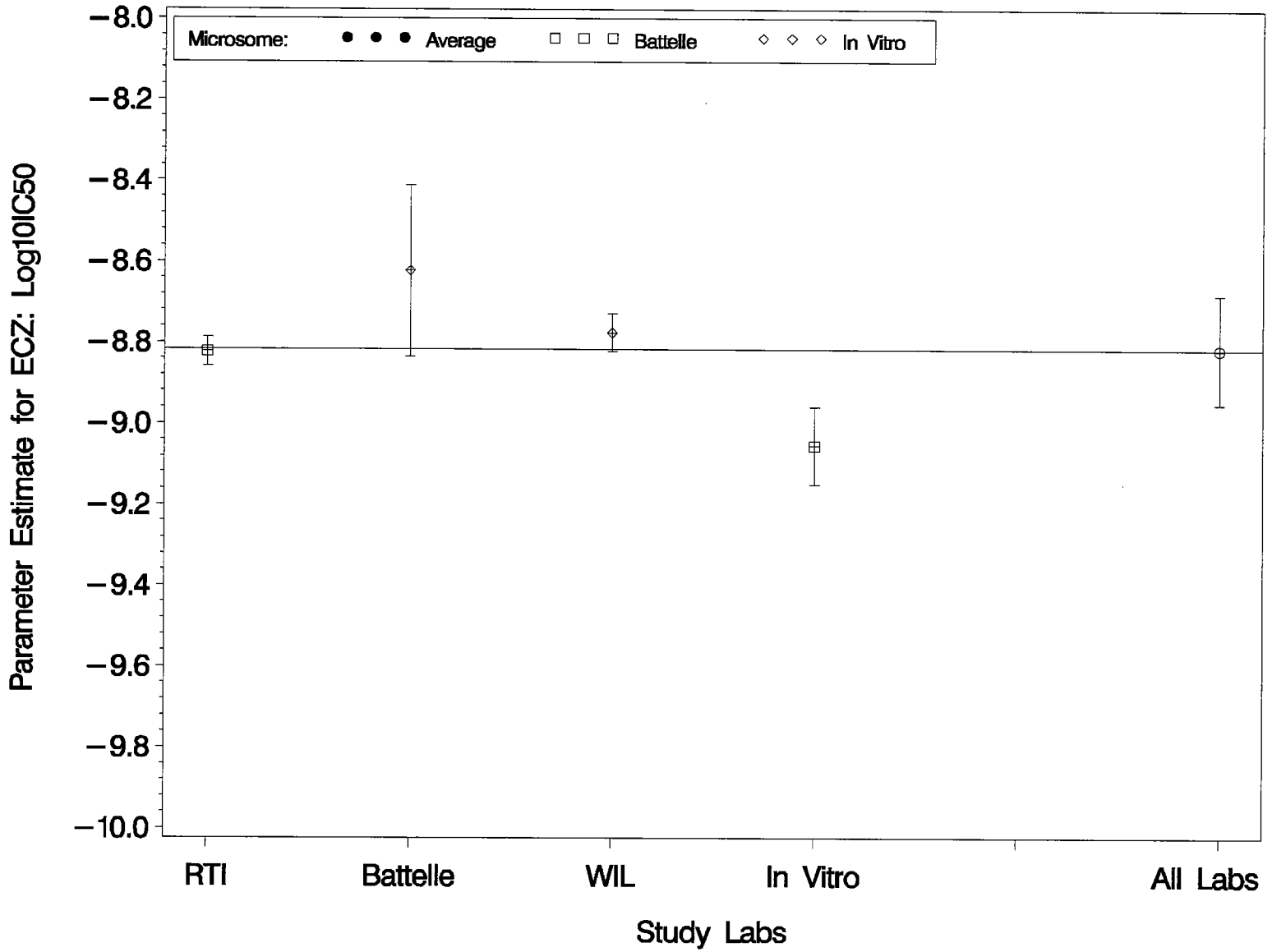

Figure 31. **Econazole: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

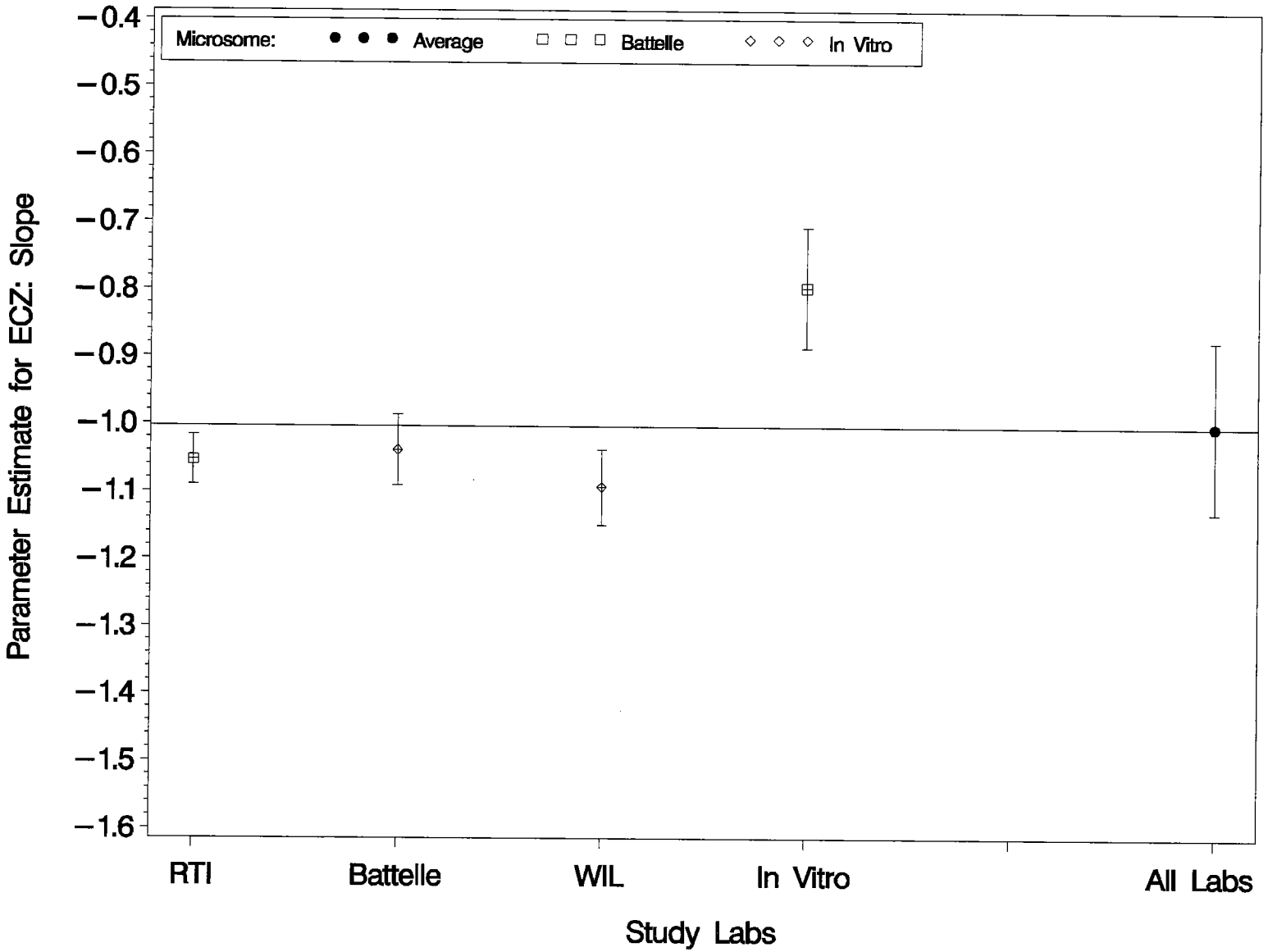

Figure 32. **Econazole: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

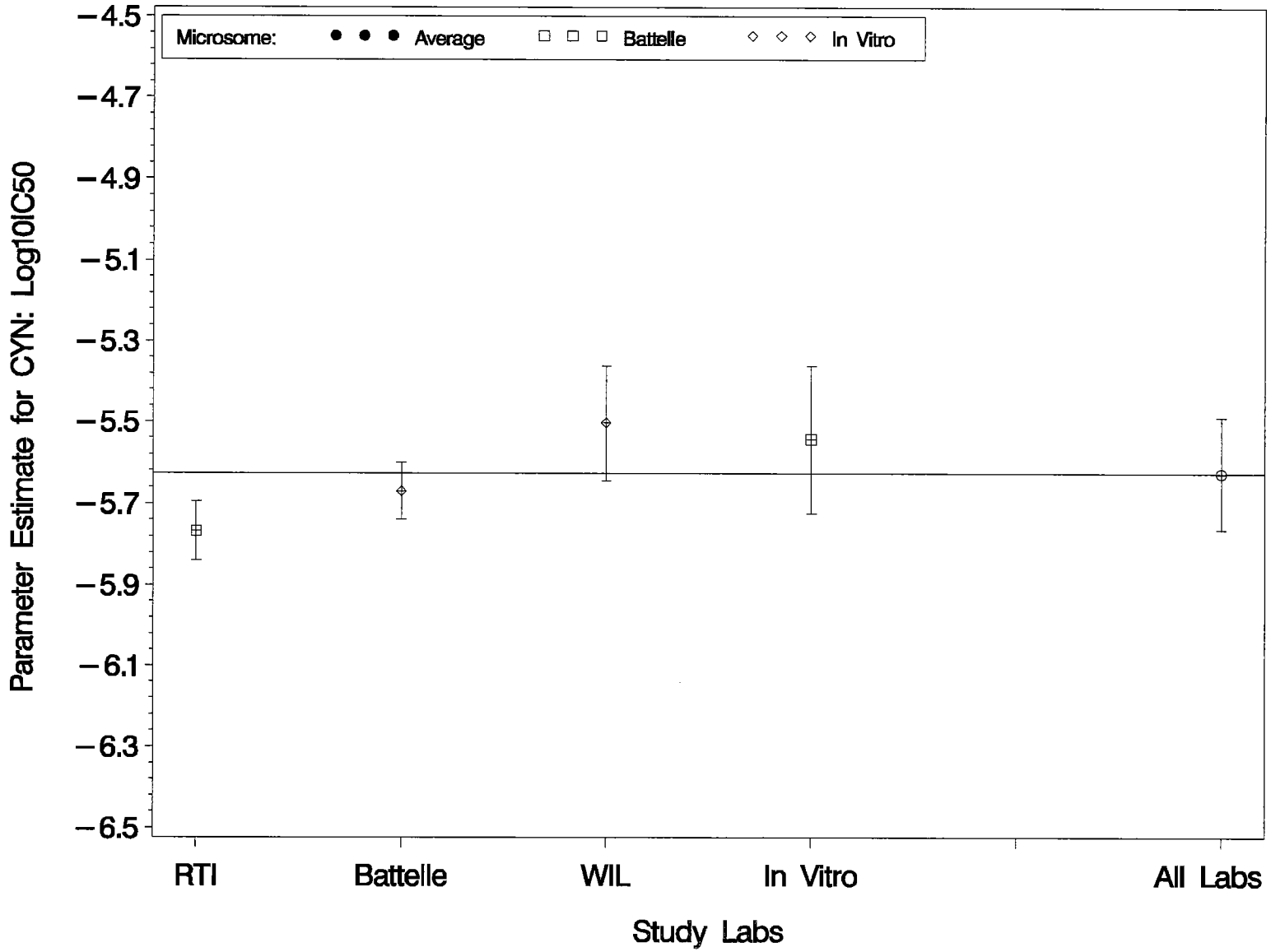

Figure 33. **Chrysin: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

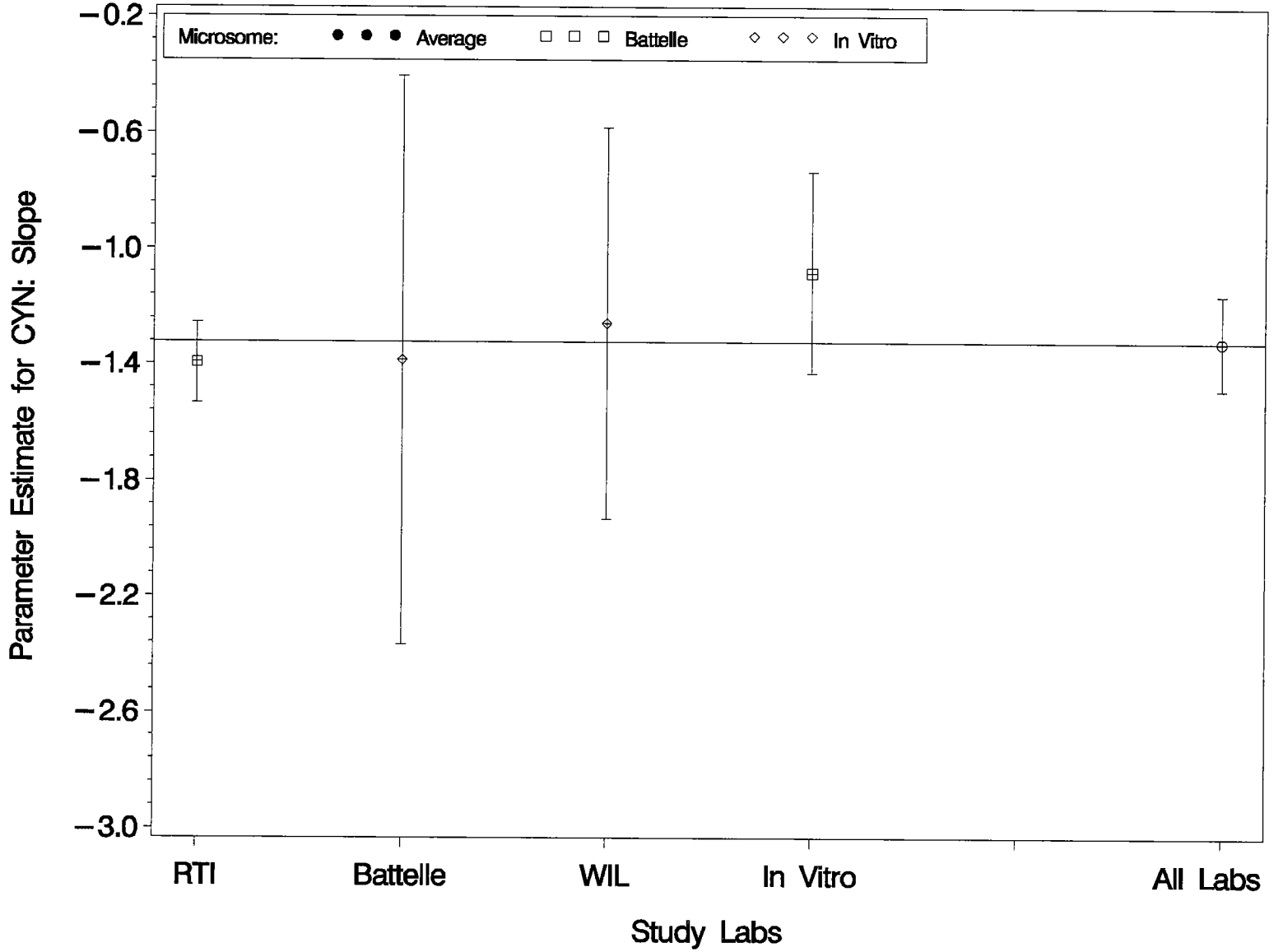

Figure 34. **Chrysin: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

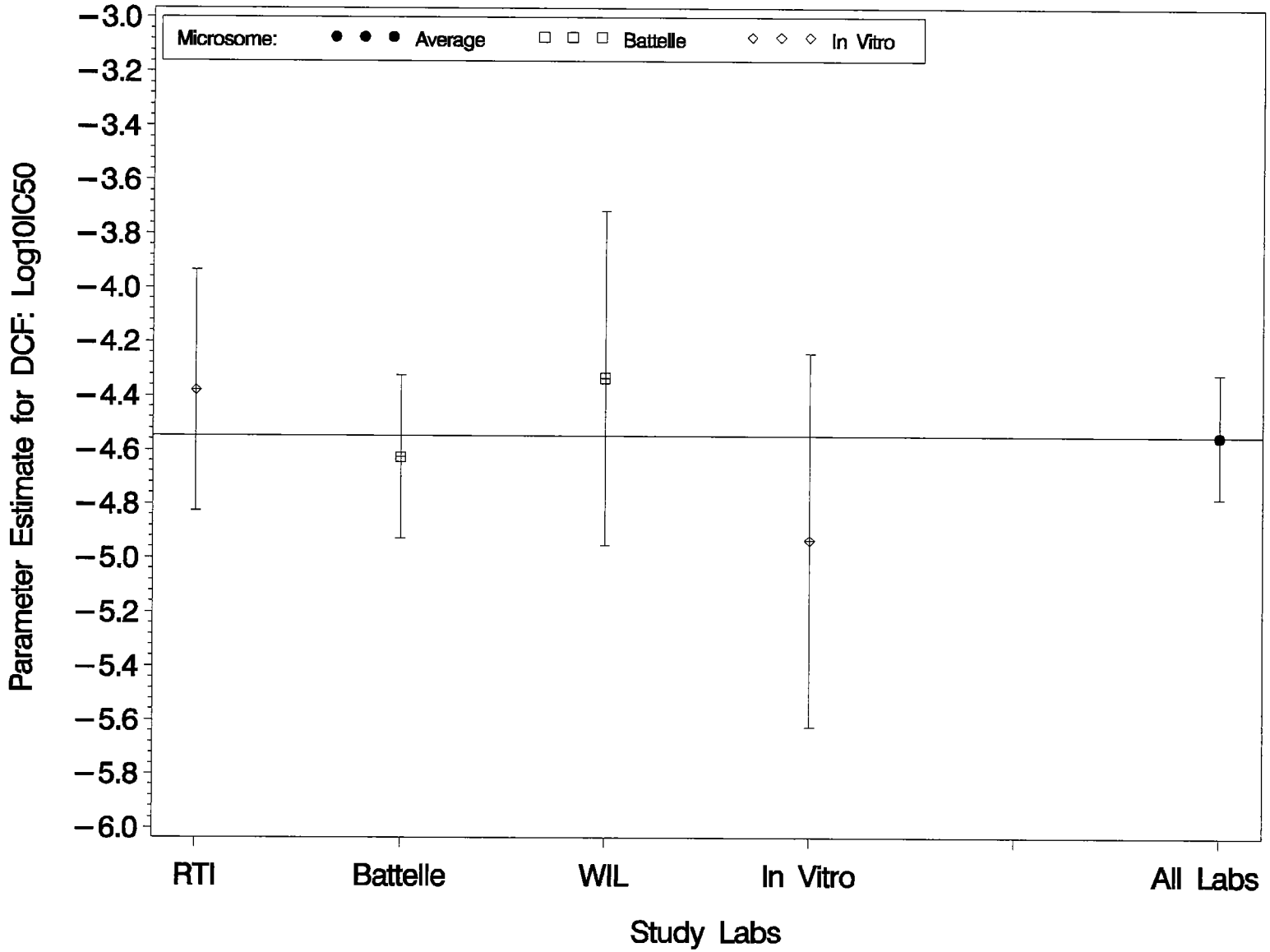

Figure 35. **Dicofol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

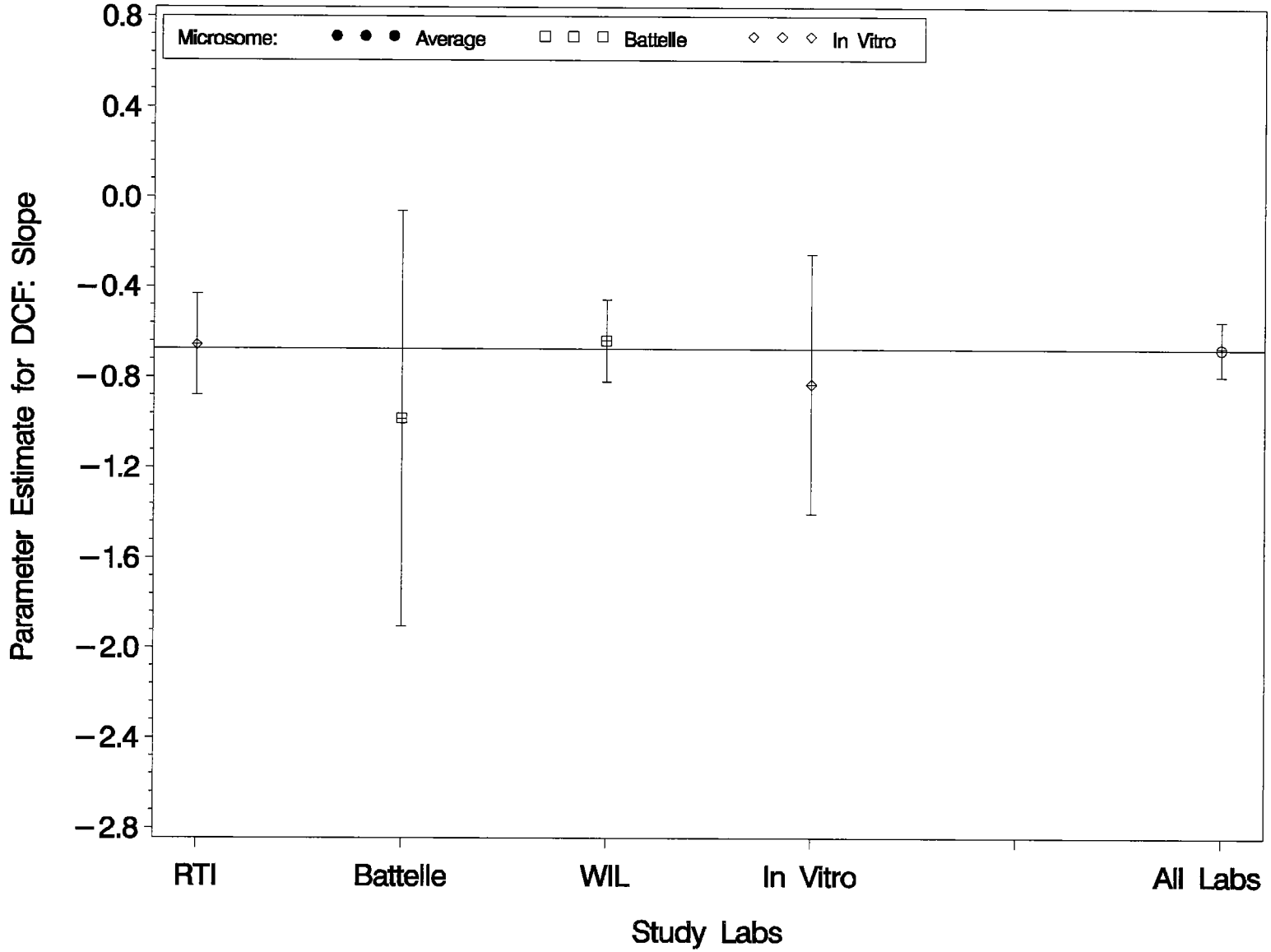

Figure 36. **Dicofol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

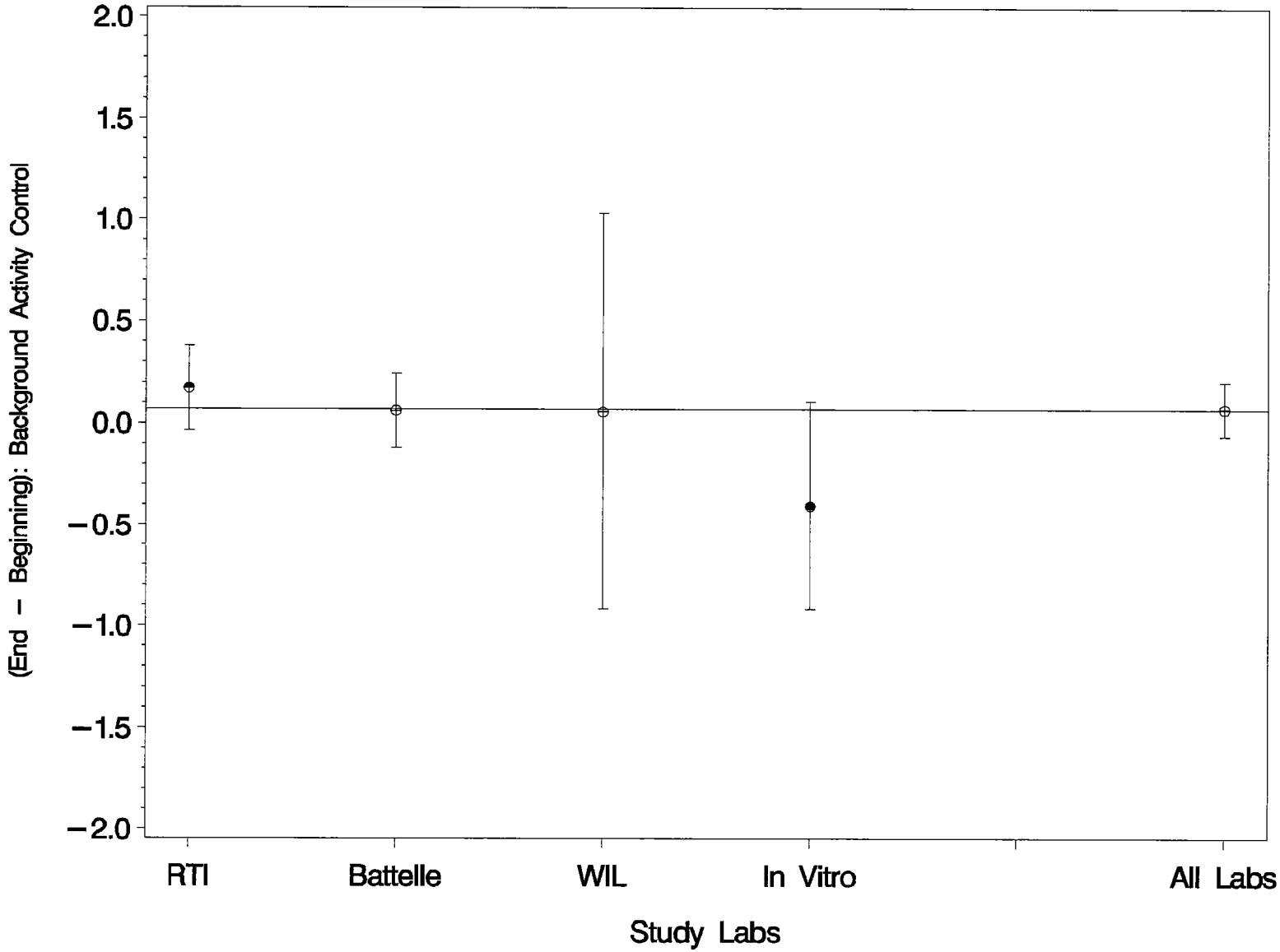

Figure 37. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference (End Minus Beginning) for Background Activity Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

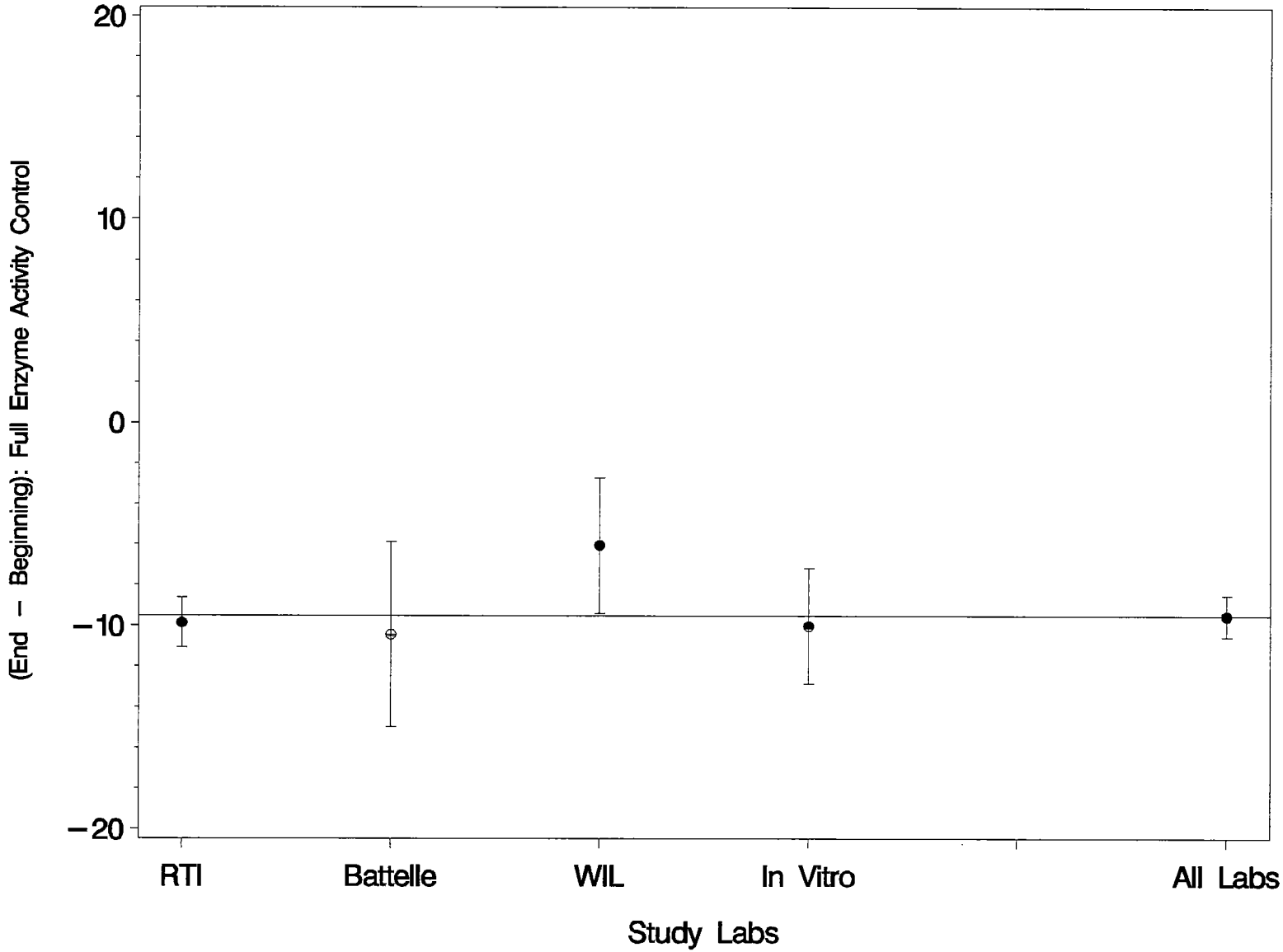

Figure 38. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference (End Minus Beginning) for Full Enzyme Activity Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

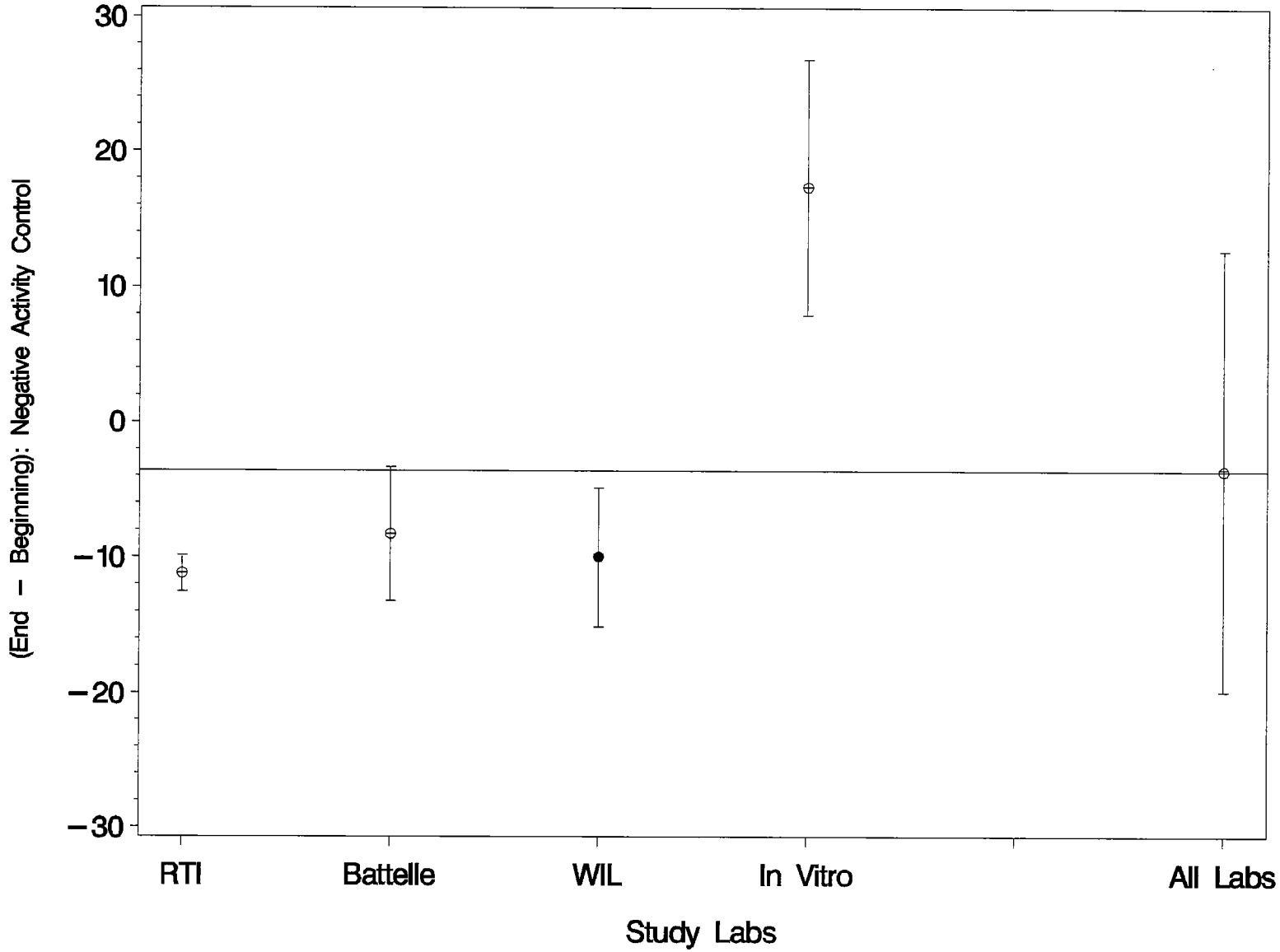

Figure 39. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference (End Minus Beginning) for Negative Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

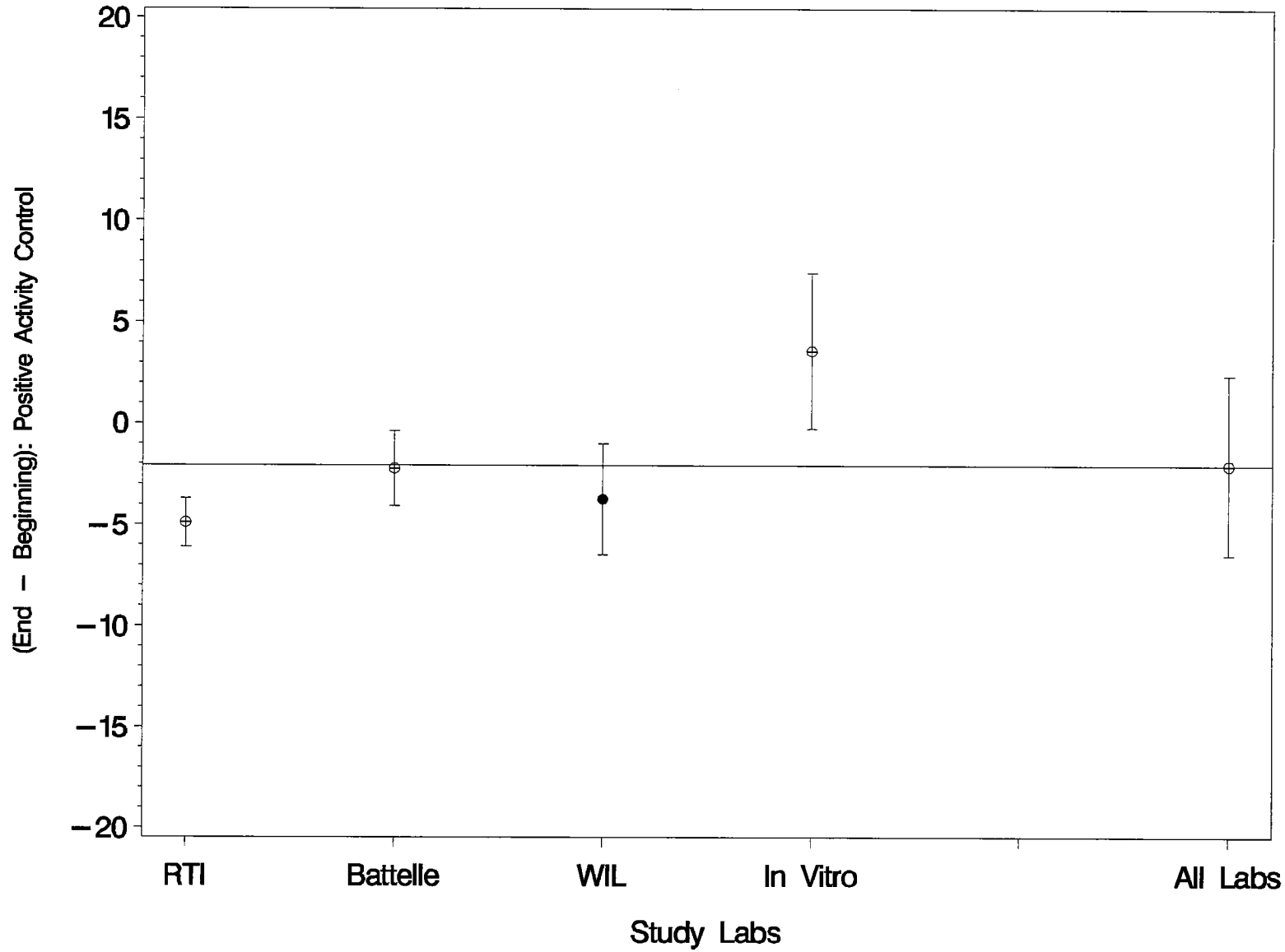

Figure 40. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference (End Minus Beginning) for Positive Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

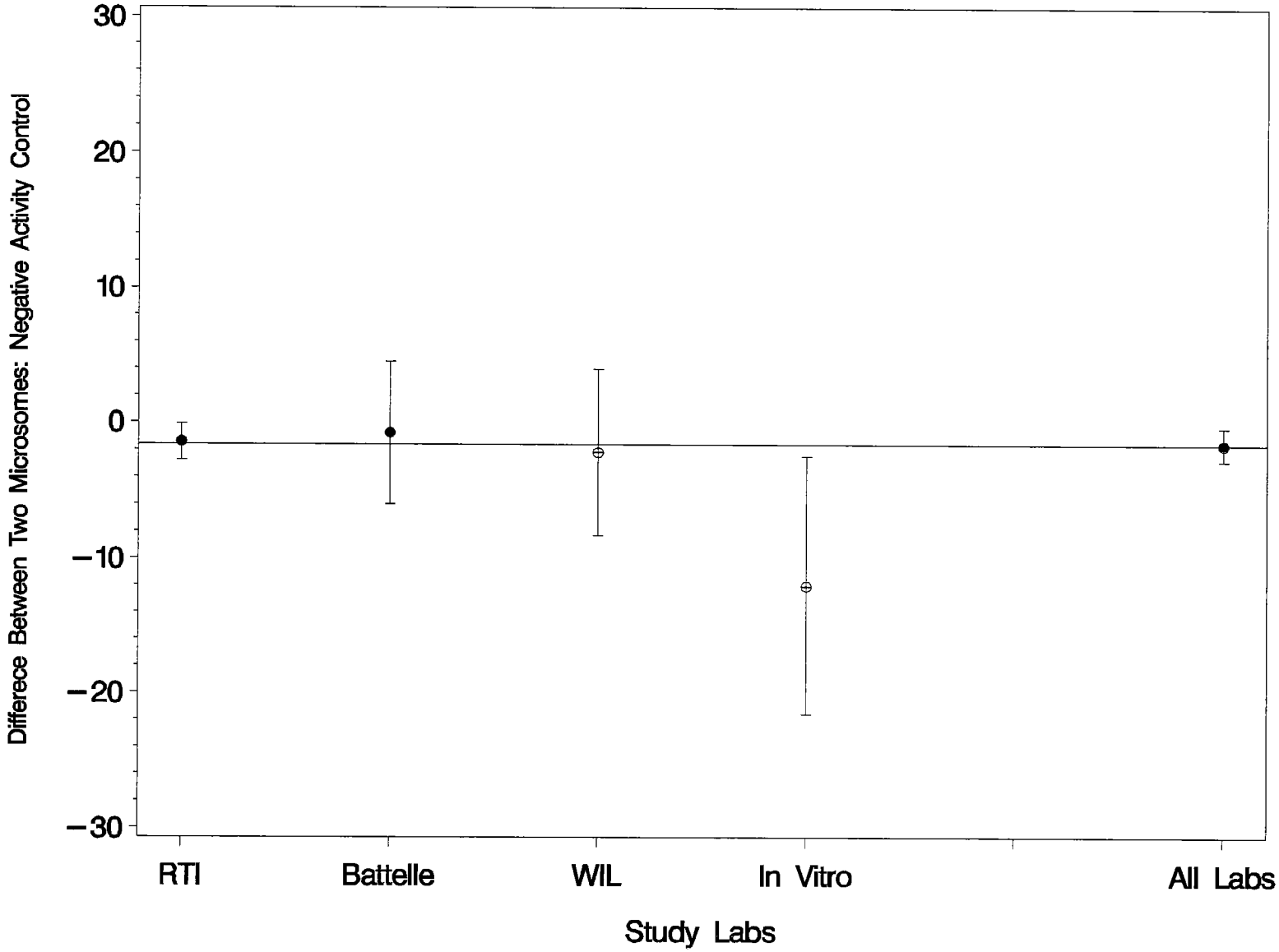

Figure 41. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference Between Sources (Battelle Minus In Vitro) for Negative Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

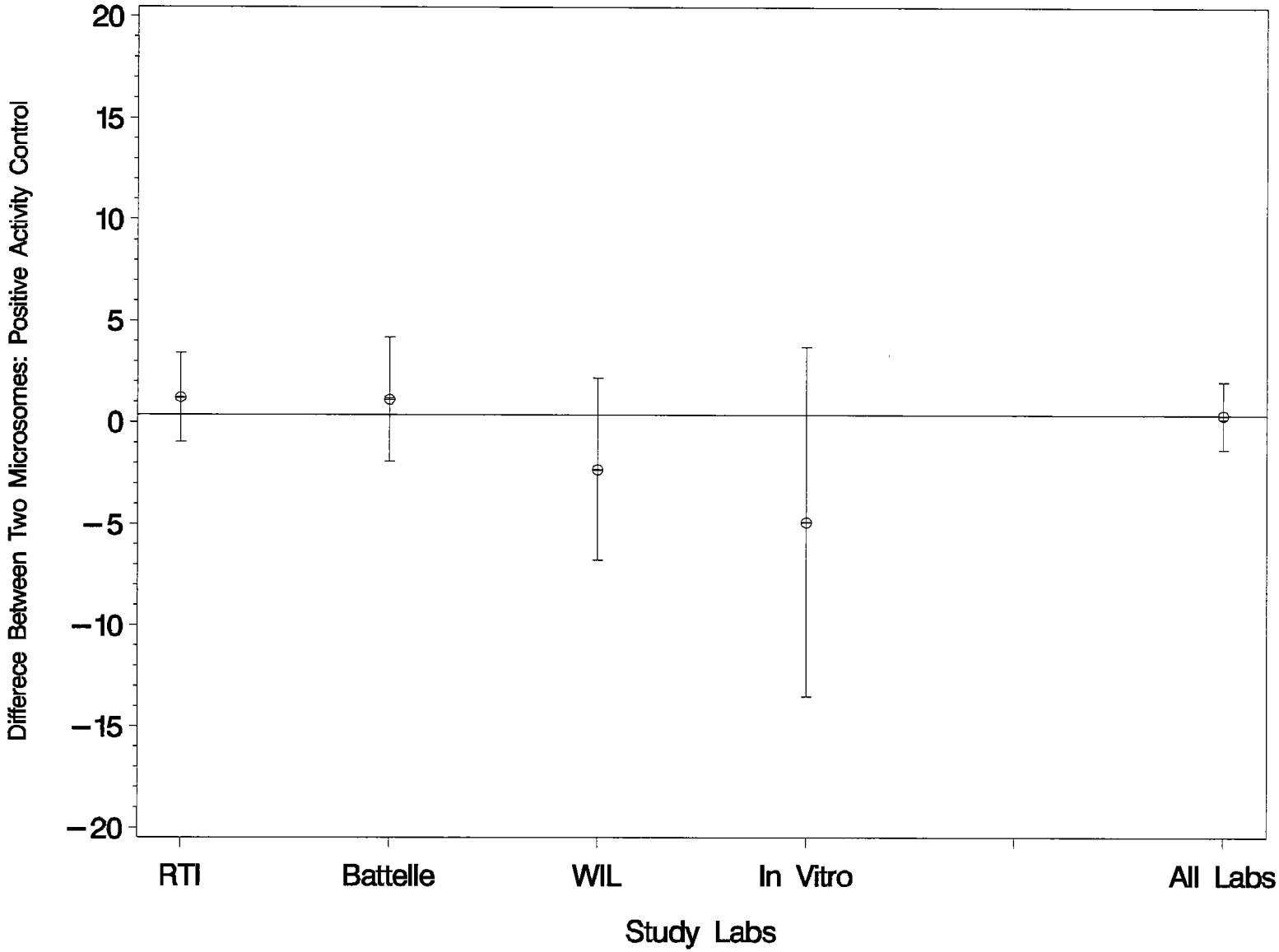

Figure 42. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference Between Sources (Battelle Minus In Vitro) for Positive Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

4.0 DISCUSSION

In this task, the responsiveness of the human placental microsomal aromatase assay to ten reference chemicals and positive and negative control test substances (4-OH ASDN and lindane) were evaluated. The assay was conducted by a lead laboratory (RTI) and three participating laboratories using microsomes prepared and distributed by two of the participating laboratories (Battelle and In Vitro). As planned, this task provided information about the utility of the low and high protein QC standards, intralaboratory and interlaboratory variability for enzyme activity and background activity controls, aromatase inhibition (as a percent of control) by the positive and negative controls, and aromatase inhibition (percent of control and IC_{50} values) for each of the reference chemicals.

Protein QC standards were used in this work assignment to evaluate protein assay variability from replicate-to-replicate within a given laboratory and from laboratory-to-laboratory. RTI prepared and distributed the QC standards to the participating laboratories, thereby removing the variability that would have occurred if each laboratory had prepared their own QCs. Two QC standards were prepared with target concentrations of 10 and 100 μg/mL. RTI reported the concentrations prior to distribution to be 12 and 110 μg/mL, respectively. These levels were selected because they: a) bracket the target protein concentration of the assay (0.0125 mg/mL) and b) fall just within the lowest and highest protein standard curve standards, i.e. 5 and 125 μg/mL (excluding the 250 μg/mL standard that was not used by most laboratories). The results from this work assignment clearly indicated that all laboratories were able to achieve accurate and precise measurements of the high QC standard (% RE and CV were within ± 15 percent). Similar results were obtained for the low QC standard with regard to accuracy (3 of 4 laboratories had % RE values within ± 15 percent) but, in regard to precision, the results were not acceptable (1 of 4 laboratories had % CV values within ± 15 percent). Based on the low QC precision results but acceptable accuracy results, as well as the acceptable precision and accuracy results obtained for the high QC standard, these levels of QCs appear to provide the information needed to evaluate the replicate and laboratory performance in the conduct of the protein assay; although the laboratories will need to provide particular attention to their procedures in order to achieve acceptable levels of precision at the low QC level. It will also be important for laboratories to prepare a sufficient volume of QC standards for an entire project and, if laboratory-to-laboratory comparisons are made, for a central laboratory to prepare and distribute the QC standards in order to minimize variability.

Placental microsomes were prepared and distributed by two participating laboratories – Battelle and In Vitro. The microsomes used by all of the laboratories in this task were prepared as described in the report for WA 4-16, Task 6. In this previous task, Battelle and In Vitro reported the protein concentrations of their microsomal preparations to be 22.5 ± 0.9 and 8.4 ± 0.3 mg/mL. Similar results were obtained in the present task, i.e. 23.6 ± 1.8 mg/mL and 8.7 ± 1.0 mg/mL, thereby providing further confirmation about these initial measurements and, although limited, some information about the stability of the microsomes.

The full enzyme activity control value for this assay should be at least 0.03 nmol/mg protein/min according to the acceptance criterium in the study plan. Regardless of the source of microsomes, this criteria was achieved. The overall task mean ± SEM full enzyme activity control value for the Battelle-prepared microsomes was 0.055 ± 0.002 nmol/mg protein/min and,

for the In Vitro-prepared microsomes, was 0.052 ± 0.008 nmol/mg protein/min. The overall full enzyme activity with both sources combined was 0.054 ± 0.005 nmol/mg protein/min. These results are similar to the results reported following preparation (WA 4-16, Task 6). In this previous task, the Battelle- and In Vitro-prepared microsomes had activities of 0.058 ± 0.005 and 0.036 ± 0.003 nmol/mg protein/min, respectively. These results also provide information about the stability of the microsomes over a fairly short storage period of a few months. For most labs, the precision (as measured by % CV) of the full enzyme activity control values for a given replicate was generally less than 10 percent, thereby indicating that the intra-assay precision was high. There was a time or two when a laboratory had a much higher % CV for a given replicate, e.g. >25 percent, but these instances were few, thereby suggesting that the assay performed consistently on a given day for a given laboratory. The inter-assay precision for a given laboratory tended to be higher. Although the lead laboratory mostly had inter-assay precision values less than 10 percent, the other laboratories had inter-assay variability values that tended to range between 10 and 20 percent (one laboratory had a value of approximately 40 percent). These results indicated that the assay was also consistent from day to day for multiple laboratories. Using RTI's results as the standard, then a measure of accuracy (as measured by % RE) can be determined, which for Battelle, In Vitro, and WIL were 17.2, 53.3, and 26.1 percent, respectively. The high values for In Vitro were attributed to a few samples that were high but no account for the exaggerated response could be found. Thus, the precision of the assay to determine enzyme activity approximated 10 to 20 percent and, for accuracy, was approximately 20 percent (excluding the one laboratory with the few high values).

There was a decrease in the full enzyme activity when the beginning repetitions were compared to the ending repetitions. This finding suggested that the time required to analyze the samples decreased the enzyme activity and may have implications on the number of samples that can be analyzed at a given time.

The positive control, 4-OH ASDN, is a known aromatase inhibitor and inhibited aromatase activity in a consistent manner for all laboratories. 4-OH ASDN, at a final concentration of 5×10^{-8} M, resulted in laboratory overall group mean (± SEM) inhibition values (as a percent of control) of 48.0 ± 0.4, 48.7 ± 1.0, 40.5 ± 7.4, and 42.7 ± 3.1 percent for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM percent of control value was 45.0 ± 2.0 percent. These results indicated that the target concentration, which was selected to inhibit aromatase activity by approximately 50 percent, produced the desired degree of inhibition. The precision (% CV) of the assay for measuring 4-OH ASDN-dependent inhibition for RTI, Battelle, In Vitro and WIL was 2.3, 6.8, 44.5, and 17.5 percent, respectively (Table 11). Using RTI for comparison in order to obtain a measure of accuracy, resulted in % RE values of 1.4, -15.7, and -11.1 percent for Battelle, In Vitro, and WIL, respectively. Thus, with the exception of one high % CV value, the precision and accuracy of the assay to measure 4-OH ASDN inhibition was less than 20 percent.

The negative control, lindane, is known to <u>not</u> inhibit aromatase and this outcome was clearly demonstrated for most laboratories. Lindane, at a final concentration of 10^{-6} M, resulted in laboratory overall average (± SEM) enzyme activity values of ≥ 97 percent for RTI, Battelle, and WIL but approximately 75 percent for In Vitro. It is unclear why In Vitro observed this decrease in aromatase activity. The researchers re-examined their dilution scheme, procedures, and results for a possible explanation but none could be found. Although conjecture, it appears

that there was a systematic error in the procedure followed for the negative control samples because the lindane-induced inhibition was consistently produced by In Vitro throughout the task but no similar responses were observed by the other laboratories. In addition, In Vitro's other controls responded in a manner consistent with known responses, previous assays, and results from other laboratories. Aside from the In Vitro results, lindane at the concentration tested produced the desired response for use as a negative control. The precision (% CV) of the assay for measuring lindane inhibition for RTI, Battelle, In Vitro and WIL was 2.1, 2.8, 42.4, and 21.6 percent, respectively (Table 13). Using RTI for comparison in order to obtain a measure of accuracy, resulted in % RE values of -0.7, -23.6, and 1.8 percent for Battelle, In Vitro, and WIL, respectively. Thus, with the exception of one high % CV value, the precision and accuracy of the assay to measure 4-OH ASDN inhibition was approximately 20 percent or less.

The reference chemicals used on this task were selected for their known effect on aromatase activity (Table 1). In addition, the selection was based on chemical classes and, within a class, according to their varying aromatase inhibitory strengths. The IC_{50} results from the present task, together with some values reported from previous work assignments and in the literature, are summarized in the Table 40. Qualitatively, the laboratories in the present study produced results that agreed with information presented in the literature for the reference chemicals, with the exception of 4-nonylphenol. It was unexpected for 4-nonylphenol to inhibit aromatase as this reference chemical reportedly produces its effects at the level of the androgen and estrogen receptors (AR/ER). Although no inhibition was detected in a previous work assignment (WA 2-24), the lack of an inhibitory effect at that time may have been attributed to the limited number of concentrations selected for testing. Clearly, an explanation for the inhibitory effects of 4-nonylphenol reported in the present task is needed. It is possible that the aromatase assay has identified an inhibitor of one aspect of the steroidogenesis pathway that was not identified as such before; alternatively, it is possible that this was a false positive response, which is also an important finding in the evaluation of this assay for screening chemicals for aromatase inhibitory activity.

In contrast to the qualitative outcome of testing the aromatase assay with the reference chemicals, the IC_{50} values were also quantitatively similar to previously reported values. The similar IC_{50} values with historical values indicate that laboratories with extensive or marginal experience with the assay are able to obtain accurate information about the inhibitory effect of the test substance on aromatase activity.

Further explanation is required for the error described at the beginning of this report. An examination of the individual laboratory reports indicates that aminogluethimide has an IC_{50} value that is approximately 10-fold higher, and ketoconazole has an IC_{50} value that is approximately 10-fold lower than what had been obtained in previous tasks and is reported in the literature. Upon further investigation for an explanation for these discrepancies, it was discovered that these two reference chemicals were shipped together and were mislabeled by the CR as to their correct identity and stock solution concentrations. Since the stock solution for aminogluethimide was prepared as a 0.1 M solution and the ketoconazole was prepared as a 0.01 M stock solution (due to insolubility at 0.1 M), the 10-fold difference in stock concentration

Table 40. Summary of Previous and Present Task and Literature IC_{50} Values

Reference Compound	IC_{50} Values (µM or nM)[a]			Literature Reference
	Present Task[b]	Previous Tasks[c]	Literature	
Aminoglutethimide	4.30 ± 0.23	4.4 ± 0.1 5.6 ± NA[d]	1.0 1.6 5.0 5.5 130.0	LeBail, et. al., 2001 Jeong, et. al., 1999 Wang, et. al., 1994 Chen, et. al., 1997 Pelissero, et. al., 1996
Atrazine	No inhibition	--	None	Roberge, et. al., 2004
Chrysin	2.5 ± 0.3	4.1 ± 0.3 3.2 ± NA[d]	0.7 4.4 11.0	LeBail, et. al., 2001 Jeong, et. al., 1999 Chen, et. al., 1997
Dibenz[a,h]anthracene	No inhibition	--	--	--
Dicofol	29.1 ± 8.6	--	>50	Vinggaard, et. al., 2000
Econazole	1.6 ± 0.3	2.1 ± 0.1 2.8 ± NA[d]	0.03	Mason, et. al., 1987
Fenarimol	5.8 ± 0.6	--	10	Vinggaard, et. al., 2000
Ketoconazole	6.47 ± 0.72	7.5 ± 0.6 15.0 ± NA[d]	6 15 60 7.3	White, et. al., 1999 Rowlands, et al., 1995 Mason, et. al., 1987 Ayub and Levell, 1988
4-Nonylphenol	29.1 ± 3.0	No inhibition	None	Laws, et. al., 2000
Prochloraz	28.4 ± 4.4	--	40 – 340 700	Vinggaard, et. al., 2000 Mason, et. al., 1987

a. Reported values are the mean (± SEM). Concentration units are µM, except for econazole and prochloraz, which are reported in nM.
b. Overall task values.
c. Previous tasks include WA 2-24, WA 4-10, and WA 4-16.
d. Measure of variability not available.

together with the misidentification, resulted in the laboratories testing different concentrations than they thought were being tested. For example, aminogluethemide at a concentration of 0.1 M was planned to be tested at concentrations ranging from 10^{-3} to 10^{-10} M. Instead, aminogluethimide was incorrectly identified as ketoconazole (concentration of 0.01 M), which resulted in the laboratories actually testing concentrations ranging from 10^{-3} to 10^{-9} M. A similar error occurred for ketoconazole. Thus, the data for these two chemicals were corrected for presentation in the overall report so that the appropriate intralaboratory and interlaboratory comparisons could be made without concern for the error. However, it is important to note that this change has not yet be made to the individual laboratory reports found in the appendices.

One of the uncertainties of this assay was how well could the laboratories characterize the inhibitory effects of an unknown chemical. In order to evaluate this question, the laboratories were directed to test each of the reference chemicals at eight predefined target concentrations in triplicate repetitions (tubes) per replicate of the assay. Percent of control results from the first replicate of a given reference chemical were reviewed and the target concentrations were adjusted to better define the concentration response curve. Concentrations were selected to define the upper and lower plateaus with intermediate concentrations chosen to distribute as evenly as possible along the slope of the curve. This resulted in some different concentrations

being tested in replicate 1 than were tested in replicates 2 and 3. This adjustment in concentrations may also explain some of the differences and variability of the replicates for a given laboratory since the latter replicates were optimized to better characterize the curve and estimate the IC_{50}. Exclusion of the first replicate and analysis of three replicates where the concentrations have been settled upon, may substantially improve the precision and accuracy of the assay.

Another issue that was addressed in the conduct of this task involved fitting the observed data. The four-parameter model, in which the top and bottom plateaus are allowed to vary to fit the data, resulted in a more satisfactory curve fit than the two-parameter model tested in WA 4-16, Task 5. In addition, the present task did not use any weighting nor were there any limitations imposed on percent of control values below 0 or above 100 percent. This approach successfully addressed how to analyze percent of control curves when the concentrations tested do not identify or fully characterize the 0 and/or 100 percent levels of inhibition.

5.0 CONCLUSIONS

A lead laboratory and three participating laboratories used human placental microsomes prepared by two of the participating laboratories to conduct the aromatase assay to test ten reference chemicals for aromatase inhibition. This task provided information about the utility of the low and high QC standards, intralaboratory and interlaboratory variability for endpoints such as full enzyme activity and background activity controls, aromatase inhibition (as a percent of control) by the positive and negative controls and aromatase inhibition (percent of control and IC_{50} values) for each of the reference chemicals. The assay produced results by all four laboratories that were in good agreement with historical information about the IC_{50} values for nine of ten reference chemicals, the exception being 4-nonylphenol.

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APPENDIX A

RESEARCH TRIANGLE INSTITUTE REPORT

ERRATA

To the Draft Final Report
For WA 4-16, Task 7

The aminoglutethimide and ketoconazole IC_{50} values reported in the individual laboratory reports are incorrect by a factor of 10, i.e., the IC_{50} for aminoglutethimide is 10-fold high and for ketoconazole is 10-fold low. This error occurred due to a miscommunication by the Chemical Repository as to the identity of these two reference chemicals and their respective stock concentrations. In this overall report, the errors were corrected for each of the laboratories in order for an evaluation of the intralaboratory and interlaboratory results to be made without having to be concerned about this discrepancy. The individual laboratory reports will be corrected by their respective study directors when the draft final reports are finalized.

AUDITED DRAFT TASK REPORT

PLACENTAL AROMATASE VALIDATION STUDY

WA 4-16 Task 7: Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories

EPA Contract Number 68-W-01-023
Work Assignment 4-16

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DRAFT REPORT

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Experimental Dates: July 14, 2005 – September 30, 2005

Draft Task Report Date: March 24, 2006

RTI Identification Number: 08055.003.032 – Task 7

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1.0 EXECUTIVE SUMMARY

In this task, RTI, as one of four participating laboratories, conducted studies with ten potential aromatase inhibitors (reference chemicals, RC) using two human placental microsome preparations (5 chemicals were tested in each microsome preparation) in order to produce data that could be compared with data from other participating laboratories as the final phase of the aromatase assay validation study. The reference chemicals were aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, dibenz[a,h]anthracene, fenarimol, econazole, chrysin, dicofol, and atrazine.

Human placental microsomes prepared by Battelle and In Vitro were used in this task. A freshly diluted microsomal suspension was prepared each day the aromatase assay was conducted and the protein concentration in the microsomal suspension was measured. The average measured protein content of the Battelle-supplied microsome stock was 27.0 ± 1.4 mg/mL (5.3% CV), while that of the In Vitro-supplied microsomes was 10.9 ± 1.2 mg/mL (10.8% CV).

Quality control (QC) standards of 12 and 110 μg/mL were included with each of the sixteen protein assays. Overall, the data show acceptable correlation of calculated and known amounts (-5.7 and 3.5% relative error, respectively) for the low and high QC standards.

Three independent replicates were conducted (on separate days) for each reference chemical. Each reference chemical was tested at eight concentrations and there were three (triplicate) repetitions for each concentration of a given replicate. Four types of control samples were included for each replicate. These included full enzyme (aromatase) activity controls, background controls, positive controls (with a known aromatase inhibitor) and negative controls (with a known aromatase non-inhibitor).

Aromatase activities for each type of control had coefficients of variance (CV) on the order of 8 and 12%, respectively for the Battelle and In Vitro microsomes. These coefficients of variance are lower than the approximately 20% found in a previous WA 4-16 task for aromatase activity. This improvement may be correlated to improved control of the protein assay in this Task versus the previous one through the use of a different range for the standard curve and the incorporation of QC standards. Interestingly, the coefficient of variance for the protein concentration of the Battelle microsomes was 5.3% and that for the In Vitro microsomes was

10.8%, which mirrors the CVs for the aromatase activity, suggesting that variance in aromatase activity is closely tied to variance in protein content determination.

For each type of control, there is a discernable pattern in which the percent of control values for the controls run at the beginning of the assay set (beginning portion) were higher than the percent of control values for the controls run at the end (end portion). The data show decreases between beginning and end percent of control activities generally on the order of 5-15%. Given that only about 25 minutes elapsed between the beginning and end of the assay set, these decreases are notable, and support the need for minimizing the amount of time between thawing and use of the microsomes in the assay.

IC_{50}s, slope, and top and bottom plateaus were calculated for each reference chemical using a four-parameter equation. There was very little variance (generally 5-15%) in IC_{50} data over three replicates for each reference chemical. 4-Nonylphenol was identified as an aromatase inhibitor in this study, whereas in WA 2-24, it was classified as a non-inhibitor. The inclusion of more concentrations between 10^{-4} and 10^{-5} M in this task allowed for the detection of the inhibition of aromatase at those higher concentrations and for appropriate curve-fitting to estimate IC_{50}. When the percent of control values do not span the range 0-100%, the value returned as logIC_{50} by Prism (Version 3.02) is actually equivalent to (top + bottom)/2. Therefore, caution should be exercised in the reporting of IC_{50} estimates generated from data sets that do not span the 0-100% range for percent of control. Data from chrysin, where the percent of control values ranged from about 23-95%, confirm this limitation of the software. Dibenz[a,h]anthracene and atrazine were identified as non-inhibitors of aromatase, which is as expected based on the literature and the results of WA 2-24.

2.0 INTRODUCTION

2.1 Background

The Food Quality Protection Act of 1996 was enacted by Congress to authorize the U. S Environmental Protection Agency (EPA) to implement a screening program on pesticides and other chemicals found in food or water sources for endocrine effects in humans. Thus, the EPA is implementing an Endocrine Disruptor Screening Program (EDSP). In this program, comprehensive toxicological and ecotoxicological screens and tests are being developed for identifying and characterizing the endocrine effects of various environmental contaminants,

industrial chemicals, and pesticides. The program's aim is to develop a two-tiered approach, e.g., a combination of *in vitro* and *in vivo* mammalian and ecotoxicological screens (Tier 1) and a set of *in vivo* tests (Tier 2) for identifying and characterizing endocrine effects of pesticides, industrial chemicals, and environmental contaminants. Validation of the individual screens and tests is required, and the Endocrine Disruptor Method Validation Committee (EDMVAC) will provide advice and counsel on the validation assays.

Estrogens are sex steroid hormones that are necessary for female reproduction and affect the development of secondary sex characteristics of females. Estrogens are biosynthesized from cholesterol by a series of enzymatic steps, with the last step involving the conversion of androgens into estrogens by the enzyme aromatase. Estrogen biosynthesis occurs primarily in the ovary in mature, premenopausal women. During pregnancy, the placenta is the main source of estrogen biosynthesis and pathways for production change. Small amounts of these hormones are also synthesized by the testes in the male and by the adrenal cortex, the hypothalamus, and the anterior pituitary in both sexes. The major source of estrogens in both postmenopausal women and men occurs in extraglandular sites, particularly in adipose tissue. One potential endocrine target for environmental chemicals is the enzyme aromatase, which catalyzes the biosynthesis of estrogens. An aromatase assay is proposed as one of the Tier 1 Screening Battery Alternate Methods. A detailed literature review on aromatase was performed and encompassed (1) searching the literature databases, (2) contacting individuals to obtain information on unpublished research, and (3) evaluating the literature and personal communications.

Aromatase is a cytochrome P450 enzyme complex responsible for estrogen biosynthesis and converts androgens, such as testosterone and androstenedione, into the estrogens estradiol and estrone. Aromatase is present in the ovary, placenta, uterus, testis, brain, and extraglandular adipose tissues. Two proteins, cytochrome $P450_{arom}$ and NADPH-cytochrome P450 reductase, are necessary for enzymatic activity, and the enzyme complex is localized in the smooth endoplasmic reticulum. The aromatase gene, designated CYP19, encodes the cytochrome $P450_{arom}$ and consists of 10 exons, with the exact size of the gene exceeding 70 kilobases. Aromatase is also found in breast tissue, and the importance of intratumoral aromatase and local estrogen production is the subject of current research efforts. Aromatase inhibitors have been developed as efficacious therapeutic agents for estrogen-dependent breast cancer to reduce the

stimulatory effects of estrogens in the progression of breast cancer. Investigations on the development of aromatase inhibitors began in the 1970's and have expanded greatly in the past three decades.

An *in vitro* aromatase assay could easily be utilized as an alternative screening method in the Tier 1 Screening Battery to assess the potential effects of various environmental toxicants on aromatase activity. Both *in vitro* subcellular (microsomal) assays and cell-based assays are available for measuring aromatase activity. The *in vitro* subcellular assay using human placental microsomes is commonly used to evaluate the ability of pharmaceuticals and environmental chemicals to inhibit aromatase activity. In addition, human JEG-3 and JAR choriocarcinoma cell culture lines, originally isolated from cytotrophoblasts of malignant placental tissues, have been used as *in vitro* systems for measuring the effects of compounds on aromatase activity. These cell lines are also utilized for investigations on the effects of agents in placental toxicology.

Numerous flavonoids and related phytoestrogen derivatives have been extensively evaluated for their ability to inhibit aromatase activity for two primary reasons: (1) these natural plant products can serve as possible leads for the development of new nonsteroidal aromatase inhibitors; and (2) humans and other animals are exposed to these agents through the diet. In general, the flavonoids and related analogs demonstrate aromatase inhibition with IC_{50} values in the micromolar range; however, these compounds lack both the potency and specificity of aromatase inhibitors developed for breast cancer therapy. Several pesticides have also demonstrated inhibition of aromatase activity in the human placental microsomal assay system, with IC_{50} values for aromatase inhibition as low as 0.04 μM.

The human placental microsomal aromatase assay was recommended as the *in vitro* aromatase screening assay to be included in the Tier 1 Screening Battery. This assay will detect environmental contaminants that possess the ability to inhibit aromatase activity. Prevalidation studies on recombinant aromatase (WA 2-24) were conducted to optimize the microsomal aromatase assay protocol for human placenta, demonstrate the utility of the microsomal assay to detect known aromatase inhibitors, and compare the performance of a recombinant assay system and the placental microsomal assays. Concerns with this initial work involving high variability in some experiments and partial inhibition curves were addressed in a supplemental prevalidation study (WA 4-10). The objective of the current work assignment is to use the now optimized

assay to obtain intra- and interlaboratory assay variability estimates to complete the validation of the human placental microsome aromatase assay.

2.2 Task Description and Objectives

In this task, RTI, as one of four participating laboratories, conducted studies with ten potential aromatase inhibitors (reference chemicals, RC) using microsomes prepared by two participating laboratories (Battelle and In Vitro) in Task 6 and determine the intra- and interlaboratory variability of the assay.

3.0 MATERIALS AND METHODS

3.1 Substrate

The substrate for the aromatase assay was androstenedione (ASDN). Non-radiolabeled and radiolabeled ASDN were used. The non-radiolabeled ASDN (Lot # 024K0809) was obtained from Sigma (St. Louis, MO) by the Sponsor's Chemical Repository and was then distributed to the participating laboratories. The radiolabeled androstenedione ([1-^{3}H]-androstenedione, [^{3}H]ASDN, Lot # 3538496), was obtained from Perkin Elmer Life Science (Boston) and had a reported specific activity of 25.3 Ci/mmol. Radiochemical purity was reported by the supplier to be > 97%. Radiochemical purity was assessed by HPLC in an earlier Task and results of that analysis are presented in the report contained in Appendix B.

The substrate solution was prepared fresh each day of assay by combining solutions of [^{3}H]ASDN and non-radiolabeled ASDN in order to prepare a solution containing 2 μM ASDN with ca. 1 μCi/mL. A 1:100 dilution of the radiolabeled [^{3}H]ASDN stock in buffer was prepared fresh each day of assay. A solution (1 mg/mL) of ASDN in ethanol was prepared (fresh each day) and then diluted in buffer to a final concentration of 1 μg/mL. The substrate solution was prepared by combining 4.5 mL of the 1 μg/mL solution of ASDN, 800 μL of the [^{3}H]ASDN dilution and 2.7 mL buffer to make 8 mL of substrate solution. The addition of 100 μL of the substrate solution to each 2 mL assay volume yielded a final [^{3}H]ASDN concentration of 100 nM with ca. 0.1 μCi/tube.

3.2 Reference and Control Substances

Battelle's Chemical Repository (CR) was responsible for chemistry activities required to perform this study. Their responsibilities included chemical procurement, determination of

solubility and formulation stability, formulation preparation, formulation analysis and shipment of stock formulation to the participating laboratories. These chemistry activities and results are described in the Sponsor's Chemistry Reports.

3.2.1 Reference Chemicals

The RC formulations and vehicles were received from Battelle's CR by RTI's Materials Handling Facility (MHF). Each solution and vehicle was assigned an RTI log number. The RC formulations were coded prior to distribution to the laboratory for use in the aromatase assay.

Table 1. Reference Chemicals for Task 7

Chemical Code	Reference chemical	Abbreviation	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Basis for Selection
1	aminoglutethimide	AG	125-84-8	$C_{13}H_{16}N_2O_2$	232.3	Non-steroidal aromatase inhibitor
2	ketoconazole	KCZ	65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	531.43	Weak imidazole anti-fungal
3	prochloraz	PCZ	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	conazole fungicide
4	4-nonylphenol	NYP	104-40-5	$C_{15}H_{24}O$	220.4	Affects AR/ER; no known aromatase inhibition
5	dibenz [*a,h*] anthracene	DBA	53-70-3	$C_{22}H_{14}$	278.35	Known non-aromatase inhibitor; Ah receptor agonist
6	fenarimol	FRM	60168-88-9	$C_{17}H_{12}Cl_2N_2O$	331.2	pyrimidine fungicide
7	econazole (nitrate)	ECZ	24169-02-6	$C_{18}H_{15}Cl_3N_2O$-HNO_3	444.7	Potent imidazole anti-fungal
8	chrysin	CYN	480-40-0	$C_{15}H_{10}O_4$	254.2	Potent flavonoid
9	dicofol	DCF	115-32-2	$C_{14}H_9Cl_5O$	370.47	Organochlorine
10	atrazine	ATZ	1912-24-9	$C_8H_{14}ClN_5$	215.69	Affects aromatase gene expression; no aromatase inhibition

Dilutions of each RC were prepared fresh each day for use in the assay. In each case, the same vehicle used to prepare the stock solution was used in the preparation of the dilutions. Dimethyl sulfoxide (DMSO) of the same lot number used in the preparation of the stock solutions for RCs 3, 4, 5, 6, 9, and 10 was not provided, so DMSO of lot 2969A46428 was used

in the preparation of dilutions of those chemicals. Suppliers and lot numbers for reference chemical vehicles are presented in Table 8 (Section 3.4). The dilution scheme described in Table 2 was used in the preparation of dilutions for the first replicate of each RC that was supplied as a 0.1 M solution. The scheme described in Table 3 was used for the first replicates of ketoconazole (RC-2), dibenz[a,h]anthracene (RC-5) and chrysin (RC-8), which were each supplied as 0.01 M solutions. The results of each replicate 1 were reviewed and adjustments were made as necessary (at the direction of the Study Director) to the concentrations tested in replicates 2 and 3 of the assay in order to obtain data that better described the IC_{50} curve. The concentrations of each RC tested in all replicates are included in Table 14 and Appendix D.

Table 2. Dilution Scheme for the First Replicate of all Reference Chemicals Supplied as 0.1 M Solutions

Dilution ID	µL vehicle	µL sample	Sample used	Solution concentration (M)	Final Concentration in assay (M)
1	0	1000	Stock	1.00E-01	1.00E-03
2	900	100	Diln. 1	1.00E-02	1.00E-04
3	900	100	Diln. 2	1.00E-03	1.00E-05
4	900	100	Diln. 3	1.00E-04	1.00E-06
5	900	100	Diln. 4	1.00E-05	1.00E-07
6	900	100	Diln. 5	1.00E-06	1.00E-08
7	900	100	Diln. 6	1.00E-07	1.00E-09
8	900	100	Diln. 7	1.00E-08	1.00E-10

Table 3. Dilution Scheme for the First Replicate of Reference Chemicals Supplied as 0.01 M Solutions

Dilution ID	µL vehicle	µL sample	Sample used	Solution concentration (M)	Final Concentration in assay (M)
1	0	1000	Stock	1.00E-02	1.00E-04
2	900	100	Diln. 1	1.00E-03	1.00E-05
3	900	100	Diln. 2	1.00E-04	1.00E-06
4	500	500	Diln. 3	5.00E-05	5.00E-07
5	900	100	Diln. 3	1.00E-05	1.00E-07
6	900	100	Diln. 5	1.00E-06	1.00E-08
7	900	100	Diln. 6	1.00E-07	1.00E-09
8	900	100	Diln. 7	1.00E-08	1.00E-10

3.2.2 Control Chemicals

The known aromatase inhibitor, 4-hydroxyandrostenedione (4-OH ASDN), was used as a positive control and the known aromatase non-inhibitor, lindane, was used as a negative control. Stock solutions (Table 4) were supplied by Battelle's CR and were stored refrigerated and protected from light. Dilutions were made fresh each day of use in the same vehicle and lot number that was used to prepare the stock solutions (see Table 8, Section 3.4 for details). Tables 5 and 6 describe the dilution scheme for 4-OH ASDN and lindane, respectively.

Table 4. Control Chemicals for Task 7

Test Substance	Mfr Purity	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Stock Solution ID	Stock Formulation Concentration (M)	Vehicle	Storage Conditions
4-OH ASDN	99%	566-48-3	$C_{19}H_{26}O_3$	302.4	4-ASDN-1	0.01	95% ethanol	Refrigerate, Dark
Lindane	99.6%	58-89-9	$C_6H_6Cl_6$	290.8	3-LIN-1	0.1	DMSO	Refrigerate, Dark

Table 5. Dilution Scheme for 4-OH ASDN Control

Dilution ID	µL EtOH	µL sample	Sample used	Solution concentration (M)	Final Concentration in assay (M)
1	9900	100	Stock	1.00E-04	
2	9500	500	Diln. 1	5.00E-06	5.00E-08

Table 6. Dilution Scheme for Lindane Control

Dilution ID	µL DMSO	µL sample	Sample used	Solution concentration (M)	Final Concentration in assay (M)
1	9900	100	Stock	1.00E-03	
2	900	100	Diln. 1	1.00E-04	1.00E-06

3.3 Microsomes

Human placental microsomes, prepared at Battelle or In Vitro, were used on this task. Twenty vials of microsomes (Lot # BAA, ca 150 µL/vial, 8 mg protein /mL) were received from In Vitro. Microsomes from Battelle (Lot # 6-041305) were supplied in 15 vials containing about 200 µL each with a reported protein concentration of 21 mg/mL. The microsomes were stored at approximately -70°C. Prior to assay, microsomes were thawed rapidly in a 37 ± 1°C water bath,

rehomogenized by brief vortexing and then kept on ice until used. The microsome stock was diluted with buffer and maintained on ice until used. The microsomes supplied by Battelle were serially diluted 1:80 and then 1:10 for a total dilution of 1:800. For each assay (which included the testing of two chemicals), two tubes of microsomes from In Vitro were vortex mixed and combined in a single tube before they were diluted in a single step to a final dilution of 1:320 (0.25 mL stock microsomes + 79.75 mL buffer). The time between thawing of the microsomes and their use in the assay was limited to less than 2 h and, in most cases was about 30 min.

The source of the microsomes used in the testing of each RC was defined in the protocol and is presented in Table 7.

Table 7. Reference Chemicals and Microsome Sources for Assays Conducted at RTI

Chemical Code	Chemical Name	Microsome Source
1	aminoglutethimide	Battelle
2	ketoconazole	Battelle
3	prochloraz	In Vitro
4	4-nonylphenol	Battelle
5	dibenz [*a,h*] anthracene	In Vitro
6	fenarimol	In Vitro
7	econazole (nitrate)	Battelle
8	chrysin	Battelle
9	dicofol	In Vitro
10	atrazine	In Vitro

3.4 Other Assay Components

In addition to substrate, reference or control chemicals or vehicle, and microsomes, the aromatase assay contained NADPH, propylene glycol and phosphate buffer. Supplier and lot numbers for other aromatase assay components are presented in Table 8. The lot numbers for the DMSO used in the preparation of the stock solutions for RCs 3, 4, 5, 6, 9 and 10 were not included with the data sheets that accompanied these materials. In all of these cases, DMSO of lot number 2969A46428 was used in the preparation of the dilutions.

Table 8. Supplier and Lot Numbers for Aromatase Assay Components

Chemical	Supplier	Lot Number
NADPH	Sigma	103K7046, 074K7057
Propylene glycol	J.T.Baker	Y18600, B01647
Sodium phosphate dibasic	J.T.Baker	A08H50
Sodium phosphate monobasic	J.T.Baker	A12H20
Vehicle (DMSO) for Lindane (negative control chemical), RC1, 2, 7	Battelle CR	2969A46428
Vehicle (DMSO) for Reference Chemicals 3, 4, 5, 6, 9, 10	Not supplied[a]	00743BD
Vehicle (DMSO) for Reference Chemical 8	Battelle CR	44155
Vehicle (95% EtOH) for 4-OH ASDN (positive control chemical)	Battelle CR	SW0045

[a]This lot number of DMSO was used in the preparation of the stock solutions of these reference chemicals, but it was not supplied to RTI for use in the preparation of dilutions. DMSO, lot number 2969A46428, was used in the preparation of dilutions of these reference chemicals.

3.4.1 NADPH

A solution of NADPH (6 mM) in pH 7.4 sodium phosphate buffer (see Section 3.4.2) was prepared fresh each day of assay and was kept on ice until used.

3.4.2 Assay Buffer

The assay buffer was 0.1 M sodium phosphate buffer, pH 7.4. Sodium phosphate monobasic (JT Baker, catolog # 4011-01, 137.99 g/mol) and sodium phosphate dibasic (JT Baker, catolog # 4062-01, 141.96 g/mol) were used in the preparation of the buffer. Solutions of each reagent at 0.1 M were prepared in distilled, deionized water and then the solutions were combined to a final pH of 7.4. The assay buffer was stored for up to one month in the refrigerator (2-8 °C).

3.5 Protein Determination

The protein concentration of the microsome preparation was determined on each day of use of the microsomes in the aromatase assay. A 6-point standard curve was prepared, ranging from 5 to 250 µg protein/mL. The protein standards were made from bovine serum albumin (BSA). QC standards were supplied by RTI and had reported concentrations of 12 and 110 µg/mL (see data sheets in Appendix C). Unknown and curve standards were run in triplicate

and QC standards were run in duplicate. Protein was determined by using a DC Protein Assay kit purchased from BioRad (Hercules, CA). To a 200 µL aliquot of unknown or standard, 100 µL of BioRad DC Protein Kit Reagent A was added and mixed. Next, 0.8 mL of BioRad DC Protein Kit Reagent B was added to each standard or unknown and the samples were vortex mixed. The samples were allowed to sit at room temperature for at least 15 min to allow for color development. Each sample (unknown and standard) was transferred to a disposable polystyrene cuvette and the absorbance (@ 750 nm) was measured using a spectrophotometer. The protein concentration of the microsomal sample was determined by interpolation of the absorbance value using the curve developed using the protein standards.

3.6 Cytochrome P450 Aromatase (CYP19) Activity

Three independent replicates were conducted (on separate days) for each RC with all replicates for a given RC conducted by the same technician. The RCs were paired according to the microsome preparation in which they were to be assayed. In most cases, two references chemicals were tested on each day of assay and the two RCs sets shared a set of control tubes. The following pairs of RCs were run concurrently and shared control tubes: 1 and 2, 3 and 6, 7 and 8, and 9 and 10. The first replicate of chemical 1 was run alone, as was the third replicate of RC 2, so these sets have their own controls. Replicate 2 of chemical 1 and replicate 1 of chemical 2 were run concurrently, as were replicate 3 of chemical 1 and replicate 2 of chemical 2 and the concurrent sets shared controls. RCs 4 and 5 were assayed on the same days, but since they required testing in different microsomes, a separate set of control tubes was prepared for each. Each RC was tested at eight concentrations and there were three (triplicate) repetitions for each concentration of a given replicate. A single replicate study of an example RC is described in Table 9.

Four types of control samples were included for each assay. These included:

- full enzyme (aromatase) activity controls which contained: substrate, NADPH, propylene glycol, buffer, vehicle [used for preparation of reference chemical solutions] and microsomes
- background activity controls which contained: all components that are in the full aromatase activity controls, except NADPH

- positive controls which contained: all components that are in the full aromatase activity controls, except vehicle, and with the addition of 4-OH ASDN at 5×10^{-8} M
- negative controls which contained: all components that are in the full aromatase activity controls, except vehicle, and with the addition of lindane at a 1×10^{-6} M.

Four test tubes of each type of control were included with each replicate and were treated the same as the other samples. The controls sets were split so that two tubes (of each control type) were run at the beginning and two at the end of each replicate set.

Table 9. Reference Chemical Study Design

Sample type	Repetitions (test tubes)	Description	Test Chemical concentration (M, final)[a]
Full Enzyme Activity Control	4	Complete assay[b] with reference chemical vehicle control	N/A
Background Activity Control	4	Complete assay with reference chemical vehicle control omitting NADPH	N/A
Positive Control	4	Complete assay with positive control chemical (4-OH ASDN) added	5×10^{-8}
Negative Control	4	Complete assay with negative control chemical (lindane) added	1×10^{-6}
RC Concentration 1	3	Complete assay with RC added	1×10^{-3}
RC Concentration 2	3	Complete assay with RC added	1×10^{-4}
RC Concentration 3	3	Complete assay with RC added	1×10^{-5}
RC Concentration 4	3	Complete assay with RC added	1×10^{-6}
RC Concentration 5	3	Complete assay with RC added	1×10^{-7}
RC Concentration 6	3	Complete assay with RC added	1×10^{-8}
RC Concentration 7	3	Complete assay with RC added	1×10^{-9}
RC Concentration 8	3	Complete assay with RC added	1×10^{-10}

[a] The actual reference chemical concentrations tested varied with the concentration of the stock and the results of the first replicate of the assay. The range of concentrations described here was used for the first replicate of all reference chemicals that were supplied as 0.1 M solutions.

[b] The Complete Assay contained buffer, propylene glycol, microsomal protein, [^{3}H]ASDN and NADPH

The assays were performed in 13x100 mm test tubes maintained at 37 ± 1°C in a shaking water bath. Propylene glycol (100 µL), [^{3}H]ASDN, NADPH (as applicable) and RC (or control or vehicle), and buffer (0.1 M sodium phosphate buffer, pH 7.4) were combined in the test tubes (total volume 1 mL). The volume of the reference or control chemical solutions or vehicle used

was 20 µL (1% of total assay volume). The final concentrations for the assay components are presented in Table 10. The tubes and the microsomal suspension were placed in the 37 ± 1°C water bath for five minutes prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume was 2 mL, and the tubes were incubated for 15 min. The incubations were stopped by the addition of methylene chloride (2 mL); the tubes were vortex-mixed for ca. 5 s and placed on ice. The tubes were then vortex-mixed an additional 20-25 s. The tubes were then centrifuged using a Beckman GS-6R centrifuge with GH-3.8 rotor for 10 min at a setting of 1000 rpm to aid in the separation of the organic and aqueous phases. The methylene chloride layer was removed and discarded; the aqueous layers were extracted again with methylene chloride (2 mL). This extraction procedure was performed one additional time, each time discarding the methylene chloride layer. The aqueous layers were transferred to vials and duplicate aliquots (0.5 mL) were transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Packard, 10 mL) was added to each counting vial and shaken to mix the solution. The radiochemical content of each aliquot was determined by using liquid scintillation spectrometry (LSS). Radiolabel found in the aqueous fractions represented tritiated water formed. The aromatization of one mole of [^{3}H]ASDN resulted in the production of one mole of estrone (non-radiolabeled) and one mole of tritium associated with water.

Table 10. Optimized Aromatase Assay Conditions

Assay factor (units)	Human Placental
Microsomal Protein (mg/mL)[a]	0.0125
NADPH (mM)[a]	0.3
[^{3}H]ASDN (nM)[a]	100
Incubation Time (min)	15

[a] Final concentrations

3.7 Data Analysis

3.7.1 Aromatase Activity and Percent of Control Calculations

Data were entered into an Excel spreadsheet for calculation of aromatase activity and percent of control. The master spreadsheet used was titled Aromatase_Master_Version1.4.xls.

For each repeat tube (full enzyme activity, background activity, positive, and negative controls and each RC concentration) the Excel spreadsheet included total observed (uncorrected) disintegrations per minute (DPMs) per tube and total aromatase activity per tube. The DPM and aromatase activity values were corrected for the background DPMs, as measured by the average of the background activity control tubes. The aromatase activity was calculated as the corrected DPM, normalized by the specific activity of the [^{3}H]ASDN, the mg of protein of the aromatase, and the incubation time. The average (corrected) DPMs and aromatase activity across the four background activity control repeat tubes were necessarily equal to 0 within each replicate.

For each tube, percent of control was determined by dividing the background corrected aromatase activity for that tube by the average background corrected aromatase activity for the four full enzyme activity control tubes and multiplying by 100. Nominally one might expect for an inhibitor the percent of control activity values to vary between approximately 0% near the high inhibition concentrations and approximately 100% near the low inhibition concentrations. However, due to experimental variation individual observed percent of control values sometimes extended below 0% or above 100%.

3.7.2 Statistical Analyses

3.7.2.1 Concentration-Response Fits for the Reference Chemicals. Concentration response trend curves were fitted to the percent of control activity values within each of the repeat tubes at each reference chemical concentration. Concentration was expressed on the log scale. In agreement with past convention, logarithms were common logarithms (i.e., base 10). Let X denote the logarithm of the concentration of reference chemical (e.g., if concentration = 10^{-5} then X = -5). Let

Y ≡ percent of control activity in the inhibitor tube

X ≡ logarithm (base 10) of the concentration

T ≡ top of plateau

B ≡ bottom of plateau

H ≡ slope of the concentration response curve (H will be negative)

$\mu \equiv \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

The following concentration response curve was fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$Y = B + (T-B)/[1 + 10^{(\mu - X)H}]$$

The variance was approximately proportional to Y.

The response curve was fitted by nonweighted least squares nonlinear regression analysis. Model fits were carried out using Prism software (Version 3.02).

3.7.2.2 Graphical and Analysis of Variance Comparisons Among Concentration Response Curve Fits. For each replicate the individual percent of control values were plotted versus logarithm of the RC concentration. The fitted concentration response curve was superimposed on the plot. Individual plots were prepared for each replicate.

Additional plots were prepared to compare the percent of control activity values across replicates. For each replicate the average percent of control values were plotted versus logarithm of RC concentration on the same plot. Plotting symbols distinguished among replicates. The fitted concentration response curves for each replicate were superimposed on the plots. On a separate plot the average percent of control values for each replicate was plotted versus logarithm of RC concentration. The average concentration response curve across replicates was superimposed on the same plot.

Graphs were prepared for the parameter estimates from the response curve model fits to visually assess the estimates and their variations.

3.7.2.3 Graphical and Analysis of Variance Comparisons of Full Enzyme Activity, Background Activity, and Positive and Negative Control Percent of Control Across Reference Chemicals and Replicates. The means of the full enzyme activity control values within each replicate and chemical were calculated as the reference value for 100% of control for the replicate and chemical. The percent of control for each repetition within each replicate and chemical was calculated as the ratio of the repetition value divided by the corresponding 100% of control reference value. Graphs of the percent of control values

indicating the repetition portion by replicate and chemical for background activity controls, full enzyme activity controls, negative and positive controls were prepared. Graphs displaying the differences of the means of the beginning repetitions and the means of the end repetitions within each replicate and chemical were prepared. A mixed effects model was fit to the percent of control values for each control. The fixed effects were assigned as the chemical type, portion, and portion by chemical interaction. The random effects were assigned as replicated within reference chemical and portion by replicate interaction within chemical.

3.7.2.4 Statistical Software. Concentration response curves were fitted to the data using the non-linear regression analysis features in the PRISM statistical analysis package, Version 3.02. Supplemental statistical analyses and displays such as summary tables, graphical displays, analysis of variance, and multiple comparisons were carried out using the SAS statistical analysis system, Version 8.

4.0 RESULTS

4.1 Stock Formulation Analysis

The formulation stability and formulation analysis results for all ten RCs and the positive and negative control chemicals from the Battelle CR are reported separately. Some of the formulation analysis data are summarized in Table 11.

Table 11. Formulation Analysis Data

RC Code	Chemical ID	Stock Solution ID	RTI Log #	Manufacturer's Purity (%)	Stock Solution Prep Date	Exp Date	Date of Last Use on WA 4-16, Task 7
RC-1	Aminoglutethimide	1	11007-107-03	>99	6/30/2005	8/27/2005	7/20/2005
RC-2	Ketoconazole	2	11007-107-05	>99	6/29/2005	8/27/2005	7/22/2005
RC-3	Prochloraz	3	11007-116-01	99.5	7/25/2005	9/26/2005	8/5/2005
RC-4	4-Nonylphenol	4	11007-132-01	>98.5	9/13/2005	10/3/2005	9/30/2005
RC-5	Dibenz[a,h]anthracene	5	11007-132-02	97	9/13/2005	10/3/2005	9/30/2005
RC-6	Fenarimol	6	11007-116-02	99	7/25/2005	8/24/2005	8/5/2005
RC-7	Econazole	7	11007-122-01	98	8/8/2005	10/2/2005	8/26/2005
RC-8	Chrysin	8	11007-122-03	98.2	8/9/2005	11/16/2005	8/26/2005
RC-9	Dicofol	9	11007-126-01	96.5	8/22/2005	9/20/2005	9/15/2005
RC-10	Atrazine	10	11007-126-02	98	8/23/2005	9/19/2005	9/15/2005
N/A	Lindane	3-LIN-1	11007-107-01	99.6	7/1/2005	12/15/2005	9/30/2005
N/A	4- OH ASDN	4-ASDN-1	11007-107-07	99	6/27/2005	12/16/2005	9/30/2005

4.2 Protein Analysis

Protein content of the human placental microsomes was measured each day of the aromatase assay as described in Section 3.5. Protein assay spreadsheets are presented in Appendix D. The final dilution of the stock microsomes was assayed for protein content and then the protein concentration of the stock microsomes was calculated from the data. The measured protein concentration for the Battelle and In Vitro human placental microsomes for each day of use is presented in Table 13.

The protein assay included standards ranging from 5 to 250 µg protein/mL. QC standards at concentrations of 12 and 110 µg/mL were also included in each assay set. Both a 6-point and a 5-point (which omitted the 250 µg/mL sample) concentration-absorbance response curve were calculated for each assay. Generally, the 5-point curve better fit the data as evidenced by lower deviation of the QC results from the expected values. Therefore, the protein values for the placental microsomes as determined using the 5-point standard curve were used in calculation of aromatase activity.

The QC results are presented in Table 12. The measured values for the high QC sample (110 µg/mL) showed an overall percent relative error of 3.5%, whereas, for the low QC sample (12 µg/mL) the overall percent relative error was -5.7%. There was a much higher variability in the values for the low QC (36.2% CV) than the high QC (4.5% CV). There were a few determinations of the low QC standard with a high percent relative error which contributed greatly to the high %CV. These variances in the low QC standard had little effect on the determination of protein concentration of the microsomal samples, which, in most cases had %CV from the means of less than 10%.

Table 12. Protein QC Results

Reference Chemical	Replicate	Date	Low QC					High QC				
			Measured Concentration[a]	Mean	SD	% RE	% CV	Measured Concentration[a]	Mean	SD	% RE	% CV
AG	1	7/14/2005	0.011	0.011	0.001	-6.3	8.5	0.118	0.111	0.006	0.7	5.7
AG, KCZ	2, 1	7/18/2005	0.010					0.105				
AG, KCZ	3, 2	7/20/2005	0.012					0.114				
KCZ	3	7/22/2005	0.012					0.106				
PCZ, FRM	1	8/2/2005	0.011	0.011	0.001	-5.6	5.1	0.116	0.114	0.002	3.9	1.3
	2	8/4/2005	0.012					0.114				
	3	8/5/2005	0.011					0.113				
NYP, DBA	1	9/21/2005	0.013	0.016	0.008	33.3	49.6	0.120	0.116	0.003	5.5	3.0
	2	9/23/2005	0.010					0.114				
	3	9/30/2005	0.025					0.114				
ECZ, CYN	1	8/22/2005	0.009	0.010	0.002	-19.4	21.5	0.112	0.116	0.009	5.5	7.5
	2	8/24/2005	0.012					0.110				
	3	8/26/2005	0.008					0.126				
DCF, ATZ	1	9/8/2005	0.008	0.008	0.003	-30.6	30.2	0.117	0.113	0.004	2.7	3.5
	2	9/13/2005	0.006					0.109				
	3	9/15/2005	0.011					0.113				
Overall				**0.011**	**0.004**	**-5.7**	**36.2**		**0.114**	**0.005**	**3.5**	**4.5**

[a]Concentration = mg/mL

The protein concentration measured for each day of assay for each RC is presented in Table 13. The measured protein concentrations showed low day to day variation (CV% < 10%) across the three replicates of a given RC. The overall average measured protein concentration for the Battelle microsomes was 27.0 ± 1.4 mg/mL (5.3 % CV), which is slightly higher than the 21 mg/mL concentration reported on the data sheets for these microsomes. Similarly, the overall measured average protein concentration for the In Vitro microsomes (10.9 ± 1.2 mg/mL, 10.8% CV) was slightly higher than the 8 mg/mL reported by the supplier of that preparation.

Table 13. Measured Protein Concentrations

Reference Chemical	Microsome Source	Replicate	Date	Protein Concentration[a]	Mean	Standard Deviation	% CV
AG	Battelle	1	7/14/2005	27.2	26.1	1.0	3.8
		2	7/18/2005	25.3			
		3	7/20/2005	25.9			
KCZ	Battelle	1	7/18/2005	25.3	26.4	1.3	5.0
		2	7/20/2005	25.9			
		3	7/22/2005	27.9			
PCZ, FRM	In Vitro	1	8/2/2005	9.9	10.0	0.5	4.8
		2	8/4/2005	10.6			
		3	8/5/2005	9.7			
NYP	Battelle	1	9/21/2005	27.5	28.7	1.2	4.0
		2	9/23/2005	28.7			
		3	9/30/2005	29.8			
DBA	In Vitro	1	9/21/2005	10.8	12.1	1.2	9.9
		2	9/23/2005	12.2			
		3	9/30/2005	13.2			
ECZ, CYN	Battelle	1	8/22/2005	26.1	26.1	0.3	1.3
		2	8/24/2005	25.7			
		3	8/26/2005	26.4			
DCF, ATZ	In Vitro	1	9/8/2005	10.1	10.5	0.7	6.7
		2	9/13/2005	11.3			
		3	9/15/2005	10.2			
Overall	**Battelle**				**27.0**	**1.4**	**5.3**
	In Vitro				**10.9**	**1.2**	**10.8**

[a]Concentration units =– mg/mL

4.3 Aromatase Activity

Aromatase activity was measured in the presence of eight concentrations of each of ten reference chemicals. Each assay set also included four types of controls (see Section 3.6). Three replicates were run of each RC. Information regarding assay dates, protein concentration,

substrate specific activity and tested RC concentration ranges is presented in Table 14. Radioactivity remaining in each assay tube after extraction of the unreacted substrate represented the reaction product, estrone. The aromatase activity was calculated by normalizing the radioactivity present in each tube by the amount of microsomal protein and the reaction time and has the units nmol/mg protein/min. Aromatase activity calculation spreadsheets are presented in Appendix D.

Table 14. Aromatase Assay Summary

RC Code	Test Chemical ID	Replicate	Assay Date	Protein Stock Concentration (measured) mg/mL	Substrate Soln SA µCi/µg ASDN	Reference Chemical Concentration Range (M)
RC-1	Aminoglutethimide	1	7/14/2005	27.2	1.323	1.00E-03 to 1.00E-10
RC-1	Aminoglutethimide	2	7/18/2005	25.3	1.524	1.00E-03 to 1.00E-07
RC-1	Aminoglutethimide	3	7/20/2005	25.9	1.525	1.00E-03 to 1.00E-07
RC-2	Ketoconazole	1	7/18/2005	25.3	1.524	1.00E-04 to 1.00E-10
RC-2	Ketoconazole	2	7/20/2005	25.9	1.525	1.00E-04 to 1.00E-10
RC-2	Ketoconazole	3	7/22/2005	27.9	1.384	1.00E-04 to 1.00E-10
RC-3	Prochoraz	1	8/2/2005	9.9	1.438	1.00E-03 to 1.00E-10
RC-3	Prochoraz	2	8/4/2005	10.6	1.465	1.00E-05 to 1.00E-10
RC-3	Prochoraz	3	8/5/2005	9.7	1.456	1.00E-05 to 1.00E-10
RC-4	4-Nonylphenol	1	9/21/2005	27.5	1.816	1.00E-03 to 1.00E-10
RC-4	4-Nonylphenol	2	9/23/2005	28.7	1.392	1.00E-03 to 1.00E-07
RC-4	4-Nonylphenol	3	9/30/2005	29.8	1.627	1.00E-03 to 1.00E-07
RC-5	Dibenz[a,h]anthracene	1	9/21/2005	10.8	1.816	1.00E-04 to 1.00E-10
RC-5	Dibenz[a,h]anthracene	2	9/23/2005	12.2	1.392	1.00E-04 to 1.00E-10
RC-5	Dibenz[a,h]anthracene	3	9/30/2005	13.2	1.627	1.00E-04 to 1.00E-10
RC-6	Fenarimol	1	8/2/2005	9.9	1.438	1.00E-03 to 1.00E-10
RC-6	Fenarimol	2	8/4/2005	10.6	1.465	1.00E-03 to 1.00E-08
RC-6	Fenarimol	3	8/5/2005	9.7	1.456	1.00E-03 to 1.00E-08
RC-7	Econazole	1	8/22/2005	26.1	1.318	1.00E-03 to 1.00E-10
RC-7	Econazole	2	8/24/2005	25.7	1.396	1.00E-06 to 1.00E-10
RC-7	Econazole	3	8/26/2005	26.4	1.426	1.00E-06 to 1.00E-10
RC-8	Chrysin	1	8/22/2005	26.1	1.318	1.00E-04 to 1.00E-10
RC-8	Chrysin	2	8/24/2005	25.7	1.396	1.00E-04 to 1.00E-08
RC-8	Chrysin	3	8/26/2005	26.4	1.426	1.00E-04 to 1.00E-08
RC-9	Dicofol	1	9/8/2005	10.1	1.342	1.00E-03 to 1.00E-10
RC-9	Dicofol	2	9/13/2005	11.3	1.386	1.00E-03 to 1.00E-08
RC-9	Dicofol	3	9/15/2005	10.2	1.327	1.00E-03 to 1.00E-08
RC-10	Atrazine	1	9/8/2005	10.1	1.342	1.00E-03 to 1.00E-10
RC-10	Atrazine	2	9/13/2005	11.3	1.386	1.00E-03 to 1.00E-07

RC-10	Atrazine	3	9/15/2005	10.2	1.327	1.00E-03 to 1.00E-07

4.3.1 Control Results

Each replicate set for each chemical included four types of controls, each run in quadruplicate. The control types were full aromatase activity, background activity, positive and negative controls. The positive control tubes contained the known aromatase inhibitor, 4-OH ASDN, at a concentration of 5×10^{-8} M and the negative control tubes contained the known aromatase non-inhibitor, lindane, at a concentration of 1×10^{-6} M. The control tubes were divided so that two of each type were run at the beginning of the set and two were run at the end of the set. In general, there was about a 25 minute interval between the beginning and the end of each replicate set. The aromatase activity in the full aromatase activity controls represented 100% activity and since all aromatase activities were corrected for background, the background activity controls necessarily were set to 0%. The mean activities for each type of control (except background) for the beginning and end groups and the overall means, SD, SEM and CVs across replicates and across chemicals are presented in Tables 15 and 16, for the Battelle and In Vitro microsomes, respectively. Appendix E contains graphical representations of the beginning and end percent of control values for each type of control for each reference chemical and replicate. The percent of control values for background activity controls were fairly consistent and close to 0% (generally ranging between -0.3% and 0.3%) of control across the replicates and chemicals. The beginning and end percent of control values for full enzyme activity and negative control were fairly consistent and close to 100% of control (ranging between 90 and 110% and 85 and 110%, respectively) across the replicates and chemicals. The beginning and end percent of control values of the positive control were fairly consistent and close to 50% of control (ranging between 40 and 60%) across all replicates and chemicals.

Table 15. Control Activities Battelle Microsomes[a]

Reference Chemical	Replicate	Mean Beginning Control	Mean End Control	Overall Mean	SD	SEM	%CV	Overall (by chemical) Mean	±SD	SEM	%CV
Full Aromatase Activity											
AG	1	0.0520	0.0494	0.0507	0.0016	0.0008	3.09	0.0499	0.0011	0.0006	2.15
	2	0.0515	0.0458	0.0486	0.0033	0.0017	6.82				
	3	0.0540	0.0464	0.0502	0.0045	0.0022	8.90				
KCZ	1	0.0515	0.0458	0.0486	0.0033	0.0017	6.82	0.0492	0.0009	0.0005	1.76
	2	0.0540	0.0464	0.0502	0.0045	0.0022	8.90				
	3	0.0500	0.0475	0.0487	0.0018	0.0009	3.69				
NYP	1	0.0376	0.0355	0.0366	0.0013	0.0007	3.59	0.0440	0.0069	0.0040	15.67
	2	0.0478	0.0426	0.0452	0.0030	0.0015	6.55				
	3	0.0505	0.0499	0.0502	0.0004	0.0002	0.86				
ECZ	1	0.0582	0.0520	0.0551	0.0037	0.0018	6.69	0.0508	0.0037	0.0021	7.22
	2	0.0527	0.0450	0.0488	0.0047	0.0023	9.52				
	3	0.0514	0.0459	0.0486	0.0032	0.0016	6.60				
CYN	1	0.0582	0.0520	0.0551	0.0037	0.0018	6.69	0.0508	0.0037	0.0021	7.22
	2	0.0527	0.0450	0.0488	0.0047	0.0023	9.52				
	3	0.0514	0.0459	0.0486	0.0032	0.0016	6.60				
Overall								0.0489	0.0042	0.0011	8.64
Positive Control											
AG	1	0.0258	0.0226	0.0242	0.0020	0.0010	8.07	0.0244	0.0006	0.0004	2.64
	2	0.0269	0.0234	0.0252	0.0022	0.0011	8.79				
	3	0.0253	0.0226	0.0239	0.0019	0.0009	7.88				
KCZ	1	0.0269	0.0234	0.0252	0.0022	0.0011	8.79	0.0242	0.0009	0.0005	3.77
	2	0.0253	0.0226	0.0239	0.0019	0.0009	7.88				
	3	0.0244	0.0223	0.0234	0.0014	0.0007	6.01				
NYP	1	0.0184	0.0170	0.0177	0.0009	0.0004	5.04	0.0207	0.0026	0.0015	12.79
	2	0.0229	0.0206	0.0218	0.0014	0.0007	6.41				
	3	0.0234	0.0220	0.0227	0.0009	0.0005	4.04				
ECZ	1	0.0277	0.0251	0.0264	0.0015	0.0008	5.82	0.0250	0.0015	0.0009	6.18
	2	0.0278	0.0225	0.0252	0.0032	0.0016	12.88				
	3	0.0245	0.0222	0.0233	0.0015	0.0007	6.36				

(continued)

Table 15. Control Activities Battelle Microsomes[a] (continued)

Reference Chemical	Replicate	Mean Beginning Control	Mean End Control	Overall Mean	SD	SEM	%CV	Overall (by chemical)			
								Mean	±SD	SEM	%CV
CYN	1	0.0277	0.0251	0.0264	0.0015	0.0008	5.82	0.0250	0.0015	0.0009	6.18
	2	0.0278	0.0225	0.0252	0.0032	0.0016	12.88				
	3	0.0245	0.0222	0.0233	0.0015	0.0007	6.36				
Overall								0.0239	0.0021	0.0006	8.99
Negative Control											
AG	1	0.0514	0.0464	0.0489	0.0029	0.0015	5.95	0.0482	0.0006	0.0004	1.33
	2	0.0509	0.0443	0.0476	0.0038	0.0019	8.05				
	3	0.0519	0.0443	0.0481	0.0044	0.0022	9.16				
KCZ	1	0.0509	0.0443	0.0476	0.0038	0.0019	8.05	0.0477	0.0003	0.0002	0.63
	2	0.0519	0.0443	0.0481	0.0044	0.0022	9.16				
	3	0.0510[b]	0.0458	0.0475	0.0032	0.0018	6.74				
NYP	1	0.0366	0.0353	0.0360	0.0009	0.0004	2.39	0.0437	0.0070	0.0040	15.97
	2	0.0482	0.0430	0.0456	0.0031	0.0016	6.91				
	3	0.0506	0.0485	0.0496	0.0014	0.0007	2.74				
ECZ	1	0.0562	0.0495	0.0529	0.0040	0.0020	7.48	0.0485	0.0038	0.0022	7.93
	2	0.0507	0.0425	0.0466	0.0052	0.0026	11.19				
	3	0.0487	0.0431	0.0459	0.0033	0.0016	7.15				
CYN	1	0.0562	0.0495	0.0529	0.0040	0.0020	7.48	0.0485	0.0038	0.0022	7.93
	2	0.0507	0.0425	0.0466	0.0052	0.0026	11.19				
	3	0.0487	0.0431	0.0459	0.0033	0.0016	7.15				
Overall								0.0473	0.0038	0.0010	8.13

[a] Units are nmol/mg protein/min

[b] Data from Tube 1 of the negative control was excluded from the calculation of mean, SD, SEM and % CV since it was an outlier.

Table 16. Control Activities – In Vitro Microsomes[a]

Reference Chemical	Replicate	Mean Beginning Control	Mean End Control	Overall Mean	SD	SEM	%CV	Overall (by chemical) Mean	±SD	SEM	%CV
Full Aromatase Activity											
Prochloraz	1	0.0399	0.0336	0.0368	0.0037	0.0018	9.99	0.0394	0.0026	0.0015	6.57
	2	0.0419	0.0370	0.0394	0.0029	0.0014	7.34				
	3	0.0439	0.0400	0.0419	0.0027	0.0014	6.55				
Dibenz[a,h]anthracene	1	0.0274	0.0277	0.0276	0.0005	0.0002	1.78	0.0295	0.0018	0.0010	6.07
	2	0.0317	0.0304	0.0311	0.0009	0.0004	2.85				
	3	0.0287	0.0311	0.0299	0.0019	0.0009	6.31				
Fenarimol	1	0.0399	0.0336	0.0368	0.0037	0.0018	9.99	0.0394	0.0026	0.0015	6.57
	2	0.0419	0.0370	0.0394	0.0029	0.0014	7.34				
	3	0.0439	0.0400	0.0419	0.0027	0.0014	6.55				
Dicofol	1	0.0399	0.0351	0.0375	0.0028	0.0014	7.47	0.0398	0.0025	0.0015	6.40
	2	0.0455	0.0396	0.0426	0.0035	0.0017	8.12				
	3	0.0404	0.0384	0.0394	0.0012	0.0006	3.04				
Atrazine	1	0.0399	0.0351	0.0375	0.0028	0.0014	7.47	0.0398	0.0025	0.0015	6.40
	2	0.0455	0.0396	0.0426	0.0035	0.0017	8.12				
	3	0.0404	0.0384	0.0394	0.0012	0.0006	3.04				
Overall								0.0376	0.0047	0.0012	12.41
Positive Control											
Prochloraz	1	0.0190	0.0155	0.0172	0.0021	0.0011	12.22	0.0190	0.0018	0.0010	9.42
	2	0.0187	0.0193	0.0190	0.0011	0.0006	6.02				
	3	0.0222	0.0194	0.0208	0.0016	0.0008	7.80				
Dibenz[a,h]anthracene	1	0.0127	0.0130	0.0128	0.0003	0.0002	2.63	0.0138	0.0011	0.0007	8.22
	2	0.0154	0.0147	0.0151	0.0004	0.0002	2.99				
	3	0.0137	0.0135	0.0136	0.0004	0.0002	2.81				
Fenarimol	1	0.0190	0.0155	0.0172	0.0021	0.0011	12.22	0.0190	0.0018	0.0010	9.42
	2	0.0187	0.0193	0.0190	0.0011	0.0006	6.02				
	3	0.0222	0.0194	0.0208	0.0016	0.0008	7.80				
Dicofol	1	0.0204	0.0181	0.0193	0.0016	0.0008	8.36	0.0187	0.0010	0.0006	5.18
	2	0.0184	0.0169	0.0176	0.0010	0.0005	5.55				
	3	0.0202	0.0185	0.0193	0.0011	0.0006	5.80				

(continued)

Table 16. Control Activities – In Vitro Microsomes[a] (continued)

Reference Chemical	Replicate	Mean Beginning Control	Mean End Control	Overall Mean	SD	SEM	%CV	Overall (by chemical)			
								Mean	±SD	SEM	%CV
Atrazine	1	0.0204	0.0181	0.0193	0.0016	0.0008	8.36	0.0187	0.0010	0.0006	5.18
	2	0.0184	0.0169	0.0176	0.0010	0.0005	5.55				
	3	0.0202	0.0185	0.0193	0.0011	0.0006	5.80				
Overall								0.0179	0.0024	0.0006	13.42
Negative Control											
Prochloraz	1	0.0398	0.0344[b]	0.0380	0.0031	0.0018	8.21	0.0391	0.0018	0.0010	4.61
	2	0.0403	0.0360	0.0381	0.0030	0.0015	7.76				
	3	0.0443	0.0381	0.0412	0.0036	0.0018	8.78				
Dibenz[a,h]anthracene	1	0.0287	0.0278	0.0282	0.0006	0.0003	2.23	0.0299	0.0015	0.0009	4.93
	2	0.0322	0.0297	0.0309	0.0016	0.0008	5.14				
	3	0.0309	0.0303	0.0306	0.0006	0.0003	2.07				
Fenarimol	1	0.0398	0.0344[b]	0.0380	0.0031	0.0018	8.21	0.0391	0.0018	0.0010	4.61
	2	0.0403	0.0360	0.0381	0.0030	0.0015	7.76				
	3	0.0443	0.0381	0.0412	0.0036	0.0018	8.78				
Dicofol	1	0.0396	0.0334	0.0365	0.0039	0.0019	10.57	0.0384	0.0018	0.0010	4.71
	2	0.0419	0.0383	0.0401	0.0022	0.0011	5.45				
	3	0.0399	0.0373	0.0386	0.0017	0.0008	4.35				
Atrazine	1	0.0396	0.0334	0.0365	0.0039	0.0019	10.57	0.0384	0.0018	0.0010	4.71
	2	0.0419	0.0383	0.0401	0.0022	0.0011	5.45				
	3	0.0399	0.0373	0.0386	0.0017	0.0008	4.35				
Overall								0.0370	0.0040	0.0010	10.69

[a] Units are nmol/mg protein/min

[b] Data from Tube 4 of the negative control was excluded from the calculation of mean, SD, SEM and % CV since it was an outlier.

There was a consistent and statistically significant decrease in activity of about 5-15% for all controls (except background) between the beginning and end control tubes. This difference in beginning and end percent of control activities is presented graphically in the statisticians report in Appendix E. The coefficients of variance for the full aromatase activity, positive and negative controls across all chemicals and replicates where the microsome source was Battelle were approximately 8-9%; the corresponding coefficients of variance for the controls where In Vitro was the microsome source were approximately 10-13%.

4.3.2 Percent of Control Values

The aromatase activity found in each assay tube was normalized to percent of control by dividing by the average full enzyme activity for the replicate. The percent of control values for each RC replicate and tube along with the mean, SD, SEM and %CV of the percent of control across tubes within a replicate are presented in Table 17.

Table 17. Percent of Control Values

RC	Rep.	Log[RC]	Tube 1	Tube 2	Tube 3	Mean	SD	SEM	% CV
AG	1	-3.00	5.05	4.79	4.98	4.94	0.14	0.08	2.79
		-4.00	38.11	38.33	36.74	37.73	0.86	0.50	2.28
		-5.00	84.42	81.83	78.07	81.44	3.19	1.84	3.92
		-6.00	98.62	95.93	93.68	96.07	2.47	1.43	2.58
		-7.00	101.25	96.15	95.58	97.66	3.12	1.80	3.20
		-8.00	100.07	95.10	93.29	96.15	3.51	2.03	3.65
		-9.00	97.84	95.29	93.02	95.39	2.41	1.39	2.53
		-10.00	98.60	90.82	94.03	94.48	3.91	2.26	4.14
	2	-3.00	5.13	5.54	5.92	5.53	0.40	0.23	7.20
		-3.30	12.13	11.83	11.67	11.88	0.23	0.13	1.95
		-3.60	21.98	22.52	21.93	22.14	0.33	0.19	1.50
		-4.00	41.18	40.55	39.98	40.57	0.60	0.35	1.48
		-4.30	59.58	53.43	56.57	56.53	3.08	1.78	5.44
		-5.00	90.64	89.19	86.62	88.82	2.03	1.17	2.29
		-6.00	102.32	100.58	99.26	100.72	1.53	0.89	1.52
		-7.00	101.69	101.32	98.58	100.53	1.70	0.98	1.69
	3	-3.00	3.83	3.52	3.54	3.63	0.17	0.10	4.79
		-3.30	10.29	10.61	9.92	10.27	0.34	0.20	3.34
		-3.60	20.28	18.69	20.72	19.90	1.06	0.61	5.34
		-4.00	40.25	39.58	39.39	39.74	0.45	0.26	1.14
		-4.30	56.80	54.24	53.20	54.75	1.85	1.07	3.38
		-5.00	88.56	84.88	84.89	86.11	2.12	1.23	2.46
		-6.00	97.55	101.06	94.78	97.80	3.15	1.82	3.22
		-7.00	101.26	98.52	96.53	98.77	2.37	1.37	2.40

(continued)

Table 17. Percent of Control Values (continued)

RC	Rep.	Log[RC]	Tube 1	Tube 2	Tube 3	Mean	SD	SEM	% CV
KCZ	1	-4.00	0.74	0.36	0.54	0.55	0.19	0.11	35.13
		-5.00	4.66	4.26	4.63	4.52	0.22	0.13	4.98
		-6.00	29.91	28.84	28.73	29.16	0.65	0.38	2.23
		-6.30	46.04	44.83	44.09	44.98	0.98	0.57	2.19
		-7.00	80.62	77.63	75.42	77.89	2.61	1.51	3.35
		-8.00	100.35	97.14	93.39	96.96	3.48	2.01	3.59
		-9.00	100.19	97.79	94.99	97.66	2.60	1.50	2.67
		-10.00	98.24	97.32	94.88	96.81	1.74	1.00	1.80
	2	-4.00	-1.09	-1.12	-1.13	-1.11	0.02	0.01	-2.02
		-5.00	1.95	2.29	2.46	2.23	0.26	0.15	11.57
		-6.00	27.92	28.42	28.74	28.36	0.41	0.24	1.46
		-6.30	41.90	40.45	39.40	40.59	1.25	0.72	3.09
		-7.00	73.49	71.08	68.41	70.99	2.54	1.47	3.58
		-8.00	89.34	87.96	87.21	88.17	1.08	0.62	1.22
		-9.00	94.54	91.14	90.31	92.00	2.24	1.29	2.44
		-10.00	93.70	91.35	89.94	91.66	1.90	1.10	2.07
	3	-4.00	0.36	0.07	0.19	0.21	0.15	0.09	72.01
		-5.00	4.38	3.77	4.03	4.06	0.30	0.18	7.50
		-6.00	28.93	27.11	27.27	27.77	1.01	0.58	3.62
		-6.30	45.19	43.28	42.41	43.63	1.42	0.82	3.25
		-7.00	78.53	74.36	73.80	75.56	2.58	1.49	3.42
		-8.00	99.72	95.86	92.74	96.11	3.50	2.02	3.64
		-9.00	100.54	100.61	96.40	99.18	2.41	1.39	2.43
		-10.00	101.12	99.13	96.67	98.97	2.23	1.29	2.25
PCZ	1	-3.00	-0.06	0.05	0.04	0.01	0.06	0.04	538.13
		-4.00	-0.20	-0.13	0.13	-0.07	0.17	0.10	-267.81
		-5.00	0.05	0.43	0.26	0.25	0.19	0.11	75.46
		-6.00	1.85	2.25	2.02	2.04	0.20	0.12	9.80
		-7.00	16.36	15.80	16.25	16.14	0.29	0.17	1.82
		-8.00	61.57	64.09	60.30	61.99	1.93	1.11	3.11
		-9.00	94.99	89.68	88.71	91.13	3.38	1.95	3.71
		-10.00	100.22	97.97	92.51	96.90	3.96	2.29	4.09
	2	-5.00	0.11	0.15	0.21	0.15	0.05	0.03	32.02
		-6.00	2.04	2.37	2.28	2.23	0.17	0.10	7.65
		-7.00	18.43	19.74	19.09	19.09	0.65	0.38	3.42
		-7.60	49.73	49.47	47.43	48.87	1.26	0.73	2.58
		-8.00	69.81	67.69	66.28	67.93	1.78	1.03	2.62
		-8.30	84.09	84.19	79.09	82.46	2.92	1.69	3.54
		-9.00	98.71	95.97	92.25	95.64	3.24	1.87	3.39
		-10.00	90.35	102.10	99.84	97.43	6.23	3.60	6.40

(continued)

Table 17. Percent of Control Values (continued)

RC	Rep.	Log[RC]	Tube 1	Tube 2	Tube 3	Mean	SD	SEM	% CV
PCZ	3	-5.00	-0.01	-0.15	0.16	0.00	0.15	0.09	-5411.10
		-6.00	2.95	2.97	2.94	2.96	0.02	0.01	0.59
		-7.00	18.65	18.33	17.49	18.15	0.60	0.35	3.30
		-7.60	47.26	47.17	44.77	46.40	1.41	0.81	3.04
		-8.00	68.21	69.86	71.54	69.87	1.66	0.96	2.38
		-8.30	85.16	86.32	85.81	85.77	0.58	0.34	0.68
		-9.00	102.84	100.71	99.85	101.13	1.54	0.89	1.52
		-10.00	108.98	108.84	106.14	107.99	1.60	0.92	1.48
NYP	1	-3.00	0.02	0.18	0.10	0.10	0.08	0.05	83.72
		-4.00	7.42	5.66	6.30	6.46	0.89	0.52	13.82
		-5.00	89.07	88.35	84.86	87.43	2.25	1.30	2.58
		-6.00	98.21	98.60	97.75	98.19	0.43	0.25	0.44
		-7.00	102.00	98.60	99.06	99.89	1.85	1.07	1.85
		-8.00	103.55	97.86	98.90	100.10	3.03	1.75	3.03
		-9.00	101.29	100.30	96.84	99.48	2.34	1.35	2.35
		-10.00	102.26	99.20	97.30	99.58	2.50	1.45	2.51
	2	-3.00	0.15	0.14	0.01	0.10	0.08	0.04	75.10
		-4.00	7.03	7.23	6.96	7.07	0.14	0.08	1.99
		-4.30	22.65	22.65	21.95	22.42	0.41	0.24	1.82
		-4.60	48.84	48.47	45.33	47.55	1.92	1.11	4.05
		-5.00	88.53	87.95	84.24	86.91	2.33	1.34	2.68
		-5.30	97.19	94.59	96.20	96.00	1.31	0.76	1.37
		-6.00	104.38	97.14	96.48	99.33	4.38	2.53	4.41
		-7.00	100.75	99.97	96.94	99.22	2.01	1.16	2.03
	3	-3.00	-0.03	-0.07	0.11	0.01	0.10	0.05	1655.54
		-4.00	7.11	6.65	6.65	6.80	0.26	0.15	3.86
		-4.30	23.89	23.63	22.47	23.33	0.76	0.44	3.24
		-4.60	45.10	47.14	44.94	45.72	1.23	0.71	2.68
		-5.00	84.59	86.06	82.84	84.50	1.62	0.93	1.91
		-5.30	94.26	95.51	92.82	94.20	1.35	0.78	1.43
		-6.00	95.38	96.94	98.78	97.03	1.70	0.98	1.75
		-7.00	93.36	96.78	97.24	95.79	2.12	1.22	2.21
DBA	1	-4.00	104.86	103.34	97.23	101.81	4.04	2.33	3.97
		-5.00	105.37	104.05	101.68	103.70	1.87	1.08	1.80
		-6.00	101.28	104.34	99.46	101.69	2.46	1.42	2.42
		-6.30	103.02	99.22	100.03	100.76	2.00	1.15	1.98
		-7.00	106.77	104.73	101.37	104.29	2.73	1.57	2.61
		-8.00	104.68	107.45	100.37	104.17	3.57	2.06	3.43
		-9.00	101.32	105.29	103.13	103.25	1.99	1.15	1.92
		-10.00	106.52	103.10	102.60	104.07	2.13	1.23	2.05

(continued)

Table 17. Percent of Control Values (continued)

RC	Rep.	Log[RC]	Tube 1	Tube 2	Tube 3	Mean	SD	SEM	% CV
DBA	2	-4.00	89.24	89.23	84.16	87.55	2.93	1.69	3.35
		-5.00	99.85	100.87	98.91	99.88	0.98	0.57	0.98
		-6.00	102.96	102.73	100.30	101.99	1.47	0.85	1.45
		-6.30	99.75	100.89	98.45	99.70	1.22	0.71	1.23
		-7.00	101.53	101.91	98.92	100.79	1.63	0.94	1.62
		-8.00	102.02	102.23	98.46	100.90	2.12	1.22	2.10
		-9.00	104.23	98.98	99.60	100.94	2.87	1.66	2.84
		-10.00	99.99	102.68	99.96	100.88	1.56	0.90	1.55
	3	-4.00	100.14	100.99	98.58	99.90	1.22	0.70	1.22
		-5.00	102.63	106.23	99.16	102.67	3.54	2.04	3.45
		-6.00	104.03	104.85	103.84	104.24	0.54	0.31	0.52
		-6.30	104.14	106.24	103.51	104.63	1.43	0.83	1.37
		-7.00	102.82	104.58	105.37	104.25	1.31	0.75	1.25
		-8.00	104.84	105.71	106.35	105.63	0.76	0.44	0.72
		-9.00	103.29	105.75	103.10	104.05	1.48	0.86	1.42
		-10.00	82.21	105.05	102.98	96.75	12.64	7.29	13.06
FRM	1	-3.00	0.99	1.01	1.10	1.03	0.06	0.03	5.43
		-4.00	5.71	6.01	5.22	5.65	0.40	0.23	7.11
		-5.00	33.93	32.48	32.66	33.02	0.79	0.46	2.40
		-6.00	75.20	70.14	69.56	71.63	3.10	1.79	4.33
		-7.00	88.42	80.71	77.31	82.14	5.69	3.29	6.93
		-8.00	86.55	81.16	82.02	83.24	2.90	1.67	3.48
		-9.00	84.54	84.11	81.98	83.54	1.37	0.79	1.64
		-10.00	83.68	81.89	78.87	81.48	2.43	1.40	2.98
	2	-3.00	1.27	1.46	2.64	1.79	0.74	0.43	41.57
		-4.00	6.36	6.31	5.88	6.19	0.26	0.15	4.28
		-4.60	19.62	19.48	18.73	19.28	0.48	0.28	2.49
		-5.00	35.85	36.66	34.72	35.74	0.97	0.56	2.72
		-5.30	51.10	51.14	45.67	49.30	3.15	1.82	6.38
		-6.00	82.97	81.24	77.72	80.64	2.68	1.55	3.32
		-7.00	96.98	90.81	91.74	93.18	3.33	1.92	3.57
		-8.00	98.17	95.26	95.29	96.24	1.67	0.97	1.74
	3	-3.00	1.00	0.86	0.74	0.86	0.13	0.08	15.12
		-4.00	6.51	6.34	6.45	6.43	0.09	0.05	1.35
		-4.60	21.06	22.12	20.54	21.24	0.81	0.47	3.79
		-5.00	39.09	37.11	37.95	38.05	0.99	0.57	2.61
		-5.30	56.20	56.02	55.66	55.96	0.27	0.16	0.49
		-6.00	79.24	84.96	83.31	82.50	2.94	1.70	3.57
		-7.00	101.99	98.12	97.14	99.09	2.57	1.48	2.59
		-8.00	101.44	99.49	97.27	99.40	2.09	1.20	2.10

(continued)

Table 17. Percent of Control Values (continued)

RC	Rep.	Log[RC]	Tube 1	Tube 2	Tube 3	Mean	SD	SEM	% CV
ECZ	1	-3.00	0.06	0.12	0.09	0.09	0.03	0.02	34.54
		-4.00	0.31	-0.03	-0.09	0.06	0.22	0.13	345.85
		-5.00	-0.04	0.09	-0.02	0.01	0.07	0.04	698.10
		-6.00	0.16	0.13	0.23	0.18	0.05	0.03	30.81
		-7.00	1.20	1.24	1.27	1.24	0.04	0.02	2.89
		-8.00	11.29	11.09	10.99	11.12	0.15	0.09	1.37
		-9.00	58.97	8.79[a]	57.03	58.00	1.37	0.97	2.36
		-10.00	96.91	91.47	91.52	93.30	3.13	1.81	3.35
	2	-6.00	0.10	0.10	0.12	0.11	0.02	0.01	15.75
		-7.00	1.43	1.43	1.42	1.42	0.01	0.00	0.39
		-8.00	12.87	13.69	12.86	13.14	0.47	0.27	3.60
		-8.30	23.41	23.56	23.09	23.35	0.24	0.14	1.05
		-9.00	63.58	58.80	59.80	60.72	2.52	1.45	4.15
		-9.30	78.20	77.93	74.87	77.00	1.85	1.07	2.41
		-9.60	89.51	87.30	87.36	88.06	1.26	0.73	1.43
		-10.00	96.34	93.41	93.41	94.39	1.69	0.97	1.79
	3	-6.00	-0.17	0.03	0.18	0.01	0.18	0.10	1466.50
		-7.00	1.26	1.29	1.51	1.35	0.13	0.08	9.89
		-8.00	12.24	12.54	12.19	12.32	0.19	0.11	1.52
		-8.30	21.76	20.87	21.25	21.29	0.45	0.26	2.10
		-9.00	60.31	60.99	58.93	60.08	1.05	0.61	1.75
		-9.30	76.17	74.15	74.99	75.10	1.01	0.59	1.35
		-9.60	86.84	84.87	82.69	84.80	2.08	1.20	2.45
		-10.00	94.02	91.28	91.12	92.14	1.63	0.94	1.77
CYN	1	-4.00	22.97	23.03	23.62	23.21	0.36	0.21	1.56
		-5.00	22.79	22.59	22.91	22.76	0.16	0.09	0.71
		-6.00	69.94	70.98	69.12	70.01	0.94	0.54	1.34
		-6.30	78.77	81.03	80.25	80.02	1.15	0.66	1.43
		-7.00	91.80	92.07	92.97	92.28	0.62	0.36	0.67
		-8.00	92.97	94.87	96.37	94.74	1.70	0.98	1.80
		-9.00	94.71	95.77	95.74	95.41	0.61	0.35	0.63
		-10.00	91.11	93.45	95.34	93.30	2.12	1.22	2.27
	2	-4.00	22.12	23.74	21.50	22.45	1.16	0.67	5.15
		-5.00	23.55	22.47	22.50	22.84	0.61	0.35	2.68
		-5.30	36.84	34.60	34.24	35.23	1.41	0.81	4.01
		-5.60	51.46	50.40	48.14	50.00	1.69	0.98	3.38
		-6.00	72.39	70.48	66.83	69.90	2.82	1.63	4.04
		-6.30	80.49	79.81	79.58	79.96	0.47	0.27	0.59
		-7.00	95.22	88.83	83.04	89.03	6.09	3.52	6.84
		-8.00	90.73	90.85	89.71	90.43	0.63	0.36	0.69

(continued)

Table 17. Percent of Control Values (continued)

RC	Rep.	Log[RC]	Tube 1	Tube 2	Tube 3	Mean	SD	SEM	% CV
CYN	3	-4.00	23.20	21.73	21.81	22.25	0.83	0.48	3.73
		-5.00	22.72	23.15	22.43	22.77	0.36	0.21	1.58
		-5.30	37.00	35.65	34.39	35.68	1.30	0.75	3.65
		-5.60	50.84	52.36	49.71	50.97	1.33	0.77	2.60
		-6.00	71.22	72.04	70.11	71.12	0.97	0.56	1.37
		-6.30	81.64	83.32	81.42	82.13	1.03	0.60	1.26
		-7.00	91.70	95.23	94.53	93.82	1.87	1.08	1.99
		-8.00	103.78	94.19	93.75	97.24	5.67	3.27	5.83
DCF	1	-3.00	2.95	4.28	4.71	3.98	0.92	0.53	23.12
		-4.00	35.46	45.01	38.85	39.77	4.84	2.80	12.18
		-5.00	70.70	67.43	66.80	68.31	2.09	1.21	3.06
		-6.00	91.22	91.30	88.63	90.38	1.52	0.88	1.68
		-7.00	99.69	99.48	94.86	98.01	2.73	1.57	2.78
		-8.00	105.23	99.83	98.23	101.10	3.67	2.12	3.63
		-9.00	102.45	102.22	98.01	100.89	2.50	1.44	2.48
		-10.00	103.29	100.68	96.40	100.13	3.48	2.01	3.48
	2	-3.00	1.88	1.99	2.04	1.97	0.08	0.05	4.17
		-3.30	3.91	4.83	5.64	4.79	0.86	0.50	18.03
		-3.60	7.83	11.67	11.30	10.27	2.12	1.22	20.64
		-4.00	19.11	25.89	20.88	21.96	3.52	2.03	16.02
		-5.00	74.20	68.36	60.56	67.71	6.84	3.95	10.11
		-6.00	86.77	87.46	87.41	87.21	0.39	0.22	0.44
		-7.00	93.45	98.26	90.19	93.97	4.06	2.35	4.32
		-8.00	102.94	101.07	86.76	96.93	8.85	5.11	9.13
	3	-3.00	2.46	2.56	2.18	2.40	0.20	0.11	8.16
		-3.30	6.51	5.63	7.02	6.39	0.70	0.41	11.00
		-3.60	14.65	11.07	15.81	13.84	2.47	1.43	17.87
		-4.00	25.62	25.12	21.99	24.24	1.96	1.13	8.10
		-5.00	68.36	64.47	60.20	64.34	4.08	2.35	6.34
		-6.00	92.76	93.18	92.33	92.76	0.42	0.24	0.46
		-7.00	98.41	99.11	98.73	98.75	0.35	0.20	0.35
		-8.00	101.40	102.60	99.09	101.03	1.78	1.03	1.76
ATZ	1	-3.00	81.24	80.64	79.85	80.58	0.70	0.40	0.87
		-4.00	89.99	90.78	91.68	90.82	0.85	0.49	0.93
		-5.00	98.04	97.25	93.18	96.16	2.61	1.51	2.71
		-6.00	93.30	96.07	94.56	94.64	1.39	0.80	1.47
		-7.00	99.54	98.18	96.84	98.19	1.35	0.78	1.38
		-8.00	97.24	90.24	94.94	94.14	3.57	2.06	3.79
		-9.00	95.63	96.96	90.83	94.48	3.22	1.86	3.41
		-10.00	93.33	94.37	91.67	93.12	1.36	0.79	1.46

(continued)

Table 17. Percent of Control Values (continued)

RC	Rep.	Log[RC]	Tube 1	Tube 2	Tube 3	Mean	SD	SEM	% CV
ATZ	2	-3.00	73.89	75.52	72.93	74.11	1.31	0.76	1.77
		-3.30	76.91	77.44	76.86	77.07	0.32	0.18	0.41
		-3.60	85.39	81.67	83.83	83.63	1.87	1.08	2.24
		-4.00	84.76	86.61	88.97	86.78	2.11	1.22	2.43
		-4.30	90.79	95.20	91.97	92.65	2.28	1.32	2.46
		-5.00	93.61	95.36	92.38	93.78	1.50	0.87	1.60
		-6.00	95.22	93.96	93.72	94.30	0.80	0.46	0.85
		-7.00	95.34	96.92	90.87	94.37	3.14	1.81	3.32
	3	-3.00	80.56	80.91	80.19	80.55	0.36	0.21	0.45
		-3.30	79.67	80.43	79.89	80.00	0.39	0.23	0.49
		-3.60	88.17	89.54	85.91	87.87	1.83	1.06	2.09
		-4.00	98.48	94.74	91.84	95.02	3.33	1.92	3.50
		-4.30	96.53	95.59	94.96	95.69	0.79	0.46	0.83
		-5.00	100.02	95.11	96.48	97.20	2.53	1.46	2.61
		-6.00	99.25	96.87	95.99	97.37	1.68	0.97	1.73
		-7.00	99.71	97.28	95.12	97.37	2.30	1.33	2.36

[a]This value was excluded as an outlier from the calculation of mean, SD, SEM and %CV.

The mean percent of control values across tubes for each replicate and the overall mean, SD, SEM and %CV across replicates are presented in Table 18. Generally, the %CV at each tested concentration was less than 10% indicating low replicate to replicate variance. At some high concentrations where the percent of control was near zero small variances in percent of control yielded high %CVs, but this had little effect on the curve-fitting.

Table 18. Mean Percent of Control per Replicate and Percent of Control Across Replicates

		Mean Percent of Control			Overall			
RC	Log[RC]	Rep 1	Rep 2	Rep 3	Mean	±SD	SEM	%CV
AG	-3.00	4.94	5.53	3.63	4.70	0.97	0.56	20.69
	-3.30	NA	11.88	10.27	11.08	1.13	0.80	10.23
	-3.60	NA	22.14	19.90	21.02	1.59	1.12	7.56
	-4.00	37.73	40.57	39.74	39.35	1.46	0.84	3.71
	-4.30	NA	56.53	54.75	55.64	1.26	0.89	2.26
	-5.00	81.44	88.82	86.11	85.46	3.73	2.15	4.36
	-6.00	96.07	100.72	97.80	98.20	2.35	1.36	2.39
	-7.00	97.66	100.53	98.77	98.98	1.45	0.84	1.46
	-8.00	96.15	NA	NA	96.15	NC	NC	NC
	-9.00	95.39	NA	NA	95.39	NC	NC	NC
	-10.00	94.48	NA	NA	94.48	NC	NC	NC

(continued)

Table 18. Mean Percent of Control per Replicate and Percent of Control Across Replicates (continued)

RC	Log[RC]	Mean Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	±SD	SEM	%CV
KCZ	-4.00	0.55	-1.11	0.21	-0.12	0.88	0.51	-736.32
	-5.00	4.52	2.23	4.06	3.60	1.21	0.70	33.52
	-6.00	29.16	28.36	27.77	28.43	0.70	0.40	2.45
	-6.30	44.98	40.59	43.63	43.07	2.25	1.30	5.23
	-7.00	77.89	70.99	75.56	74.82	3.51	2.03	4.69
	-8.00	96.96	88.17	96.11	93.74	4.85	2.80	5.17
	-9.00	97.66	92.00	99.18	96.28	3.79	2.19	3.93
	-10.00	96.81	91.66	98.97	95.82	3.76	2.17	3.92
PCZ	-3.00	0.01	NA	NA	0.01	NC	NC	NC
	-4.00	-0.07	NA	NA	-0.07	NC	NC	NC
	-5.00	0.25	0.15	0.00	0.13	0.13	0.07	95.06
	-6.00	2.04	2.23	2.96	2.41	0.48	0.28	20.04
	-7.00	16.14	19.09	18.15	17.79	1.51	0.87	8.47
	-7.60	NA	48.87	46.40	47.64	1.75	1.24	3.67
	-8.00	61.99	67.93	69.87	66.59	4.11	2.37	6.17
	-8.30	NA	82.46	85.77	84.11	2.34	1.65	2.78
	-9.00	91.13	95.64	101.13	95.97	5.01	2.89	5.22
	-10.00	96.90	97.43	107.99	100.77	6.25	3.61	6.20
NYP	-3.00	0.10	0.10	0.01	0.07	0.06	0.03	79.51
	-4.00	6.46	7.07	6.80	6.78	0.31	0.18	4.52
	-4.30	NA	22.42	23.33	22.87	0.64	0.46	2.82
	-4.60	NA	47.55	45.72	46.63	1.29	0.91	2.76
	-5.00	87.43	86.91	84.50	86.28	1.56	0.90	1.81
	-5.30	NA	96.00	94.20	95.10	1.27	0.90	1.34
	-6.00	98.19	99.33	97.03	98.18	1.15	0.66	1.17
	-7.00	99.89	99.22	95.79	98.30	2.20	1.27	2.24
	-8.00	100.10	NA	NA	100.10	NC	NC	NC
	-9.00	99.48	NA	NA	99.48	NC	NC	NC
	-10.00	99.58	NA	NA	99.58	NC	NC	NC
DBA	-4.00	101.81	87.55	99.90	96.42	7.74	4.47	8.03
	-5.00	103.70	99.88	102.67	102.08	1.98	1.14	1.94
	-6.00	101.69	101.99	104.24	102.64	1.39	0.80	1.36
	-6.30	100.76	99.70	104.63	101.70	2.60	1.50	2.55
	-7.00	104.29	100.79	104.25	103.11	2.01	1.16	1.95
	-8.00	104.17	100.90	105.63	103.57	2.42	1.40	2.34
	-9.00	103.25	100.94	104.05	102.74	1.61	0.93	1.57
	-10.00	104.07	100.88	96.75	100.57	3.67	2.12	3.65

(continued)

Table 18. Mean Percent of Control per Replicate and Percent of Control Across Replicates (continued)

RC	Log[RC]	Mean Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	±SD	SEM	%CV
FRM	-3.00	1.03	1.79	0.86	1.23	0.49	0.28	40.17
	-4.00	5.65	6.19	6.43	6.09	0.40	0.23	6.61
	-4.60	NA	19.28	21.24	20.26	1.39	0.98	6.86
	-5.00	33.02	35.74	38.05	35.61	2.52	1.45	7.08
	-5.30	NA	49.30	55.96	52.63	4.71	3.33	8.94
	-6.00	71.63	80.64	82.50	78.26	5.81	3.36	7.43
	-7.00	82.14	93.18	99.09	91.47	8.60	4.96	9.40
	-8.00	83.24	96.24	99.40	92.96	8.56	4.94	9.21
	-9.00	83.54	NA	NA	83.54	NC	NC	NC
	-10.00	81.48	NA	NA	81.48	NC	NC	NC
ECZ	-3.00	0.09	NA	NA	0.09	NC	NC	NC
	-4.00	0.06	NA	NA	0.06	NC	NC	NC
	-5.00	0.01	NA	NA	0.01	NC	NC	NC
	-6.00	0.18	0.11	0.01	0.10	0.08	0.05	83.80
	-7.00	1.24	1.42	1.35	1.34	0.09	0.05	7.08
	-8.00	11.12	13.14	12.32	12.20	1.02	0.59	8.33
	-8.30	NA	23.35	21.29	22.32	1.46	1.03	6.52
	-9.00	58.00	60.72	60.08	59.60	1.42	0.82	2.39
	-9.30	NA	77.00	75.10	76.05	1.34	0.95	1.77
	-9.60	NA	88.06	84.80	86.43	2.30	1.63	2.66
	-10.00	93.30	94.39	92.14	93.28	1.12	0.65	1.21
CYN	-4.00	23.21	22.45	22.25	22.63	0.51	0.29	2.24
	-5.00	22.76	22.84	22.77	22.79	0.04	0.03	0.19
	-5.30	NA	35.23	35.68	35.45	0.32	0.23	0.91
	-5.60	NA	50.00	50.97	50.48	0.69	0.49	1.36
	-6.00	70.01	69.90	71.12	70.35	0.68	0.39	0.96
	-6.30	80.02	79.96	82.13	80.70	1.23	0.71	1.53
	-7.00	92.28	89.03	93.82	91.71	2.45	1.41	2.67
	-8.00	94.74	90.43	97.24	94.14	3.45	1.99	3.66
	-9.00	95.41	NA	NA	95.41	NC	NC	NC
	-10.00	93.30	NA	NA	93.30	NC	NC	NC
DCF	-3.00	3.98	1.97	2.40	2.78	1.06	0.61	37.97
	-3.30	NA	4.79	6.39	5.59	1.12	0.80	20.12
	-3.60	NA	10.27	13.84	12.05	2.53	1.79	20.98
	-4.00	39.77	21.96	24.24	28.66	9.69	5.60	33.82
	-5.00	68.31	67.71	64.34	66.79	2.14	1.23	3.20
	-6.00	90.38	87.21	92.76	90.12	2.78	1.61	3.08
	-7.00	98.01	93.97	98.75	96.91	2.58	1.49	2.66
	-8.00	101.10	96.93	101.03	99.69	2.39	1.38	2.40
	-9.00	100.89	NA	NA	100.89	NC	NC	NC
	-10.00	100.13	NA	NA	100.13	NC	NC	NC

(continued)

Table 18. Mean Percent of Control per Replicate and Percent of Control Across Replicates (continued)

RC	Log[RC]	Mean Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	±SD	SEM	%CV
ATZ	-3.00	80.58	74.11	80.55	78.41	3.73	2.15	4.75
	-3.30	NA	77.07	80.00	78.54	2.07	1.46	2.63
	-3.60	NA	83.63	87.87	85.75	3.00	2.12	3.50
	-4.00	90.82	86.78	95.02	90.87	4.12	2.38	4.53
	-4.30	NA	92.65	95.69	94.17	2.15	1.52	2.28
	-5.00	96.16	93.78	97.20	95.71	1.75	1.01	1.83
	-6.00	94.64	94.30	97.37	95.44	1.68	0.97	1.76
	-7.00	98.19	94.37	97.37	96.64	2.01	1.16	2.08
	-8.00	94.14	NA	NA	94.14	NC	NC	NC
	-9.00	94.48	NA	NA	94.48	NC	NC	NC
	-10.00	93.12	NA	NA	93.12	NC	NC	NC

NA Not applicable – this test concentration was not assayed in this replicate
NC Not calculated – only one data point – no SD, SEM or CV calculated.

4.3.3 IC_{50} Values

For each RC and replicate the percent of control values were fitted to the equation presented in Section 3.7.2.1 using Prism 3.02 and the top, bottom, IC_{50} and slope were calculated. The percent of control values for the tested concentrations of all RCs except chrysin, dibenz[a,h]anthracene and atrazine essentially span the range 0-100%. The model fits the data well for each of these reference chemicals. For chrysin the percent of control data for the tested concentrations ranges from approximately 22-95% and the current 4-parameter model curve fits these data well, which is in contrast to the two-parameter model used in a previous task that did not fit this type of data satisfactorily. However, as pointed out previously by Dr. Paul Feder of Battelle, when the percent of control values do not span the range 0-100%, the value returned as $logIC_{50}$ by Prism is actually equivalent to (top + bottom)/2. Therefore, caution should be exercised in the reporting of IC_{50} estimates generated from data sets that do not span the 0-100% range for percent of control. In the case of chrysin, the point estimate for the concentration corresponding to 50% activity returned by Prism for the data averaged across all three replicates is -5.633. This value is not encompassed by the 95% confidence interval (-5.839 to -5.695) for LogIC50 (see Table 20). Dibenz[a,h]anthracene and atrazine did not inhibit aromatase more than 20% at concentrations up to 1 mM, and thus were both categorized as non-inhibitors according to the study criteria. The individual replicate concentration response curves for the eight reference chemicals for which the fit converged are presented in Appendix F.

Figure 1 shows the concentration response curve fits of each replicate per RC superimposed on one plot. It is clear that the replicate to replicate variation in curve fit is low. No plots are presented for the non-inhibitors, dibenz[a,h]anthracene and atrazine.

Figure 2 presents the results of curve fitting across the replicates for each RC. The mean percent of control data for the repetitions within a replicate (as presented in Table 18) were fit to the model. No plots are presented for the non-inhibitors, dibenz[a,h]anthracene and atrazine.

The top, bottom, IC_{50} and slope data are summarized in Table 19 by chemical and replicate. The status of each response is also indicated in the table. All of the tested chemicals, except dibenz[a,h]anthracene and atrazine, were classified as inhibitors.

The summary statistics for the response model parameters (overall means, SE and 95% confidence intervals) for each chemical (across replicates) are presented in Table 20. Graphs of the top, bottom, IC_{50} and slope means, standard errors and confidence intervals are included in statistician's report in Appendix E.

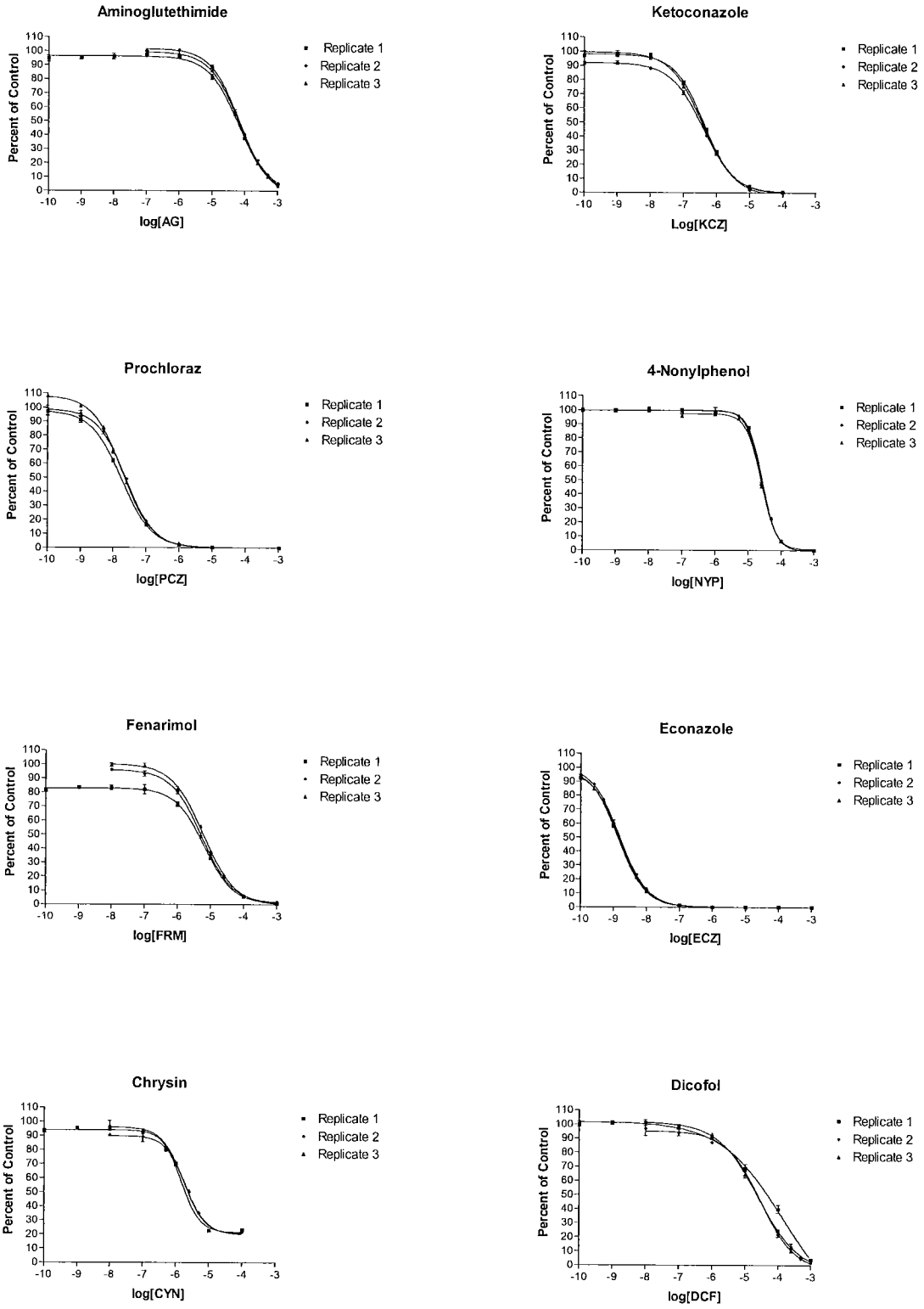

Figure 1. Concentration-Response Curve Fits – Overlay of Individual Replicate Results

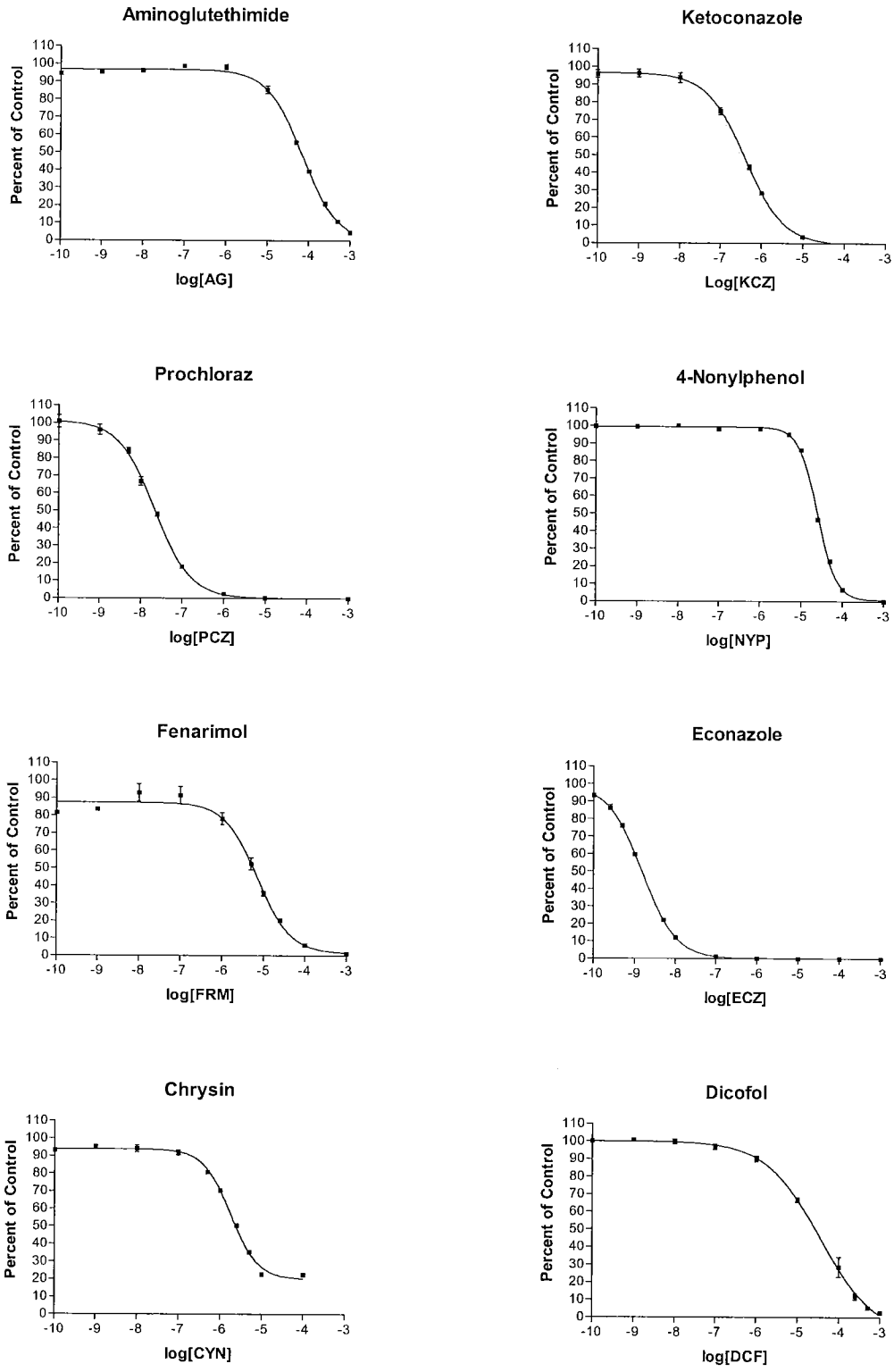

Figure 2. Average Concentration-Response Curve Fits (Across Replicates)

Table 19. Summary of Top, Bottom, LogIC_{50} and Slope and their Associated SE

RC	Code	Rep.	Top	SE Top	Bottom	SE Bottom	$log_{10}IC_{50}$	SE $log_{10}IC_{50}$	Slope	SE Slope	Status
AG	1	1	96.27	0.7558	-1.984	2.941	-4.182	0.04567	-0.9437	0.07679	Inhibitor
		2	101.7	0.7431	-0.5217	1.624	-4.178	0.01973	-0.9842	0.04441	Inhibitor
		3	99.40	0.7882	-3.500	1.830	-4.159	0.02204	-0.9547	0.04587	Inhibitor
KCZ	2	1	97.94	0.7048	0.2081	0.9492	-6.381	0.01886	-0.9772	0.04268	Inhibitor
		2	91.97	0.5661	-2.476	0.7907	-6.378	0.01678	-0.8726	0.03115	Inhibitor
		3	99.39	0.7244	-0.6192	0.9722	-6.429	0.01980	-0.9062	0.03819	Inhibitor
PCZ	3	1	97.44	0.9589	-0.07196	0.5527	-7.743	0.02266	-0.9392	0.03994	Inhibitor
		2	98.70	1.332	-0.05843	1.239	-7.621	0.02643	-0.9971	0.05585	Inhibitor
		3	108.1	0.6502	0.2152	0.5643	-7.725	0.01124	-0.9697	0.02336	Inhibitor
NYP	4	1	99.48	0.4728	0.01138	1.075	-4.574	0.02684	-2.017	0.1120	Inhibitor
		2	99.90	0.8444	0.7939	1.134	-4.608	0.01230	-1.948	0.09056	Inhibitor
		3	97.38	0.8158	0.7179	1.099	-4.606	0.01233	-1.908	0.08676	Inhibitor
DBA	5	1									no inhibition
		2									no inhibition
		3									no inhibition
FRM	6	1	82.93	0.7732	0.5092	1.358	-5.188	0.03603	-0.9924	0.07539	Inhibitor
		2	96.07	0.8917	0.9139	1.037	-5.266	0.02056	-0.9430	0.04181	Inhibitor
		3	100.2	0.8354	-0.5225	1.020	-5.213	0.01867	-0.9089	0.03605	Inhibitor
ECZ	7	1	99.24	1.088	0.06986	0.2891	-8.859	0.01745	-1.048	0.03602	Inhibitor
		2	100.7	1.187	0.1160	0.5851	-8.811	0.01661	-1.030	0.03087	Inhibitor
		3	96.88	0.8613	0.1432	0.446	-8.804	0.01259	-1.073	0.02527	Inhibitor
CYN	8	1	94.02	0.6105	21.27	1.100	-5.831	0.03025	-1.528	0.1406	Inhibitor
		2	89.84	1.241	20.27	1.568	-5.714	0.02965	-1.432	0.1124	Inhibitor
		3	96.10	1.327	19.60	1.645	-5.757	0.02984	-1.302	0.09583	Inhibitor
DCF	9	1	101.8	1.13	-34.12	13.34	-3.871	0.2096	-0.4643	0.04879	Inhibitor
		2	95.20	1.733	-3.624	3.744	-4.534	0.07350	-0.8303	0.09404	Inhibitor
		3	101.5	0.8974	-4.540	2.050	-4.604	0.03790	-0.7067	0.03725	Inhibitor
ATZ	10	1									no inhibition
		2									no inhibition
		3									no inhibition

Table 20. Summary Statistics for Response Model Parameters by Chemical

Chemical	Dependent	Mean	Standard Error	Lower 95% CI	Upper 95% CI
AG	Top	99.12	1.58	95.78	102.5
AG	Bottom	-1.86	1.14	-4.27	0.55
AG	Log10(IC50)	-4.17	0.01	-4.20	-4.14
AG	Slope	-0.97	0.03	-1.03	-0.90
KCZ	Top	96.41	2.28	91.58	101.2
KCZ	Bottom	-1.04	0.83	-2.79	0.72
KCZ	Log10(IC50)	-6.39	0.02	-6.43	-6.36
KCZ	Slope	-0.91	0.03	-0.98	-0.85
PCZ	Top	101.5	3.40	94.27	108.7
PCZ	Bottom	0.06	0.38	-0.74	0.85
PCZ	Log10(IC50)	-7.70	0.04	-7.78	-7.62
PCZ	Slope	-0.97	0.02	-1.01	-0.93
NYP	Top	98.97	0.73	97.43	100.5
NYP	Bottom	0.49	0.64	-0.85	1.84
NYP	Log10(IC50)	-4.60	0.01	-4.62	-4.59
NYP	Slope	-1.95	0.05	-2.06	-1.83
FRM	Top	93.06	5.2	82.02	104.1
FRM	Bottom	0.26	0.64	-1.10	1.62
FRM	Log10(IC50)	-5.23	0.02	-5.27	-5.18
FRM	Slope	-0.93	0.03	-0.99	-0.88
ECZ	Top	98.83	1.14	96.41	101.2
ECZ	Bottom	0.10	0.22	-0.38	0.57
ECZ	Log10(IC50)	-8.82	0.02	-8.86	-8.79
ECZ	Slope	-1.05	0.02	-1.09	-1.02
CYN	Top	93.33	1.77	89.59	97.08
CYN	Bottom	20.63	0.79	18.96	22.31
CYN	Log10(IC50)	-5.77	0.03	-5.84	-5.69
CYN	Slope	-1.39	0.06	-1.53	-1.26
DCF	Top	99.71	2.03	95.40	104.0
DCF	Bottom	-4.86	1.78	-8.64	-1.08
DCF	Log10(IC50)	-4.38	0.21	-4.82	-3.93
DCF	Slope	-0.66	0.11	-0.88	-0.43

5.0 DISCUSSION

This WA 4-16, Task 7 involved testing the response of human placental microsome aromatase activity to inhibition by ten reference chemicals using two different microsome preparations. This task was performed in the four aromatase validation laboratories (RTI, Battelle, WIL and In Vitro) and only the results obtained by RTI are presented here.

The microsomes used in this task were prepared at Battelle and In Vitro on a previous task of this WA. At the time of preparation, the protein content of the microsomal suspensions were determined to be 8 and 21 mg/mL for the microsomes prepared at In Vitro and Battelle, respectively. At RTI, during the conduct of Task 7, the average measured protein content of the In Vitro microsome stock was 10.9 ± 1.2 mg/mL (10.8% CV) and that of the Battelle microsomes was 27.0 ± 1.4 mg/mL (5.3% CV).

QC standards of 12 and 110 µg/mL were included with each of the sixteen protein assays. Overall, the data show acceptable correlation of calculated and known amounts (-5.7 and 3.5% relative error, respectively) for the low and high QC standards.

Four types of controls were used for the aromatase assay – a full activity control which served as the 100% activity control, a background activity control which was used to correct for non-enzymatic product formation and other artifactual radiochemical content in the assay mixture, a positive control which employed a known aromatase inhibitor and a negative control which employed a known aromatase non-inhibitor.

All calculated aromatase activities were corrected for the radioactivity measured in the background activity controls, so the average background control activity for a replicate necessarily was zero. The average full aromatase activity for the Battelle microsomes was 0.0489 ± 0.0042 nmol/mg/min (8.64 % CV), the positive control activity averaged 0.0239 ± 0.0021 nmol/mg/min (8.99 %CV) and the negative control activity averaged 0.0473 ± 0.0038 nmol/mg/min (8.13 %CV). For the In Vitro microsomes, the average full aromatase activity was 0.0376 ± 0.0047 nmol/mg/min (12.41 % CV), the average positive control activity was 0.0179 ± 0.0024 nmol/mg/min (13.42 %CV) and the average negative control activity was 0.0370 ± 0.0040 nmol/mg/min (10.69 %CV). For all controls, a decrease in aromatase activity was noted between the controls run at the beginning of each replicate and those run at the end of the replicate. While these differences were small (generally less than 15% difference in portion means), the trend to lower activity between the beginning and end of the assay was extremely

consistent and may indicate an issue with decreasing aromatase activity over time. Given that only about 25 minutes elapsed between the beginning and end of the assay set, these decreases are notable, and support the need for minimizing the amount of time between thawing and use of the microsomes in the assay.

Aromatase activities were expressed as percent of control after normalizing by the average full enzyme activity for a given replicate. The background and full enzyme activity controls were set at 0 and 100% by definition. The beginning and end positive and negative control percent of control activities generally ranged from 40-60% and 90-110%, respectively, across all runs of the assay. Average positive and negative control values across all assays were 48.1 ± 2.6% and 97.7 ± 2.6%, respectively,.

Each of the ten reference chemicals was added to the aromatase assay at eight predefined concentrations in triplicate repetitions (tubes) per replicate of the assay. Data from the first replicate of each reference chemical were reviewed and the target concentrations were adjusted as necessary to better define the concentration response curve. Concentrations were selected to define the upper and lower plateaus with intermediate concentrations chosen to distribute as evenly as possible along the slope of the curve. The aromatase assay was conducted in triplicate for each reference chemical and all replicates of a given reference chemical were conducted by the same technician.

The response curve was fitted by nonweighted least squares nonlinear regression analysis. The four-parameter model used in this work assignment leads to satisfactory curve fits for all of the data sets where inhibitions of at least 80% were found.

IC_{50}s, slope, and top and bottom plateaus were estimated for each reference chemical using a four-parameter equation. There was very little variance (generally 5-15%) in IC_{50} data over three replicates for each reference chemical. Dibenz[a,h]anthracene and atrazine were identified as non-inhibitors of aromatase, which is as expected based on the literature and the results of WA 2-24. All other reference chemicals tested were classified as aromatase inhibitors. 4-Nonylphenol was identified as an aromatase inhibitor in this study, whereas in WA 2-24, it was classified as a non-inhibitor. The inclusion of more concentrations between 10^{-4} and 10^{-5} M in this task allowed for the detection of the inhibition of aromatase at those higher concentrations and for appropriate curve-fitting to estimate IC_{50}.

6.0 CONCLUSION

In this WA 4-16 Task 7, the performance of the aromatase assay was assessed using two human placental microsome preparations and ten reference chemicals. The data reported here will be compared with data obtained from other participating laboratories using the same microsomal preparations and reference chemicals.

Aromatase activities for each type of control had coefficients of variance on the order of 8 and 12%, respectively for the Battelle and In Vitro microsomes. These coefficients of variance are lower than the approximately 20% found in a previous WA 4-16 task for aromatase activity. This improvement may be correlated to improved control of the protein assay in this Task versus the previous one through the use of a different range for the standard curve and the incorporation of QC standards. Interestingly, the coefficient of variance for the protein concentration of the Battelle microsomes was 5.3% and that for the In Vitro microsomes was 10.8%, which mirrors the CVs for the aromatase activity, suggesting that variance in aromatase activity is closely tied to variance in protein content determination. Decreases in control activities were notable over the approximately 25 minutes that elapsed between the beginning and end of the assay, suggesting that it is important to minimize the length of time between thawing and use of microsomes.

There was very little variance in the top, bottom, IC_{50} and slope estimates over the three replicates for any of the ten tested reference chemicals. Data from chrysin point out the need for caution in the interpretation of IC_{50} values reported by Prism for data sets where the percent of control values do not encompass the entire range 0-100%.

The results of this study show that the aromatase assay can be used to reliably detect aromatase inhibitors and non-inhibitors and to produce appropriate concentration response data, including IC_{50}s, for those chemicals with acceptable reproducibility.

Appendix A
Study Protocol

EPA Contract No.: 68-W-01-023
EPA Work Assignment No.: 4-16
Task Number: 7
RTI Study Code: An05-948

TITLE: WA 4-16 Task 7: Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories

SPONSOR: Battelle
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Columbus, OH 43201

TESTING FACILITY: RTI International
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Research Triangle Park, NC 27709

PROPOSED EXPERIMENTAL START DATE: July 1, 2005
PROPOSED EXPERIMENTAL END DATE: August 30, 2005

AMENDMENTS:

Number	Date	Section(s)	Page(s)
1			
2			
3			

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1.0 OBJECTIVES

TASK 7: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES

The objectives of this task are to test 10 reference chemicals using microsomes prepared by two participating laboratories (Battelle and In Vitro) in Task 6 and determine the intra- and interlaboratory variability.

The microsomes prepared in Task 6 by Battelle and In Vitro will be distributed to each laboratory and tested as outlined in Section 2.2.

1.1 Justification for Test System

The test system for this study is human placental microsomes. This test system was selected because it provides a biological source of the aromatase enzyme and since the assay is being evaluated for its potential to serve as a screening assay, the use of human tissue enhances its predictive potential.

1.2 Test Method

This *in vitro* test method involves combining microsomes, substrate, appropriate co-factors and reference chemicals in a common reaction vessel. The effect of the reference chemicals on microsomal enzyme activity is evaluated by measuring the amount of the product of the enzyme-catalyzed substrate oxidation that is formed.

There is no applicable route of administration in the sense of a dose administration route for this *in vitro* test.

2.0 MATERIALS RECEIPT AND/OR PREPARATION

A sufficient supply of chemical reagents, radiolabeled and non-radiolabeled androstenedione, and human placental microsomal preparations will be obtained prior to initiation of the first set of experiments to ensure that sufficient quantities are available to conduct the studies.

2.1 Substrate

2.1.1 Substrate Name/Supplier

The substrate for the aromatase assay is androstenedione (ASDN). Nonradiolabeled and radiolabeled ASDN will be used. The nonradiolabeled ASDN and the radiolabeled androstenedione ([1β-^{3}H]-androstenedione, [^{3}H]ASDN) will be provided to the laboratories by Battelle's Chemical Repository (CR). The CR will forward all applicable information regarding supplier, lot numbers, and reported/measured purity for the substrate to the laboratories and this information will be included in study reports. The radiochemical purity of the [^{3}H]ASDN was

assessed by the lead laboratory in a previous Task and was found to be 97%.

2.1.2 Preparation of Substrate Solution for use in Aromatase Assay

Since the specific activity of the stock [^{3}H]ASDN is too high for use directly in the assay, a solution containing a mixture of nonradiolabeled and radiolabeled [^{3}H]ASDN must be prepared such that the final concentration of ASDN in the assay is 100 nM and the amount of tritium added to each incubation is about 0.1 µCi. This substrate solution should have a concentration of 2 µM with a radiochemical content of about 1 µCi/mL.

The following illustrates the preparation of a substrate solution using a stock of [^{3}H]ASDN with a specific activity of 25.3 Ci/mmol and a concentration of 1 mCi/mL. Prepare a 1:100 dilution of the radiolabeled stock in buffer. Prepare a 1 mg/mL solution of ASDN in ethanol and then prepare dilutions in buffer to a final concentration of 1 µg/mL. Combine 4.5 mL of the 1 µg/mL solution of ASDN, 800 µL of the [^{3}H]ASDN dilution and 2.7 mL buffer to make 8 mL of substrate solution (enough for 80 tubes). Record the weight of each component added to the substrate solution. After mixing the solution well, weigh aliquots (ca 20 µL) and combine with scintillation cocktail for radiochemical content analysis. The addition of 100 µL of the substrate solution to each 2 mL assay volume yields a final [^{3}H]ASDN concentration of 100 nM with 0.1 µCi/tube.

2.2 *Reference Chemicals*

The reference chemicals for Task 7, their properties and rationale for selection are listed in Table 1. Each chemical will initially be tested over the concentration range 10^{-3} to 10^{-10} M (final concentration) but the range may be adjusted as described in Section 5.

Table 1. Reference Chemicals for Aromatase Assay Validation

Reference chemical	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Basis for Selection
aminoglutethimide	125-84-8	$C_{13}H_{16}N_2O_2$	232.3	Non-steroidal aromatase inhibitor
chrysin	480-40-0	$C_{15}H_{10}O_4$	254.2	Potent flavonoid
dicofol	115-32-2	$C_{14}H_9Cl_5O$	370.47	Organochlorine
econazole (nitrate)	24169-02-6	$C_{18}H_{15}Cl_3N_2O$-HNO_3	444.7	Potent imidazole anti-fungal
ketoconazole	65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	531.43	Weak imidazole anti-fungal
atrazine	1912-24-9	$C_8H_{14}ClN_5$	215.69	Affects aromatase gene expression; no aromatase inhibition
fenarimol	60168-88-9	$C_{17}H_{12}Cl_2N_2O$	331.2	pyrimidine fungicide

Reference chemical	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Basis for Selection
4-nonylphenol	104-40-5	$C_{15}H_{24}O$	220.4	Affects AR/ER; no aromatase inhibition
prochloraz	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	conazole fungicide
dibenz [*a,h*] anthracene	53-70-3	$C_{22}H_{14}$	278.35	Known non-aromatase inhibitor; Ah receptor agonist

2.2.1 Reference Chemical Formulation and Analysis

Reference chemical stock solutions will be prepared and analyzed by the CR and distributed to the laboratories. Reference chemicals will be formulated in buffer, absolute ethanol or dimethylsulfoxide (DMSO). The total volume of reference chemical formulation used in each assay should be no more than 1% of the total assay volume (i.e., 20 µL in a 2 mL assay) in order to minimize the potential of the solvent to inhibit the enzyme. Fresh dilutions of the stock solution will be prepared in the same solvent as the stock solution on the day of use such that the target concentration of reference chemical can be achieved by the addition of 20 µL of the dilution to a 2 mL assay volume. Information on storage conditions for reference chemical stock solutions will be provided by the CR.

The reference chemicals will be numbered 1-10 by the CR and these same numeric designations will be used when the samples are coded prior to distribution to the assaying laboratories. This will ensure that, for example, Chemical 1, is always the same chemical in each laboratory. This is important for the proper balancing of the study as outlined in Table 4.

2.3 *Control Substances*

The known aromatase inhibitor, 4-hydroxyandrostendione (4-OH ASDN), is used as the positive control substance. A known aromatase non-inhibitor, lindane, will be used as the negative control substance. Table 2 contains identity and property information for these substances.

Table 2. Control Substances

Control Substance	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Target Concentration in Assay (M)	Basis for Selection
4-OH ASDN	566-48-3	$C_{19}H_{26}O_3$	302.4	5E-8	Known aromatase inhibitor
Lindane	58-89-9	$C_6H_6Cl_6$	290.8	1E-6	Affects StAR and cholesterol metabolism; no aromatase activity

2.3.1 Control Substance Formulation and Analysis

Control substance stock solutions will be prepared and analyzed by the CR and distributed to the laboratories. Control substances will be formulated in buffer, absolute ethanol or DMSO. The total volume of control substance formulation used in each assay should be no more than 1% of the total assay volume (i.e., 20 µL in a 2 mL assay) in order to minimize the potential of the solvent to inhibit the enzyme. Fresh dilutions of the stock solution will be prepared in the same solvent as the stock solution on the day of use. Dilutions will be prepared such that the target concentration of control substance (Table 2) can be achieved by the addition of 20 µL of the dilution to a 2 mL assay volume. Information on storage conditions for control substance stock solutions will be provided by the CR.

2.4 Microsomes

Human placental microsomes (prepared in Task 6) will be supplied to each laboratory by Battelle and In Vitro. These samples should be treated as potentially infectious and appropriate precautions must be employed. The microsomes must be stored at -70 to -80 °C. The approximate protein content of the microsomes will be provided by the supplier.

Caution: Microsomes can be denatured by detergents. Therefore, it is important to ensure that all glassware, etc. that is used in the preparation or usage of microsomes is free of detergent residue. New disposable test tubes, bottles, vials, pipets and pipet tips may be used directly in the assay. Durable labware that may have been exposed to detergents should be rinsed with water and/or buffer prior to use in the assay.

If the human placental microsomes are supplied in aliquots in excess of what is required to conduct a single experiment, they will be thawed, pooled, homogenized, divided into appropriate aliquots for conduct of a single experiment and refrozen as described below in order to minimize and standardize the number of freeze/thaw cycles each preparation undergoes. Microsomes will be thawed quickly in a 37 ± 1 °C water bath and then will be immediately transferred to an ice bath. The microsomes will be pooled and rehomogenized using a Potter-Elvejhem homogenizer (about 5–10 passes). The pooled sample will be aliquoted into portions appropriate for use in a single experiments (ca. 160 µL, dependant on the protein concentration of the preparation) and the samples will be flash frozen and stored at -70 to -80 °C for future use. Each tube will provide enough protein for a single experiment and any excess thawed microsomal preparation will be discarded.

On the day of use, microsomes will be thawed quickly in a 37 ± 1 °C water bath and then will be immediately transferred to an ice bath. The microsomes will be rehomogenized using a Potter-Elvejhem homogenizer (about 5-10 passes) or by vortexing about 5 seconds prior to use. The microsomes will be diluted in buffer (serial dilutions may be necessary) to an approximate protein concentration of 0.025 mg/mL. The addition of 1 mL of that microsome dilution will result in a final approximate protein concentration of 0.0125 mg/mL in the assay tubes. All microsome samples must be kept on ice until they are placed in the water bath just prior to their addition to the aromatase assay. Microsomes are not be left on ice for longer than approximately 1 h before proceeding with the assay. Appropriate documentation of time from

thaw to use must be maintained.

Diluted microsomes must be used only on the day of preparation. Under no conditions should diluted microsomes be refrozen for later use in the assay.

2.5 *Other Assay Components*

2.5.1 Buffer

The assay buffer will be 0.1 M sodium phosphate buffer, pH 7.4. Sodium phosphate monobasic (JT Baker, cat # 4011-01, 137.99 g/mol) and sodium phosphate dibasic (JT Baker, cat # 4062-01, 141.96 g/mol) will be used in the preparation of the buffer. Solutions of each reagent at 0.1 M will be prepared in distilled, deionized water and then the solutions will be combined to a final pH of 7.4. The assay buffer may be stored for up to one month in the refrigerator (2–8 °C).

2.5.2 Propylene Glycol

Propylene glycol (JT Baker, cat # 9402-01, 76.1 g/mol) will be added to the assay directly as described below.

2.5.3 NADPH

NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt, Sigma, cat # 1630, 833.4 g/mol) is the required co-factor for CYP19. The final concentration in the assay is 0.3 mM. Typically, a 6 mM stock solution will be prepared in assay buffer and then 100 µL of the stock will be added to the 2 mL assay volume. NADPH must be prepared fresh each day and will be kept on ice.

3.0 PROTEIN ASSAY

The protein concentration of the microsome preparation will be determined on each day of use of the microsomes in the aromatase assay. QC standards (nominal protein concentrations of 10 and 100 µg/mL) will be prepared by the lead laboratory and distributed to each participating laboratory. Each of these QC standards will be run in duplicate with each run of the protein assay. A 6-point standard curve will be prepared, ranging from 5 to 250 µg protein/mL. The protein curve standards will be made from bovine serum albumin (BSA). Protein will be determined by using a DC Protein Assay kit purchased from Bio-Rad (Hercules, CA). To a 200 µL aliquot of unknown, QC or curve standard, 100 µL of BioRad DC Protein Kit Reagent A will be added and mixed. Next, 800 µL of BioRad DC Protein Kit Reagent B will be added to each sample and the samples will be vortex mixed. The samples will be allowed to sit at room temperature for at least 15 min to allow for color development. The absorbances are stable for about 1 h. Each sample (unknown and standards) will be transferred to disposable polystyrene cuvettes and the absorbance (@ 750 nm) will be measured using a spectrophotometer. The protein concentration of the microsomal sample will be determined by interpolation of the absorbance value using the curve developed from the protein standards.

4.0 AROMATASE ASSAY METHOD

The assays will be performed in 13 x 100 mm test tubes maintained at 37 ± 1 °C in a shaking water bath. Each test tube will be uniquely identified by applying a label or writing directly on the test tube. Propylene glycol (100 µL), [^{3}H]ASDN, NADPH, and buffer (0.1 M sodium phosphate buffer, pH 7.4) will be combined in the test tubes (total volume 1 mL). The final concentrations for the assay components are presented in Table 3. The tubes and the microsomal suspension will be placed at 37 ± 1 °C in the water bath for 5 min prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume will be 2 mL, and the tubes will be incubated for 15 min. The incubations will be stopped by the addition of methylene chloride (2 mL); the tubes will be vortex-mixed for ca. 5 s and placed on ice. The tubes will then be vortex-mixed an additional 20-25 s. The tubes will then be centrifuged using a Beckman GS-6R centrifuge with GH-3.8 rotor for 10 min at a setting of 1000 rpm. The methylene chloride layer will be removed and discarded; the aqueous layers will be extracted again with methylene chloride (2 mL). This extraction procedure will be performed one additional time, each time discarding the methylene chloride layer. The aqueous layers will be transferred to vials and duplicate aliquots (0.5 mL) will be transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Packard, 10 mL) will be added to each counting vial and shaken to mix the solution. The radiochemical content of each aliquot will be determined as described below.

Table 3. Optimized Aromatase Assay Conditions

Assay factor (units)	Human Placental
Microsomal Protein (mg/mL)[a]	0.0125
NADPH (mM)[a]	0.3
[^{3}H]ASDN (nM)[a]	100
Incubation Time (min)	15
[a] Final concentrations	

Analysis of the samples will be performed using liquid scintillation spectrometry (LSS). Radioactivity found in the aqueous fractions represents ^3H_2O formed.

5.0 DETERMINATION OF THE RESPONSE OF AROMATASE ACTIVITY TO REFERENCE CHEMICALS

This task is similar in structure to Task 5 except that the test laboratories will use the microsomes prepared in laboratory 2 or in laboratory 4, according to the matrix shown below (Table 4). Ten inhibitor compounds will be tested, including the four inhibitor compounds in Task 5. The test is organized so that across the ten chemicals the allocation of microsome sources to reference chemicals is approximately balanced.

For each chemical (1 to 10) laboratory 2 (Battelle) will supply two of laboratories RTI, Battelle, WIL and In Vitro (sometimes itself and sometimes not) and laboratory 4 (In Vitro) will supply the other two. The distribution of microsomal preparations will differ for different chemicals. Let M_2 denote the microsomal preparation from laboratory 2 and let $\mathcal{M}_4$ denote the microsomal preparation from laboratory 4. The four laboratories will use microsomal preparations from the laboratories as shown in Table 4.

Table 4. Microsome Distribution and Testing Matrix

	Analysis Laboratory			
Chemical	**Laboratory 1 RTI**	**Laboratory 2 Battelle**	**Laboratory 3 WIL**	**Laboratory 4 In Vitro**
Chemical 1	M_2	M_2	$\mathcal{M}_4$	$\mathcal{M}_4$
Chemical 2	M_2	M_2	$\mathcal{M}_4$	$\mathcal{M}_4$
Chemical 3	$\mathcal{M}_4$	$\mathcal{M}_4$	M_2	M_2
Chemical 4	M_2	$\mathcal{M}_4$	M_2	$\mathcal{M}_4$
Chemical 5	$\mathcal{M}_4$	M_2	$\mathcal{M}_4$	M_2
Chemical 6	$\mathcal{M}_4$	$\mathcal{M}_4$	M_2	M_2
Chemical 7	M_2	$\mathcal{M}_4$	$\mathcal{M}_4$	M_2
Chemical 8	M_2	$\mathcal{M}_4$	$\mathcal{M}_4$	M_2
Chemical 9	$\mathcal{M}_4$	M_2	M_2	$\mathcal{M}_4$
Chemical 10	$\mathcal{M}_4$	M_2	M_2	$\mathcal{M}_4$

Each test laboratory will analyze five chemicals with microsomal preparation made in Laboratory 2 and five chemicals with microsomal preparation made in Laboratory 4. Across the 10 chemicals the laboratories are approximately balanced for comparison of M_2 and $\mathcal{M}_4$. Thus if there is no interaction between laboratory or microsome source and chemical, the laboratory effects are excluded from the comparison between microsomal preparations.

Each replicate will test the response of aromatase activity to the presence of eight concentrations of a reference chemical. The reference chemicals must be coded prior to distribution to the assaying technicians in order that the replicates are conducted blind for reference chemical identity. This task will be conducted in three independent replicates. All three replicates for a given reference chemical must be conducted by the same technician. However, the same technician is not required to perform the three replicates for all 10 reference chemicals. Multiple reference chemicals may be conducted by a single technician in a given day and, in that case, the control tubes may be shared across the reference chemical sets; however,

microsome source and reference chemical vehicle may not vary between the reference chemicals that share control tubes. Each replicate for a given reference chemical must be conducted entirely independently of the other replicates for that reference chemical. Thus, it is recommended that if multiple replicates are conducted on a given day by a single technician, those replicates should use different reference chemicals. Each reference chemical will be tested at eight concentrations and there will be three (triplicate) repetitions for each concentration of a given replicate. A single replicate study of a given reference chemical is described in Table 5.

Four types of control samples will be included for each replicate. These include:

- full enzyme (aromatase) activity controls (substrate, NADPH, propylene glycol, buffer, vehicle [used for preparation of reference chemical solutions] and microsomes)
- background activity controls (all components that are in the full aromatase activity controls, except NADPH)
- positive controls (all components that are in the full aromatase activity controls, except vehicle, and with the addition of 4-OH ASDN at 5×10^{-8} M)
- negative controls (all components that are in the full aromatase activity controls, except vehicle, and with the addition of lindane at 1×10^{-6} M).

Four test tubes of each type of control are included with each replicate and are treated the same as the other samples. The controls sets will be split so that two tubes (of each control type) are run at the beginning and two at the end of each replicate set.

The assay will be conducted as described in Section 4.0 with the following modification. Reference chemical solution (or vehicle) will be added to the mixture of propylene glycol, substrate, NADPH and buffer in a volume not to exceed 20 µL prior to preincubation of that mixture. The volume of buffer used will be adjusted so the total incubation volume remains at 2 mL.

After completion of the first replicate, the data will be reviewed and, if necessary, the concentration of reference chemical used in the second and third replicates can be adjusted. The decision whether to adjust test concentrations rests with the Study Director. The decision should be based on the results from the first replicate with the following guidelines in mind:

- If insolubility is observed at the high concentration (10^{-3} M), then set the highest concentration for the second and third replicates at the highest concentration that appeared to be soluble (limited to 10^{-4} or 10^{-5} M). Do not use a concentration lower than 10^{-5} M for the highest concentration tested.
- If the highest concentration to be tested is lowered to 10^{-4} or 10^{-5} M, then add mid-log concentration(s) near the estimated IC_{50} based on the replicate one results in order to keep eight concentrations in the test set.
- The lowest concentration to be tested is 10^{-10} M.

Table 5. Reference Chemical Study Design

Sample type	Repetitions (test tubes)	Description	Reference Chemical concentration (M final)
Full Enzyme Activity Control	4	Complete assay[a] with reference chemical vehicle control	N/A
Background Activity Control	4	Complete assay with reference chemical vehicle control omitting NADPH	N/A
Positive Control	4	Complete assay with positive control chemical (4-OH ASDN) added	5×10^{-8}
Negative Control	4	Complete assay with negative control chemical (lindane) added	1×10^{-6}
Reference Chemical Concentration 1	3	Complete assay with Reference Chemical added	1×10^{-3}
Reference Chemical Concentration 2	3	Complete assay with Reference Chemical added	1×10^{-4}
Reference Chemical Concentration 3	3	Complete assay with Reference Chemical added	1×10^{-5}
Reference Chemical Concentration 4	3	Complete assay with Reference Chemical added	1×10^{-6}
Reference Chemical Concentration 5	3	Complete assay with Reference Chemical added	1×10^{-7}
Reference Chemical Concentration 6	3	Complete assay with Reference Chemical added	1×10^{-8}
Reference Chemical Concentration 7	3	Complete assay with Reference Chemical added	1×10^{-9}
Reference Chemical Concentration 8	3	Complete assay with Reference Chemical added	1×10^{-10}

[a]The complete assay contains buffer, propylene glycol, microsomal protein, [^{3}H]ASDN and NADPH

6.0 DATA ANALYSIS

6.1 Aromatase Activity and Percent of Control Calculations

Relevant data are entered into the latest version of the spreadsheet Aromatase_Master_Versionx.y.xls (where x and y denote version number designation) for calculation of aromatase activity and percent of control. The version of the spreadsheet used will be included in the reports. A working document detailing the use of this spreadsheet has been distributed previously.

6.2 *Statistical Analyses*

6.2.1 Concentration Response Fits for the Reference Chemicals

For the reference chemicals, three independent replicates of the concentration response curve fit will be carried out.

For each replicate two repeat tubes of the full enzyme activity controls, the background activity controls and the positive and negative controls will be run prior to the to repetitions of the graded concentrations of the reference chemical and two repeat tubes of each control will be run following the repetition of the reference chemical. Three repetitions will be prepared for each concentration of the reference chemical.

For each repeat tube (full enzyme activity, background activity, positive, and negative controls and each reference chemical concentration) the Excel database spreadsheet will include total observed (uncorrected) disintegrations per minute (DPMs) per tube and total aromatase activity per tube. The DPM and aromatase activity values are corrected for the background DPMs, as measured by the average of the background activity control tubes. The aromatase activity is calculated as the corrected DPM, normalized by the specific activity of the [^{3}H]ASDN, the mg of protein of the aromatase, and the incubation time. The average (corrected) DPMs and aromatase activity across the four background activity control repeat tubes must necessarily be equal to 0 within each replicate.

For each tube percent of control is determined by dividing the background corrected aromatase activity for that tube by the average background corrected aromatase activity for the four full enzyme activity control tubes and multiplying by 100.

Concentration response trend curves will be fitted to the percent of control activity values within each of the repeat tubes at each reference chemical concentration. Concentration is expressed on the log scale. In agreement with past convention, logarithms will be common logarithms (i.e., base 10). Let X denote the logarithm of the concentration of reference chemical (e.g., if concentration = 10^{-5} then X = -5). Let

Y ≡ percent of control activity in the inhibitor tube
X ≡ logarithm (base 10) of the concentration
DAVG ≡ average DPMs across the repeat tubes with the same reference chemical concentration
T ≡ top of plateau
B ≡ bottom of plateau
β ≡ slope of the concentration response curve (β will be negative)
$\mu \equiv \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

The following concentration response curve will be fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$Y = B + (T-B)/[1 + [(T-B)/(50-B) -1]10^{(\mu-X)\beta}] + \varepsilon$$

where ε is the variation among repetitions, distributed with mean 0 and variance proportional to DAVG (based on Poisson distribution theory for radiation counts). The variance is approximately proportional to Y.

The response curve will be fitted by weighted least squares nonlinear regression analysis with weights equal to 1/Y. Model fits will be carried out using Prism software (Version 3 or higher).

Concentration response models will be fitted for each replicate test within each reference chemical. Based on the results of the fit within each replicate the extent of aromatase inhibition will be summarized as top (T), bottom (B), $\log_{10}IC_{50}$ (μ), and slope (β). The estimated T, B, $\log_{10}IC_{50}$, and β for a reference chemical will be (weighted) means across the replicates. The estimated overall standard errors will be based on the standard errors within each replicate and the replicate-to-replicate variability. The average values and standard errors of T, B, $\log_{10}IC_{50}$, or β and the replicate-to-replicate components of variation will be calculated based on one-way random effects analysis of variance model fits. For each reference chemical and replicate the estimated top (T), the within replicate standard error of T, bottom (B), the within replicate standard error of B, $\log_{10}IC_{50}$ (μ), the within replicate standard error of μ, the IC_{50}, the slope (β), the within replicate standard error of β, and the "Status" of each replicate of each response curve will be displayed in a table. The "Status" of each replicate of each response curve is indicated as:

- Complete curve – "inhibitor" – data are available up to at least 80% inhibition – Calculate IC_{50}.
- Incomplete curve – "presumed inhibitor" – Data are available up to at least 50% inhibition but not beyond 80% inhibition – Calculate IC_{50}.
- Incomplete curve -"equivocal" – Data are available to between 20% and 50% inhibition – Do not calculate IC_{50}.
- "No inhibition" – No data are available above 20% inhibition - Do not calculate IC_{50}.

6.2.2 Graphical and Analysis of Variance Comparisons Among Concentration Response Curve Fits

For each replicate the individual percent of control values will be plotted versus logarithm of the reference chemical concentration. The fitted concentration response curve will be superimposed on the plot. Individual plots will be prepared for each replicate.

Additional plots will be prepared to compare the percent of control activity values across replicates. For each replicate the average percent of control values will be plotted versus logarithm of reference chemical concentration on the same plot. Plotting symbols will distinguish among replicates. The fitted concentration response curves for each replicate will be superimposed on the plots. On a separate plot the average percent of control values for each replicate will be plotted versus logarithm of reference chemical concentration. The average

concentration response curve across replicates will be superimposed on the same plot. The average concentration response curve will be the unweighted average of the response curves within each replicate.

Top (T), bottom (B), slope (β) and $\log_{10}IC_{50}$ (μ) will be compared across replicates based on one-way random effects analysis of variance, treating the replicates as random effects. For each of T, B, β and μ, plots will be prepared that display the parameters within each replicate with associated 95% confidence intervals based on the within replicate standard error and the average across replicates with associated 95% confidence interval incorporating replicate-to-replicate variation.

6.2.3 Graphical and Analysis of Variance Comparisons of Full Enzyme Activity, Background Activity, Positive and Negative Control Percent of Control Across Reference Chemicals and Replicates

Within each replicate of each reference chemical quadruplicate repetitions will be made of the full enzyme activity control, background activity control, and negative and positive control tubes. Half the repetitions will be carried out at the beginning of the replicate and half at the end. If the conditions are consistent throughout the replicate test, the control tubes at the beginning should be equivalent to those at the end.

To assess whether this is the case the control responses will be adjusted for background DPMs, divided by the average of the (background adjusted) full enzyme activity control values, and expressed as percent of control. The average of the four background activity controls within a replicate must necessarily be 0 percent and the average of the four full enzyme activity controls within a replicate must necessarily be 100 percent. The full enzyme activity controls percent of control, the background activity controls percent of control, and the negative and positive controls percent of control values will be plotted across reference chemical and replicate within reference chemical, with plotting symbol distinguishing between beginning and end, and with reference line 0% (background activity control) or 100% (full enzyme activity control) respectively. These plots will display the extent of consistency across reference chemicals and replicates with respect to average value and variability and will provide comparisons of beginning versus end of each replicate. Additional plots will be prepared displaying the difference of the average of the first two percent of control values (i.e., those based on the "beginning" tubes) and the average of the last two percent of control values (i.e., those based on the "end" tubes) (end minus beginning) across reference chemicals and replicates within reference chemicals. Each plot will have a reference line of 0.

Three-factor mixed effects analysis of variance models will be fitted, separately for the full enzyme activity control, the background activity control, and the positive and negative control tubes. The fixed effect factors in the analysis of variance will be

- reference chemical,
- portion (beginning or end), and
- portion by reference chemical interaction.

The random effects will be

- replicate nested within reference chemical and
- portion by replicate within reference chemical interaction.

The residual error variation corresponds to repetition within reference chemical, replicate, and portion. The response will be percent of control. Since for the background activity and full enzyme activity controls the average of the repetitions within a reference chemical and replicate are constrained to be 0 and 100, respectively, by the way in which "percent of control" is defined, the variation associated with the reference chemical effect and the replication within reference chemcial effect are both necessarily constrained to be 0.

If the daily replicates are in control the portion main effect, the portion by reference chemical interaction, and the portion by replicate within reference chemical interaction should be nonsignificant. If the portion by reference chemical interaction is significant the nature of the effect will be assessed by comparing the portion effect (averaged across replicates) within each reference chemical to the portion main effect. If the portion by replicate within reference chemical interaction is significant the nature of the effect will be assessed by comparing the portion effect within each replicate within a reference chemical to the portion effect averaged across replicates within the same reference chemical. Simulataneity of inference will be adjusted for by Bonferroni's method.

6.2.4 Statistical Software

Concentration response curves will be fitted to the data using the non-linear regression analysis features in the PRISM statistical analysis package, Version 3 or higher. Supplemental statistical analyses and displays such as summary tables, graphical displays, analysis of variance, and multiple comparisons will be carried out using PRISM, the SAS statistical analysis system, Version 8 or higher, or other general purpose statistical packages (e.g., SPSS), as convenient.

6.2.5 Interlaboratory Statistical Analysis

The lead laboratory and each of the participating laboratories will carry out "intra-laboratory" statistical analyses based on their test data, according to this common statistical analysis plan, developed by the Data Coordination Center (Battelle). The Data Coordination Center will carry out the "inter-laboratory" statistical analysis. It will combine summary values developed in each of the intra-laboratory analyses to assess relationships among the laboratory results, the extent of laboratory-to-laboratory variation, and overall consensus estimates among the laboratories.

7.0 RETENTION OF RECORDS

All records that remain the responsibility of the testing laboratory will be retained in the archives for the life of the contract.

8.0 QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES

Quality control (QC) and quality assurance (QA) procedures will follow those outlined in the Quality Assurance Project Plan (QAPP) that was prepared for this study. The study will be conducted in compliance with the Federal Register, 40 CFR Part 160. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practices Standards.

9.0 REPORTS

Interim data summaries, draft and final reports will be submitted as described in Section 9.5 of the QAPP.

The data to be reported in the interim data summaries will include (but is not limited to) the following information: assay date and run number, technician code, chemical code and log chemical concentration, background corrected aromatase activity (for each control and reference chemical repetition), percent of control activity, IC_{50}, slope and graphs of activity versus log chemical concentration.

In addition, draft and final reports will contain tables and graphs, as appropriate, containing the results of the intra- and inter-laboratory statistical analyses described in Section 6 of this document.

10.0 STUDY RECORDS TO BE MAINTAINED

- All records that document the conduct of the laboratory experiments and results obtained, as well as the equipment and chemicals used
- Protocol and any Amendments
- List of any Protocol Deviations
- List of Standard Operating Procedures
- QAPP and any Amendments
- List of any QAPP Deviations

EPA Contract No.: 68-W-01-023
EPA Work Assignment No.: 4-16
Task Number: 7
RTI Study Code: An05-948

AMENDMENT 1

TITLE: WA 4-16 Task 7: Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories

SPONSOR: Battelle
505 King Ave.
Columbus, OH 43201

TESTING FACILITY: RTI International
DMPK
3040 Cornwallis Rd.
Research Triangle Park, NC 27709

PROPOSED EXPERIMENTAL START DATE: July 1, 2005
PROPOSED EXPERIMENTAL END DATE: August 30, 2005

Approved By:

James M. Mathews, Ph.D., DABT — Date 11-16-05
RTI Study Director

Jerry Johnson, Ph.D. DABT — Date 11/22/05
Work Assignment Leader, Battelle

David P. Houchens, Ph.D. — Date 11/22/05
EDSP Program Manager, Battelle

Reviewed By:

Kim Collier, B.A. — Date 11/17/05
RTI, Quality Assurance Specialist

Terri L. Pollock, B.A. — Date 11-22-05
EDSP Quality Assurance Manager, Battelle

Item 1

Section 2.2.1 Reference Chemical Formulation and Analysis

Is hereby amended as follows:

Addition of the following:

Analysis of the reference chemical stock solutions will occur before the laboratories use the formulations in the assay. The analytical method used to analyze each of the reference chemicals in the stock solutions will be gas chromatography (4-OH ASDN and aminoglutethimide), gas chromatography with flame ionization detection (lindane, fenarimol, dicofol, atrazine, and dibenz[a,h]anthracene), HPLC (ketoconazole, econazole, and chrysin), HPLC with UV-Vis detection (prochloraz), and a combination of mass spectrometry and gas chromatography with flame ionization detection (4-nonylphenol). The chemistry procedures and results will be given to the laboratories in reports prepared and submitted to the laboratories by the CR.

Justification

This change is made to clarify timing and methods of analysis and reporting procedures.

Item 2

Section 6.2.1 Concentration Response Fits for the Reference Chemicals, paragraphs 5-7, which read:

Concentration response trend curves will be fitted to the percent of control activity values within each of the repeat tubes at each reference chemical concentration. Concentration is expressed on the log scale. In agreement with past convention, logarithms will be common logarithms (i.e., base 10). Let X denote the logarithm of the concentration of reference chemical (e.g., if concentration = 10^{-5} then X = -5). Let

Y ≡ percent of control activity in the inhibitor tube
X ≡ logarithm (base 10) of the concentration
DAVG ≡ average DPMs across the repeat tubes with the same reference chemical concentration
T ≡ top of plateau
B ≡ bottom of plateau
β ≡ slope of the concentration response curve (β will be negative)
$\mu \equiv \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

The following concentration response curve will be fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$Y = B + (T-B)/[1 + [(T-B)/(50-B) -1]10^{(\mu-X)\beta}] + \varepsilon$$

where ε is the variation among repetitions, distributed with mean 0 and variance proportional to DAVG (based on Poisson distribution theory for radiation counts). The variance is approximately proportional to Y.

The response curve will be fitted by weighted least squares nonlinear regression analysis with weights equal to 1/Y. Model fits will be carried out using Prism software (Version 3 or higher).

Is hereby amended as follows: (changes in **bold**)

Concentration response trend curves will be fitted to the percent of control activity values within each of the repeat tubes at each reference chemical concentration. Concentration is expressed on the log scale. In agreement with past convention, logarithms will be common logarithms (i.e., base 10). Let X denote the logarithm of the concentration of reference chemical (e.g., if concentration = 10^{-5} then X = -5). Let

Y ≡ percent of control activity in the inhibitor tube
X ≡ logarithm (base 10) of the concentration
T ≡ top of plateau
B ≡ bottom of plateau
H ≡ slope of the concentration response curve (H will be negative)
$\mu \equiv \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

The following concentration response curve will be fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$\mathbf{Y = B + (T-B)/[1 + 10^{(X-\mu)H}]}$$

The variance is approximately proportional to Y.

The response curve will be fitted by **nonweighted** least squares nonlinear regression analysis. Model fits will be carried out using Prism software (Version 3 or higher).

Justification

This change is made at the direction of the Sponsor.

EPA Contract No.: 68-W-01-023
EPA Work Assignment No.: 4-16
Task Number: 7
RTI Study Code: An05-948

AMENDMENT 2

TITLE: WA 4-16 Task 7: Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories

SPONSOR: Battelle
505 King Ave.
Columbus, OH 43201

TESTING FACILITY: RTI International
DMPK
3040 Cornwallis Rd.
Research Triangle Park, NC 27709

PROPOSED EXPERIMENTAL START DATE: July 1, 2005
PROPOSED EXPERIMENTAL END DATE: August 30, 2005

Approved By:

J M. Mathews 12-21-05

James M. Mathews, Ph.D., DABT Date
RTI Study Director

Jerry D Johnson 1-3-06

Jerry Johnson, Ph.D. DABT Date
Work Assignment Leader, Battelle

David P Houchens 1/6/06

David P. Houchens, Ph.D. Date
EDSP Program Manager, Battelle

Reviewed By:

K. Collier 12/19/05

Kim Collier, B.A. Date
RTI, Quality Assurance Specialist

Terri L Pollock 1-9-06

Terri L. Pollock, B.A. Date
EDSP Quality Assurance Manager, Battelle

Item 1

Section 6.2.1 Concentration Response Fits for the Reference Chemicals, paragraphs 5-7, which read (as amended in Protocol Amendment 1):

Concentration response trend curves will be fitted to the percent of control activity values within each of the repeat tubes at each reference chemical concentration. Concentration is expressed on the log scale. In agreement with past convention, logarithms will be common logarithms (i.e., base 10). Let X denote the logarithm of the concentration of reference chemical (e.g., if concentration = 10^{-5} then X = -5). Let

Y ≡ percent of control activity in the inhibitor tube
X ≡ logarithm (base 10) of theconcentration
T ≡ top of plateau
B ≡ bottom of plateau
H ≡ slope of the concentration response curve (H will be negative)
$\mu \equiv \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

The following concentration response curve will be fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$Y = B + (T-B)/[1 + 10^{(X-\mu)H}]$$

The variance is approximately proportional to Y.

The response curve will be fitted by nonweighted least squares nonlinear regression analysis. Model fits will be carried out using Prism software (Version 3 or higher).

Is hereby amended as follows: (changes in **bold**)

Concentration response trend curves will be fitted to the percent of control activity values within each of the repeat tubes at each reference chemical concentration. Concentration is expressed on the log scale. In agreement with past convention, logarithms will be common logarithms (i.e., base 10). Let X denote the logarithm of the concentration of reference chemical (e.g., if concentration = 10^{-5} then X = -5). Let

Y ≡ percent of control activity in the inhibitor tube
X ≡ logarithm (base 10) of the concentration
T ≡ top of plateau
B ≡ bottom of plateau
H ≡ slope of the concentration response curve (H will be negative)

$\mu \equiv \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

The following concentration response curve will be fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$Y = B + (T-B)/[1 + 10^{(\mu-X)H}]$$

The variance is approximately proportional to Y.

The response curve will be fitted by nonweighted least squares nonlinear regression analysis. Model fits will be carried out using Prism software (Version 3 or higher).

Justification

This change is made to correct an error in the equation.

ITEM 1

ORIGINAL DOCUMENT SPECIFICATIONS:

Page 9, Section 5.0 Determination Of The Response Of Aromatase Activity To Reference Chemicals

The highest concentration to be tested in the first replicate of each reference chemical is specified as 1E-3M. (Table 5).

DEVIATION:

The highest concentration tested in the first replicate of ketoconazole, dibenzanthracene and chrysin was 1E-4M. The solubility of these chemicals in DMSO limited the concentration of the stock solution supplied by Battelle to 1E-2M. Therefore, 1E-4M was the highest achievable concentration in the assay within the restriction of limiting the amount of organic solvent added to the assay to 20μL.

REASON/IMPACT OF CHANGE:
No impact.

ITEM 2

ORIGINAL DOCUMENT SPECIFICATIONS:

Page 9, Section 5.0 Determination Of The Response Of Aromatase Activity To Reference Chemicals

The highest concentration to be tested for any reference chemical is not to be lower than 1E-5M

DEVIATION:

The highest concentration tested in the replicates 2 and 3 of econazole was 1E-6M. This concentration was chosen based on data obtained in replicate 1 so as to allow sufficient other concentrations for description of the inhibition curve.

REASON/IMPACT OF CHANGE:
No impact.

J Matt
Study Director

3-15-06
Date

Appendix B
Radiochemical Analysis Report

FINAL ANALYSIS REPORT

PLACENTAL AROMATASE VALIDATION STUDY

[^{3}H]ASDN Radiochemical Purity Determination

EPA Contract Number 68-W-01-023
Work Assignment 4-16

Sponsor:

Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

Performing Laboratory:
Drug Metabolism and Pharmacokinetics
RTI International
Post Office Box 12194
Research Triangle Park, NC 27709

RTI INTERNATIONAL

FINAL REPORT

Title:	PLACENTAL AROMATASE VALIDATION STUDY [^{3}H]ASDN Radiochemical Purity Determination
Author:	Sherry Black
Performing Laboratory:	Drug Metabolism and Pharmacokinetics RTI International Post Office Box 12194 Research Triangle Park, NC 27709
Sponsor:	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693
Sponsor's Representatives:	David Houchens, Ph.D. EDSP Program Manager Battelle Jerry D. Johnson, Ph.D. Diplomate, A.B.T. Work Assignment Leader Battelle
Analysis Date:	January 5, 2005
Final Report Date	September 28, 2005

Author:

Sherry Black 9/28/05

Sherry Black Date
Research Chemist

Approved:

9-28-05

James Mathews, Ph. D, DABT Date
Study Director

RTI INTERNATIONAL

Quality Assurance Statement

Study Title:	**[^{3}H] ASDN Radiochemical Purity Determination WA 4-16 and WA 4-17**
Sponsor:	**Battelle Memorial Institute**
Study Code:	**An05-928**
Protocol Number:	**RTI-928-AN**

This study was audited by the Science and Engineering – Health Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the study director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Data and Report Audit	March 24, 2005	March 25, 2005

K. Collier
Quality Assurance Specialist

9/28/2005
Date

Approval:

Carrie Ingalls
Quality Assurance Assistant Manager

09/28/2005
Date

Introduction

The objective of this work is to determine the radiochemical purity of the [3H]ASDN to be used in the conduct of WA 4-16 and WA 4-17. The criteria for acceptance of the material for this use is 95% radiochemical purity as determined by high performance liquid chromatography (HPLC) and liquid scintillation counting.

Materials and Methods

[^{3}H]Androstenedione ([^{3}H]ASDN) of lot number 3538496 was received from Perkin Elmer Life Science (Boston, MA).

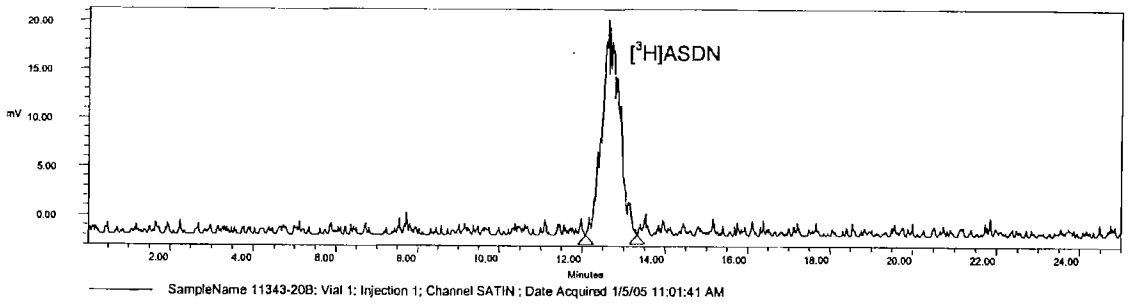
The radiochemical purity of the [^{3}H]ASDN (1:100 dilution in ethanol) was determined using high performance liquid chromatography (HPLC) and liquid scintillation counting. The HPLC system consists of a Waters 2690 Separations Module, a Waters 2487 Dual λ Absorbance Detector and a β-RAM Model 3 flow-through radioactivity detector (IN/US, Inc., Tampa, FL) with a 250 µL glass scintillant cell. Data was collected using Waters Millennium^{32} Client/Server Chromatography Data System Software, Version 4.0.

The HPLC method used a Zorbax Rx-C_{18} column (4.6 x 250 mm) with a mobile phase of 55:15:30 (v:v:v) distilled, deionized water: tetrahydrofuran: methanol and a flow rate of 1 mL/min. The eluant was monitored by ultraviolet (UV) absorbance at 240 nm and by a flow-through radiochemical detector. Eluant fractions were collected manually into vials containing ca. 10 mL Ultima Gold and assayed for radiochemical content by liquid scintillation spectrometry (LSS)

Results

The HPLC radiochromatogram of the [^{3}H]ASDN, lot number 3538496, is presented in Figure 1. The measured radiochemical purity of the [^{3}H]ASDN was 97%.

Figure 1. HPLC Radiochromatogram of [^{3}H]ASDN



Conclusion

[^{3}H]ASDN, lot number 3538496, is acceptable for use on WA 4-16 and WA 4-17.

Appendix C
Protein QC Standard Data Sheet

RTI International

Data Sheet

Sample 1:

Name: BSA Standard 100 µg/mL

Lot #: 11540-6A

Composition: Bovine serum albumin in 0.1 M sodium phosphate buffer, pH 7.4 with 0.1% sodium azide

Measured Concentration: 110 µg BSA/mL

Volume: 50 mL

Preparation Date: 7/8/2005

Expiration Date: TBD

Storage Conditions: 2-8 °C.

Sample 2:

Name: BSA Standard 10 µg/mL

Lot #: 11540-6B

Composition: Bovine serum albumin in 0.1 M sodium phosphate buffer, pH 7.4 with 0.1% sodium azide

Measured Concentration: 12 µg BSA/mL

Volume: 50 mL

Preparation Date: 7/8/2005

Expiration Date: TBD

Storage Conditions: 2-8 °C.

Sherry L Black

Sherry Black, Research Chemist

10/26/05

Date

Appendix D
Aromatase Assay Spreadsheets

Appendix D Notes

1. On each of the Protein spreadsheets, the units for standards are μg/mL.

Aromatase Assay Spreadsheet

Assay Date	7/14/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0205	33327	1625707	
2	0.0201	33085	1646020	
3	0.0201	33259	1654677	
4	0.0201	34255	1704229	
5	0.0196	33508	1709592	
			Average DPM/g soln	1668045
			SD	37053
			CV	2.22
			µCi/g soln	0.751

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0101 g
Mass of dilution B used in substrate prep	4.4826 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.559618 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00851 µg/g soln.
µg/g soln.

a. µCi/g soln	0.751
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.559618 + 0.00851
= 0.568124 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.323 µCi/µg ASDN

840887 dpm/nmol

Assay Date	7/14/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22

	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.547	0.357	0.234	0.175	0.129	0.120	0.111	25.2	100	
	0.534	0.350	0.253	0.183	0.140	0.118	0.114			
	0.537	0.376	0.239	0.183	0.143	0.119	0.118			

	11540-6A	11540-6B	11345-11I	11540-30C
Samples:	0.363	0.154	0.134	0.188
	0.350	0.134	0.138	0.187
			0.141	0.193

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0504	0.539	0.425	0.0483
125	100	200	0.00013	200	0.0252	0.361	0.247	0.0272
50	200	1000	0.00005	200	0.0101	0.242	0.128	0.0131
25	100	1000	0.00003	200	0.0050	0.180	0.066	0.0058
10	40	1000	0.00001	200	0.0020	0.137	0.023	0.0007
5	20	1000	0.00001	200	0.0010	0.119	0.005	-0.0014

Variables	Regression results	
m, b	0.118	-0.002
se_m, se_b	0.007	0.001
r^2, se_y	0.986	0.003
F, df	281	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

			r^2=	0.986
Blank	0.114		m=	0.118
			b=	-0.002

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.363	0.249	0.027	200	1	1	0.137	0.133	0.11
11540-6A	0.350	0.236	0.026	200	1	1	0.129		
11540-6A									
11540-6B	0.154	0.040	0.003	200	1	1	0.014	0.008	0.012
11540-6B	0.134	0.020	0.000	200	1	1	0.002		
11540-6B									
11345-11I	0.134	0.020	0.000	200	1	600	1.0	2.3	
11345-11I	0.138	0.024	0.001	200	1	600	2.4		
11345-11I	0.141	0.027	0.001	200	1	600	3.5		
11540-30C	0.188	0.074	0.007	200	1	800	26.9	27.6	
11540-30C	0.187	0.073	0.007	200	1	800	26.4		
11540-30C	0.193	0.079	0.007	200	1	800	29.3		

Assay Date	7/14/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.2	100	
	0.547	0.357	0.234	0.175	0.129	0.120	0.111			
	0.534	0.350	0.253	0.183	0.140	0.118	0.114			
	0.537	0.376	0.239	0.183	0.143	0.119	0.118			

Samples:	11540-6A	11540-6B	11345-11I	11540-30C
	0.363	0.154	0.134	0.188
	0.350	0.134	0.138	0.187
			0.141	0.193

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0504	0.539	0.425	0.0419
125	100	200	0.00013	200	0.0252	0.361	0.247	0.0240
50	200	1000	0.00005	200	0.0101	0.242	0.128	0.0121
25	100	1000	0.00003	200	0.0050	0.180	0.066	0.0059
10	40	1000	0.00001	200	0.0020	0.137	0.023	0.0016
5	20	1000	0.00001	200	0.0010	0.119	0.005	-0.0002

Variables		Regression results		
m, b		0.100		-0.001
se_m, se_b		0.008		0.001
r^2, se_y		0.980		0.002
F, df		145		3
ss_{reg}, ss_{resid}		0.000		0.000

Blank 0.114

r^2= 0.980
m= 0.100
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.363	0.249	0.024	200	1	1	0.121	0.118	0.11
11540-6A	0.350	0.236	0.023	200	1	1	0.114		
11540-6A									
11540-6B	0.154	0.040	0.003	200	1	1	0.016	0.011	0.012
11540-6B	0.134	0.020	0.001	200	1	1	0.006		
11540-6B									
11345-11I	0.134	0.020	0.001	200	1	600	3.8	4.9	
11345-11I	0.138	0.024	0.002	200	1	600	5.0		
11345-11I	0.141	0.027	0.002	200	1	600	5.9		
11540-30C	0.188	0.074	0.007	200	1	800	26.7	27.2	
11540-30C	0.187	0.073	0.007	200	1	800	26.3		
11540-30C	0.193	0.079	0.007	200	1	800	28.7		

Assay Date	7/14/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	1

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	27.2
Protein Concentration (dilution added to assay, mg/mL):	0.034032

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	7/14/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed						
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5515	11030	11300	22600	0.1	166804	13.55	22498	0.0268	1	0.017	15	0.0524
			0.5	2	5785	11570											
	2	2	0.5	1	5529	11058	11137	22274	0.1	166804	13.35	22172	0.0264	1	0.017	15	0.0517
			0.5	2	5608	11216											
	3	2	0.5	1	5300	10600	10683	21366	0.1	166804	12.81	21264	0.0253	1	0.017	15	0.0495
			0.5	2	5383	10766											
	4	2	0.5	1	5378	10756	10617	21234	0.1	166804	12.73	21132	0.0251	1	0.017	15	0.0492
			0.5	2	5239	10478											
Background control	1	2	0.5	1	34	68	50	100	0.1	166804	0.06	-2	0.0000	1	0.017	15	0.0000
			0.5	2	16	32											
	2	2	0.5	1	13	26	37	74	0.1	166804	0.04	-26	0.0000	1	0.017	15	-0.0001
			0.5	2	24	48											
	3	2	0.5	1	39	78	64	128	0.1	166804	0.08	26	0.0000	1	0.017	15	0.0001
			0.5	2	25	50											
	4	2	0.5	1	32	64	53	106	0.1	166804	0.06	4	0.0000	1	0.017	15	0.0000
			0.5	2	21	42											
Positive control	1	2	0.5	1	2809	5618	5643	11286	0.1	166804	6.77	11184	0.0133	1	0.017	15	0.0261
			0.5	2	2834	5668											
	2	2	0.5	1	2733	5466	5521	11042	0.1	166804	6.62	10940	0.0130	1	0.017	15	0.0255
			0.5	2	2788	5576											
	3	2	0.5	1	2383	4766	4725	9450	0.1	166804	5.67	9348	0.0111	1	0.017	15	0.0218
			0.5	2	2342	4684											
	4	2	0.5	1	2521	5042	5097	10194	0.1	166804	6.11	10092	0.0120	1	0.017	15	0.0235
			0.5	2	2576	5152											
Negative Control	1	2	0.5	1	5759	11518	11131	22262	0.1	166804	13.35	22160	0.0264	1	0.017	15	0.0516
			0.5	2	5372	10744											
	2	2	0.5	1	5464	10928	11032	22064	0.1	166804	13.23	21962	0.0261	1	0.017	15	0.0512
			0.5	2	5568	11136											
	3	2	0.5	1	5032	10064	10045	20090	0.1	166804	12.04	19988	0.0238	1	0.017	15	0.0466
			0.5	2	5013	10026											
	4	2	0.5	1	4917	9834	9962	19924	0.1	166804	11.94	19822	0.0236	1	0.017	15	0.0462
			0.5	2	5045	10090											
Ref Chem 1	1-1	2	0.5	1	315	630	601	1202	0.1	166804	0.72	1100	0.0013	1	0.017	15	0.0026
			0.5	2	286	572											
	1-2	2	0.5	1	289	578	572	1144	0.1	166804	0.69	1042	0.0012	1	0.017	15	0.0024
			0.5	2	283	566											
	1-3	2	0.5	1	304	608	593	1186	0.1	166804	0.71	1084	0.0013	1	0.017	15	0.0025
			0.5	2	289	578											
	2-1	2	0.5	1	2089	4178	4199	8398	0.1	166804	5.03	8296	0.0099	1	0.017	15	0.0193
			0.5	2	2110	4220											
	2-2	2	0.5	1	2120	4240	4223	8446	0.1	166804	5.06	8344	0.0099	1	0.017	15	0.0194
			0.5	2	2103	4206											
	2-3	2	0.5	1	2025	4050	4050	8100	0.1	166804	4.86	7998	0.0095	1	0.017	15	0.0186
			0.5	2	2025	4050											
	3-1	2	0.5	1	4574	9148	9239	18478	0.1	166804	11.08	18376	0.0219	1	0.017	15	0.0428
			0.5	2	4665	9330											
	3-2	2	0.5	1	4534	9068	8957	17914	0.1	166804	10.74	17812	0.0212	1	0.017	15	0.0415
			0.5	2	4423	8846											
	3-3	2	0.5	1	4243	8486	8548	17096	0.1	166804	10.25	16994	0.0202	1	0.017	15	0.0396
			0.5	2	4305	8610											
	4-1	2	0.5	1	5394	10788	10784	21568	0.1	166804	12.93	21466	0.0255	1	0.017	15	0.0500
			0.5	2	5390	10780											
	4-2	2	0.5	1	5232	10464	10491	20982	0.1	166804	12.58	20890	0.0248	1	0.017	15	0.0486
			0.5	2	5259	10518											
	4-3	2	0.5	1	5092	10184	10246	20492	0.1	166804	12.29	20390	0.0242	1	0.017	15	0.0475
			0.5	2	5154	10308											
	5-1	2	0.5	1	5534	11068	11070	22140	0.1	166804	13.27	22038	0.0262	1	0.017	15	0.0513
			0.5	2	5536	11072											
	5-2	2	0.5	1	5146	10292	10515	21030	0.1	166804	12.61	20928	0.0249	1	0.017	15	0.0488
			0.5	2	5369	10738											
	5-3	2	0.5	1	5211	10422	10453	20906	0.1	166804	12.53	20804	0.0247	1	0.017	15	0.0485
			0.5	2	5242	10484											
	6-1	2	0.5	1	5481	10962	10942	21884	0.1	166804	13.12	21782	0.0259	1	0.017	15	0.0507
			0.5	2	5461	10922											
	6-2	2	0.5	1	5248	10496	10401	20802	0.1	166804	12.47	20700	0.0246	1	0.017	15	0.0482
			0.5	2	5153	10306											
	6-3	2	0.5	1	5159	10318	10204	20408	0.1	166804	12.23	20306	0.0241	1	0.017	15	0.0473
			0.5	2	5045	10090											
	7-1	2	0.5	1	5364	10728	10699	21398	0.1	166804	12.83	21296	0.0253	1	0.017	15	0.0496
			0.5	2	5335	10670											
	7-2	2	0.5	1	5219	10438	10422	20844	0.1	166804	12.50	20742	0.0247	1	0.017	15	0.0483
			0.5	2	5203	10406											
	7-3	2	0.5	1	5124	10248	10175	20350	0.1	166804	12.20	20248	0.0241	1	0.017	15	0.0472
			0.5	2	5051	10102											
	8-1	2	0.5	1	5396	10792	10782	21564	0.1	166804	12.93	21462	0.0255	1	0.017	15	0.0500
			0.5	2	5386	10772											
	8-2	2	0.5	1	4823	9646	9935	19870	0.1	166804	11.91	19768	0.0235	1	0.017	15	0.0461
			0.5	2	5112	10224											
	8-3	2	0.5	1	5142	10284	10284	20568	0.1	166804	12.33	20466	0.0243	1	0.017	15	0.0477
			0.5	2	5142	10284											

Assay Date	7/14/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0520	0.0005
Full activity	End	0.0494	0.0002
Full activity	Overall	0.0507	0.0016
Background	Beginning	0.0000	4.28298E-05
Background	End	0.0000	3.62406E-05
Background	Overall	0.0000	5.17438E-05
Positive	Beginning	0.0258	0.0004
Positive	End	0.0226	0.0012
Positive	Overall	0.0242	0.0020
Negative	Beginning	0.0514	0.0003
Negative	End	0.0464	0.0003
Negative	Overall	0.0489	0.0029

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 1	1	1	1.00E-03	-3.00	0.0026
Ref Chem 1	1	2	1.00E-03	-3.00	0.0024
Ref Chem 1	1	3	1.00E-03	-3.00	0.0025
Ref Chem 1	2	1	1.00E-04	-4.00	0.0193
Ref Chem 1	2	2	1.00E-04	-4.00	0.0194
Ref Chem 1	2	3	1.00E-04	-4.00	0.0186
Ref Chem 1	3	1	1.00E-05	-5.00	0.0428
Ref Chem 1	3	2	1.00E-05	-5.00	0.0415
Ref Chem 1	3	3	1.00E-05	-5.00	0.0396
Ref Chem 1	4	1	1.00E-06	-6.00	0.0500
Ref Chem 1	4	2	1.00E-06	-6.00	0.0486
Ref Chem 1	4	3	1.00E-06	-6.00	0.0475
Ref Chem 1	5	1	1.00E-07	-7.00	0.0513
Ref Chem 1	5	2	1.00E-07	-7.00	0.0488
Ref Chem 1	5	3	1.00E-07	-7.00	0.0485
Ref Chem 1	6	1	1.00E-08	-8.00	0.0507
Ref Chem 1	6	2	1.00E-08	-8.00	0.0482
Ref Chem 1	6	3	1.00E-08	-8.00	0.0473
Ref Chem 1	7	1	1.00E-09	-9.00	0.0496
Ref Chem 1	7	2	1.00E-09	-9.00	0.0483
Ref Chem 1	7	3	1.00E-09	-9.00	0.0472
Ref Chem 1	8	1	1.00E-10	-10.00	0.0500
Ref Chem 1	8	2	1.00E-10	-10.00	0.0461
Ref Chem 1	8	3	1.00E-10	-10.00	0.0477

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	5.05	4.79	4.98
2	-4.00	38.11	38.33	36.74
3	-5.00	84.42	81.83	78.07
4	-6.00	98.62	95.93	93.68
5	-7.00	101.25	96.15	95.58
6	-8.00	100.07	95.10	93.29
7	-9.00	97.84	95.29	93.02
8	-10.00	98.60	90.82	94.03

Aromatase Assay Spreadsheet

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8		
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0203	39034	1922857	
2	0.0198	37462	1892020	
3	0.0199	37954	1907236	
4	0.0198	39619	2000960	
5	0.0199	39396	1979698	
			Average DPM/g soln	1940554
			SD	47328
			CV	2.44
			µCi/g soln	0.874

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1346 g
Mass of dilution B used in substrate prep	4.5842 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563543 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00990 µg/g soln.
µg/g soln.

a. µCi/g soln 0.874
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.563543 + 0.00990
= 0.573439 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.524 µCi/µg ASDN

969197 dpm/nmol

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.6	100	
	0.534	0.398	0.235	0.172	0.143	0.119	0.112			
	0.573	0.395	0.236	0.178	0.147	0.133	0.118			
	0.574	0.397	0.244	0.175	0.138	0.119	0.117			

Samples:	11540-6A	11540-6B	11345-12I	11540-31C
	0.351	0.135	0.139	0.183
	0.349	0.147	0.131	0.192
			0.136	0.189

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00026	200	0.0512	0.560	0.445	0.0482	m, b	0.112	-0.002
125	100	200	0.00013	200	0.0256	0.397	0.281	0.0298	se_m, se_b	0.008	0.002
50	200	1000	0.00005	200	0.0102	0.238	0.123	0.0120	r^2, se_y	0.982	0.003
25	100	1000	0.00003	200	0.0051	0.175	0.059	0.0049	F, df	219	4
10	40	1000	0.00001	200	0.0020	0.143	0.027	0.0013	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.124	0.008	-0.0009			
			Blank	0.116	r^2=	0.982					
					m=	0.112					
					b=	-0.002					

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.351	0.235	0.025	200	1	1	0.123	0.123	0.11
11540-6A	0.349	0.233	0.024	200	1	1	0.122		
11540-6A									
11540-6B	0.135	0.019	0.000	200	1	1	0.002	0.005	0.012
11540-6B	0.147	0.031	0.002	200	1	1	0.009		
11540-6B									
11345-12I	0.139	0.023	0.001	200	1	600	2.5	1.3	
11345-12I	0.131	0.015	0.000	200	1	600	-0.2		
11345-12I	0.136	0.020	0.001	200	1	600	1.5		
11540-31C	0.183	0.067	0.006	200	1	800	23.1	25.4	
11540-31C	0.192	0.076	0.007	200	1	800	27.2		
11540-31C	0.189	0.073	0.006	200	1	800	25.8		

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.534	0.398	0.235	0.172	0.143	0.119	0.112	25.6	100	
	0.573	0.395	0.236	0.178	0.147	0.133	0.118			
	0.574	0.397	0.244	0.175	0.138	0.119	0.117			

Samples:	11540-6A	11540-6B	11345-12I	11540-31C
	0.351	0.135	0.139	0.183
	0.349	0.147	0.131	0.192
			0.136	0.189

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00026	200	0.0512	0.560	0.445	0.0402	m, b	0.091	0.000
125	100	200	0.00013	200	0.0256	0.397	0.281	0.0253	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0102	0.238	0.123	0.0109	r^2, se_y	0.998	0.001
25	100	1000	0.00003	200	0.0051	0.175	0.059	0.0051	F, df	1416	3
10	40	1000	0.00001	200	0.0020	0.143	0.027	0.0022	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.124	0.008	0.0005			
			Blank	0.116	r^2=	0.998					
					m=	0.091					
					b=	0.000					

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.351	0.235	0.021	200	1	1	0.106	0.105	0.11
11540-6A	0.349	0.233	0.021	200	1	1	0.105		
11540-6A									
11540-6B	0.135	0.019	0.002	200	1	1	0.008	0.010	0.012
11540-6B	0.147	0.031	0.003	200	1	1	0.013		
11540-6B									
11345-12I	0.139	0.023	0.002	200	1	600	5.6	4.6	
11345-12I	0.131	0.015	0.001	200	1	600	3.4		
11345-12I	0.136	0.020	0.002	200	1	600	4.8		
11540-31C	0.183	0.067	0.006	200	1	800	23.5	25.3	
11540-31C	0.192	0.076	0.007	200	1	800	26.8		
11540-31C	0.189	0.073	0.006	200	1	800	25.7		

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	2

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	25.3
Protein Concentration (dilution added to assay, mg/mL):	0.031632

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	5.00E-04
3	2.50E-04
4	1.00E-04
5	5.00E-05
6	1.00E-05
7	1.00E-06
8	1.00E-07

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5972	11944	11710	23420	0.1	194055	12.07	23291	0.0240	1	0.016	15	0.0505
			0.5	2	5738	11476											
	2	2	0.5	1	6120	12240	12081	24162	0.1	194055	12.45	24033	0.0248	1	0.016	15	0.0523
			0.5	2	5961	11922											
	3	2	0.5	1	5472	10944	10688	21376	0.1	194055	11.02	21247	0.0219	1	0.016	15	0.0462
			0.5	2	5216	10432											
	4	2	0.5	1	5331	10662	10522	21044	0.1	194055	10.84	20915	0.0216	1	0.016	15	0.0455
			0.5	2	5191	10382											
Background control	1	2	0.5	1	20	40	58	116	0.1	194055	0.06	-13	0.0000	1	0.016	15	0.0000
			0.5	2	38	76											
	2	2	0.5	1	17	34	51	102	0.1	194055	0.05	-27	0.0000	1	0.016	15	-0.0001
			0.5	2	34	68											
	3	2	0.5	1	46	92	79	158	0.1	194055	0.08	29	0.0000	1	0.016	15	0.0001
			0.5	2	33	66											
	4	2	0.5	1	37	74	70	140	0.1	194055	0.07	11	0.0000	1	0.016	15	0.0000
			0.5	2	33	66											
Positive control	1	2	0.5	1	3343	6686	6514	13028	0.1	194055	6.71	12899	0.0133	1	0.016	15	0.0280
			0.5	2	3171	6342											
	2	2	0.5	1	2995	5990	5976	11952	0.1	194055	6.16	11823	0.0122	1	0.016	15	0.0257
			0.5	2	2981	5962											
	3	2	0.5	1	2747	5494	5521	11042	0.1	194055	5.69	10913	0.0113	1	0.016	15	0.0237
			0.5	2	2774	5548											
	4	2	0.5	1	2723	5446	5390	10780	0.1	194055	5.56	10651	0.0110	1	0.016	15	0.0232
			0.5	2	2667	5334											
Negative Control	1	2	0.5	1	6121	12242	11914	23828	0.1	194055	12.28	23699	0.0245	1	0.016	15	0.0515
			0.5	2	5793	11586											
	2	2	0.5	1	5815	11630	11616	23232	0.1	194055	11.97	23103	0.0238	1	0.016	15	0.0502
			0.5	2	5801	11602											
	3	2	0.5	1	5247	10494	10358	20716	0.1	194055	10.68	20587	0.0212	1	0.016	15	0.0448
			0.5	2	5111	10222											
	4	2	0.5	1	5048	10096	10162	20324	0.1	194055	10.47	20195	0.0208	1	0.016	15	0.0439
			0.5	2	5114	10228											
Ref Chem 1	1-1	2	0.5	1	333	666	638	1276	0.1	194055	0.66	1147	0.0012	1	0.016	15	0.0025
			0.5	2	305	610											
	1-2	2	0.5	1	353	706	684	1368	0.1	194055	0.70	1239	0.0013	1	0.016	15	0.0027
			0.5	2	331	662											
	1-3	2	0.5	1	374	748	727	1454	0.1	194055	0.75	1325	0.0014	1	0.016	15	0.0029
			0.5	2	353	706											
	2-1	2	0.5	1	699	1398	1421	2842	0.1	194055	1.46	2713	0.0028	1	0.016	15	0.0059
			0.5	2	722	1444											
	2-2	2	0.5	1	709	1418	1388	2776	0.1	194055	1.43	2647	0.0027	1	0.016	15	0.0058
			0.5	2	679	1358											
	2-3	2	0.5	1	661	1322	1370	2740	0.1	194055	1.41	2611	0.0027	1	0.016	15	0.0057
			0.5	2	709	1418											
	3-1	2	0.5	1	1261	2522	2523	5046	0.1	194055	2.60	4917	0.0051	1	0.016	15	0.0107
			0.5	2	1262	2524											
	3-2	2	0.5	1	1299	2598	2584	5168	0.1	194055	2.66	5039	0.0052	1	0.016	15	0.0110
			0.5	2	1285	2570											
	3-3	2	0.5	1	1284	2568	2517	5034	0.1	194055	2.59	4905	0.0051	1	0.016	15	0.0107
			0.5	2	1233	2466											
	4-1	2	0.5	1	2369	4738	4671	9342	0.1	194055	4.81	9213	0.0095	1	0.016	15	0.0200
			0.5	2	2302	4604											
	4-2	2	0.5	1	2304	4608	4600	9200	0.1	194055	4.74	9071	0.0094	1	0.016	15	0.0197
			0.5	2	2296	4592											
	4-3	2	0.5	1	2266	4532	4537	9074	0.1	194055	4.68	8945	0.0092	1	0.016	15	0.0195
			0.5	2	2271	4542											
	5-1	2	0.5	1	3330	6660	6729	13458	0.1	194055	6.94	13329	0.0138	1	0.016	15	0.0290
			0.5	2	3399	6798											
	5-2	2	0.5	1	2998	5996	6041	12082	0.1	194055	6.23	11953	0.0123	1	0.016	15	0.0260
			0.5	2	3043	6086											
	5-3	2	0.5	1	3229	6458	6392	12784	0.1	194055	6.59	12655	0.0131	1	0.016	15	0.0275
			0.5	2	3163	6326											
	6-1	2	0.5	1	5123	10246	10203	20406	0.1	194055	10.52	20277	0.0209	1	0.016	15	0.0441
			0.5	2	5080	10160											
	6-2	2	0.5	1	4998	9996	10041	20082	0.1	194055	10.35	19953	0.0206	1	0.016	15	0.0434
			0.5	2	5043	10086											
	6-3	2	0.5	1	4866	9732	9754	19508	0.1	194055	10.05	19379	0.0200	1	0.016	15	0.0421
			0.5	2	4888	9776											
	7-1	2	0.5	1	5834	11668	11510	23020	0.1	194055	11.86	22891	0.0236	1	0.016	15	0.0498
			0.5	2	5676	11352											
	7-2	2	0.5	1	5734	11468	11315	22630	0.1	194055	11.66	22501	0.0232	1	0.016	15	0.0489
			0.5	2	5581	11162											
	7-3	2	0.5	1	5609	11218	11168	22336	0.1	194055	11.51	22207	0.0229	1	0.016	15	0.0483
			0.5	2	5559	11118											
	8-1	2	0.5	1	5686	11372	11439	22878	0.1	194055	11.79	22749	0.0235	1	0.016	15	0.0495
			0.5	2	5753	11506											
	8-2	2	0.5	1	5720	11440	11398	22796	0.1	194055	11.75	22667	0.0234	1	0.016	15	0.0493
			0.5	2	5678	11356											
	8-3	2	0.5	1	5600	11200	11091	22182	0.1	194055	11.43	22053	0.0228	1	0.016	15	0.0480
			0.5	2	5491	10982											

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0515	0.0011
Full activity	End	0.0458	0.0005
Full activity	Overall	0.0486	0.0033
Background	Beginning	0.0000	2.15271E-05
Background	End	0.0000	2.76777E-05
Background	Overall	0.0000	5.41463E-05
Positive	Beginning	0.0269	0.0017
Positive	End	0.0234	0.0004
Positive	Overall	0.0252	0.0022
Negative	Beginning	0.0509	0.0009
Negative	End	0.0443	0.0006
Negative	Overall	0.0476	0.0038

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 1	1	1	1.00E-03	-3.00	0.0025
Ref Chem 1	1	2	1.00E-03	-3.00	0.0027
Ref Chem 1	1	3	1.00E-03	-3.00	0.0029
Ref Chem 1	2	1	5.00E-04	-3.30	0.0059
Ref Chem 1	2	2	5.00E-04	-3.30	0.0058
Ref Chem 1	2	3	5.00E-04	-3.30	0.0057
Ref Chem 1	3	1	2.50E-04	-3.60	0.0107
Ref Chem 1	3	2	2.50E-04	-3.60	0.0110
Ref Chem 1	3	3	2.50E-04	-3.60	0.0107
Ref Chem 1	4	1	1.00E-04	-4.00	0.0200
Ref Chem 1	4	2	1.00E-04	-4.00	0.0197
Ref Chem 1	4	3	1.00E-04	-4.00	0.0195
Ref Chem 1	5	1	5.00E-05	-4.30	0.0290
Ref Chem 1	5	2	5.00E-05	-4.30	0.0260
Ref Chem 1	5	3	5.00E-05	-4.30	0.0275
Ref Chem 1	6	1	1.00E-05	-5.00	0.0441
Ref Chem 1	6	2	1.00E-05	-5.00	0.0434
Ref Chem 1	6	3	1.00E-05	-5.00	0.0421
Ref Chem 1	7	1	1.00E-06	-6.00	0.0498
Ref Chem 1	7	2	1.00E-06	-6.00	0.0489
Ref Chem 1	7	3	1.00E-06	-6.00	0.0483
Ref Chem 1	8	1	1.00E-07	-7.00	0.0495
Ref Chem 1	8	2	1.00E-07	-7.00	0.0493
Ref Chem 1	8	3	1.00E-07	-7.00	0.0480

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	5.13	5.54	5.92
2	-3.30	12.13	11.83	11.67
3	-3.60	21.98	22.52	21.93
4	-4.00	41.18	40.55	39.98
5	-4.30	59.58	53.43	56.57
6	-5.00	90.64	89.19	86.62
7	-6.00	102.32	100.58	99.26
8	-7.00	101.69	101.32	98.58

Aromatase Assay Spreadsheet

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0210	41364	1969714	
2	0.0212	38688	1824906	
3	0.0209	39457	1887895	
4	0.0208	41815	2010337	
5	0.0210	41647	1983190	
			Average DPM/g soln	1935208
			SD	76718
			CV	3.96
			µCi/g soln	0.872

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.123 g
Mass of dilution B used in substrate prep	4.5644 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.561911 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00987 µg/g soln.
µg/g soln.

a. µCi/g soln 0.872
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.561911 + 0.00987
= 0.571779 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.525 µCi/µg ASDN

969333 dpm/nmol

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8						
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.579	0.382	0.250	0.188	0.138	0.123	0.112	25.4	100	
	0.577	0.383	0.243	0.185	0.137	0.124	0.109			
	0.581	0.385	0.245	0.182	0.139	0.126	0.116			

Samples:	11540-6A	11540-6B	11540-32C	11345-13I
	0.366	0.144	0.189	0.143
	0.365	0.154	0.197	0.133
			0.188	0.139

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0508	0.579	0.467	0.0489	m, b	0.109	-0.002
125	100	200	0.00013	200	0.0254	0.383	0.271	0.0275	se_m, se_b	0.006	0.001
50	200	1000	0.00005	200	0.0102	0.246	0.134	0.0125	r^2, se_y	0.990	0.002
25	100	1000	0.00003	200	0.0051	0.185	0.073	0.0058	F, df	386	4
10	40	1000	0.00001	200	0.0020	0.138	0.026	0.0006	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.124	0.012	-0.0009			

Regression results are calculated using the function LINEST

	Blank	0.112	r^2=	0.990
			m=	0.109
			b=	-0.002

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.366	0.254	0.026	200	1	1	0.128	0.128	0.11
11540-6A	0.365	0.253	0.025	200	1	1	0.127		
11540-6A									
11540-6B	0.144	0.032	0.001	200	1	1	0.007	0.009	0.012
11540-6B	0.154	0.042	0.002	200	1	1	0.012		
11540-6B									
11540-32C	0.189	0.077	0.006	200	1	800	24.9	25.9	
11540-32C	0.197	0.085	0.007	200	1	800	28.4		
11540-32C	0.188	0.076	0.006	200	1	800	24.5		
11345-13I	0.143	0.031	0.001	200	1	600	3.6	2.0	
11345-13I	0.133	0.021	0.000	200	1	600	0.3		
11345-13I	0.139	0.027	0.001	200	1	600	2.3		

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8						
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.579	0.382	0.250	0.188	0.138	0.123	0.112	25.4	100	
	0.577	0.383	0.243	0.185	0.137	0.124	0.109			
	0.581	0.385	0.245	0.182	0.139	0.126	0.116			

Samples:	11540-6A	11540-6B	11540-32C	11345-13I
	0.366	0.144	0.189	0.143
	0.365	0.154	0.197	0.133
			0.188	0.139

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0508	0.579	0.467	0.0430
125	100	200	0.00013	200	0.0254	0.383	0.271	0.0246
50	200	1000	0.00005	200	0.0102	0.246	0.134	0.0116
25	100	1000	0.00003	200	0.0051	0.185	0.073	0.0059
10	40	1000	0.00001	200	0.0020	0.138	0.026	0.0015
5	20	1000	0.00001	200	0.0010	0.124	0.012	0.0002

Variables	Regression results	
m, b	0.094	-0.001
se_m, se_b	0.006	0.001
r^2, se_y	0.989	0.001
F, df	259	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

Blank	0.112	r^2=	0.989
		m=	0.094
		b=	-0.001

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.366	0.254	0.023	200	1	1	0.115	0.114	0.11
11540-6A	0.365	0.253	0.023	200	1	1	0.114		
11540-6A									
11540-6B	0.144	0.032	0.002	200	1	1	0.010	0.012	0.012
11540-6B	0.154	0.042	0.003	200	1	1	0.015		
11540-6B									
11540-32C	0.189	0.077	0.006	200	1	800	25.0	25.9	
11540-32C	0.197	0.085	0.007	200	1	800	28.0		
11540-32C	0.188	0.076	0.006	200	1	800	24.7		
11345-13I	0.143	0.031	0.002	200	1	600	5.8	4.5	
11345-13I	0.133	0.021	0.001	200	1	600	3.0		
11345-13I	0.139	0.027	0.002	200	1	600	4.7		

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	3

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	25.9
Protein Concentration (dilution added to assay, mg/mL):	0.03239

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	5.00E-04
3	2.50E-04
4	1.00E-04
5	5.00E-05
6	1.00E-05
7	1.00E-06
8	1.00E-07

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	6384	12768	12805	25612	0.1	193521	13.23	25120	0.0259	1	0.016	15	0.0533
			0.5	2	6422	12844											
	2	2	0.5	1	6618	13236	13132	26264	0.1	193521	13.57	25772	0.0266	1	0.016	15	0.0547
			0.5	2	6514	13028											
	3	2	0.5	1	5549	11098	11103	22206	0.1	193521	11.47	21714	0.0224	1	0.016	15	0.0461
			0.5	2	5554	11108											
	4	2	0.5	1	5653	11306	11225	22450	0.1	193521	11.60	21958	0.0227	1	0.016	15	0.0466
			0.5	2	5572	11144											
Background control	1	2	0.5	1	38	76	67	134	0.1	193521	0.07	-359	-0.0004	1	0.016	15	-0.0008
			0.5	2	29	58											
	2	2	0.5	1	31	62	73	146	0.1	193521	0.08	-347	-0.0004	1	0.016	15	-0.0007
			0.5	2	42	84											
	3	2	0.5	1	217	434	426	852	0.1	193521	0.44	360	0.0004	1	0.016	15	0.0008
			0.5	2	209	418											
	4	2	0.5	1	206	412	419	838	0.1	193521	0.43	346	0.0004	1	0.016	15	0.0007
			0.5	2	213	426											
Positive control	1	2	0.5	1	3191	6382	6460	12920	0.1	193521	6.68	12428	0.0128	1	0.016	15	0.0264
			0.5	2	3269	6538											
	2	2	0.5	1	3010	6020	5957	11914	0.1	193521	6.16	11422	0.0118	1	0.016	15	0.0243
			0.5	2	2947	5894											
	3	2	0.5	1	2677	5354	5415	10830	0.1	193521	5.60	10338	0.0107	1	0.016	15	0.0220
			0.5	2	2738	5476											
	4	2	0.5	1	2889	5778	5698	11396	0.1	193521	5.89	10904	0.0112	1	0.016	15	0.0232
			0.5	2	2809	5618											
Negative Control	1	2	0.5	1	6248	12496	12540	25080	0.1	193521	12.96	24588	0.0254	1	0.016	15	0.0522
			0.5	2	6292	12584											
	2	2	0.5	1	6156	12312	12380	24760	0.1	193521	12.79	24268	0.0250	1	0.016	15	0.0515
			0.5	2	6224	12448											
	3	2	0.5	1	5297	10594	10750	21500	0.1	193521	11.11	21008	0.0217	1	0.016	15	0.0446
			0.5	2	5453	10906											
	4	2	0.5	1	5364	10728	10592	21184	0.1	193521	10.95	20692	0.0213	1	0.016	15	0.0439
			0.5	2	5228	10456											
Ref Chem 1	1-1	2	0.5	1	344	688	699	1398	0.1	193521	0.72	906	0.0009	1	0.016	15	0.0019
			0.5	2	355	710											
	1-2	2	0.5	1	357	714	662	1324	0.1	193521	0.68	832	0.0009	1	0.016	15	0.0018
			0.5	2	305	610											
	1-3	2	0.5	1	346	692	665	1330	0.1	193521	0.69	838	0.0009	1	0.016	15	0.0018
			0.5	2	319	638											
	2-1	2	0.5	1	746	1492	1463	2926	0.1	193521	1.51	2434	0.0025	1	0.016	15	0.0052
			0.5	2	717	1434											
	2-2	2	0.5	1	725	1450	1500	3000	0.1	193521	1.55	2508	0.0026	1	0.016	15	0.0053
			0.5	2	775	1550											
	2-3	2	0.5	1	686	1372	1419	2838	0.1	193521	1.47	2346	0.0024	1	0.016	15	0.0050
			0.5	2	733	1466											
	3-1	2	0.5	1	1323	2646	2643	5286	0.1	193521	2.73	4794	0.0049	1	0.016	15	0.0102
			0.5	2	1320	2640											
	3-2	2	0.5	1	1248	2496	2456	4912	0.1	193521	2.54	4420	0.0046	1	0.016	15	0.0094
			0.5	2	1208	2416											
	3-3	2	0.5	1	1367	2734	2695	5390	0.1	193521	2.79	4898	0.0051	1	0.016	15	0.0104
			0.5	2	1328	2656											
	4-1	2	0.5	1	2506	5012	5004	10008	0.1	193521	5.17	9516	0.0098	1	0.016	15	0.0202
			0.5	2	2498	4996											
	4-2	2	0.5	1	2441	4882	4925	9850	0.1	193521	5.09	9358	0.0097	1	0.016	15	0.0199
			0.5	2	2484	4968											
	4-3	2	0.5	1	2438	4876	4902	9804	0.1	193521	5.07	9312	0.0096	1	0.016	15	0.0198
			0.5	2	2464	4928											
	5-1	2	0.5	1	3473	6946	6960	13920	0.1	193521	7.19	13428	0.0139	1	0.016	15	0.0285
			0.5	2	3487	6974											
	5-2	2	0.5	1	3209	6418	6657	13314	0.1	193521	6.88	12822	0.0132	1	0.016	15	0.0272
			0.5	2	3448	6896											
	5-3	2	0.5	1	3223	6446	6535	13070	0.1	193521	6.75	12578	0.0130	1	0.016	15	0.0267
			0.5	2	3312	6624											
	6-1	2	0.5	1	5429	10858	10714	21428	0.1	193521	11.07	20936	0.0216	1	0.016	15	0.0445
			0.5	2	5285	10570											
	6-2	2	0.5	1	5247	10494	10279	20558	0.1	193521	10.62	20066	0.0207	1	0.016	15	0.0426
			0.5	2	5032	10064											
	6-3	2	0.5	1	5143	10286	10280	20560	0.1	193521	10.62	20068	0.0207	1	0.016	15	0.0426
			0.5	2	5137	10274											
	7-1	2	0.5	1	5841	11682	11777	23554	0.1	193521	12.17	23062	0.0238	1	0.016	15	0.0490
			0.5	2	5936	11872											
	7-2	2	0.5	1	6129	12258	12192	24384	0.1	193521	12.60	23892	0.0246	1	0.016	15	0.0507
			0.5	2	6063	12126											
	7-3	2	0.5	1	5823	11646	11449	22898	0.1	193521	11.83	22406	0.0231	1	0.016	15	0.0476
			0.5	2	5626	11252											
	8-1	2	0.5	1	6032	12064	12215	24430	0.1	193521	12.62	23938	0.0247	1	0.016	15	0.0508
			0.5	2	6183	12366											
	8-2	2	0.5	1	5864	11728	11891	23782	0.1	193521	12.29	23290	0.0240	1	0.016	15	0.0495
			0.5	2	6027	12054											
	8-3	2	0.5	1	5885	11770	11656	23312	0.1	193521	12.05	22820	0.0235	1	0.016	15	0.0485
			0.5	2	5771	11542											

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0540	0.0010
Full activity	End	0.0464	0.0004
Full activity	Overall	0.0502	0.0045
Background	Beginning	-0.0007	1.80172E-05
Background	End	0.0007	2.10201E-05
Background	Overall	0.0000	0.000864419
Positive	Beginning	0.0253	0.0015
Positive	End	0.0226	0.0008
Positive	Overall	0.0239	0.0019
Negative	Beginning	0.0519	0.0005
Negative	End	0.0443	0.0005
Negative	Overall	0.0481	0.0044

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 1	1	1	1.00E-03	-3.00	0.0019
Ref Chem 1	1	2	1.00E-03	-3.00	0.0018
Ref Chem 1	1	3	1.00E-03	-3.00	0.0018
Ref Chem 1	2	1	5.00E-04	-3.30	0.0052
Ref Chem 1	2	2	5.00E-04	-3.30	0.0053
Ref Chem 1	2	3	5.00E-04	-3.30	0.0050
Ref Chem 1	3	1	2.50E-04	-3.60	0.0102
Ref Chem 1	3	2	2.50E-04	-3.60	0.0094
Ref Chem 1	3	3	2.50E-04	-3.60	0.0104
Ref Chem 1	4	1	1.00E-04	-4.00	0.0202
Ref Chem 1	4	2	1.00E-04	-4.00	0.0199
Ref Chem 1	4	3	1.00E-04	-4.00	0.0198
Ref Chem 1	5	1	5.00E-05	-4.30	0.0285
Ref Chem 1	5	2	5.00E-05	-4.30	0.0272
Ref Chem 1	5	3	5.00E-05	-4.30	0.0267
Ref Chem 1	6	1	1.00E-05	-5.00	0.0445
Ref Chem 1	6	2	1.00E-05	-5.00	0.0426
Ref Chem 1	6	3	1.00E-05	-5.00	0.0426
Ref Chem 1	7	1	1.00E-06	-6.00	0.0490
Ref Chem 1	7	2	1.00E-06	-6.00	0.0507
Ref Chem 1	7	3	1.00E-06	-6.00	0.0476
Ref Chem 1	8	1	1.00E-07	-7.00	0.0508
Ref Chem 1	8	2	1.00E-07	-7.00	0.0495
Ref Chem 1	8	3	1.00E-07	-7.00	0.0485

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	3.83	3.52	3.54
2	-3.30	10.29	10.61	9.92
3	-3.60	20.28	18.69	20.72
4	-4.00	40.25	39.58	39.39
5	-4.30	56.80	54.24	53.20
6	-5.00	88.56	84.88	84.89
7	-6.00	97.55	101.06	94.78
8	-7.00	101.26	98.52	96.53

Aromatase Assay Spreadsheet

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0203	39034	1922857	
2	0.0198	37462	1892020	
3	0.0199	37954	1907236	
4	0.0198	39619	2000960	
5	0.0199	39396	1979698	
			Average DPM/g soln	1940554
			SD	47328
			CV	2.44
			µCi/g soln	0.874

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1346 g
Mass of dilution B used in substrate prep	4.5842 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563543 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00990 µg/g soln.
µg/g soln.

a. µCi/g soln 0.874
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.563543 + 0.00990
= 0.573439 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.524 µCi/µg ASDN

969197 dpm/nmol

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.534	0.398	0.235	0.172	0.143	0.119	0.112	25.6	100	
	0.573	0.395	0.236	0.178	0.147	0.133	0.118			
	0.574	0.397	0.244	0.175	0.138	0.119	0.117			

Samples:	11540-6A	11540-6B	11345-12I	11540-31C
	0.351	0.135	0.139	0.183
	0.349	0.147	0.131	0.192
			0.136	0.189

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0512	0.560	0.445	0.0482
125	100	200	0.00013	200	0.0256	0.397	0.281	0.0298
50	200	1000	0.00005	200	0.0102	0.238	0.123	0.0120
25	100	1000	0.00003	200	0.0051	0.175	0.059	0.0049
10	40	1000	0.00001	200	0.0020	0.143	0.027	0.0013
5	20	1000	0.00001	200	0.0010	0.124	0.008	-0.0009

Variables	Regression results	
m, b	0.112	-0.002
se_m, se_b	0.008	0.002
r^2, se_y	0.982	0.003
F, df	219	4
ss_{regr}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank 0.116

r^2= 0.982
m= 0.112
b= -0.002

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.351	0.235	0.025	200	1	1	0.123	0.123	0.11
11540-6A	0.349	0.233	0.024	200	1	1	0.122		
11540-6A									
11540-6B	0.135	0.019	0.000	200	1	1	0.002	0.005	0.012
11540-6B	0.147	0.031	0.002	200	1	1	0.009		
11540-6B									
11345-12I	0.139	0.023	0.001	200	1	600	2.5	1.3	
11345-12I	0.131	0.015	0.000	200	1	600	-0.2		
11345-12I	0.136	0.020	0.001	200	1	600	1.5		
11540-31C	0.183	0.067	0.006	200	1	800	23.1	25.4	
11540-31C	0.192	0.076	0.007	200	1	800	27.2		
11540-31C	0.189	0.073	0.006	200	1	800	25.8		

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.534	0.398	0.235	0.172	0.143	0.119	0.112	25.6	100	
	0.573	0.395	0.236	0.178	0.147	0.133	0.118			
	0.574	0.397	0.244	0.175	0.138	0.119	0.117			

Samples:	11540-6A	11540-6B	11345-12I	11540-31C
	0.351	0.135	0.139	0.183
	0.349	0.147	0.131	0.192
			0.136	0.189

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0512	0.560	0.445	0.0402
125	100	200	0.00013	200	0.0256	0.397	0.281	0.0253
50	200	1000	0.00005	200	0.0102	0.238	0.123	0.0109
25	100	1000	0.00003	200	0.0051	0.175	0.059	0.0051
10	40	1000	0.00001	200	0.0020	0.143	0.027	0.0022
5	20	1000	0.00001	200	0.0010	0.124	0.008	0.0005

Variables	Regression results	
m, b	0.091	0.000
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.001
F, df	1416	3
ss_{regr}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

		r^2=	0.998
Blank	0.116	m=	0.091
		b=	0.000

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.351	0.235	0.021	200	1	1	0.106	0.105	0.11
11540-6A	0.349	0.233	0.021	200	1	1	0.105		
11540-6A									
11540-6B	0.135	0.019	0.002	200	1	1	0.008	0.010	0.012
11540-6B	0.147	0.031	0.003	200	1	1	0.013		
11540-6B									
11345-12I	0.139	0.023	0.002	200	1	600	5.6	4.6	
11345-12I	0.131	0.015	0.001	200	1	600	3.4		
11345-12I	0.136	0.020	0.002	200	1	600	4.8		
11540-31C	0.183	0.067	0.006	200	1	800	23.5	25.3	
11540-31C	0.192	0.076	0.007	200	1	800	26.8		
11540-31C	0.189	0.073	0.006	200	1	800	25.7		

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	1

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	25.3
Protein Concentration (dilution added to assay, mg/mL):	0.031632

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	5.00E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5972	11944	11710	23420	0.1	194055	12.07	23291	0.0240	1	0.016	15	0.0506
			0.5	2	5738	11476											
	2	2	0.5	1	6120	12240	12081	24162	0.1	194055	12.45	24033	0.0248	1	0.016	15	0.0523
			0.5	2	5961	11922											
	3	2	0.5	1	5472	10944	10688	21376	0.1	194055	11.02	21247	0.0219	1	0.016	15	0.0462
			0.5	2	5216	10432											
	4	2	0.5	1	5331	10662	10522	21044	0.1	194055	10.84	20915	0.0216	1	0.016	15	0.0455
			0.5	2	5191	10382											
Background control	1	2	0.5	1	21	42	59	118	0.1	194055	0.06	-12	0.0000	1	0.016	15	0.0000
			0.5	2	38	76											
	2	2	0.5	1	17	34	51	102	0.1	194055	0.05	-28	0.0000	1	0.016	15	-0.0001
			0.5	2	34	68											
	3	2	0.5	1	46	92	79	158	0.1	194055	0.08	29	0.0000	1	0.016	15	0.0001
			0.5	2	33	66											
	4	2	0.5	1	37	74	70	140	0.1	194055	0.07	11	0.0000	1	0.016	15	0.0000
			0.5	2	33	66											
Positive control	1	2	0.5	1	3343	6686	6514	13028	0.1	194055	6.71	12899	0.0133	1	0.016	15	0.0280
			0.5	2	3171	6342											
	2	2	0.5	1	2995	5990	5976	11952	0.1	194055	6.16	11823	0.0122	1	0.016	15	0.0257
			0.5	2	2981	5962											
	3	2	0.5	1	2747	5494	5521	11042	0.1	194055	5.69	10913	0.0113	1	0.016	15	0.0237
			0.5	2	2774	5548											
	4	2	0.5	1	2723	5446	5390	10780	0.1	194055	5.56	10651	0.0110	1	0.016	15	0.0232
			0.5	2	2667	5334											
Negative Control	1	2	0.5	1	6121	12242	11914	23828	0.1	194055	12.28	23699	0.0245	1	0.016	15	0.0515
			0.5	2	5793	11586											
	2	2	0.5	1	5815	11630	11616	23232	0.1	194055	11.97	23103	0.0238	1	0.016	15	0.0502
			0.5	2	5801	11602											
	3	2	0.5	1	5247	10494	10358	20716	0.1	194055	10.68	20587	0.0212	1	0.016	15	0.0448
			0.5	2	5111	10222											
	4	2	0.5	1	5048	10096	10162	20324	0.1	194055	10.47	20195	0.0208	1	0.016	15	0.0439
			0.5	2	5114	10228											
Ref Chem 2	1-1	2	0.5	1	70	140	148	296	0.1	194055	0.15	167	0.0002	1	0.016	15	0.0004
			0.5	2	78	156											
	1-2	2	0.5	1	69	138	105	210	0.1	194055	0.11	81	0.0001	1	0.016	15	0.0002
			0.5	2	36	72											
	1-3	2	0.5	1	67	134	125	250	0.1	194055	0.13	121	0.0001	1	0.016	15	0.0003
			0.5	2	58	116											
	2-1	2	0.5	1	304	608	586	1172	0.1	194055	0.60	1043	0.0011	1	0.016	15	0.0023
			0.5	2	282	564											
	2-2	2	0.5	1	266	532	541	1082	0.1	194055	0.56	953	0.0010	1	0.016	15	0.0021
			0.5	2	275	550											
	2-3	2	0.5	1	293	586	583	1166	0.1	194055	0.60	1037	0.0011	1	0.016	15	0.0023
			0.5	2	290	580											
	3-1	2	0.5	1	1743	3486	3410	6820	0.1	194055	3.51	6691	0.0069	1	0.016	15	0.0145
			0.5	2	1667	3334											
	3-2	2	0.5	1	1634	3268	3291	6582	0.1	194055	3.39	6453	0.0067	1	0.016	15	0.0140
			0.5	2	1657	3314											
	3-3	2	0.5	1	1649	3298	3278	6556	0.1	194055	3.38	6427	0.0066	1	0.016	15	0.0140
			0.5	2	1629	3258											
	4-1	2	0.5	1	2565	5130	5214	10428	0.1	194055	5.37	10299	0.0106	1	0.016	15	0.0224
			0.5	2	2649	5298											
	4-2	2	0.5	1	2592	5184	5079	10158	0.1	194055	5.23	10029	0.0103	1	0.016	15	0.0218
			0.5	2	2487	4974											
	4-3	2	0.5	1	2527	5054	4996	9992	0.1	194055	5.15	9863	0.0102	1	0.016	15	0.0214
			0.5	2	2469	4938											
	5-1	2	0.5	1	4601	9202	9083	18166	0.1	194055	9.36	18037	0.0186	1	0.016	15	0.0392
			0.5	2	4482	8964											
	5-2	2	0.5	1	4411	8822	8748	17496	0.1	194055	9.02	17367	0.0179	1	0.016	15	0.0378
			0.5	2	4337	8674											
	5-3	2	0.5	1	4244	8488	8501	17002	0.1	194055	8.76	16873	0.0174	1	0.016	15	0.0367
			0.5	2	4257	8514											
	6-1	2	0.5	1	5665	11330	11289	22578	0.1	194055	11.63	22449	0.0232	1	0.016	15	0.0488
			0.5	2	5624	11248											
	6-2	2	0.5	1	5569	11138	10930	21860	0.1	194055	11.26	21731	0.0224	1	0.016	15	0.0473
			0.5	2	5361	10722											
	6-3	2	0.5	1	5276	10552	10511	21022	0.1	194055	10.83	20893	0.0216	1	0.016	15	0.0454
			0.5	2	5235	10470											
	7-1	2	0.5	1	5688	11376	11272	22544	0.1	194055	11.62	22415	0.0231	1	0.016	15	0.0487
			0.5	2	5584	11168											
	7-2	2	0.5	1	5532	11064	11003	22006	0.1	194055	11.34	21877	0.0226	1	0.016	15	0.0476
			0.5	2	5471	10942											
	7-3	2	0.5	1	5397	10794	10690	21380	0.1	194055	11.02	21251	0.0219	1	0.016	15	0.0462
			0.5	2	5293	10586											
	8-1	2	0.5	1	5563	11126	11053	22106	0.1	194055	11.39	21977	0.0227	1	0.016	15	0.0478
			0.5	2	5490	10980											
	8-2	2	0.5	1	5499	10998	10951	21902	0.1	194055	11.29	21773	0.0225	1	0.016	15	0.0473
			0.5	2	5452	10904											
	8-3	2	0.5	1	5453	10906	10677	21354	0.1	194055	11.00	21225	0.0219	1	0.016	15	0.0462
			0.5	2	5224	10448											

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0515	0.0011
Full activity	End	0.0458	0.0005
Full activity	Overall	0.0486	0.0033
Background	Beginning	0.0000	2.46024E-05
Background	End	0.0000	2.76777E-05
Background	Overall	0.0000	5.34283E-05
Positive	Beginning	0.0269	0.0017
Positive	End	0.0234	0.0004
Positive	Overall	0.0252	0.0022
Negative	Beginning	0.0509	0.0009
Negative	End	0.0443	0.0006
Negative	Overall	0.0476	0.0038

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 2	1	1	1.00E-04	-4.00	0.0004
Ref Chem 2	1	2	1.00E-04	-4.00	0.0002
Ref Chem 2	1	3	1.00E-04	-4.00	0.0003
Ref Chem 2	2	1	1.00E-05	-5.00	0.0023
Ref Chem 2	2	2	1.00E-05	-5.00	0.0021
Ref Chem 2	2	3	1.00E-05	-5.00	0.0023
Ref Chem 2	3	1	1.00E-06	-6.00	0.0145
Ref Chem 2	3	2	1.00E-06	-6.00	0.0140
Ref Chem 2	3	3	1.00E-06	-6.00	0.0140
Ref Chem 2	4	1	5.00E-07	-6.30	0.0224
Ref Chem 2	4	2	5.00E-07	-6.30	0.0218
Ref Chem 2	4	3	5.00E-07	-6.30	0.0214
Ref Chem 2	5	1	1.00E-07	-7.00	0.0392
Ref Chem 2	5	2	1.00E-07	-7.00	0.0378
Ref Chem 2	5	3	1.00E-07	-7.00	0.0367
Ref Chem 2	6	1	1.00E-08	-8.00	0.0488
Ref Chem 2	6	2	1.00E-08	-8.00	0.0473
Ref Chem 2	6	3	1.00E-08	-8.00	0.0454
Ref Chem 2	7	1	1.00E-09	-9.00	0.0487
Ref Chem 2	7	2	1.00E-09	-9.00	0.0476
Ref Chem 2	7	3	1.00E-09	-9.00	0.0462
Ref Chem 2	8	1	1.00E-10	-10.00	0.0478
Ref Chem 2	8	2	1.00E-10	-10.00	0.0473
Ref Chem 2	8	3	1.00E-10	-10.00	0.0462

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.74	0.36	0.54
2	-5.00	4.66	4.26	4.63
3	-6.00	29.91	28.84	28.73
4	-6.30	46.04	44.83	44.09
5	-7.00	80.62	77.63	75.42
6	-8.00	100.35	97.14	93.39
7	-9.00	100.19	97.79	94.99
8	-10.00	98.24	97.32	94.88

Aromatase Assay Spreadsheet

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8		
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0210	41364	1969714	
2	0.0212	38688	1824906	
3	0.0209	39457	1887895	
4	0.0208	41815	2010337	
5	0.0210	41647	1983190	
			Average DPM/g soln	1935208
			SD	76718
			CV	3.96
			µCi/g soln	0.872

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.123 g
Mass of dilution B used in substrate prep	4.5644 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.561911 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00987 µg/g soln.
µg/g soln.

a. µCi/g soln 0.872
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.561911 + 0.00987
= 0.571779 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.525 µCi/µg ASDN

969333 dpm/nmol

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8						
Technician ID		Replicate #	1	2	Microsome type	Placental	Microsome ID	11540-22			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.4	100	
	0.579	0.382	0.250	0.188	0.138	0.123	0.112			
	0.577	0.383	0.243	0.185	0.137	0.124	0.109			
	0.581	0.385	0.245	0.182	0.139	0.126	0.116			

Samples:	11540-6A	11540-6B	11540-32C	11345-13I
	0.366	0.144	0.189	0.143
	0.365	0.154	0.197	0.133
			0.188	0.139

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0508	0.579	0.467	0.0489
125	100	200	0.00013	200	0.0254	0.383	0.271	0.0275
50	200	1000	0.00005	200	0.0102	0.246	0.134	0.0125
25	100	1000	0.00003	200	0.0051	0.185	0.073	0.0058
10	40	1000	0.00001	200	0.0020	0.138	0.026	0.0006
5	20	1000	0.00001	200	0.0010	0.124	0.012	-0.0009

Variables	Regression results				
m, b	0.109	-0.002			
se_m, se_b	0.006	0.001			
r^2, se_y	0.990	0.002			
F, df	386	4			
ss_{reg}, ss_{resid}	0.002	0.000			

Regression results are calculated using the function LINEST

Blank	0.112	r^2=	0.990		
		m=	0.109		
		b=	-0.002		

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.366	0.254	0.026	200	1	1	0.128	0.128	0.11
11540-6A	0.365	0.253	0.025	200	1	1	0.127		
11540-6A									
11540-6B	0.144	0.032	0.001	200	1	1	0.007	0.009	0.012
11540-6B	0.154	0.042	0.002	200	1	1	0.012		
11540-6B									
11540-32C	0.189	0.077	0.006	200	1	800	24.9	25.9	
11540-32C	0.197	0.085	0.007	200	1	800	28.4		
11540-32C	0.188	0.076	0.006	200	1	800	24.5		
11345-13I	0.143	0.031	0.001	200	1	600	3.6	2.0	
11345-13I	0.133	0.021	0.000	200	1	600	0.3		
11345-13I	0.139	0.027	0.001	200	1	600	2.3		

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8						
Technician ID		Replicate #	1	2	Microsome type	Placental	Microsome ID	11540-22			
									Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
									25.4	100	

Standards:	250	125	50	25	10	5	0
	0.579	0.382	0.250	0.188	0.138	0.123	0.112
	0.577	0.383	0.243	0.185	0.137	0.124	0.109
	0.581	0.385	0.245	0.182	0.139	0.126	0.116

Samples:	11540-6A	11540-6B	11540-32C	11345-13I
	0.366	0.144	0.189	0.143
	0.365	0.154	0.197	0.133
			0.188	0.139

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0508	0.579	0.467	0.0430
125	100	200	0.00013	200	0.0254	0.383	0.271	0.0246
50	200	1000	0.00005	200	0.0102	0.246	0.134	0.0116
25	100	1000	0.00003	200	0.0051	0.185	0.073	0.0059
10	40	1000	0.00001	200	0.0020	0.138	0.026	0.0015
5	20	1000	0.00001	200	0.0010	0.124	0.012	0.0002

Variables	Regression results	
m, b	0.094	-0.001
se_m, se_b	0.006	0.001
r^2, se_y	0.989	0.001
F, df	259	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

Blank	0.112

r^2= 0.989
m= 0.094
b= -0.001

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.366	0.254	0.023	200	1	1	0.115	0.114	0.11
11540-6A	0.365	0.253	0.023	200	1	1	0.114		
11540-6A									
11540-6B	0.144	0.032	0.002	200	1	1	0.010	0.012	0.012
11540-6B	0.154	0.042	0.003	200	1	1	0.015		
11540-6B									
11540-32C	0.189	0.077	0.006	200	1	800	25.0	25.9	
11540-32C	0.197	0.085	0.007	200	1	800	28.0		
11540-32C	0.188	0.076	0.006	200	1	800	24.7		
11345-13I	0.143	0.031	0.002	200	1	600	5.8	4.5	
11345-13I	0.133	0.021	0.001	200	1	600	3.0		
11345-13I	0.139	0.027	0.002	200	1	600	4.7		

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate #	2

Microsome Dilution Details

Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	25.9
Protein Concentration (dilution added to assay, mg/mL):	0.03239

Test Chemical Concentrations

Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	5.00E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	6384	12768	12806	25612	0.1	193521	13.23	25120	0.0259	1	0.016	15	0.0533
			0.5	2	6422	12844											
	2	2	0.5	1	6618	13236	13132	26264	0.1	193521	13.57	25772	0.0266	1	0.016	15	0.0547
			0.5	2	6514	13028											
	3	2	0.5	1	5549	11098	11103	22206	0.1	193521	11.47	21714	0.0224	1	0.016	15	0.0461
			0.5	2	5554	11108											
	4	2	0.5	1	5653	11306	11225	22450	0.1	193521	11.60	21958	0.0227	1	0.016	15	0.0466
			0.5	2	5572	11144											
Background control	1	2	0.5	1	38	76	67	134	0.1	193521	0.07	-359	-0.0004	1	0.016	15	-0.0008
			0.5	2	29	58											
	2	2	0.5	1	31	62	73	146	0.1	193521	0.08	-347	-0.0004	1	0.016	15	-0.0007
			0.5	2	42	84											
	3	2	0.5	1	217	434	426	852	0.1	193521	0.44	360	0.0004	1	0.016	15	0.0008
			0.5	2	209	418											
	4	2	0.5	1	206	412	419	838	0.1	193521	0.43	346	0.0004	1	0.016	15	0.0007
			0.5	2	213	426											
Positive control	1	2	0.5	1	3191	6382	6460	12920	0.1	193521	6.68	12428	0.0128	1	0.016	15	0.0264
			0.5	2	3269	6538											
	2	2	0.5	1	3010	6020	5957	11914	0.1	193521	6.16	11422	0.0118	1	0.016	15	0.0243
			0.5	2	2947	5894											
	3	2	0.5	1	2677	5354	5415	10830	0.1	193521	5.60	10338	0.0107	1	0.016	15	0.0220
			0.5	2	2738	5476											
	4	2	0.5	1	2889	5778	5698	11396	0.1	193521	5.89	10904	0.0112	1	0.016	15	0.0232
			0.5	2	2809	5618											
Negative Control	1	2	0.5	1	6248	12496	12540	25080	0.1	193521	12.96	24586	0.0254	1	0.016	15	0.0522
			0.5	2	6292	12584											
	2	2	0.5	1	6156	12312	12380	24760	0.1	193521	12.79	24288	0.0250	1	0.016	15	0.0515
			0.5	2	6224	12448											
	3	2	0.5	1	5297	10594	10750	21500	0.1	193521	11.11	21008	0.0217	1	0.016	15	0.0446
			0.5	2	5453	10906											
	4	2	0.5	1	5364	10728	10592	21184	0.1	193521	10.95	20692	0.0213	1	0.016	15	0.0439
			0.5	2	5228	10456											
Ref Chem 2	1-1	2	0.5	1	58	116	118	236	0.1	193521	0.12	-257	-0.0003	1	0.016	15	-0.0005
			0.5	2	60	120											
	1-2	2	0.5	1	54	108	114	228	0.1	193521	0.12	-265	-0.0003	1	0.016	15	-0.0006
			0.5	2	60	120											
	1-3	2	0.5	1	61	122	113	226	0.1	193521	0.12	-267	-0.0003	1	0.016	15	-0.0006
			0.5	2	52	104											
	2-1	2	0.5	1	237	474	477	954	0.1	193521	0.49	462	0.0005	1	0.016	15	0.0010
			0.5	2	240	480											
	2-2	2	0.5	1	253	506	517	1034	0.1	193521	0.53	542	0.0006	1	0.016	15	0.0011
			0.5	2	264	528											
	2-3	2	0.5	1	266	532	537	1074	0.1	193521	0.55	582	0.0006	1	0.016	15	0.0012
			0.5	2	271	542											
	3-1	2	0.5	1	1773	3546	3546	7092	0.1	193521	3.66	6600	0.0068	1	0.016	15	0.0140
			0.5	2	1773	3546											
	3-2	2	0.5	1	1786	3572	3606	7212	0.1	193521	3.73	6720	0.0069	1	0.016	15	0.0143
			0.5	2	1820	3640											
	3-3	2	0.5	1	1840	3680	3643	7286	0.1	193521	3.76	6794	0.0070	1	0.016	15	0.0144
			0.5	2	1803	3606											
	4-1	2	0.5	1	2574	5148	5199	10398	0.1	193521	5.37	9906	0.0102	1	0.016	15	0.0210
			0.5	2	2625	5250											
	4-2	2	0.5	1	2516	5032	5028	10056	0.1	193521	5.20	9564	0.0099	1	0.016	15	0.0203
			0.5	2	2512	5024											
	4-3	2	0.5	1	2403	4806	4904	9808	0.1	193521	5.07	9316	0.0096	1	0.016	15	0.0198
			0.5	2	2501	5002											
	5-1	2	0.5	1	4362	8724	8933	17866	0.1	193521	9.23	17374	0.0179	1	0.016	15	0.0369
			0.5	2	4571	9142											
	5-2	2	0.5	1	4318	8636	8648	17296	0.1	193521	8.94	16804	0.0173	1	0.016	15	0.0357
			0.5	2	4330	8660											
	5-3	2	0.5	1	4205	8410	8332	16664	0.1	193521	8.61	16172	0.0167	1	0.016	15	0.0343
			0.5	2	4127	8254											
	6-1	2	0.5	1	5498	10996	10806	21612	0.1	193521	11.17	21120	0.0219	1	0.016	15	0.0449
			0.5	2	5308	10616											
	6-2	2	0.5	1	5274	10548	10643	21286	0.1	193521	11.00	20794	0.0215	1	0.016	15	0.0442
			0.5	2	5369	10738											
	6-3	2	0.5	1	5236	10472	10555	21110	0.1	193521	10.91	20618	0.0213	1	0.016	15	0.0438
			0.5	2	5319	10638											
	7-1	2	0.5	1	5718	11436	11421	22842	0.1	193521	11.80	22350	0.0231	1	0.016	15	0.0475
			0.5	2	5703	11406											
	7-2	2	0.5	1	5610	11220	11019	22038	0.1	193521	11.39	21546	0.0222	1	0.016	15	0.0457
			0.5	2	5409	10818											
	7-3	2	0.5	1	5435	10870	10921	21842	0.1	193521	11.29	21350	0.0220	1	0.016	15	0.0453
			0.5	2	5486	10972											
	8-1	2	0.5	1	5677	11354	11322	22644	0.1	193521	11.70	22152	0.0229	1	0.016	15	0.0470
			0.5	2	5645	11290											
	8-2	2	0.5	1	5545	11090	11044	22088	0.1	193521	11.41	21596	0.0223	1	0.016	15	0.0459
			0.5	2	5499	10998											
	8-3	2	0.5	1	5459	10918	10877	21754	0.1	193521	11.24	21262	0.0219	1	0.016	15	0.0451
			0.5	2	5418	10836											

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0540	0.0010
Full activity	End	0.0464	0.0004
Full activity	Overall	0.0502	0.0045
Background	Beginning	-0.0007	1.80172E-05
Background	End	0.0007	2.10201E-05
Background	Overall	0.0000	0.000864419
Positive	Beginning	0.0253	0.0015
Positive	End	0.0226	0.0008
Positive	Overall	0.0239	0.0019
Negative	Beginning	0.0519	0.0005
Negative	End	0.0443	0.0005
Negative	Overall	0.0481	0.0044

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 2	1	1	1.00E-04	-4.00	-0.0005
Ref Chem 2	1	2	1.00E-04	-4.00	-0.0006
Ref Chem 2	1	3	1.00E-04	-4.00	-0.0006
Ref Chem 2	2	1	1.00E-05	-5.00	0.0010
Ref Chem 2	2	2	1.00E-05	-5.00	0.0011
Ref Chem 2	2	3	1.00E-05	-5.00	0.0012
Ref Chem 2	3	1	1.00E-06	-6.00	0.0140
Ref Chem 2	3	2	1.00E-06	-6.00	0.0143
Ref Chem 2	3	3	1.00E-06	-6.00	0.0144
Ref Chem 2	4	1	5.00E-07	-6.30	0.0210
Ref Chem 2	4	2	5.00E-07	-6.30	0.0203
Ref Chem 2	4	3	5.00E-07	-6.30	0.0198
Ref Chem 2	5	1	1.00E-07	-7.00	0.0369
Ref Chem 2	5	2	1.00E-07	-7.00	0.0357
Ref Chem 2	5	3	1.00E-07	-7.00	0.0343
Ref Chem 2	6	1	1.00E-08	-8.00	0.0448
Ref Chem 2	6	2	1.00E-08	-8.00	0.0442
Ref Chem 2	6	3	1.00E-08	-8.00	0.0438
Ref Chem 2	7	1	1.00E-09	-9.00	0.0475
Ref Chem 2	7	2	1.00E-09	-9.00	0.0457
Ref Chem 2	7	3	1.00E-09	-9.00	0.0453
Ref Chem 2	8	1	1.00E-10	-10.00	0.0470
Ref Chem 2	8	2	1.00E-10	-10.00	0.0459
Ref Chem 2	8	3	1.00E-10	-10.00	0.0451

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	-1.09	-1.12	-1.13
2	-5.00	1.95	2.29	2.46
3	-6.00	27.92	28.42	28.74
4	-6.30	41.90	40.45	39.40
5	-7.00	73.49	71.08	68.41
6	-8.00	89.34	87.96	87.21
7	-9.00	94.54	91.14	90.31
8	-10.00	93.70	91.35	89.94

Aromatase Assay Spreadsheet

Assay Date	7/22/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0217	39221	1807419	
2	0.0212	35886	1692736	
3	0.0211	37667	1785166	
4	0.0211	38623	1830474	
5	0.0208	38553	1853510	
			Average DPM/g soln	1793861
			SD	62017
			CV	3.46
			µCi/g soln	0.808

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.2	10		1020.00
Dilution A			100	10.20
Dilution B			10	1.02

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1568 g
Mass of dilution B used in substrate prep	4.5965 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.574788 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00915 µg/g soln.

µg/g soln.

a. µCi/g soln 0.808

b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000

c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.574788 + 0.00915

= 0.583935 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)

= 1.384 µCi/µg ASDN

879827 dpm/nmol

Assay Date	7/22/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8						
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.565	0.358	0.248	0.180	0.142	0.124	0.107	26.6	100	
	0.576	0.366	0.247	0.181	0.141	0.119	0.112			
	0.570	0.379	0.247	0.176	0.139	0.124	0.110			

Samples:	11540-6A	11540-6B	11540-33C	11345-14I
	0.332	0.144	0.189	0.144
	0.322	0.148	0.191	0.142
			0.191	0.136

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00027	200	0.0532	0.570	0.461	0.0517
125	100	200	0.00013	200	0.0266	0.368	0.258	0.0278
50	200	1000	0.00005	200	0.0106	0.247	0.138	0.0137
25	100	1000	0.00003	200	0.0053	0.179	0.069	0.0056
10	40	1000	0.00001	200	0.0021	0.141	0.031	0.0011
5	20	1000	0.00001	200	0.0011	0.122	0.013	-0.0010

Variables	Regression results	
m, b	0.118	-0.003
se_m, se_b	0.006	0.001
r^2, se_y	0.991	0.002
F, df	439	4
ss_{reg}, ss_{resid}	0.002	0.000

Blank 0.110

r^2= 0.991
m= 0.118
b= -0.003

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.332	0.222	0.024	200	1	1	0.118	0.115	0.11
11540-6A	0.322	0.212	0.022	200	1	1	0.112		
11540-6A									
11540-6B	0.144	0.034	0.002	200	1	1	0.008	0.009	0.012
11540-6B	0.148	0.038	0.002	200	1	1	0.010		
11540-6B									
11540-33C	0.189	0.079	0.007	200	1	800	27.3	27.9	
11540-33C	0.191	0.081	0.007	200	1	800	28.2		
11540-33C	0.191	0.081	0.007	200	1	800	28.2		
11345-14I	0.144	0.034	0.002	200	1	600	4.6	3.4	
11345-14I	0.142	0.032	0.001	200	1	600	3.9		
11345-14I	0.136	0.026	0.001	200	1	600	1.8		

Assay Date 7/22/2005 | Test Chemical ID Ref Chem 2 | # Concentrations tested 8

Technician ID 1 | Replicate # 3 | Microsome type Placental | Microsome ID 11540-22

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	26.6	100	
	0.565	0.358	0.248	0.180	0.142	0.124	0.107			
	0.576	0.366	0.247	0.181	0.141	0.119	0.112			
	0.570	0.379	0.247	0.176	0.139	0.124	0.110			

Samples:	11540-6A	11540-6B	11540-33C	11345-14I
	0.332	0.144	0.189	0.144
	0.322	0.148	0.191	0.142
			0.191	0.136

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00027	200	0.0532	0.570	0.461	0.0464
125	100	200	0.00013	200	0.0266	0.368	0.258	0.0254
50	200	1000	0.00005	200	0.0106	0.247	0.138	0.0129
25	100	1000	0.00003	200	0.0053	0.179	0.069	0.0058
10	40	1000	0.00001	200	0.0021	0.141	0.031	0.0018
5	20	1000	0.00001	200	0.0011	0.122	0.013	-0.0001
			Blank	0.110				

Variables	Regression results	
m, b	0.104	-0.001
se_m, se_b	0.008	0.001
r^2, se_y	0.981	0.002
F, df	157	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.981
m= 0.104
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.332	0.222	0.022	200	1	1	0.108	0.106	0.11
11540-6A	0.322	0.212	0.021	200	1	1	0.103		
11540-6A									
11540-6B	0.144	0.034	0.002	200	1	1	0.011	0.012	0.012
11540-6B	0.148	0.038	0.003	200	1	1	0.013		
11540-6B									
11540-33C	0.189	0.079	0.007	200	1	800	27.3	27.9	
11540-33C	0.191	0.081	0.007	200	1	800	28.1		
11540-33C	0.191	0.081	0.007	200	1	800	28.1		
11345-14I	0.144	0.034	0.002	200	1	600	6.5	5.4	
11345-14I	0.142	0.032	0.002	200	1	600	5.8		
11345-14I	0.136	0.026	0.001	200	1	600	4.0		

Assay Date	7/22/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	3

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	27.9
Protein Concentration (dilution added to assay, mg/mL):	0.034816

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	5.00E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	7/22/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5721	11442	11419	22838	0.1	179386	12.73	22690	0.0258	1	0.017	15	0.0494
			0.5	2	5698	11396											
	2	2	0.5	1	5872	11744	11726	23452	0.1	179386	13.07	23304	0.0265	1	0.017	15	0.0507
			0.5	2	5854	11708											
	3	2	0.5	1	5372	10744	10743	21486	0.1	179386	11.98	21338	0.0243	1	0.017	15	0.0464
			0.5	2	5371	10742											
	4	2	0.5	1	5635	11270	11208	22416	0.1	179386	12.50	22268	0.0253	1	0.017	15	0.0485
			0.5	2	5573	11146											
Background control	1	2	0.5	1	46	92	85	170	0.1	179386	0.09	22	0.0000	1	0.017	15	0.0000
			0.5	2	39	78											
	2	2	0.5	1	30	60	66	132	0.1	179386	0.07	-17	0.0000	1	0.017	15	0.0000
			0.5	2	36	72											
	3	2	0.5	1	47	94	73	146	0.1	179386	0.08	-3	0.0000	1	0.017	15	0.0000
			0.5	2	26	52											
	4	2	0.5	1	43	86	73	146	0.1	179386	0.08	-3	0.0000	1	0.017	15	0.0000
			0.5	2	30	60											
Positive control	1	2	0.5	1	2909	5818	5889	11778	0.1	179386	6.57	11630	0.0132	1	0.017	15	0.0253
			0.5	2	2980	5960											
	2	2	0.5	1	2795	5590	5485	10970	0.1	179386	6.12	10822	0.0123	1	0.017	15	0.0236
			0.5	2	2690	5380											
	3	2	0.5	1	2623	5246	5208	10416	0.1	179386	5.81	10268	0.0117	1	0.017	15	0.0223
			0.5	2	2585	5170											
	4	2	0.5	1	2621	5242	5204	10408	0.1	179386	5.80	10260	0.0117	1	0.017	15	0.0223
			0.5	2	2583	5166											
Negative Control	1	2	0.5	1	2918	5836	5848	11696	0.1	179386	6.52	11548	0.0131	1	0.017	15	0.0251
			0.5	2	2930	5860											
	2	2	0.5	1	5827	11654	11782	23564	0.1	179386	13.14	23416	0.0266	1	0.017	15	0.0510
			0.5	2	5955	11910											
	3	2	0.5	1	5395	10790	10834	21668	0.1	179386	12.08	21520	0.0245	1	0.017	15	0.0468
			0.5	2	5439	10878											
	4	2	0.5	1	5184	10368	10347	20694	0.1	179386	11.54	20546	0.0234	1	0.017	15	0.0447
			0.5	2	5163	10326											
Ref Chem 2	1-1	2	0.5	1	55	110	115	230	0.1	179386	0.13	82	0.0001	1	0.017	15	0.0002
			0.5	2	60	120											
	1-2	2	0.5	1	39	78	82	164	0.1	179386	0.09	16	0.0000	1	0.017	15	0.0000
			0.5	2	43	86											
	1-3	2	0.5	1	51	102	95	190	0.1	179386	0.11	42	0.0000	1	0.017	15	0.0001
			0.5	2	44	88											
	2-1	2	0.5	1	295	590	565	1130	0.1	179386	0.63	982	0.0011	1	0.017	15	0.0021
			0.5	2	270	540											
	2-2	2	0.5	1	248	496	497	994	0.1	179386	0.55	846	0.0010	1	0.017	15	0.0018
			0.5	2	249	498											
	2-3	2	0.5	1	266	532	526	1052	0.1	179386	0.59	904	0.0010	1	0.017	15	0.0020
			0.5	2	260	520											
	3-1	2	0.5	1	1655	3310	3314	6628	0.1	179386	3.69	6480	0.0074	1	0.017	15	0.0141
			0.5	2	1659	3318											
	3-2	2	0.5	1	1572	3144	3111	6222	0.1	179386	3.47	6074	0.0069	1	0.017	15	0.0132
			0.5	2	1539	3078											
	3-3	2	0.5	1	1571	3142	3128	6256	0.1	179386	3.49	6108	0.0069	1	0.017	15	0.0133
			0.5	2	1557	3114											
	4-1	2	0.5	1	2527	5054	5135	10270	0.1	179386	5.73	10122	0.0115	1	0.017	15	0.0220
			0.5	2	2608	5216											
	4-2	2	0.5	1	2429	4858	4922	9844	0.1	179386	5.49	9696	0.0110	1	0.017	15	0.0211
			0.5	2	2493	4986											
	4-3	2	0.5	1	2383	4766	4824	9648	0.1	179386	5.38	9500	0.0108	1	0.017	15	0.0207
			0.5	2	2441	4882											
	5-1	2	0.5	1	4468	8936	8869	17738	0.1	179386	9.89	17590	0.0200	1	0.017	15	0.0383
			0.5	2	4401	8802											
	5-2	2	0.5	1	4224	8448	8402	16804	0.1	179386	9.37	16656	0.0189	1	0.017	15	0.0362
			0.5	2	4178	8356											
	5-3	2	0.5	1	4147	8294	8340	16680	0.1	179386	9.30	16532	0.0188	1	0.017	15	0.0360
			0.5	2	4193	8386											
	6-1	2	0.5	1	5587	11174	11243	22486	0.1	179386	12.53	22338	0.0254	1	0.017	15	0.0486
			0.5	2	5656	11312											
	6-2	2	0.5	1	5466	10932	10810	21620	0.1	179386	12.05	21472	0.0244	1	0.017	15	0.0467
			0.5	2	5344	10688											
	6-3	2	0.5	1	5221	10442	10461	20922	0.1	179386	11.66	20774	0.0236	1	0.017	15	0.0452
			0.5	2	5240	10480											
	7-1	2	0.5	1	5713	11426	11335	22670	0.1	179386	12.64	22522	0.0256	1	0.017	15	0.0490
			0.5	2	5622	11244											
	7-2	2	0.5	1	5690	11380	11342	22684	0.1	179386	12.65	22536	0.0256	1	0.017	15	0.0490
			0.5	2	5652	11304											
	7-3	2	0.5	1	5378	10756	10871	21742	0.1	179386	12.12	21594	0.0245	1	0.017	15	0.0470
			0.5	2	5493	10986											
	8-1	2	0.5	1	5720	11440	11399	22798	0.1	179386	12.71	22650	0.0257	1	0.017	15	0.0493
			0.5	2	5679	11358											
	8-2	2	0.5	1	5531	11062	11177	22354	0.1	179386	12.46	22206	0.0252	1	0.017	15	0.0483
			0.5	2	5646	11292											
	8-3	2	0.5	1	5463	10926	10901	21802	0.1	179386	12.15	21654	0.0246	1	0.017	15	0.0471
			0.5	2	5438	10876											

Assay Date	7/22/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0500	0.0009
Full activity	End	0.0475	0.0014
Full activity	Overall	0.0487	0.0018
Background	Beginning	0.0000	5.84791E-05
Background	End	0.0000	0
Background	Overall	0.0000	3.43425E-05
Positive	Beginning	0.0244	0.0012
Positive	End	0.0223	0.0000
Positive	Overall	0.0234	0.0014
Negative	Beginning	0.0510	NA
Negative	End	0.0458	0.0015
Negative	Overall	0.0475	0.0032

Negative Tube 1 excluded as outlier

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 2	1	1	1.00E-04	-4.00	0.0002
Ref Chem 2	1	2	1.00E-04	-4.00	0.0000
Ref Chem 2	1	3	1.00E-04	-4.00	0.0001
Ref Chem 2	2	1	1.00E-05	-5.00	0.0021
Ref Chem 2	2	2	1.00E-05	-5.00	0.0018
Ref Chem 2	2	3	1.00E-05	-5.00	0.0020
Ref Chem 2	3	1	1.00E-06	-6.00	0.0141
Ref Chem 2	3	2	1.00E-06	-6.00	0.0132
Ref Chem 2	3	3	1.00E-06	-6.00	0.0133
Ref Chem 2	4	1	5.00E-07	-6.30	0.0220
Ref Chem 2	4	2	5.00E-07	-6.30	0.0211
Ref Chem 2	4	3	5.00E-07	-6.30	0.0207
Ref Chem 2	5	1	1.00E-07	-7.00	0.0383
Ref Chem 2	5	2	1.00E-07	-7.00	0.0362
Ref Chem 2	5	3	1.00E-07	-7.00	0.0360
Ref Chem 2	6	1	1.00E-08	-8.00	0.0486
Ref Chem 2	6	2	1.00E-08	-8.00	0.0467
Ref Chem 2	6	3	1.00E-08	-8.00	0.0452
Ref Chem 2	7	1	1.00E-09	-9.00	0.0490
Ref Chem 2	7	2	1.00E-09	-9.00	0.0490
Ref Chem 2	7	3	1.00E-09	-9.00	0.0470
Ref Chem 2	8	1	1.00E-10	-10.00	0.0493
Ref Chem 2	8	2	1.00E-10	-10.00	0.0483
Ref Chem 2	8	3	1.00E-10	-10.00	0.0471

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.36	0.07	0.19
2	-5.00	4.38	3.77	4.03
3	-6.00	28.93	27.11	27.27
4	-6.30	45.19	43.28	42.41
5	-7.00	78.53	74.36	73.80
6	-8.00	99.72	95.86	92.74
7	-9.00	100.54	100.61	96.40
8	-10.00	101.12	99.13	96.67

Aromatase Assay Spreadsheet

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0210	39340	1873333	
2	0.0217	37568	1731244	
3	0.0218	38959	1787110	
4	0.0217	40419	1862627	
5	0.0214	39999	1869112	
			Average DPM/g soln	1824685
			SD	63093
			CV	3.46
			µCi/g soln	0.822

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1457 g
Mass of dilution B used in substrate prep	4.5807 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562346 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00930 µg/g soln.
µg/g soln.

a. µCi/g soln 0.822
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.562346 + 0.00930
= 0.571650 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.438 µCi/µg ASDN

914178 dpm/nmol

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.592	0.398	0.252	0.176	0.138	0.122	0.101	25.4	100	
	0.592	0.396	0.246	0.180	0.135	0.125	0.119			
	0.600	0.393	0.248	0.183	0.147	0.124	0.114			

Samples:	11540-6A	11540-6B	11540-40B	11345-15I
	0.371	0.144	0.189	0.133
	0.384	0.147	0.188	0.143
			0.190	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0508	0.595	0.483	0.0488
125	100	200	0.00013	200	0.0254	0.396	0.284	0.0279
50	200	1000	0.00005	200	0.0102	0.249	0.137	0.0124
25	100	1000	0.00003	200	0.0051	0.180	0.068	0.0052
10	40	1000	0.00001	200	0.0020	0.140	0.029	0.0010
5	20	1000	0.00001	200	0.0010	0.124	0.012	-0.0007
			Blank	0.111				

Variables	Regression results	
m, b	0.105	-0.002
se_m, se_b	0.005	0.001
r^2, se_y	0.990	0.002
F, df	383	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

			r^2=	0.990
			m=	0.105
			b=	-0.002

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.371	0.260	0.025	200	1	1	0.126	0.130	0.11
11540-6A	0.384	0.273	0.027	200	1	1	0.133		
11540-6A									
11540-6B	0.144	0.033	0.001	200	1	1	0.007	0.008	0.012
11540-6B	0.147	0.036	0.002	200	1	1	0.009		
11540-6B									
11540-40B	0.189	0.078	0.006	200	1	320	9.8	9.8	
11540-40B	0.188	0.077	0.006	200	1	320	9.6		
11540-40B	0.190	0.079	0.006	200	1	320	10.0		
11345-15I	0.133	0.022	0.000	200	1	600	0.7	2.0	
11345-15I	0.143	0.032	0.001	200	1	600	3.9		
11345-15I	0.135	0.024	0.000	200	1	600	1.4		

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8					
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20			

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.592	0.398	0.252	0.176	0.138	0.122	0.101	25.4	100	
	0.592	0.396	0.246	0.180	0.135	0.125	0.119			
	0.600	0.393	0.248	0.183	0.147	0.124	0.114			

Samples:	11540-6A	11540-6B	11540-40B	11345-15I
	0.371	0.144	0.189	0.133
	0.384	0.147	0.188	0.143
			0.190	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0508	0.595	0.483	0.0426
125	100	200	0.00013	200	0.0254	0.396	0.284	0.0247
50	200	1000	0.00005	200	0.0102	0.249	0.137	0.0115
25	100	1000	0.00003	200	0.0051	0.180	0.068	0.0053
10	40	1000	0.00001	200	0.0020	0.140	0.029	0.0018
5	20	1000	0.00001	200	0.0010	0.124	0.012	0.0003

Variables	Regression results	
m, b	0.090	-0.001
se_m, se_b	0.004	0.001
r^2, se_y	0.993	0.001
F, df	400	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

			r^2=	0.993
Blank	0.111		m=	0.090
			b=	-0.001

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.371	0.260	0.023	200	1	1	0.113	0.116	0.11
11540-6A	0.384	0.273	0.024	200	1	1	0.118		
11540-6A									
11540-6B	0.144	0.033	0.002	200	1	1	0.011	0.011	0.012
11540-6B	0.147	0.036	0.002	200	1	1	0.012		
11540-6B									
11540-40B	0.189	0.078	0.006	200	1	320	9.9	9.9	
11540-40B	0.188	0.077	0.006	200	1	320	9.7		
11540-40B	0.190	0.079	0.006	200	1	320	10.0		
11345-15I	0.133	0.022	0.001	200	1	600	3.4	4.5	
11345-15I	0.143	0.032	0.002	200	1	600	6.1		
11345-15I	0.135	0.024	0.001	200	1	600	4.0		

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate #	1

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	9.9
Protein Concentration (dilution added to assay, mg/mL):	0.030879

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4243	8486	8459	16918	0.1	182469	9.27	16783	0.0184	1	0.015	15	0.0396
			0.5	2	4216	8432											
	2	2	0.5	1	4362	8724	8568	17176	0.1	182469	9.41	17041	0.0186	1	0.015	15	0.0402
			0.5	2	4226	8452											
	3	2	0.5	1	3609	7218	7233	14466	0.1	182469	7.93	14331	0.0157	1	0.015	15	0.0338
			0.5	2	3624	7248											
	4	2	0.5	1	3535	7070	7131	14262	0.1	182469	7.82	14127	0.0155	1	0.015	15	0.0334
			0.5	2	3596	7192											
Background control	1	2	0.5	1	42	84	62	124	0.1	182469	0.07	-12	0.0000	1	0.015	15	0.0000
			0.5	2	20	40											
	2	2	0.5	1	40	80	68	136	0.1	182469	0.07	1	0.0000	1	0.015	15	0.0000
			0.5	2	28	56											
	3	2	0.5	1	34	68	70	140	0.1	182469	0.08	5	0.0000	1	0.015	15	0.0000
			0.5	2	36	72											
	4	2	0.5	1	36	72	71	142	0.1	182469	0.08	7	0.0000	1	0.015	15	0.0000
			0.5	2	35	70											
Positive control	1	2	0.5	1	2089	4178	4225	8450	0.1	182469	4.63	8315	0.0091	1	0.015	15	0.0196
			0.5	2	2136	4272											
	2	2	0.5	1	1987	3974	3942	7884	0.1	182469	4.32	7749	0.0085	1	0.015	15	0.0183
			0.5	2	1955	3910											
	3	2	0.5	1	1687	3374	3320	6640	0.1	182469	3.64	6505	0.0071	1	0.015	15	0.0154
			0.5	2	1633	3266											
	4	2	0.5	1	1697	3394	3359	6718	0.1	182469	3.68	6583	0.0072	1	0.015	15	0.0155
			0.5	2	1662	3324											
Negative Control	1	2	0.5	1	4239	8478	8524	17046	0.1	182469	9.34	16913	0.0185	1	0.015	15	0.0399
			0.5	2	4285	8570											
	2	2	0.5	1	4255	8510	8469	16938	0.1	182469	9.28	16803	0.0184	1	0.015	15	0.0397
			0.5	2	4214	8428											
	3	2	0.5	1	3724	7449	7353	14706	0.1	182469	8.06	14571	0.0159	1	0.015	15	0.0344
			0.5	2	3629	7258											
	4	2	0.5	1	733	1466	1477	2954	0.1	182469	1.62	2819	0.0031	1	0.015	15	0.0067
			0.5	2	744	1488											
Ref Chem 3	1-1	2	0.5	1	25	50	63	126	0.1	182469	0.07	-10	0.0000	1	0.015	15	0.0000
			0.5	2	38	76											
	1-2	2	0.5	1	39	78	72	144	0.1	182469	0.08	9	0.0000	1	0.015	15	0.0000
			0.5	2	33	66											
	1-3	2	0.5	1	28	56	71	142	0.1	182469	0.08	7	0.0000	1	0.015	15	0.0000
			0.5	2	43	86											
	2-1	2	0.5	1	26	52	52	104	0.1	182469	0.06	-32	0.0000	1	0.015	15	-0.0001
			0.5	2	26	52											
	2-2	2	0.5	1	29	58	58	116	0.1	182469	0.06	-20	0.0000	1	0.015	15	0.0000
			0.5	2	29	58											
	2-3	2	0.5	1	39	78	78	156	0.1	182469	0.09	21	0.0000	1	0.015	15	0.0000
			0.5	2	39	78											
	3-1	2	0.5	1	34	68	72	144	0.1	182469	0.08	9	0.0000	1	0.015	15	0.0000
			0.5	2	38	76											
	3-2	2	0.5	1	52	104	101	202	0.1	182469	0.11	67	0.0001	1	0.015	15	0.0002
			0.5	2	49	98											
	3-3	2	0.5	1	40	80	88	176	0.1	182469	0.10	41	0.0000	1	0.015	15	0.0001
			0.5	2	48	96											
	4-1	2	0.5	1	102	204	212	424	0.1	182469	0.23	289	0.0003	1	0.015	15	0.0007
			0.5	2	110	220											
	4-2	2	0.5	1	123	246	243	486	0.1	182469	0.27	351	0.0004	1	0.015	15	0.0008
			0.5	2	120	240											
	4-3	2	0.5	1	100	200	225	450	0.1	182469	0.25	315	0.0003	1	0.015	15	0.0007
			0.5	2	125	250											
	5-1	2	0.5	1	686	1372	1341	2682	0.1	182469	1.47	2547	0.0026	1	0.015	15	0.0060
			0.5	2	655	1310											
	5-2	2	0.5	1	654	1308	1298	2596	0.1	182469	1.42	2461	0.0027	1	0.015	15	0.0058
			0.5	2	644	1288											
	5-3	2	0.5	1	665	1330	1333	2666	0.1	182469	1.46	2531	0.0028	1	0.015	15	0.0060
			0.5	2	668	1336											
	6-1	2	0.5	1	2411	4822	4861	9722	0.1	182469	5.33	9587	0.0105	1	0.015	15	0.0226
			0.5	2	2450	4900											
	6-2	2	0.5	1	2514	5028	5057	10114	0.1	182469	5.54	9979	0.0109	1	0.015	15	0.0236
			0.5	2	2543	5086											
	6-3	2	0.5	1	2397	4794	4762	9524	0.1	182469	5.22	9389	0.0103	1	0.015	15	0.0222
			0.5	2	2365	4730											
	7-1	2	0.5	1	3746	7492	7463	14926	0.1	182469	8.18	14791	0.0162	1	0.015	15	0.0349
			0.5	2	3717	7434											
	7-2	2	0.5	1	3497	6994	7049	14098	0.1	182469	7.73	13963	0.0153	1	0.015	15	0.0330
			0.5	2	3552	7104											
	7-3	2	0.5	1	3449	6898	6974	13948	0.1	182469	7.64	13813	0.0151	1	0.015	15	0.0326
			0.5	2	3525	7050											
	8-1	2	0.5	1	3890	7780	7870	15740	0.1	182469	8.63	15605	0.0171	1	0.015	15	0.0369
			0.5	2	3980	7960											
	8-2	2	0.5	1	3890	7780	7695	15390	0.1	182469	8.43	15255	0.0167	1	0.015	15	0.0360
			0.5	2	3805	7610											
	8-3	2	0.2	1	1453	7265	7270	14540	0.1	182469	7.97	14405	0.0156	1	0.015	15	0.0340
			0.2	2	1455	7275											

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0399	0.0004
Full activity	End	0.0336	0.0003
Full activity	Overall	0.0368	0.0037
Background	Beginning	0.0000	2.00395E-05
Background	End	0.0000	3.33991E-06
Background	Overall	0.0000	1.90404E-05
Positive	Beginning	0.0190	0.0009
Positive	End	0.0155	0.0001
Positive	Overall	0.0172	0.0021
Negative	Beginning	0.0398	0.0002
Negative	End	0.0344	NA
Negative	Overall	0.0380	0.0031

Exclude Neg Tube 4

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 3	1	1	1.00E-03	-3.00	0.0000
Ref Chem 3	1	2	1.00E-03	-3.00	0.0000
Ref Chem 3	1	3	1.00E-03	-3.00	0.0000
Ref Chem 3	2	1	1.00E-04	-4.00	-0.0001
Ref Chem 3	2	2	1.00E-04	-4.00	0.0000
Ref Chem 3	2	3	1.00E-04	-4.00	0.0000
Ref Chem 3	3	1	1.00E-05	-5.00	0.0000
Ref Chem 3	3	2	1.00E-05	-5.00	0.0002
Ref Chem 3	3	3	1.00E-05	-5.00	0.0001
Ref Chem 3	4	1	1.00E-06	-6.00	0.0007
Ref Chem 3	4	2	1.00E-06	-6.00	0.0008
Ref Chem 3	4	3	1.00E-06	-6.00	0.0007
Ref Chem 3	5	1	1.00E-07	-7.00	0.0060
Ref Chem 3	5	2	1.00E-07	-7.00	0.0058
Ref Chem 3	5	3	1.00E-07	-7.00	0.0060
Ref Chem 3	6	1	1.00E-08	-8.00	0.0226
Ref Chem 3	6	2	1.00E-08	-8.00	0.0236
Ref Chem 3	6	3	1.00E-08	-8.00	0.0222
Ref Chem 3	7	1	1.00E-09	-9.00	0.0349
Ref Chem 3	7	2	1.00E-09	-9.00	0.0330
Ref Chem 3	7	3	1.00E-09	-9.00	0.0326
Ref Chem 3	8	1	1.00E-10	-10.00	0.0369
Ref Chem 3	8	2	1.00E-10	-10.00	0.0360
Ref Chem 3	8	3	1.00E-10	-10.00	0.0340

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	-0.06	0.05	0.04
2	-4.00	-0.20	-0.13	0.13
3	-5.00	0.05	0.43	0.26
4	-6.00	1.85	2.25	2.02
5	-7.00	16.36	15.80	16.25
6	-8.00	61.57	64.09	60.30
7	-9.00	94.99	89.68	88.71
8	-10.00	100.22	97.97	92.51

Aromatase Assay Spreadsheet

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8		
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	36889	1835274	
2	0.0204	36211	1775049	
3	0.0204	38433	1883971	
4	0.0204	39411	1931912	
5	0.0203	39588	1950148	
			Average DPM/g soln	1875271
			SD	71650
			CV	3.82
			µCi/g soln	0.845

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.1	10		1010.00
Dilution A			100	10.10
Dilution B			10	1.01

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0912 g
Mass of dilution B used in substrate prep	4.5439 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.567201 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00956 µg/g soln.
µg/g soln.

a. µCi/g soln 0.845
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.567201 + 0.00956
= 0.576764 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.465 µCi/µg ASDN

931192 dpm/nmol

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8					
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20			

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.584	0.393	0.253	0.182	0.136	0.124	0.108	25.4	100	11540-42
	0.593	0.386	0.246	0.192	0.146	0.129	0.110			
	0.593	0.392	0.251	0.177	0.142	0.126	0.120			

Samples:	11540-6A	11540-6B	11540-42B	11345-16I
	0.366	0.149	0.196	0.139
	0.374	0.151	0.194	0.137
			0.196	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0508	0.590	0.477	0.0490
125	100	200	0.00013	200	0.0254	0.390	0.278	0.0275
50	200	1000	0.00005	200	0.0102	0.250	0.137	0.0125
25	100	1000	0.00003	200	0.0051	0.184	0.071	0.0054
10	40	1000	0.00001	200	0.0020	0.141	0.029	0.0009
5	20	1000	0.00001	200	0.0010	0.126	0.014	-0.0008

Variables	Regression results	
m, b	0.107	-0.002
se_m, se_b	0.005	0.001
r^2, se_y	0.990	0.002
F, df	409	4
ss_{reg}, ss_{resid}	0.002	0.000

Blank 0.113

r^2= 0.990
m= 0.107
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.366	0.253	0.025	200	1	1	0.125	0.127	0.11
11540-6A	0.374	0.261	0.026	200	1	1	0.129		
11540-6A									
11540-6B	0.149	0.036	0.002	200	1	1	0.008	0.009	0.012
11540-6B	0.151	0.038	0.002	200	1	1	0.009		
11540-6B									
11540-42B	0.196	0.083	0.007	200	1	320	10.7	10.6	
11540-42B	0.194	0.081	0.006	200	1	320	10.4		
11540-42B	0.196	0.083	0.007	200	1	320	10.7		
11345-16I	0.139	0.026	0.001	200	1	600	1.8	1.2	
11345-16I	0.137	0.024	0.000	200	1	600	1.2		
11345-16I	0.135	0.022	0.000	200	1	600	0.5		

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.584	0.393	0.253	0.182	0.136	0.124	0.108	25.4	100	11540-42
	0.593	0.386	0.246	0.192	0.146	0.129	0.110			
	0.593	0.392	0.251	0.177	0.142	0.126	0.120			

Samples:	11540-6A	11540-6B	11540-42B	11345-16I
	0.366	0.149	0.196	0.139
	0.374	0.151	0.194	0.137
			0.196	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0508	0.590	0.477	0.0431
125	100	200	0.00013	200	0.0254	0.390	0.278	0.0246
50	200	1000	0.00005	200	0.0102	0.250	0.137	0.0117
25	100	1000	0.00003	200	0.0051	0.184	0.071	0.0055
10	40	1000	0.00001	200	0.0020	0.141	0.029	0.0016
5	20	1000	0.00001	200	0.0010	0.126	0.014	0.0002
			Blank	0.113				

Variables	Regression results	
m, b	0.092	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.990	0.001
F, df	308	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.990
m= 0.092
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.366	0.253	0.022	200	1	1	0.112	0.114	0.11
11540-6A	0.374	0.261	0.023	200	1	1	0.116		
11540-6A									
11540-6B	0.149	0.036	0.002	200	1	1	0.012	0.012	0.012
11540-6B	0.151	0.038	0.003	200	1	1	0.013		
11540-6B									
11540-42B	0.196	0.083	0.007	200	1	320	10.7	10.6	
11540-42B	0.194	0.081	0.006	200	1	320	10.4		
11540-42B	0.196	0.083	0.007	200	1	320	10.7		
11345-16I	0.139	0.026	0.001	200	1	600	4.2	3.7	
11345-16I	0.137	0.024	0.001	200	1	600	3.7		
11345-16I	0.135	0.022	0.001	200	1	600	3.1		

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	2

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	10.6
Protein Concentration (dilution added to assay, mg/mL):	0.033069

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	2.50E-08
5	1.00E-08
6	5.00E-09
7	1.00E-09
8	1.00E-10

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed						
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.2	1	1929	9645	9670	19340	0.1	187527	10.31	19160	0.0206	1	0.017	15	0.0415
			0.2	2	1939	9695											
	2	2	0.1	1	990	9900	9850	19700	0.1	187527	10.51	19520	0.0210	1	0.017	15	0.0423
			0.07	2	686	9800											
	3	2	0.5	1	4450	8900	8817	17634	0.1	187527	9.40	17454	0.0187	1	0.017	15	0.0378
			0.5	2	4367	8734											
	4	2	0.5	1	4229	8458	8458	16916	0.1	187527	9.02	16736	0.0180	1	0.017	15	0.0362
			0.5	2	4229	8458											
Background control	1	2	0.5	1	51	102	105	210	0.1	187527	0.11	30	0.0000	1	0.017	15	0.0001
			0.5	2	54	108											
	2	2	0.5	1	44	88	97	194	0.1	187527	0.10	14	0.0000	1	0.017	15	0.0000
			0.5	2	53	106											
	3	2	0.5	1	40	80	72	144	0.1	187527	0.08	-37	0.0000	1	0.017	15	-0.0001
			0.5	2	32	64											
	4	2	0.5	1	39	78	87	174	0.1	187527	0.09	-7	0.0000	1	0.017	15	0.0000
			0.5	2	48	96											
Positive control	1	2	0.5	1	2332	4664	4709	9418	0.1	187527	5.02	9238	0.0099	1	0.017	15	0.0200
			0.5	2	2377	4754											
	2	2	0.5	1	2019	4038	4097	8194	0.1	187527	4.37	8014	0.0086	1	0.017	15	0.0173
			0.5	2	2078	4156											
	3	2	0.5	1	2268	4536	4499	8998	0.1	187527	4.80	8818	0.0095	1	0.017	15	0.0191
			0.5	2	2231	4462											
	4	2	0.5	1	2318	4636	4576	9152	0.1	187527	4.88	8972	0.0096	1	0.017	15	0.0194
			0.5	2	2258	4516											
Negative Control	1	2	0.5	1	4762	9524	9585	19170	0.1	187527	10.22	18990	0.0204	1	0.017	15	0.0411
			0.5	2	4823	9646											
	2	2	0.5	1	4649	9298	9196	18392	0.1	187527	9.81	18212	0.0196	1	0.017	15	0.0394
			0.5	2	4547	9094											
	3	2	0.5	1	4400	8800	8820	17640	0.1	187527	9.41	17460	0.0187	1	0.017	15	0.0378
			0.5	2	4420	8840											
	4	2	0.5	1	4048	8096	7985	15970	0.1	187527	8.52	15790	0.0170	1	0.017	15	0.0342
			0.5	2	3937	7874											
Ref Chem 3	1-1	2	0.5	1	47	94	100	200	0.1	187527	0.11	20	0.0000	1	0.017	15	0.0000
			0.5	2	53	106											
	1-2	2	0.5	1	51	102	104	208	0.1	187527	0.11	28	0.0000	1	0.017	15	0.0001
			0.5	2	53	106											
	1-3	2	0.5	1	64	128	109	218	0.1	187527	0.12	38	0.0000	1	0.017	15	0.0001
			0.5	2	45	90											
	2-1	2	0.5	1	140	280	276	552	0.1	187527	0.29	372	0.0004	1	0.017	15	0.0008
			0.5	2	136	272											
	2-2	2	0.5	1	160	320	306	612	0.1	187527	0.33	432	0.0005	1	0.017	15	0.0009
			0.5	2	146	292											
	2-3	2	0.5	1	154	308	298	596	0.1	187527	0.32	416	0.0004	1	0.017	15	0.0009
			0.5	2	144	288											
	3-1	2	0.5	1	873	1746	1769	3538	0.1	187527	1.89	3358	0.0036	1	0.017	15	0.0073
			0.5	2	896	1792											
	3-2	2	0.5	1	943	1886	1888	3776	0.1	187527	2.01	3596	0.0039	1	0.017	15	0.0078
			0.5	2	945	1890											
	3-3	2	0.5	1	935	1870	1829	3658	0.1	187527	1.95	3478	0.0037	1	0.017	15	0.0075
			0.5	2	894	1788											
	4-1	2	0.5	1	2281	4562	4620	9240	0.1	187527	4.93	9060	0.0097	1	0.017	15	0.0196
			0.5	2	2339	4678											
	4-2	2	0.5	1	2260	4520	4596	9192	0.1	187527	4.90	9012	0.0097	1	0.017	15	0.0195
			0.5	2	2336	4672											
	4-3	2	0.5	1	2198	4396	4410	8820	0.1	187527	4.70	8640	0.0093	1	0.017	15	0.0187
			0.5	2	2212	4424											
	5-1	2	0.5	1	3225	6450	6449	12898	0.1	187527	6.88	12718	0.0137	1	0.017	15	0.0275
			0.5	2	3224	6448											
	5-2	2	0.5	1	3123	6246	6256	12512	0.1	187527	6.67	12332	0.0132	1	0.017	15	0.0267
			0.5	2	3133	6266											
	5-3	2	0.5	1	3053	6106	6127	12254	0.1	187527	6.53	12074	0.0130	1	0.017	15	0.0261
			0.5	2	3074	6148											
	6-1	2	0.5	1	3833	7666	7750	15500	0.1	187527	8.27	15320	0.0165	1	0.017	15	0.0332
			0.5	2	3917	7834											
	6-2	2	0.5	1	3901	7802	7759	15518	0.1	187527	8.28	15338	0.0165	1	0.017	15	0.0332
			0.5	2	3858	7716											
	6-3	2	0.5	1	3614	7228	7294	14588	0.1	187527	7.78	14408	0.0155	1	0.017	15	0.0312
			0.5	2	3680	7360											
	7-1	2	0.5	1	4443	8886	9081	18162	0.1	187527	9.69	17982	0.0193	1	0.017	15	0.0389
			0.5	2	4638	9276											
	7-2	2	0.5	1	4409	8818	8832	17664	0.1	187527	9.42	17484	0.0188	1	0.017	15	0.0379
			0.5	2	4423	8846											
	7-3	2	0.5	1	4231	8462	8493	16986	0.1	187527	9.06	16806	0.0180	1	0.017	15	0.0364
			0.5	2	4262	8524											
	8-1	2	0.5	1	4093	8186	8320	16640	0.1	187527	8.87	16460	0.0177	1	0.017	15	0.0356
			0.5	2	4227	8454											
	8-2	2	0.5	1	4655	9310	9390	18780	0.1	187527	10.01	18600	0.0200	1	0.017	15	0.0403
			0.5	2	4736	9470											
	8-3	2	0.5	1	4521	9042	9184	18366	0.1	187527	9.79	18188	0.0195	1	0.017	15	0.0394
			0.5	2	4663	9326											

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0419	0.0006
Full activity	End	0.0370	0.0011
Full activity	Overall	0.0394	0.0029
Background	Beginning	0.0000	2.44939E-05
Background	End	0.0000	4.5926E-05
Background	Overall	0.0000	6.15782E-05
Positive	Beginning	0.0187	0.0019
Positive	End	0.0193	0.0002
Positive	Overall	0.0190	0.0011
Negative	Beginning	0.0403	0.0012
Negative	End	0.0360	0.0026
Negative	Overall	0.0381	0.0030

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 3	1	1	1.00E-05	-5.00	0.0000
Ref Chem 3	1	2	1.00E-05	-5.00	0.0001
Ref Chem 3	1	3	1.00E-05	-5.00	0.0001
Ref Chem 3	2	1	1.00E-06	-6.00	0.0008
Ref Chem 3	2	2	1.00E-06	-6.00	0.0009
Ref Chem 3	2	3	1.00E-06	-6.00	0.0009
Ref Chem 3	3	1	1.00E-07	-7.00	0.0073
Ref Chem 3	3	2	1.00E-07	-7.00	0.0078
Ref Chem 3	3	3	1.00E-07	-7.00	0.0075
Ref Chem 3	4	1	2.50E-08	-7.60	0.0196
Ref Chem 3	4	2	2.50E-08	-7.60	0.0195
Ref Chem 3	4	3	2.50E-08	-7.60	0.0187
Ref Chem 3	5	1	1.00E-08	-8.00	0.0275
Ref Chem 3	5	2	1.00E-08	-8.00	0.0267
Ref Chem 3	5	3	1.00E-08	-8.00	0.0261
Ref Chem 3	6	1	5.00E-09	-8.30	0.0332
Ref Chem 3	6	2	5.00E-09	-8.30	0.0332
Ref Chem 3	6	3	5.00E-09	-8.30	0.0312
Ref Chem 3	7	1	1.00E-09	-9.00	0.0389
Ref Chem 3	7	2	1.00E-09	-9.00	0.0379
Ref Chem 3	7	3	1.00E-09	-9.00	0.0364
Ref Chem 3	8	1	1.00E-10	-10.00	0.0356
Ref Chem 3	8	2	1.00E-10	-10.00	0.0403
Ref Chem 3	8	3	1.00E-10	-10.00	0.0394

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	0.11	0.15	0.21
2	-6.00	2.04	2.37	2.28
3	-7.00	18.43	19.74	19.09
4	-7.60	49.73	49.47	47.43
5	-8.00	69.81	67.69	66.28
6	-8.30	84.09	84.19	79.09
7	-9.00	98.71	95.97	92.25
8	-10.00	90.35	102.10	99.84

Aromatase Assay Spreadsheet

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0218	40517	1858578	
2	0.0214	38144	1782430	
3	0.0214	40428	1889159	
4	0.0212	41160	1941509	
5	0.0212	37938	1789528	
			Average DPM/g soln	1852241
			SD	67414
			CV	3.64
			µCi/g soln	0.834

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1067 g
Mass of dilution B used in substrate prep	4.5677 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563448 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00944 µg/g soln.
µg/g soln.

a. µCi/g soln 0.834
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.563448 + 0.00944
= 0.572892 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.456 µCi/µg ASDN

925971 dpm/nmol

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8					
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.1	100	11540-43
	0.578	0.396	0.234	0.184	0.134	0.130	0.122			
	0.577	0.385	0.248	0.183	0.142	0.125	0.119			
	0.593	0.389	0.239	0.188	0.145	0.129	0.121			

Samples:	11540-6A	11540-6B	11540-43B	11345-17I
	0.374	0.145	0.192	0.135
	0.360	0.150	0.186	0.132
			0.188	0.134

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0502	0.583	0.462	0.0483
125	100	200	0.00013	200	0.0251	0.390	0.269	0.0276
50	200	1000	0.00005	200	0.0100	0.240	0.120	0.0115
25	100	1000	0.00003	200	0.0050	0.185	0.064	0.0056
10	40	1000	0.00001	200	0.0020	0.140	0.020	0.0008
5	20	1000	0.00001	200	0.0010	0.128	0.007	-0.0005
			Blank	0.121				

Variables	Regression results	
m, b	0.107	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.991	0.002
F, df	445	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.991
m= 0.107
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.374	0.253	0.026	200	1	1	0.130	0.126	0.11
11540-6A	0.360	0.239	0.024	200	1	1	0.122		
11540-6A									
11540-6B	0.145	0.024	0.001	200	1	1	0.006	0.008	0.012
11540-6B	0.150	0.029	0.002	200	1	1	0.009		
11540-6B									
11540-43B	0.192	0.071	0.006	200	1	320	10.2	9.6	
11540-43B	0.186	0.065	0.006	200	1	320	9.1		
11540-43B	0.188	0.067	0.006	200	1	320	9.5		
11345-17I	0.135	0.014	0.000	200	1	600	0.7	0.2	
11345-17I	0.132	0.011	0.000	200	1	600	-0.3		
11345-17I	0.134	0.013	0.000	200	1	600	0.4		

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8					
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20			

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.578	0.396	0.234	0.184	0.134	0.130	0.122	25.1	100	11540-43
	0.577	0.385	0.248	0.183	0.142	0.125	0.119			
	0.593	0.389	0.239	0.188	0.145	0.129	0.121			

Samples:	11540-6A	11540-6B	11540-43B	11345-17I
	0.374	0.145	0.192	0.135
	0.360	0.150	0.186	0.132
			0.188	0.134

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0502	0.583	0.462	0.0424
125	100	200	0.00013	200	0.0251	0.390	0.269	0.0246
50	200	1000	0.00005	200	0.0100	0.240	0.120	0.0108
25	100	1000	0.00003	200	0.0050	0.185	0.064	0.0057
10	40	1000	0.00001	200	0.0020	0.140	0.020	0.0016
5	20	1000	0.00001	200	0.0010	0.128	0.007	0.0004

Variables	Regression results	
m, b	0.092	0.000
se_m, se_b	0.004	0.000
r^2, se_y	0.995	0.001
F, df	650	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

			r^2=	0.995
Blank	0.121		m=	0.092
			b=	0.000

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.374	0.253	0.023	200	1	1	0.116	0.113	0.11
11540-6A	0.360	0.239	0.022	200	1	1	0.109		
11540-6A									
11540-6B	0.145	0.024	0.002	200	1	1	0.010	0.011	0.012
11540-6B	0.150	0.029	0.002	200	1	1	0.012		
11540-6B									
11540-43B	0.192	0.071	0.006	200	1	320	10.2	9.7	
11540-43B	0.186	0.065	0.006	200	1	320	9.3		
11540-43B	0.188	0.067	0.006	200	1	320	9.6		
11345-17I	0.135	0.014	0.001	200	1	600	3.3	2.9	
11345-17I	0.132	0.011	0.001	200	1	600	2.4		
11345-17I	0.134	0.013	0.001	200	1	600	3.0		

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate #	1	3

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	9.7
Protein Concentration (dilution added to assay, mg/mL):	0.030209

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	2.50E-08
5	1.00E-08
6	5.00E-09
7	1.00E-09
8	1.00E-10

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	6	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed						
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4440	8880	8867	17734	0.1	185224	9.57	17574	0.0190	1	0.015	15	0.0419
			0.5	2	4427	8854											
	2	2	0.5	1	4928	9856	9698	19396	0.1	185224	10.47	19236	0.0208	1	0.015	15	0.0458
			0.5	2	4770	9540											
	3	2	0.5	1	4189	8378	8512	17024	0.1	185224	9.19	16864	0.0182	1	0.015	15	0.0402
			0.5	2	4323	8646											
	4	2	0.5	1	4225	8452	8441	16882	0.1	185224	9.11	16722	0.0181	1	0.015	15	0.0399
			0.5	2	4215	8430											
Background control	1	2	0.5	1	35	70	80	160	0.1	185224	0.09	-1	0.0000	1	0.015	15	0.0000
			0.5	2	45	90											
	2	2	0.5	1	51	102	87	174	0.1	185224	0.09	14	0.0000	1	0.015	15	0.0000
			0.5	2	36	72											
	3	2	0.5	1	35	70	74	148	0.1	185224	0.08	-13	0.0000	1	0.015	15	0.0000
			0.5	2	39	78											
	4	2	0.5	1	33	66	80	160	0.1	185224	0.09	-1	0.0000	1	0.015	15	0.0000
			0.5	2	47	94											
Positive control	1	2	0.5	1	2359	4718	4747	9494	0.1	185224	5.13	9334	0.0101	1	0.015	15	0.0222
			0.5	2	2388	4776											
	2	2	0.5	1	2336	4672	4709	9418	0.1	185224	5.08	9258	0.0100	1	0.015	15	0.0221
			0.5	2	2373	4746											
	3	2	0.5	1	2106	4212	4250	8500	0.1	185224	4.59	8340	0.0090	1	0.015	15	0.0199
			0.5	2	2144	4288											
	4	2	0.5	1	2038	4076	4060	8120	0.1	185224	4.38	7960	0.0086	1	0.015	15	0.0190
			0.5	2	2022	4044											
Negative Control	1	2	0.5	1	4645	9290	9308	18616	0.1	185224	10.05	18456	0.0199	1	0.015	15	0.0440
			0.5	2	4663	9326											
	2	2	0.5	1	4753	9506	9421	18842	0.1	185224	10.17	18682	0.0202	1	0.015	15	0.0445
			0.5	2	4668	9336											
	3	2	0.5	1	4098	8196	8258	16516	0.1	185224	8.92	16356	0.0177	1	0.015	15	0.0390
			0.5	2	4160	8320											
	4	2	0.5	1	3877	7754	7897	15794	0.1	185224	8.53	15634	0.0169	1	0.015	15	0.0373
			0.5	2	4020	8040											
Ref Chem 3	1-1	2	0.5	1	42	84	79	158	0.1	185224	0.09	-3	0.0000	1	0.015	15	0.0000
			0.5	2	37	74											
	1-2	2	0.5	1	37	74	67	134	0.1	185224	0.07	-27	0.0000	1	0.015	15	-0.0001
			0.5	2	30	60											
	1-3	2	0.5	1	48	96	94	188	0.1	185224	0.10	28	0.0000	1	0.015	15	0.0001
			0.5	2	46	92											
	2-1	2	0.5	1	153	306	340	680	0.1	185224	0.37	520	0.0006	1	0.015	15	0.0012
			0.5	2	187	374											
	2-2	2	0.5	1	168	336	342	684	0.1	185224	0.37	524	0.0006	1	0.015	15	0.0012
			0.5	2	174	348											
	2-3	2	0.5	1	171	342	339	678	0.1	185224	0.37	518	0.0006	1	0.015	15	0.0012
			0.5	2	168	336											
	3-1	2	0.5	1	874	1748	1721	3442	0.1	185224	1.86	3282	0.0035	1	0.015	15	0.0078
			0.5	2	847	1694											
	3-2	2	0.5	1	870	1740	1693	3386	0.1	185224	1.83	3226	0.0035	1	0.015	15	0.0077
			0.5	2	823	1646											
	3-3	2	0.5	1	817	1634	1619	3238	0.1	185224	1.75	3078	0.0033	1	0.015	15	0.0073
			0.5	2	802	1604											
	4-1	2	0.5	1	2102	4204	4239	8478	0.1	185224	4.58	8318	0.0090	1	0.015	15	0.0198
			0.5	2	2137	4274											
	4-2	2	0.5	1	2118	4236	4231	8462	0.1	185224	4.57	8302	0.0090	1	0.015	15	0.0198
			0.5	2	2113	4226											
	4-3	2	0.5	1	2034	4068	4020	8040	0.1	185224	4.34	7880	0.0085	1	0.015	15	0.0188
			0.5	2	1986	3972											
	5-1	2	0.5	1	3023	6046	6082	12164	0.1	185224	6.57	12004	0.0130	1	0.015	15	0.0286
			0.5	2	3059	6118											
	5-2	2	0.5	1	3101	6202	6227	12454	0.1	185224	6.72	12294	0.0133	1	0.015	15	0.0293
			0.5	2	3126	6252											
	5-3	2	0.5	1	3189	6378	6375	12750	0.1	185224	6.88	12590	0.0136	1	0.015	15	0.0300
			0.5	2	3186	6372											
	6-1	2	0.5	1	3797	7594	7574	15148	0.1	185224	8.18	14988	0.0162	1	0.015	15	0.0357
			0.5	2	3777	7554											
	6-2	2	0.5	1	3853	7706	7676	15352	0.1	185224	8.29	15192	0.0164	1	0.015	15	0.0362
			0.5	2	3823	7646											
	6-3	2	0.5	1	3838	7676	7631	15262	0.1	185224	8.24	15102	0.0163	1	0.015	15	0.0360
			0.5	2	3793	7586											
	7-1	2	0.5	1	4622	9244	9129	18258	0.1	185224	9.86	18098	0.0195	1	0.015	15	0.0431
			0.5	2	4507	9014											
	7-2	2	0.5	1	4480	8960	8942	17884	0.1	185224	9.66	17724	0.0191	1	0.015	15	0.0422
			0.5	2	4462	8924											
	7-3	2	0.5	1	4392	8784	8866	17732	0.1	185224	9.57	17572	0.0190	1	0.015	15	0.0419
			0.5	2	4474	8948											
	8-1	2	0.5	1	4818	9636	9670	19340	0.1	185224	10.44	19180	0.0207	1	0.015	15	0.0457
			0.5	2	4852	9704											
	8-2	2	0.5	1	4789	9578	9657	19314	0.1	185224	10.43	19154	0.0207	1	0.015	15	0.0456
			0.5	2	4868	9736											
	8-3	2	0.5	1	4747	9494	9420	18840	0.1	185224	10.17	18680	0.0202	1	0.015	15	0.0445
			0.5	2	4673	9346											

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0439	0.0028
Full activity	End	0.0400	0.0002
Full activity	Overall	0.0419	0.0027
Background	Beginning	0.0000	2.35933E-05
Background	End	0.0000	2.02228E-05
Background	Overall	0.0000	2.53347E-05
Positive	Beginning	0.0222	0.0001
Positive	End	0.0194	0.0006
Positive	Overall	0.0208	0.0016
Negative	Beginning	0.0443	0.0004
Negative	End	0.0381	0.0012
Negative	Overall	0.0412	0.0036

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 3	1	1	1.00E-05	-5.00	0.0000
Ref Chem 3	1	2	1.00E-05	-5.00	-0.0001
Ref Chem 3	1	3	1.00E-05	-5.00	0.0001
Ref Chem 3	2	1	1.00E-06	-6.00	0.0012
Ref Chem 3	2	2	1.00E-06	-6.00	0.0012
Ref Chem 3	2	3	1.00E-06	-6.00	0.0012
Ref Chem 3	3	1	1.00E-07	-7.00	0.0078
Ref Chem 3	3	2	1.00E-07	-7.00	0.0077
Ref Chem 3	3	3	1.00E-07	-7.00	0.0073
Ref Chem 3	4	1	2.50E-08	-7.60	0.0198
Ref Chem 3	4	2	2.50E-08	-7.60	0.0198
Ref Chem 3	4	3	2.50E-08	-7.60	0.0188
Ref Chem 3	5	1	1.00E-08	-8.00	0.0286
Ref Chem 3	5	2	1.00E-08	-8.00	0.0293
Ref Chem 3	5	3	1.00E-08	-8.00	0.0300
Ref Chem 3	6	1	5.00E-09	-8.30	0.0357
Ref Chem 3	6	2	5.00E-09	-8.30	0.0362
Ref Chem 3	6	3	5.00E-09	-8.30	0.0360
Ref Chem 3	7	1	1.00E-09	-9.00	0.0431
Ref Chem 3	7	2	1.00E-09	-9.00	0.0422
Ref Chem 3	7	3	1.00E-09	-9.00	0.0419
Ref Chem 3	8	1	1.00E-10	-10.00	0.0457
Ref Chem 3	8	2	1.00E-10	-10.00	0.0456
Ref Chem 3	8	3	1.00E-10	-10.00	0.0445

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	-0.01	-0.15	0.16
2	-6.00	2.95	2.97	2.94
3	-7.00	18.65	18.33	17.49
4	-7.60	47.26	47.17	44.77
5	-8.00	68.21	69.86	71.54
6	-8.30	85.16	86.32	85.81
7	-9.00	102.84	100.71	99.85
8	-10.00	108.98	108.84	106.14

Aromatase Assay Spreadsheet

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0197	39439	2001980	
2	0.0197	39916	2026193	
3	0.0197	41062	2084365	
4	0.0197	41258	2094315	
5	0.0196	42288	2157551	
			Average DPM/g soln	2072881
			SD	61170
			CV	2.95
			µCi/g soln	0.934

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.1	10		1010.00
Dilution A			100	10.10
Dilution B			10	1.01

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0744 g
Mass of dilution B used in substrate prep	4.025 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.503474 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01057 µg/g soln.
µg/g soln.

a. µCi/g soln 0.934
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.503474 + 0.01057
= 0.514044 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.816 µCi/µg ASDN

1154907 dpm/nmol

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0
	0.489	0.324	0.234	0.181	0.138	0.119	0.123
	0.498	0.323	0.243	0.178	0.139	0.125	0.115
	0.516	0.363	0.240	0.188	0.143	0.133	0.118

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25	100	11540-60

Samples:	11540-6A	11540-6B	11540-25H	11345-25I	11540-60D	11540-60C
	0.339	0.153	0.354	0.146	0.189	0.189
	0.345	0.148	0.348	0.134	0.186	0.188
			0.351	0.142	0.190	0.191

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.501	0.382	0.0481
125	100	200	0.00013	200	0.0250	0.337	0.218	0.0264
50	200	1000	0.00005	200	0.0100	0.239	0.120	0.0135
25	100	1000	0.00003	200	0.0050	0.182	0.064	0.0060
10	40	1000	0.00001	200	0.0020	0.140	0.021	0.0004
5	20	1000	0.00001	200	0.0010	0.126	0.007	-0.0014

Variables	Regression results	
m, b	0.132	-0.002
se_m, se_b	0.008	0.002
r^2, se_y	0.985	0.003
F, df	261	4
ss_{regr}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank	0.119	r^2=	0.985
		m=	0.132
		b=	-0.002

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.339	0.220	0.027	200	1	1	0.133	0.135	0.11
11540-6A	0.345	0.226	0.027	200	1	1	0.137		
11540-6A									
11540-6B	0.153	0.034	0.002	200	1	1	0.011	0.009	0.012
11540-6B	0.148	0.029	0.002	200	1	1	0.008		
11540-6B									
11540-25H	0.354	0.235	0.029	200	1	50	7.2	7.1	
11540-25H	0.348	0.229	0.028	200	1	50	7.0		
11540-25H	0.351	0.232	0.028	200	1	50	7.1		
11345-25I	0.146	0.027	0.001	200	1	600	3.7	1.6	
11345-25I	0.134	0.015	0.000	200	1	600	-1.0		
11345-25I	0.142	0.023	0.001	200	1	600	2.1		
11540-60D	0.189	0.070	0.007	200	1	320	11.1	10.9	
11540-60D	0.186	0.067	0.007	200	1	320	10.4		
11540-60D	0.190	0.071	0.007	200	1	320	11.3		
11540-60C	0.189	0.070	0.007	200	1	800	27.6	27.8	
11540-60C	0.188	0.069	0.007	200	1	800	27.1		
11540-60C	0.191	0.072	0.007	200	1	800	28.7		

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11540-60
	0.489	0.324	0.234	0.181	0.138	0.119	0.123			
	0.498	0.323	0.243	0.178	0.139	0.125	0.115			
	0.516	0.363	0.240	0.188	0.143	0.133	0.118			

Samples:	11540-6A	11540-6B	11540-25H	11345-25I	11540-60D	11540-60C
	0.339	0.153	0.354	0.146	0.189	0.189
	0.345	0.148	0.348	0.134	0.186	0.188
			0.351	0.142	0.190	0.191

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.501	0.382	0.0419
125	100	200	0.00013	200	0.0250	0.337	0.218	0.0234
50	200	1000	0.00005	200	0.0100	0.239	0.120	0.0125
25	100	1000	0.00003	200	0.0050	0.182	0.064	0.0061
10	40	1000	0.00001	200	0.0020	0.140	0.021	0.0013
5	20	1000	0.00001	200	0.0010	0.126	0.007	-0.0003

Variables		Regression results		
m, b		0.112	-0.001	
se_m, se_b		0.012	0.001	
r^2, se_y		0.969	0.002	
F, df		95	3	
ss_{reg}, ss_{resid}		0.000	0.000	

Blank 0.119

r^2= 0.969
m= 0.112
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.339	0.220	0.024	200	1	1	0.119	0.120	0.11
11540-6A	0.345	0.226	0.024	200	1	1	0.122		
11540-6A									
11540-6B	0.153	0.034	0.003	200	1	1	0.014	0.013	0.012
11540-6B	0.148	0.029	0.002	200	1	1	0.011		
11540-6B									
11540-25H	0.354	0.235	0.025	200	1	50	6.3	6.3	
11540-25H	0.348	0.229	0.025	200	1	50	6.2		
11540-25H	0.351	0.232	0.025	200	1	50	6.3		
11345-25I	0.146	0.027	0.002	200	1	600	6.0	4.2	
11345-25I	0.134	0.015	0.001	200	1	600	1.9		
11345-25I	0.142	0.023	0.002	200	1	600	4.6		
11540-60D	0.189	0.070	0.007	200	1	320	10.9	10.8	
11540-60D	0.186	0.067	0.006	200	1	320	10.4		
11540-60D	0.190	0.071	0.007	200	1	320	11.1		
11540-60C	0.189	0.070	0.007	200	1	800	27.3	27.5	
11540-60C	0.188	0.069	0.007	200	1	800	26.9		
11540-60C	0.191	0.072	0.007	200	1	800	28.2		

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate #	1

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	27.5
Protein Concentration (dilution added to assay, mg/mL):	0.03434

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5579	11158	11121	22242	0.1	207288	10.73	22074	0.0191	1	0.017	15	0.0371
			0.5	2	5542	11084											
	2	2	0.5	1	5726	11452	11442	22884	0.1	207288	11.04	22716	0.0197	1	0.017	15	0.0382
			0.5	2	5716	11432											
	3	2	0.5	1	5289	10578	10608	21216	0.1	207288	10.24	21048	0.0182	1	0.017	15	0.0354
			0.5	2	5319	10638											
	4	2	0.5	1	5220	10440	10686	21372	0.1	207288	10.31	21204	0.0184	1	0.017	15	0.0356
			0.5	2	5466	10932											
Background control	1	2	0.5	1	42	84	86	172	0.1	207288	0.08	4	0.0000	1	0.017	15	0.0000
			0.5	2	44	88											
	2	2	0.5	1	54	108	85	170	0.1	207288	0.08	2	0.0000	1	0.017	15	0.0000
			0.5	2	31	62											
	3	2	0.5	1	34	68	77	154	0.1	207288	0.07	-15	0.0000	1	0.017	15	0.0000
			0.5	2	43	86											
	4	2	0.5	1	39	78	89	178	0.1	207288	0.09	10	0.0000	1	0.017	15	0.0000
			0.5	2	50	100											
Postive control	1	2	0.5	1	2773	5546	5538	11076	0.1	207288	5.34	10908	0.0094	1	0.017	15	0.0183
			0.5	2	2765	5530											
	2	2	0.5	1	2833	5666	5588	11176	0.1	207288	5.39	11008	0.0095	1	0.017	15	0.0185
			0.5	2	2755	5510											
	3	2	0.5	1	2612	5224	5263	10526	0.1	207288	5.08	10358	0.0090	1	0.017	15	0.0174
			0.5	2	2651	5302											
	4	2	0.5	1	2490	4980	5016	10032	0.1	207288	4.84	9864	0.0085	1	0.017	15	0.0166
			0.5	2	2526	5052											
Negative Control	1	2	0.5	1	5490	10980	11107	22214	0.1	207288	10.72	22046	0.0191	1	0.017	15	0.0371
			0.5	2	5617	11234											
	2	2	0.5	1	5391	10782	10858	21716	0.1	207288	10.48	21548	0.0187	1	0.017	15	0.0362
			0.5	2	5467	10934											
	3	2	0.5	1	5193	10386	10505	21010	0.1	207288	10.14	20842	0.0180	1	0.017	15	0.0350
			0.5	2	5312	10624											
	4	2	0.5	1	5185	10370	10693	21386	0.1	207288	10.32	21218	0.0184	1	0.017	15	0.0357
			0.5	2	5508	11016											
Ref Chem 4	1-1	2	0.5	1	45	90	86	172	0.1	207288	0.08	4	0.0000	1	0.017	15	0.0000
			0.5	2	41	82											
	1-2	2	0.5	1	49	98	104	208	0.1	207288	0.10	40	0.0000	1	0.017	15	0.0001
			0.5	2	55	110											
	1-3	2	0.5	1	38	76	95	190	0.1	207288	0.09	22	0.0000	1	0.017	15	0.0000
			0.5	2	57	114											
	2-1	2	0.5	1	447	894	892	1784	0.1	207288	0.86	1616	0.0014	1	0.017	15	0.0027
			0.5	2	445	890											
	2-2	2	0.5	1	313	626	700	1400	0.1	207288	0.68	1232	0.0011	1	0.017	15	0.0021
			0.5	2	387	774											
	2-3	2	0.5	1	413	826	770	1540	0.1	207288	0.74	1372	0.0012	1	0.017	15	0.0023
			0.5	2	357	714											
	3-1	2	0.5	1	4919	9838	9775	19550	0.1	207288	9.43	19382	0.0168	1	0.017	15	0.0326
			0.5	2	4856	9712											
	3-2	2	0.5	1	4818	9636	9697	19394	0.1	207288	9.36	19226	0.0166	1	0.017	15	0.0323
			0.5	2	4879	9758											
	3-3	2	0.5	1	4643	9286	9317	18634	0.1	207288	8.99	18466	0.0160	1	0.017	15	0.0310
			0.5	2	4674	9348											
	4-1	2	0.5	1	5375	10750	10770	21540	0.1	207288	10.39	21372	0.0185	1	0.017	15	0.0359
			0.5	2	5395	10790											
	4-2	2	0.5	1	5409	10818	10812	21624	0.1	207288	10.43	21456	0.0186	1	0.017	15	0.0361
			0.5	2	5403	10806											
	4-3	2	0.5	1	5335	10670	10719	21438	0.1	207288	10.34	21270	0.0184	1	0.017	15	0.0358
			0.5	2	5384	10768											
	5-1	2	0.5	1	5560	11120	11182	22364	0.1	207288	10.79	22196	0.0192	1	0.017	15	0.0373
			0.5	2	5622	11244											
	5-2	2	0.5	1	5484	10968	10812	21624	0.1	207288	10.43	21456	0.0186	1	0.017	15	0.0361
			0.5	2	5328	10656											
	5-3	2	0.5	1	5399	10798	10862	21724	0.1	207288	10.48	21556	0.0187	1	0.017	15	0.0362
			0.5	2	5463	10926											
	6-1	2	0.5	1	5631	11262	11351	22702	0.1	207288	10.95	22534	0.0195	1	0.017	15	0.0379
			0.5	2	5720	11440											
	6-2	2	0.5	1	5454	10908	10731	21462	0.1	207288	10.35	21294	0.0184	1	0.017	15	0.0358
			0.5	2	5277	10554											
	6-3	2	0.5	1	5454	10906	10845	21690	0.1	207288	10.46	21522	0.0186	1	0.017	15	0.0362
			0.5	2	5391	10782											
	7-1	2	0.5	1	5464	10928	11105	22210	0.1	207288	10.71	22042	0.0191	1	0.017	15	0.0371
			0.5	2	5641	11282											
	7-2	2	0.5	1	5457	10914	10997	21994	0.1	207288	10.61	21826	0.0189	1	0.017	15	0.0367
			0.5	2	5540	11080											
	7-3	2	0.5	1	5294	10588	10620	21240	0.1	207288	10.25	21072	0.0182	1	0.017	15	0.0354
			0.5	2	5326	10652											
	8-1	2	0.5	1	5690	11380	11210	22420	0.1	207288	10.82	22252	0.0193	1	0.017	15	0.0374
			0.5	2	5520	11040											
	8-2	2	0.5	1	5364	10728	10877	21754	0.1	207288	10.49	21586	0.0187	1	0.017	15	0.0363
			0.5	2	5513	11026											
	8-3	2	0.5	1	5256	10512	10670	21340	0.1	207288	10.29	21172	0.0183	1	0.017	15	0.0356
			0.5	2	5414	10828											

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0376	0.0008
Full activity	End	0.0355	0.0002
Full activity	Overall	0.0366	0.0013
Background	Beginning	0.0000	2.37725E-06
Background	End	0.0000	2.8527E-05
Background	Overall	0.0000	1.72248E-05
Positive	Beginning	0.0184	0.0001
Positive	End	0.0170	0.0006
Positive	Overall	0.0177	0.0009
Negative	Beginning	0.0366	0.0006
Negative	End	0.0353	0.0004
Negative	Overall	0.0360	0.0009

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 4	1	1	1.00E-03	-3.00	0.0000
Ref Chem 4	1	2	1.00E-03	-3.00	0.0001
Ref Chem 4	1	3	1.00E-03	-3.00	0.0000
Ref Chem 4	2	1	1.00E-04	-4.00	0.0027
Ref Chem 4	2	2	1.00E-04	-4.00	0.0021
Ref Chem 4	2	3	1.00E-04	-4.00	0.0023
Ref Chem 4	3	1	1.00E-05	-5.00	0.0326
Ref Chem 4	3	2	1.00E-05	-5.00	0.0323
Ref Chem 4	3	3	1.00E-05	-5.00	0.0310
Ref Chem 4	4	1	1.00E-06	-6.00	0.0359
Ref Chem 4	4	2	1.00E-06	-6.00	0.0361
Ref Chem 4	4	3	1.00E-06	-6.00	0.0358
Ref Chem 4	5	1	1.00E-07	-7.00	0.0373
Ref Chem 4	5	2	1.00E-07	-7.00	0.0361
Ref Chem 4	5	3	1.00E-07	-7.00	0.0362
Ref Chem 4	6	1	1.00E-08	-8.00	0.0379
Ref Chem 4	6	2	1.00E-08	-8.00	0.0358
Ref Chem 4	6	3	1.00E-08	-8.00	0.0362
Ref Chem 4	7	1	1.00E-09	-9.00	0.0371
Ref Chem 4	7	2	1.00E-09	-9.00	0.0367
Ref Chem 4	7	3	1.00E-09	-9.00	0.0354
Ref Chem 4	8	1	1.00E-10	-10.00	0.0374
Ref Chem 4	8	2	1.00E-10	-10.00	0.0363
Ref Chem 4	8	3	1.00E-10	-10.00	0.0356

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.02	0.18	0.10
2	-4.00	7.42	5.66	6.30
3	-5.00	89.07	88.35	84.86
4	-6.00	98.21	98.60	97.75
5	-7.00	102.00	98.60	99.06
6	-8.00	103.55	97.86	98.90
7	-9.00	101.29	100.30	96.84
8	-10.00	102.26	99.20	97.30

Aromatase Assay Spreadsheet

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8		
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0192	35647	1856615	
2	0.0194	33333	1718196	
3	0.0193	35234	1825596	
4	0.0195	35552	1823179	
5	0.0195	36233	1858103	
			Average DPM/g soln	1816338
			SD	57295
			CV	3.15
			µCi/g soln	0.818

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.3	10		1030.00
Dilution A			100	10.30
Dilution B			10	1.03

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1284 g
Mass of dilution B used in substrate prep	4.5646 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.578409 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00926 µg/g soln.
µg/g soln.

a. µCi/g soln 0.818
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.578409 + 0.00926
= 0.587671 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.392 µCi/µg ASDN

885188 dpm/nmol

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	26.3	100	11540-62
	0.510	0.355	0.244	0.178	0.148	0.122	0.128			
	0.572	0.334	0.230	0.193	0.142	0.128	0.124			
	0.550	0.388	0.243	0.181	0.146	0.133	0.129			

Samples:	11540-6A	11540-6B	11540-26H	11345-26I	11540-62D	11540-62C
	0.339	0.147	0.362	0.131	0.204	0.190
	0.334	0.146	0.349	0.139	0.198	0.199
			0.361	0.148	0.192	0.193

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0526	0.544	0.417	0.0510
125	100	200	0.00013	200	0.0263	0.359	0.232	0.0279
50	200	1000	0.00005	200	0.0105	0.239	0.112	0.0129
25	100	1000	0.00003	200	0.0053	0.184	0.057	0.0060
10	40	1000	0.00001	200	0.0021	0.145	0.018	0.0011
5	20	1000	0.00001	200	0.0011	0.128	0.001	-0.0011
			Blank	0.127				

Variables	Regression results	
m, b	0.125	-0.001
se_m, se_b	0.006	0.001
r^2, se_y	0.992	0.002
F, df	486	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

r^2= 0.992
m= 0.125
b= -0.001

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.339	0.212	0.025	200	1	1	0.127	0.125	0.11
11540-6A	0.334	0.207	0.025	200	1	1	0.124		
11540-6A									
11540-6B	0.147	0.020	0.001	200	1	1	0.007	0.006	0.012
11540-6B	0.146	0.019	0.001	200	1	1	0.006		
11540-6B									
11540-26H	0.362	0.235	0.028	200	1	50	7.1	6.9	
11540-26H	0.349	0.222	0.027	200	1	50	6.7		
11540-26H	0.361	0.234	0.028	200	1	50	7.0		
11345-26I	0.131	0.004	-0.001	200	1	600	-2.0	1.2	
11345-26I	0.139	0.012	0.000	200	1	600	1.0		
11345-26I	0.148	0.021	0.001	200	1	600	4.4		
11540-62D	0.204	0.077	0.008	200	1	320	13.6	12.4	
11540-62D	0.198	0.071	0.008	200	1	320	12.4		
11540-62D	0.192	0.065	0.007	200	1	320	11.2		
11540-62C	0.190	0.063	0.007	200	1	800	26.9	28.9	
11540-62C	0.199	0.072	0.008	200	1	800	31.4		
11540-62C	0.193	0.066	0.007	200	1	800	28.4		

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	26.3	100	11540-62
	0.510	0.355	0.244	0.178	0.148	0.122	0.128			
	0.572	0.334	0.230	0.193	0.142	0.128	0.124			
	0.550	0.388	0.243	0.181	0.146	0.133	0.129			

Samples:	11540-6A	11540-6B	11540-26H	11345-26I	11540-62D	11540-62C
	0.339	0.147	0.362	0.131	0.204	0.190
	0.334	0.146	0.349	0.139	0.198	0.199
			0.361	0.148	0.192	0.193

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0526	0.544	0.417	0.0457
125	100	200	0.00013	200	0.0263	0.359	0.232	0.0253
50	200	1000	0.00005	200	0.0105	0.239	0.112	0.0121
25	100	1000	0.00003	200	0.0053	0.184	0.057	0.0061
10	40	1000	0.00001	200	0.0021	0.145	0.018	0.0018
5	20	1000	0.00001	200	0.0011	0.128	0.001	-0.0001
			Blank	0.127				

Variables	Regression results	
m, b	0.110	0.000
se_m, se_b	0.007	0.001
r^2, se_y	0.987	0.001
F, df	223	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.987
m= 0.110
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.339	0.212	0.023	200	1	1	0.116	0.114	0.11
11540-6A	0.334	0.207	0.023	200	1	1	0.113		
11540-6A									
11540-6B	0.147	0.020	0.002	200	1	1	0.010	0.010	0.012
11540-6B	0.146	0.019	0.002	200	1	1	0.009		
11540-6B									
11540-26H	0.362	0.235	0.026	200	1	50	6.4	6.3	
11540-26H	0.349	0.222	0.024	200	1	50	6.1		
11540-26H	0.361	0.234	0.026	200	1	50	6.4		
11345-26I	0.131	0.004	0.000	200	1	600	0.7	3.5	
11345-26I	0.139	0.012	0.001	200	1	600	3.4		
11345-26I	0.148	0.021	0.002	200	1	600	6.3		
11540-62D	0.204	0.077	0.008	200	1	320	13.2	12.2	
11540-62D	0.198	0.071	0.008	200	1	320	12.2		
11540-62D	0.192	0.065	0.007	200	1	320	11.1		
11540-62C	0.190	0.063	0.007	200	1	800	26.9	28.7	
11540-62C	0.199	0.072	0.008	200	1	800	30.9		
11540-62C	0.193	0.066	0.007	200	1	800	28.3		

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate #	2

Microsome Dilution Details

Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	28.7
Protein Concentration (dilution added to assay, mg/mL):	0.035874

Test Chemical Concentrations

Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	5.00E-05
4	2.50E-05
5	1.00E-05
6	5.00E-06
7	1.00E-06
8	1.00E-07

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5735	11470	11371	22742	0.1	181634	12.52	22609	0.0255	1	0.018	15	0.0475
			0.5	2	5636	11272											
	2	2	0.5	1	5710	11420	11509	23018	0.1	181634	12.67	22885	0.0259	1	0.018	15	0.0480
			0.5	2	5799	11598											
	3	2	0.5	1	5144	10288	10198	20396	0.1	181634	11.23	20263	0.0229	1	0.018	15	0.0425
			0.5	2	5054	10108											
	4	2	0.5	1	5108	10216	10250	20500	0.1	181634	11.29	20367	0.0230	1	0.018	15	0.0428
			0.5	2	5142	10284											
Background control	1	2	0.5	1	29	58	56	112	0.1	181634	0.06	-21	0.0000	1	0.018	15	0.0000
			0.5	2	27	54											
	2	2	0.5	1	36	72	67	134	0.1	181634	0.07	1	0.0000	1	0.018	15	0.0000
			0.5	2	31	62											
	3	2	0.5	1	42	84	82	164	0.1	181634	0.09	31	0.0000	1	0.018	15	0.0001
			0.5	2	40	80											
	4	2	0.5	1	28	56	61	122	0.1	181634	0.07	-11	0.0000	1	0.018	15	0.0000
			0.5	2	33	66											
Positive control	1	2	0.5	1	2772	5544	5492	10984	0.1	181634	6.05	10851	0.0123	1	0.018	15	0.0228
			0.5	2	2720	5440											
	2	2	0.5	1	2740	5480	5562	11124	0.1	181634	6.12	10991	0.0124	1	0.018	15	0.0231
			0.5	2	2822	5644											
	3	2	0.5	1	2544	5088	5054	10108	0.1	181634	5.57	9975	0.0113	1	0.018	15	0.0209
			0.5	2	2510	5020											
	4	2	0.5	1	2419	4838	4880	9760	0.1	181634	5.37	9627	0.0109	1	0.018	15	0.0202
			0.5	2	2461	4922											
Negative Control	1	2	0.5	1	5689	11378	11320	22640	0.1	181634	12.46	22507	0.0254	1	0.018	15	0.0473
			0.5	2	5631	11262											
	2	2	0.5	1	5917	11834	11789	23578	0.1	181634	12.98	23445	0.0265	1	0.018	15	0.0492
			0.5	2	5872	11744											
	3	2	0.5	1	5207	10414	10358	20716	0.1	181634	11.41	20583	0.0233	1	0.018	15	0.0432
			0.5	2	5151	10302											
	4	2	0.5	1	5066	10132	10244	20488	0.1	181634	11.28	20355	0.0230	1	0.018	15	0.0427
			0.5	2	5178	10356											
Ref Chem 4	1-1	2	0.5	1	39	78	83	166	0.1	181634	0.09	33	0.0000	1	0.018	15	0.0001
			0.5	2	44	88											
	1-2	2	0.5	1	44	88	82	164	0.1	181634	0.09	31	0.0000	1	0.018	15	0.0001
			0.5	2	38	76											
	1-3	2	0.5	1	36	72	68	136	0.1	181634	0.07	3	0.0000	1	0.018	15	0.0000
			0.5	2	32	64											
	2-1	2	0.5	1	379	758	823	1646	0.1	181634	0.91	1513	0.0017	1	0.018	15	0.0032
			0.5	2	444	886											
	2-2	2	0.5	1	426	852	845	1690	0.1	181634	0.93	1557	0.0018	1	0.018	15	0.0033
			0.5	2	419	838											
	2-3	2	0.5	1	412	824	816	1632	0.1	181634	0.90	1499	0.0017	1	0.018	15	0.0031
			0.5	2	404	808											
	3-1	2	0.5	1	1269	2538	2505	5010	0.1	181634	2.76	4877	0.0055	1	0.018	15	0.0102
			0.5	2	1236	2472											
	3-2	2	0.5	1	1238	2476	2505	5010	0.1	181634	2.76	4877	0.0055	1	0.018	15	0.0102
			0.5	2	1267	2534											
	3-3	2	0.5	1	1218	2436	2429	4858	0.1	181634	2.67	4725	0.0053	1	0.018	15	0.0099
			0.5	2	1211	2422											
	4-1	2	0.5	1	2628	5256	5324	10648	0.1	181634	5.86	10515	0.0119	1	0.018	15	0.0221
			0.5	2	2696	5392											
	4-2	2	0.5	1	2629	5258	5284	10568	0.1	181634	5.82	10435	0.0118	1	0.018	15	0.0219
			0.5	2	2655	5310											
	4-3	2	0.5	1	2486	4972	4947	9894	0.1	181634	5.45	9761	0.0110	1	0.018	15	0.0205
			0.5	2	2461	4922											
	5-1	2	0.5	1	4687	9374	9597	19194	0.1	181634	10.57	19061	0.0215	1	0.018	15	0.0400
			0.5	2	4910	9820											
	5-2	2	0.5	1	4698	9396	9535	19070	0.1	181634	10.50	18937	0.0214	1	0.018	15	0.0398
			0.5	2	4837	9674											
	5-3	2	0.5	1	4631	9262	9135	18270	0.1	181634	10.06	18137	0.0205	1	0.018	15	0.0381
			0.5	2	4504	9008											
	6-1	2	0.5	1	5235	10470	10530	21060	0.1	181634	11.59	20927	0.0236	1	0.018	15	0.0439
			0.5	2	5295	10590											
	6-2	2	0.5	1	5148	10296	10250	20500	0.1	181634	11.29	20367	0.0230	1	0.018	15	0.0428
			0.5	2	5102	10204											
	6-3	2	0.5	1	5201	10402	10423	20846	0.1	181634	11.48	20713	0.0234	1	0.018	15	0.0435
			0.5	2	5222	10444											
	7-1	2	0.5	1	5718	11436	11303	22606	0.1	181634	12.45	22473	0.0254	1	0.018	15	0.0472
			0.5	2	5585	11170											
	7-2	2	0.5	1	5255	10510	10524	21048	0.1	181634	11.59	20915	0.0236	1	0.018	15	0.0439
			0.5	2	5269	10538											
	7-3	2	0.5	1	5238	10476	10453	20906	0.1	181634	11.51	20773	0.0235	1	0.018	15	0.0436
			0.5	2	5215	10430											
	8-1	2	0.5	1	5471	10942	10913	21826	0.1	181634	12.02	21693	0.0245	1	0.018	15	0.0455
			0.5	2	5442	10884											
	8-2	2	0.5	1	5326	10652	10829	21658	0.1	181634	11.92	21525	0.0243	1	0.018	15	0.0452
			0.5	2	5503	11006											
	8-3	2	0.5	1	5230	10460	10503	21006	0.1	181634	11.57	20873	0.0236	1	0.018	15	0.0438
			0.5	2	5273	10546											

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0478	0.0004
Full activity	End	0.0426	0.0002
Full activity	Overall	0.0452	0.0030
Background	Beginning	0.0000	3.26591E-05
Background	End	0.0000	6.23493E-05
Background	Overall	0.0000	4.73183E-05
Positive	Beginning	0.0229	0.0002
Positive	End	0.0206	0.0005
Positive	Overall	0.0218	0.0014
Negative	Beginning	0.0482	0.0014
Negative	End	0.0430	0.0003
Negative	Overall	0.0456	0.0031

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 4	1	1	1.00E-03	-3.00	0.0001
Ref Chem 4	1	2	1.00E-03	-3.00	0.0001
Ref Chem 4	1	3	1.00E-03	-3.00	0.0000
Ref Chem 4	2	1	1.00E-04	-4.00	0.0032
Ref Chem 4	2	2	1.00E-04	-4.00	0.0033
Ref Chem 4	2	3	1.00E-04	-4.00	0.0031
Ref Chem 4	3	1	5.00E-05	-4.30	0.0102
Ref Chem 4	3	2	5.00E-05	-4.30	0.0102
Ref Chem 4	3	3	5.00E-05	-4.30	0.0099
Ref Chem 4	4	1	2.50E-05	-4.60	0.0221
Ref Chem 4	4	2	2.50E-05	-4.60	0.0219
Ref Chem 4	4	3	2.50E-05	-4.60	0.0205
Ref Chem 4	5	1	1.00E-05	-5.00	0.0400
Ref Chem 4	5	2	1.00E-05	-5.00	0.0398
Ref Chem 4	5	3	1.00E-05	-5.00	0.0381
Ref Chem 4	6	1	5.00E-06	-5.30	0.0439
Ref Chem 4	6	2	5.00E-06	-5.30	0.0428
Ref Chem 4	6	3	5.00E-06	-5.30	0.0435
Ref Chem 4	7	1	1.00E-06	-6.00	0.0472
Ref Chem 4	7	2	1.00E-06	-6.00	0.0439
Ref Chem 4	7	3	1.00E-06	-6.00	0.0436
Ref Chem 4	8	1	1.00E-07	-7.00	0.0455
Ref Chem 4	8	2	1.00E-07	-7.00	0.0452
Ref Chem 4	8	3	1.00E-07	-7.00	0.0438

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.15	0.14	0.01
2	-4.00	7.03	7.23	6.96
3	-4.30	22.65	22.65	21.95
4	-4.60	48.84	48.47	45.33
5	-5.00	88.53	87.95	84.24
6	-5.30	97.19	94.59	96.20
7	-6.00	104.38	97.14	96.48
8	-7.00	100.75	99.97	96.94

Aromatase Assay Spreadsheet

Assay Date	9/30/2005	Test Chemical ID	Ref Chem	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0113	23447	2074956
2	0.0112	22768	2032857
3	0.0110	23955	2177727
4			
5			

Average DPM/g soln	2095180
SD	74523
CV	3.56
µCi/g soln	0.944

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.1	10		1010.00
Dilution A			100	10.10
Dilution B			10	1.01

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0731 g
Mass of dilution B used in substrate prep	4.5525 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.569549 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01068 µg/g soln.
µg/g soln.

a. µCi/g soln 0.944
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.569549 + 0.01068
= 0.580233 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.627 µCi/µg ASDN

1034171 dpm/nmol

Assay Date 9/30/2005 | Test Chemical ID Ref Chem 4 | # Concentrations tested 8

Technician ID 1 | Replicate # 3 | Microsome type Placental | Microsome ID 11540-22

	250	125	50	25	10	5	0
Standards:	0.501	0.324	0.232	0.167	0.143	0.123	0.107
	0.502	0.321	0.230	0.173	0.149	0.132	0.126
	0.526	0.349	0.231	0.176	0.138	0.119	0.121

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25.9	100	11540-64

	11540-6A	11540-6B	11540-6I	11345-64H
Samples:	0.313	0.147	0.191	0.197
	0.317	0.189	0.202	0.185
			0.192	0.183

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0518	0.510	0.392	0.0506
125	100	200	0.00013	200	0.0259	0.331	0.213	0.0267
50	200	1000	0.00005	200	0.0104	0.231	0.113	0.0132
25	100	1000	0.00003	200	0.0052	0.172	0.054	0.0053
10	40	1000	0.00001	200	0.0021	0.143	0.025	0.0015
5	20	1000	0.00001	200	0.0010	0.125	0.007	-0.0010
			Blank	0.118				

Variables	Regression results	
m, b	0.134	-0.002
se_m, se_b	0.006	0.001
r^2, se_y	0.992	0.002
F, df	517	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.992
m= 0.134
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.313	0.195	0.024	200	1	1	0.121	0.123	0.11
11540-6A	0.317	0.199	0.025	200	1	1	0.124		
11540-6A									
11540-6B	0.147	0.029	0.002	200	1	1	0.010	0.024	0.012
11540-6B	0.189	0.071	0.008	200	1	1	0.038		
11540-6B									
11540-6I	0.191	0.073	0.008	200	1	320	12.6	13.5	
11540-6I	0.202	0.084	0.009	200	1	320	15.0		
11540-6I	0.192	0.074	0.008	200	1	320	12.8		
11345-64H	0.197	0.079	0.009	200	1	800	34.7	30.1	
11345-64H	0.185	0.067	0.007	200	1	800	28.3		
11345-64H	0.183	0.065	0.007	200	1	800	27.2		

Assay Date	9/30/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8						
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.501	0.324	0.232	0.167	0.143	0.123	0.107	25.9	100	11540-62
	0.502	0.321	0.230	0.173	0.149	0.132	0.126			
	0.526	0.349	0.231	0.176	0.138	0.119	0.121			

Samples:	11540-6A	11540-6B	11540-64I	11345-64H
	0.313	0.147	0.191	0.197
	0.317	0.189	0.202	0.185
			0.192	0.183

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0518	0.510	0.392	0.0462
125	100	200	0.00013	200	0.0259	0.331	0.213	0.0247
50	200	1000	0.00005	200	0.0104	0.231	0.113	0.0126
25	100	1000	0.00003	200	0.0052	0.172	0.054	0.0055
10	40	1000	0.00001	200	0.0021	0.143	0.025	0.0020
5	20	1000	0.00001	200	0.0010	0.125	0.007	-0.0002
			Blank	0.118				

Variables	Regression results	
m, b	0.121	-0.001
se_m, se_b	0.010	0.001
r^2, se_y	0.980	0.002
F, df	149	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.980
m= 0.121
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.313	0.195	0.022	200	1	1	0.112	0.114	0.11
11540-6A	0.317	0.199	0.023	200	1	1	0.115		
11540-6A									
11540-6B	0.147	0.029	0.002	200	1	1	0.012	0.025	0.012
11540-6B	0.189	0.071	0.008	200	1	1	0.038		
11540-6B									
11540-64I	0.191	0.073	0.008	200	1	320	12.4	13.2	
11540-64I	0.202	0.084	0.009	200	1	320	14.6		
11540-64I	0.192	0.074	0.008	200	1	320	12.6		
11345-64H	0.197	0.079	0.008	200	1	800	34.0	29.8	
11345-64H	0.185	0.067	0.007	200	1	800	28.2		
11345-64H	0.183	0.065	0.007	200	1	800	27.2		

Assay Date	9/30/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	3

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	29.8
Protein Concentration (dilution added to assay, mg/mL):	0.037233

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	5.00E-05
4	2.50E-05
5	1.00E-05
6	5.00E-06
7	1.00E-06
8	1.00E-07

Assay Date	9/30/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	7193	14386	14607	29214	0.1	209518	13.94	29057	0.0281	1	0.019	15	0.0503
			0.5	2	7414	14828											
	2	2	0.5	1	7283	14566	14731	29462	0.1	209518	14.06	29305	0.0283	1	0.019	15	0.0507
			0.5	2	7448	14896											
	3	2	0.5	1	7222	14444	14433	28866	0.1	209518	13.78	28709	0.0278	1	0.019	15	0.0497
			0.5	2	7211	14422											
	4	2	0.5	1	7259	14518	14540	29080	0.1	209518	13.88	28923	0.0280	1	0.019	15	0.0501
			0.5	2	7281	14562											
Background control	1	2	0.5	1	45	90	85	170	0.1	209518	0.08	13	0.0000	1	0.019	15	0.0000
			0.5	2	40	80											
	2	2	0.5	1	31	62	62	124	0.1	209518	0.06	-33	0.0000	1	0.019	15	-0.0001
			0.5	2	31	62											
	3	2	0.5	1	49	98	94	188	0.1	209518	0.09	31	0.0000	1	0.019	15	0.0001
			0.5	2	45	90											
	4	2	0.5	1	39	78	73	146	0.1	209518	0.07	-11	0.0000	1	0.019	15	0.0000
			0.5	2	34	68											
Positive control	1	2	0.5	1	3448	6896	6856	13712	0.1	209518	6.54	13555	0.0131	1	0.019	15	0.0235
			0.5	2	3408	6816											
	2	2	0.5	1	3306	6612	6802	13604	0.1	209518	6.49	13447	0.0130	1	0.019	15	0.0233
			0.5	2	3496	6992											
	3	2	0.5	1	3237	6474	6598	13196	0.1	209518	6.30	13039	0.0125	1	0.019	15	0.0226
			0.5	2	3361	6722											
	4	2	0.5	1	3105	6210	6272	12544	0.1	209518	5.99	12387	0.0120	1	0.019	15	0.0214
			0.5	2	3167	6334											
Negative Control	1	2	0.5	1	7298	14596	14652	29304	0.1	209518	13.99	29147	0.0282	1	0.019	15	0.0505
			0.5	2	7354	14708											
	2	2	0.5	1	7302	14604	14749	29498	0.1	209518	14.08	29341	0.0284	1	0.019	15	0.0508
			0.5	2	7447	14894											
	3	2	0.5	1	7188	14376	14302	28604	0.1	209518	13.65	28447	0.0275	1	0.019	15	0.0493
			0.5	2	7114	14228											
	4	2	0.5	1	6888	13776	13885	27770	0.1	209518	13.25	27613	0.0267	1	0.019	15	0.0478
			0.5	2	6997	13994											
Ref Chem 4	1-1	2	0.5	1	29	58	74	148	0.1	209518	0.07	-9	0.0000	1	0.019	15	0.0000
			0.5	2	45	90											
	1-2	2	0.5	1	38	76	69	138	0.1	209518	0.07	-19	0.0000	1	0.019	15	0.0000
			0.5	2	31	62											
	1-3	2	0.5	1	43	86	95	190	0.1	209518	0.09	33	0.0000	1	0.019	15	0.0001
			0.5	2	52	104											
	2-1	2	0.5	1	558	1116	1109	2218	0.1	209518	1.06	2061	0.0020	1	0.019	15	0.0036
			0.5	2	551	1102											
	2-2	2	0.5	1	513	1026	1043	2086	0.1	209518	1.00	1929	0.0019	1	0.019	15	0.0033
			0.5	2	530	1060											
	2-3	2	0.5	1	521	1042	1043	2086	0.1	209518	1.00	1929	0.0019	1	0.019	15	0.0033
			0.5	2	522	1044											
	3-1	2	0.5	1	1755	3510	3542	7084	0.1	209518	3.38	6927	0.0067	1	0.019	15	0.0120
			0.5	2	1787	3574											
	3-2	2	0.5	1	1772	3544	3504	7008	0.1	209518	3.34	6851	0.0066	1	0.019	15	0.0119
			0.5	2	1732	3464											
	3-3	2	0.5	1	1637	3274	3336	6672	0.1	209518	3.18	6515	0.0063	1	0.019	15	0.0113
			0.5	2	1699	3398											
	4-1	2	0.5	1	3315	6630	6617	13234	0.1	209518	6.32	13077	0.0126	1	0.019	15	0.0226
			0.5	2	3302	6604											
	4-2	2	0.5	1	3493	6986	6913	13826	0.1	209518	6.60	13669	0.0132	1	0.019	15	0.0237
			0.5	2	3420	6840											
	4-3	2	0.5	1	3368	6736	6594	13188	0.1	209518	6.29	13031	0.0126	1	0.019	15	0.0226
			0.5	2	3226	6452											
	5-1	2	0.5	1	6146	12292	12344	24688	0.1	209518	11.78	24531	0.0237	1	0.019	15	0.0425
			0.5	2	6198	12396											
	5-2	2	0.5	1	6319	12638	12557	25114	0.1	209518	11.99	24957	0.0241	1	0.019	15	0.0432
			0.5	2	6238	12476											
	5-3	2	0.5	1	6077	12154	12089	24178	0.1	209518	11.54	24021	0.0232	1	0.019	15	0.0416
			0.5	2	6012	12024											
	6-1	2	0.5	1	6910	13820	13746	27492	0.1	209518	13.12	27335	0.0264	1	0.019	15	0.0473
			0.5	2	6836	13672											
	6-2	2	0.5	1	6960	13920	13927	27854	0.1	209518	13.29	27697	0.0268	1	0.019	15	0.0480
			0.5	2	6967	13934											
	6-3	2	0.5	1	6757	13514	13536	27072	0.1	209518	12.92	26915	0.0260	1	0.019	15	0.0466
			0.5	2	6779	13558											
	7-1	2	0.5	1	7117	14234	13908	27816	0.1	209518	13.28	27659	0.0267	1	0.019	15	0.0479
			0.5	2	6791	13582											
	7-2	2	0.5	1	7020	14040	14134	28268	0.1	209518	13.49	28111	0.0272	1	0.019	15	0.0487
			0.5	2	7114	14228											
	7-3	2	0.5	1	7156	14312	14401	28802	0.1	209518	13.75	28645	0.0277	1	0.019	15	0.0496
			0.5	2	7245	14490											
	8-1	2	0.5	1	6844	13688	13615	27230	0.1	209518	13.00	27073	0.0262	1	0.019	15	0.0469
			0.5	2	6771	13542											
	8-2	2	0.5	1	6927	13854	14111	28222	0.1	209518	13.47	28065	0.0271	1	0.019	15	0.0486
			0.5	2	7184	14368											
	8-3	2	0.5	1	7107	14214	14177	28354	0.1	209518	13.53	28197	0.0273	1	0.019	15	0.0488
			0.5	2	7070	14140											

Assay Date	9/30/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0505	0.0003
Full activity	End	0.0499	0.0003
Full activity	Overall	0.0502	0.0004
Background	Beginning	0.0000	5.63156E-05
Background	End	0.0000	5.14186E-05
Background	Overall	0.0000	4.83541E-05
Positive	Beginning	0.0234	0.0001
Positive	End	0.0220	0.0008
Positive	Overall	0.0227	0.0009
Negative	Beginning	0.0506	0.0002
Negative	End	0.0485	0.0010
Negative	Overall	0.0496	0.0014

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 4	1	1	1.00E-03	-3.00	0.0000
Ref Chem 4	1	2	1.00E-03	-3.00	0.0000
Ref Chem 4	1	3	1.00E-03	-3.00	0.0001
Ref Chem 4	2	1	1.00E-04	-4.00	0.0036
Ref Chem 4	2	2	1.00E-04	-4.00	0.0033
Ref Chem 4	2	3	1.00E-04	-4.00	0.0033
Ref Chem 4	3	1	5.00E-05	-4.30	0.0120
Ref Chem 4	3	2	5.00E-05	-4.30	0.0119
Ref Chem 4	3	3	5.00E-05	-4.30	0.0113
Ref Chem 4	4	1	2.50E-05	-4.60	0.0226
Ref Chem 4	4	2	2.50E-05	-4.60	0.0237
Ref Chem 4	4	3	2.50E-05	-4.60	0.0226
Ref Chem 4	5	1	1.00E-05	-5.00	0.0425
Ref Chem 4	5	2	1.00E-05	-5.00	0.0432
Ref Chem 4	5	3	1.00E-05	-5.00	0.0416
Ref Chem 4	6	1	5.00E-06	-5.30	0.0473
Ref Chem 4	6	2	5.00E-06	-5.30	0.0480
Ref Chem 4	6	3	5.00E-06	-5.30	0.0466
Ref Chem 4	7	1	1.00E-06	-6.00	0.0479
Ref Chem 4	7	2	1.00E-06	-6.00	0.0487
Ref Chem 4	7	3	1.00E-06	-6.00	0.0496
Ref Chem 4	8	1	1.00E-07	-7.00	0.0469
Ref Chem 4	8	2	1.00E-07	-7.00	0.0486
Ref Chem 4	8	3	1.00E-07	-7.00	0.0488

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	-0.03	-0.07	0.11
2	-4.00	7.11	6.65	6.65
3	-4.30	23.89	23.63	22.47
4	-4.60	45.10	47.14	44.94
5	-5.00	84.59	86.06	82.84
6	-5.30	94.26	95.51	92.82
7	-6.00	95.38	96.94	98.78
8	-7.00	93.36	96.78	97.24

Aromatase Assay Spreadsheet

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0197	39439	2001980	
2	0.0197	39916	2026193	
3	0.0197	41062	2084365	
4	0.0197	41258	2094315	
5	0.0196	42288	2157551	
			Average DPM/g soln	2072881
			SD	61170
			CV	2.95
			µCi/g soln	0.934

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.1	10		1010.00
Dilution A			100	10.10
Dilution B			10	1.01

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0744 g
Mass of dilution B used in substrate prep	4.025 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.503474 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01057 µg/g soln.
µg/g soln.

a. µCi/g soln 0.934
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.503474 + 0.01057
= 0.514044 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.816 µCi/µg ASDN

1154907 dpm/nmol

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0
	0.489	0.324	0.234	0.181	0.138	0.119	0.123
	0.498	0.323	0.243	0.178	0.139	0.125	0.115
	0.516	0.363	0.240	0.188	0.143	0.133	0.118

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25	100	11540-60

Samples:	11540-6A	11540-6B	11540-25H	11345-25I	11540-60D	11540-60C
	0.339	0.153	0.354	0.146	0.189	0.189
	0.345	0.148	0.348	0.134	0.186	0.188
			0.351	0.142	0.190	0.191

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.501	0.382	0.0481
125	100	200	0.00013	200	0.0250	0.337	0.218	0.0264
50	200	1000	0.00005	200	0.0100	0.239	0.120	0.0135
25	100	1000	0.00003	200	0.0050	0.182	0.064	0.0060
10	40	1000	0.00001	200	0.0020	0.140	0.021	0.0004
5	20	1000	0.00001	200	0.0010	0.126	0.007	-0.0014

Variables	Regression results	
m, b	0.132	-0.002
se_m, se_b	0.008	0.002
r^2, se_y	0.985	0.003
F, df	261	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank	0.119

r^2= 0.985
m= 0.132
b= -0.002

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.339	0.220	0.027	200	1	1	0.133	0.135	0.11
11540-6A	0.345	0.226	0.027	200	1	1	0.137		
11540-6A									
11540-6B	0.153	0.034	0.002	200	1	1	0.011	0.009	0.012
11540-6B	0.148	0.029	0.002	200	1	1	0.008		
11540-6B									
11540-25H	0.354	0.235	0.029	200	1	50	7.2	7.1	
11540-25H	0.348	0.229	0.028	200	1	50	7.0		
11540-25H	0.351	0.232	0.028	200	1	50	7.1		
11345-25I	0.146	0.027	0.001	200	1	600	3.7	1.6	
11345-25I	0.134	0.015	0.000	200	1	600	-1.0		
11345-25I	0.142	0.023	0.001	200	1	600	2.1		
11540-60D	0.189	0.070	0.007	200	1	320	11.1	10.9	
11540-60D	0.186	0.067	0.007	200	1	320	10.4		
11540-60D	0.190	0.071	0.007	200	1	320	11.3		
11540-60C	0.189	0.070	0.007	200	1	800	27.6	27.8	
11540-60C	0.188	0.069	0.007	200	1	800	27.1		
11540-60C	0.191	0.072	0.007	200	1	800	28.7		

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.489	0.324	0.234	0.181	0.138	0.119	0.123	25	100	11540-60
	0.498	0.323	0.243	0.178	0.139	0.125	0.115			
	0.516	0.363	0.240	0.188	0.143	0.133	0.118			

Samples:	11540-6A	11540-6B	11540-25H	11345-25I	11540-60D	11540-60C
	0.339	0.153	0.354	0.146	0.189	0.189
	0.345	0.148	0.348	0.134	0.186	0.188
			0.351	0.142	0.190	0.191

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}
250	200	200	0.00025	200	0.0500	0.501	0.382
125	100	200	0.00013	200	0.0250	0.337	0.218
50	200	1000	0.00005	200	0.0100	0.239	0.120
25	100	1000	0.00003	200	0.0050	0.182	0.064
10	40	1000	0.00001	200	0.0020	0.140	0.021
5	20	1000	0.00001	200	0.0010	0.126	0.007
			Blank	0.119			

Curve Output	Variables	Regression results	
0.0419	m, b	0.112	-0.001
0.0234	se_m, se_b	0.012	0.001
0.0125	r^2, se_y	0.969	0.002
0.0061	F, df	95	3
0.0013	ss_{reg}, ss_{resid}	0.000	0.000
-0.0003			

r^2= 0.969
m= 0.112
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.339	0.220	0.024	200	1	1	0.119	0.120	0.11
11540-6A	0.345	0.226	0.024	200	1	1	0.122		
11540-6A									
11540-6B	0.153	0.034	0.003	200	1	1	0.014	0.013	0.012
11540-6B	0.148	0.029	0.002	200	1	1	0.011		
11540-6B									
11540-25H	0.354	0.235	0.025	200	1	50	6.3	6.3	
11540-25H	0.348	0.229	0.025	200	1	50	6.2		
11540-25H	0.351	0.232	0.025	200	1	50	6.3		
11345-25I	0.146	0.027	0.002	200	1	600	6.0	4.2	
11345-25I	0.134	0.015	0.001	200	1	600	1.9		
11345-25I	0.142	0.023	0.002	200	1	600	4 6		
11540-60D	0.189	0.070	0.007	200	1	320	10.9	10.8	
11540-60D	0.186	0.067	0.006	200	1	320	10.4		
11540-60D	0.190	0.071	0.007	200	1	320	11.1		
11540-60C	0.189	0.070	0.007	200	1	800	27.3	27.5	
11540-60C	0.188	0.069	0.007	200	1	800	26.9		
11540-60C	0.191	0.072	0.007	200	1	800	28.2		

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	1

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	10.8
Protein Concentration (dilution added to assay, mg/mL):	0.033778

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	5.00E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3943	7886	8007	16014	0.1	207288	7.73	15821	0.0137	1	0.017	15	0.0270
			0.5	2	4064	8128											
	2	2	0.5	1	4134	8268	8242	16484	0.1	207288	7.95	16291	0.0141	1	0.017	15	0.0278
			0.5	2	4108	8216											
	3	2	0.5	1	4173	8346	8312	16624	0.1	207288	8.02	16431	0.0142	1	0.017	15	0.0281
			0.5	2	4139	8278											
	4	2	0.5	1	3983	7966	8069	16138	0.1	207288	7.79	15945	0.0138	1	0.017	15	0.0272
			0.5	2	4086	8172											
Background control	1	2	0.5	1	56	112	110	220	0.1	207288	0.11	27	0.0000	1	0.017	15	0.0000
			0.5	2	54	108											
	2	2	0.5	1	52	104	89	178	0.1	207288	0.09	-15	0.0000	1	0.017	15	0.0000
			0.5	2	37	74											
	3	2	0.5	1	42	84	104	208	0.1	207288	0.10	15	0.0000	1	0.017	15	0.0000
			0.5	2	62	124											
	4	2	0.5	1	47	94	83	166	0.1	207288	0.08	-27	0.0000	1	0.017	15	0.0000
			0.5	2	36	72											
Positive control	1	2	0.5	1	1943	3886	3908	7816	0.1	207288	3.77	7623	0.0066	1	0.017	15	0.0130
			0.5	2	1965	3930											
	2	2	0.5	1	1924	3848	3706	7412	0.1	207288	3.58	7219	0.0063	1	0.017	15	0.0123
			0.5	2	1782	3564											
	3	2	0.5	1	1932	3864	3854	7708	0.1	207288	3.72	7515	0.0065	1	0.017	15	0.0128
			0.5	2	1922	3844											
	4	2	0.5	1	1912	3824	3921	7842	0.1	207288	3.78	7649	0.0066	1	0.017	15	0.0131
			0.5	2	2009	4018											
Negative Control	1	2	0.5	1	4164	8328	8346	16692	0.1	207288	8.05	16499	0.0143	1	0.017	15	0.0282
			0.5	2	4182	8364											
	2	2	0.5	1	4308	8616	8615	17230	0.1	207288	8.31	17037	0.0148	1	0.017	15	0.0291
			0.5	2	4307	8614											
	3	2	0.5	1	4162	8324	8283	16566	0.1	207288	7.99	16373	0.0142	1	0.017	15	0.0280
			0.5	2	4121	8242											
	4	2	0.5	1	4078	8156	8186	16372	0.1	207288	7.90	16179	0.0140	1	0.017	15	0.0276
			0.5	2	4108	8216											
Ref Chem 5	1-1	2	0.5	1	4295	8590	8549	17098	0.1	207288	8.25	16905	0.0146	1	0.017	15	0.0289
			0.5	2	4254	8508											
	1-2	2	0.5	1	4227	8454	8427	16854	0.1	207288	8.13	16661	0.0144	1	0.017	15	0.0285
			0.5	2	4200	8400											
	1-3	2	0.5	1	3987	7974	7934	15868	0.1	207288	7.66	15675	0.0136	1	0.017	15	0.0268
			0.5	2	3947	7894											
	2-1	2	0.5	1	4343	8686	8590	17180	0.1	207288	8.29	16987	0.0147	1	0.017	15	0.0290
			0.5	2	4247	8494											
	2-2	2	0.5	1	4230	8460	8484	16968	0.1	207288	8.19	16775	0.0145	1	0.017	15	0.0287
			0.5	2	4254	8508											
	2-3	2	0.5	1	4060	8120	8293	16586	0.1	207288	8.00	16393	0.0142	1	0.017	15	0.0280
			0.5	2	4233	8466											
	3-1	2	0.5	1	4077	8154	8261	16522	0.1	207288	7.97	16329	0.0141	1	0.017	15	0.0279
			0.5	2	4184	8368											
	3-2	2	0.5	1	4230	8460	8507	17014	0.1	207288	8.21	16821	0.0146	1	0.017	15	0.0287
			0.5	2	4277	8554											
	3-3	2	0.5	1	4064	8128	8114	16228	0.1	207288	7.83	16035	0.0139	1	0.017	15	0.0274
			0.5	2	4050	8100											
	4-1	2	0.5	1	4140	8280	8401	16802	0.1	207288	8.11	16609	0.0144	1	0.017	15	0.0284
			0.5	2	4261	8522											
	4-2	2	0.5	1	4009	8018	8095	16190	0.1	207288	7.81	15997	0.0139	1	0.017	15	0.0273
			0.5	2	4086	8172											
	4-3	2	0.5	1	4182	8324	8160	16320	0.1	207288	7.87	16127	0.0140	1	0.017	15	0.0276
			0.5	2	3998	7996											
	5-1	2	0.5	1	4370	8740	8703	17406	0.1	207288	8.40	17213	0.0149	1	0.017	15	0.0294
			0.5	2	4333	8666											
	5-2	2	0.5	1	4283	8566	8539	17078	0.1	207288	8.24	16885	0.0146	1	0.017	15	0.0289
			0.5	2	4256	8512											
	5-3	2	0.5	1	4063	8126	8268	16536	0.1	207288	7.98	16343	0.0142	1	0.017	15	0.0279
			0.5	2	4205	8410											
	6-1	2	0.5	1	4289	8578	8535	17070	0.1	207288	8.23	16877	0.0146	1	0.017	15	0.0288
			0.5	2	4246	8492											
	6-2	2	0.5	1	4281	8562	8758	17516	0.1	207288	8.45	17323	0.0150	1	0.017	15	0.0296
			0.5	2	4477	8954											
	6-3	2	0.5	1	4044	8088	8187	16374	0.1	207288	7.90	16181	0.0140	1	0.017	15	0.0277
			0.5	2	4143	8286											
	7-1	2	0.5	1	4109	8218	8264	16528	0.1	207288	7.97	16335	0.0141	1	0.017	15	0.0279
			0.5	2	4155	8310											
	7-2	2	0.5	1	4319	8638	8584	17168	0.1	207288	8.28	16975	0.0147	1	0.017	15	0.0290
			0.5	2	4265	8530											
	7-3	2	0.5	1	4194	8388	8410	16820	0.1	207288	8.11	16627	0.0144	1	0.017	15	0.0284
			0.5	2	4216	8432											
	8-1	2	0.5	1	4271	8542	8683	17366	0.1	207288	8.38	17173	0.0149	1	0.017	15	0.0293
			0.5	2	4412	8824											
	8-2	2	0.5	1	4234	8468	8407	16814	0.1	207288	8.11	16621	0.0144	1	0.017	15	0.0284
			0.5	2	4173	8346											
	8-3	2	0.5	1	4170	8340	8367	16734	0.1	207288	8.07	16541	0.0143	1	0.017	15	0.0283
			0.5	2	4197	8394											

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0274	0.0006
Full activity	End	0.0277	0.0006
Full activity	Overall	0.0276	0.0005
Background	Beginning	0.0000	5.07533E-05
Background	End	0.0000	5.07533E-05
Background	Overall	0.0000	4.30981E-05
Positive	Beginning	0.0127	0.0005
Positive	End	0.0130	0.0002
Positive	Overall	0.0128	0.0003
Negative	Beginning	0.0287	0.0007
Negative	End	0.0278	0.0002
Negative	Overall	0.0282	0.0006

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 5	1	1	1.00E-04	-4.00	0.0289
Ref Chem 5	1	2	1.00E-04	-4.00	0.0285
Ref Chem 5	1	3	1.00E-04	-4.00	0.0268
Ref Chem 5	2	1	1.00E-05	-5.00	0.0290
Ref Chem 5	2	2	1.00E-05	-5.00	0.0287
Ref Chem 5	2	3	1.00E-05	-5.00	0.0280
Ref Chem 5	3	1	1.00E-06	-6.00	0.0279
Ref Chem 5	3	2	1.00E-06	-6.00	0.0287
Ref Chem 5	3	3	1.00E-06	-6.00	0.0274
Ref Chem 5	4	1	5.00E-07	-6.30	0.0284
Ref Chem 5	4	2	5.00E-07	-6.30	0.0273
Ref Chem 5	4	3	5.00E-07	-6.30	0.0276
Ref Chem 5	5	1	1.00E-07	-7.00	0.0294
Ref Chem 5	5	2	1.00E-07	-7.00	0.0289
Ref Chem 5	5	3	1.00E-07	-7.00	0.0279
Ref Chem 5	6	1	1.00E-08	-8.00	0.0288
Ref Chem 5	6	2	1.00E-08	-8.00	0.0296
Ref Chem 5	6	3	1.00E-08	-8.00	0.0277
Ref Chem 5	7	1	1.00E-09	-9.00	0.0279
Ref Chem 5	7	2	1.00E-09	-9.00	0.0290
Ref Chem 5	7	3	1.00E-09	-9.00	0.0284
Ref Chem 5	8	1	1.00E-10	-10.00	0.0293
Ref Chem 5	8	2	1.00E-10	-10.00	0.0284
Ref Chem 5	8	3	1.00E-10	-10.00	0.0283

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	104.86	103.34	97.23
2	-5.00	105.37	104.05	101.68
3	-6.00	101.28	104.34	99.46
4	-6.30	103.02	99.22	100.03
5	-7.00	106.77	104.73	101.37
6	-8.00	104.68	107.45	100.37
7	-9.00	101.32	105.29	103.13
8	-10.00	106.52	103.10	102.60

Aromatase Assay Spreadsheet

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8		
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0192	35647	1856615	
2	0.0194	33333	1718196	
3	0.0193	35234	1825596	
4	0.0195	35552	1823179	
5	0.0195	36233	1858103	
			Average DPM/g soln	1816338
			SD	57295
			CV	3.15
			µCi/g soln	0.818

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.3	10		1030.00
Dilution A			100	10.30
Dilution B			10	1.03

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1284 g
Mass of dilution B used in substrate prep	4.5646 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.578409 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00926 µg/g soln.
µg/g soln.

a. µCi/g soln 0.818
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.578409 + 0.00926
= 0.587671 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.392 µCi/µg ASDN

885188 dpm/nmol

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0
	0.510	0.355	0.244	0.178	0.148	0.122	0.128
	0.572	0.334	0.230	0.193	0.142	0.128	0.124
	0.550	0.388	0.243	0.181	0.146	0.133	0.129

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
26.3	100	11540-62

Samples:	11540-6A	11540-6B	11540-26H	11345-26I	11540-62D	11540-62C
	0.339	0.147	0.362	0.131	0.204	0.190
	0.334	0.146	0.349	0.139	0.198	0.199
			0.361	0.148	0.192	0.193

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0526	0.544	0.417	0.0510
125	100	200	0.00013	200	0.0263	0.359	0.232	0.0279
50	200	1000	0.00005	200	0.0105	0.239	0.112	0.0129
25	100	1000	0.00003	200	0.0053	0.184	0.057	0.0060
10	40	1000	0.00001	200	0.0021	0.145	0.018	0.0011
5	20	1000	0.00001	200	0.0011	0.128	0.001	-0.0011
			Blank	0.127				

Variables	Regression results	
m, b	0.125	-0.001
se_m, se_b	0.006	0.001
r^2, se_y	0.992	0.002
F, df	486	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.992
m= 0.125
b= -0.001

Regression resuits are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.339	0.212	0.025	200	1	1	0.127	0.125	0.11
11540-6A	0.334	0.207	0.025	200	1	1	0.124		
11540-6A									
11540-6B	0.147	0.020	0.001	200	1	1	0.007	0.006	0.012
11540-6B	0.146	0.019	0.001	200	1	1	0.006		
11540-6B									
11540-26H	0.362	0.235	0.028	200	1	50	7.1	6.9	
11540-26H	0.349	0.222	0.027	200	1	50	6.7		
11540-26H	0.361	0.234	0.028	200	1	50	7.0		
11345-26I	0.131	0.004	-0.001	200	1	600	-2.0	1.2	
11345-26I	0.139	0.012	0.000	200	1	600	1.0		
11345-26i	0.148	0.021	0.001	200	1	600	4.4		
11540-62D	0.204	0.077	0.008	200	1	320	13.6	12.4	
11540-62D	0.198	0.071	0.008	200	1	320	12.4		
11540-62D	0.192	0.065	0.007	200	1	320	11.2		
11540-62C	0.190	0.063	0.007	200	1	800	26.9	28.9	
11540-62C	0.199	0.072	0.008	200	1	800	31.4		
11540-62C	0.193	0.066	0.007	200	1	800	28.4		

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8
Technician ID	1	Replicate #	2	Microsome type	Placental

Microsome ID 11540-20

Standards:	250	125	50	25	10	5	0
	0.510	0.355	0.244	0.178	0.148	0.122	0.128
	0.572	0.334	0.230	0.193	0.142	0.128	0.124
	0.550	0.388	0.243	0.181	0.146	0.133	0.129

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
26.3	100	11540-62

Samples:	11540-6A	11540-6B	11540-26H	11345-26I	11540-62D	11540-62C
	0.339	0.147	0.362	0.131	0.204	0.190
	0.334	0.146	0.349	0.139	0.198	0.199
			0.361	0.148	0.192	0.193

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0526	0.544	0.417	0.0457
125	100	200	0.00013	200	0.0263	0.359	0.232	0.0253
50	200	1000	0.00005	200	0.0105	0.239	0.112	0.0121
25	100	1000	0.00003	200	0.0053	0.184	0.057	0.0061
10	40	1000	0.00001	200	0.0021	0.145	0.018	0.0018
5	20	1000	0.00001	200	0.0011	0.128	0.001	-0.0001
			Blank	0.127				

Variables	Regression results	
m, b	0.110	0.000
se_m, se_b	0.007	0.001
r^2, se_y	0.987	0.001
F, df	223	3
ss_{regr}, ss_{resid}	0.000	0.000

r^2= 0.987
m= 0.110
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.339	0.212	0.023	200	1	1	0.116	0.114	0.11
11540-6A	0.334	0.207	0.023	200	1	1	0.113		
11540-6A									
11540-6B	0.147	0.020	0.002	200	1	1	0.010	0.010	0.012
11540-6B	0.146	0.019	0.002	200	1	1	0.009		
11540-6B									
11540-26H	0.362	0.235	0.026	200	1	50	6.4	6.3	
11540-26H	0.349	0.222	0.024	200	1	50	6.1		
11540-26H	0.361	0.234	0.026	200	1	50	6.4		
11345-26I	0.131	0.004	0.000	200	1	600	0.7	3.5	
11345-26I	0.139	0.012	0.001	200	1	600	3.4		
11345-26I	0.148	0.021	0.002	200	1	600	6.3		
11540-62D	0.204	0.077	0.008	200	1	320	13.2	12.2	
11540-62D	0.198	0.071	0.008	200	1	320	12.2		
11540-62D	0.192	0.065	0.007	200	1	320	11.1		
11540-62C	0.190	0.063	0.007	200	1	800	26.9	28.7	
11540-62C	0.199	0.072	0.008	200	1	800	30.9		
11540-62C	0.193	0.066	0.007	200	1	800	28.3		

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	2

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	12.2
Protein Concentration (dilution added to assay, mg/mL):	0.038077

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	5.00E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4128	8256	8230	16460	0.1	181634	9.06	16316	0.0184	1	0.019	15	0.0323
			0.5	2	4102	8204											
	2	2	0.5	1	3952	7904	7956	15912	0.1	181634	8.76	15768	0.0178	1	0.019	15	0.0312
			0.5	2	4004	8008											
	3	2	0.5	1	3857	7714	7733	15466	0.1	181634	8.51	15322	0.0173	1	0.019	15	0.0303
			0.5	2	3876	7752											
	4	2	0.5	1	3894	7788	7784	15568	0.1	181634	8.57	15424	0.0174	1	0.019	15	0.0305
			0.5	2	3890	7780											
Background control	1	2	0.5	1	40	80	79	158	0.1	181634	0.09	14	0.0000	1	0.019	15	0.0000
			0.5	2	39	78											
	2	2	0.5	1	42	84	78	156	0.1	181634	0.09	12	0.0000	1	0.019	15	0.0000
			0.5	2	36	72											
	3	2	0.5	1	34	68	60	120	0.1	181634	0.07	-25	0.0000	1	0.019	15	0.0000
			0.5	2	26	52											
	4	2	0.5	1	33	66	72	144	0.1	181634	0.08	-1	0.0000	1	0.019	15	0.0000
			0.5	2	39	78											
Positive control	1	2	0.5	1	1946	3892	3968	7936	0.1	181634	4.37	7792	0.0088	1	0.019	15	0.0154
			0.5	2	2022	4044											
	2	2	0.5	1	1979	3958	3979	7958	0.1	181634	4.38	7814	0.0088	1	0.019	15	0.0155
			0.5	2	2000	4000											
	3	2	0.5	1	1914	3828	3816	7632	0.1	181634	4.20	7488	0.0085	1	0.019	15	0.0148
			0.5	2	1902	3804											
	4	2	0.5	1	1818	3636	3749	7498	0.1	181634	4.13	7354	0.0083	1	0.019	15	0.0145
			0.5	2	1931	3862											
Negative Control	1	2	0.5	1	4165	8330	8243	16486	0.1	181634	9.08	16342	0.0185	1	0.019	15	0.0323
			0.5	2	4078	8156											
	2	2	0.5	1	4011	8022	8182	16364	0.1	181634	9.01	16220	0.0183	1	0.019	15	0.0321
			0.5	2	4171	8342											
	3	2	0.5	1	3811	7622	7774	15548	0.1	181634	8.56	15404	0.0174	1	0.019	15	0.0305
			0.5	2	3963	7926											
	4	2	0.5	1	3756	7512	7378	14756	0.1	181634	8.12	14612	0.0165	1	0.019	15	0.0289
			0.5	2	3622	7244											
Ref Chem 5	1-1	2	0.5	1	3463	6926	7081	14162	0.1	181634	7.80	14018	0.0158	1	0.019	15	0.0277
			0.5	2	3618	7236											
	1-2	2	0.5	1	3509	7018	7080	14160	0.1	181634	7.80	14016	0.0158	1	0.019	15	0.0277
			0.5	2	3571	7142											
	1-3	2	0.5	1	3406	6812	6682	13364	0.1	181634	7.36	13220	0.0149	1	0.019	15	0.0261
			0.5	2	3276	6552											
	2-1	2	0.5	1	3905	7810	7914	15828	0.1	181634	8.71	15684	0.0177	1	0.019	15	0.0310
			0.5	2	4009	8018											
	2-2	2	0.5	1	3985	7970	7994	15988	0.1	181634	8.80	15844	0.0179	1	0.019	15	0.0313
			0.5	2	4009	8018											
	2-3	2	0.5	1	3926	7852	7840	15680	0.1	181634	8.63	15536	0.0176	1	0.019	15	0.0307
			0.5	2	3914	7828											
	3-1	2	0.5	1	4046	8092	8158	16316	0.1	181634	8.98	16172	0.0183	1	0.019	15	0.0320
			0.5	2	4112	8224											
	3-2	2	0.5	1	4112	8224	8140	16280	0.1	181634	8.96	16136	0.0182	1	0.019	15	0.0319
			0.5	2	4028	8056											
	3-3	2	0.5	1	3982	7964	7949	15898	0.1	181634	8.75	15754	0.0178	1	0.019	15	0.0312
			0.5	2	3967	7934											
	4-1	2	0.5	1	3956	7912	7906	15812	0.1	181634	8.71	15668	0.0177	1	0.019	15	0.0310
			0.5	2	3950	7900											
	4-2	2	0.5	1	3960	7920	7996	15992	0.1	181634	8.80	15848	0.0179	1	0.019	15	0.0313
			0.5	2	4036	8072											
	4-3	2	0.5	1	3874	7748	7804	15608	0.1	181634	8.59	15464	0.0175	1	0.019	15	0.0306
			0.5	2	3930	7860											
	5-1	2	0.5	1	4032	8064	8046	16092	0.1	181634	8.86	15948	0.0180	1	0.019	15	0.0315
			0.5	2	4014	8028											
	5-2	2	0.5	1	4016	8032	8076	16152	0.1	181634	8.89	16008	0.0181	1	0.019	15	0.0317
			0.5	2	4060	8120											
	5-3	2	0.5	1	3906	7812	7841	15682	0.1	181634	8.63	15538	0.0176	1	0.019	15	0.0307
			0.5	2	3935	7870											
	6-1	2	0.5	1	3977	7954	8084	16168	0.1	181634	8.90	16024	0.0181	1	0.019	15	0.0317
			0.5	2	4107	8214											
	6-2	2	0.5	1	3924	7848	8101	16202	0.1	181634	8.92	16058	0.0181	1	0.019	15	0.0318
			0.5	2	4177	8354											
	6-3	2	0.5	1	3924	7848	7805	15610	0.1	181634	8.59	15466	0.0175	1	0.019	15	0.0306
			0.5	2	3881	7762											
	7-1	2	0.5	1	4149	8298	8258	16516	0.1	181634	9.09	16372	0.0185	1	0.019	15	0.0324
			0.5	2	4109	8218											
	7-2	2	0.5	1	4023	8046	7846	15692	0.1	181634	8.64	15548	0.0176	1	0.019	15	0.0308
			0.5	2	3823	7646											
	7-3	2	0.5	1	3961	7922	7894	15788	0.1	181634	8.69	15644	0.0177	1	0.019	15	0.0309
			0.5	2	3933	7866											
	8-1	2	0.5	1	3928	7856	7925	15850	0.1	181634	8.73	15706	0.0177	1	0.019	15	0.0311
			0.5	2	3997	7994											
	8-2	2	0.5	1	4093	8186	8136	16272	0.1	181634	8.96	16128	0.0182	1	0.019	15	0.0319
			0.5	2	4043	8086											
	8-3	2	0.5	1	3927	7854	7923	15846	0.1	181634	8.72	15702	0.0177	1	0.019	15	0.0311
			0.5	2	3996	7992											

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0317	0.0008
Full activity	End	0.0304	0.0001
Full activity	Overall	0.0311	0.0009
Background	Beginning	0.0000	2.79724E-06
Background	End	0.0000	3.35669E-05
Background	Overall	0.0000	3.45434E-05
Positive	Beginning	0.0154	0.0000
Positive	End	0.0147	0.0002
Positive	Overall	0.0151	0.0004
Negative	Beginning	0.0322	0.0002
Negative	End	0.0297	0.0011
Negative	Overall	0.0309	0.0016

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 5	1	1	1.00E-04	-4.00	0.0277
Ref Chem 5	1	2	1.00E-04	-4.00	0.0277
Ref Chem 5	1	3	1.00E-04	-4.00	0.0261
Ref Chem 5	2	1	1.00E-05	-5.00	0.0310
Ref Chem 5	2	2	1.00E-05	-5.00	0.0313
Ref Chem 5	2	3	1.00E-05	-5.00	0.0307
Ref Chem 5	3	1	1.00E-06	-6.00	0.0320
Ref Chem 5	3	2	1.00E-06	-6.00	0.0319
Ref Chem 5	3	3	1.00E-06	-6.00	0.0312
Ref Chem 5	4	1	5.00E-07	-6.30	0.0310
Ref Chem 5	4	2	5.00E-07	-6.30	0.0313
Ref Chem 5	4	3	5.00E-07	-6.30	0.0306
Ref Chem 5	5	1	1.00E-07	-7.00	0.0315
Ref Chem 5	5	2	1.00E-07	-7.00	0.0317
Ref Chem 5	5	3	1.00E-07	-7.00	0.0307
Ref Chem 5	6	1	1.00E-08	-8.00	0.0317
Ref Chem 5	6	2	1.00E-08	-8.00	0.0318
Ref Chem 5	6	3	1.00E-08	-8.00	0.0306
Ref Chem 5	7	1	1.00E-09	-9.00	0.0324
Ref Chem 5	7	2	1.00E-09	-9.00	0.0308
Ref Chem 5	7	3	1.00E-09	-9.00	0.0309
Ref Chem 5	8	1	1.00E-10	-10.00	0.0311
Ref Chem 5	8	2	1.00E-10	-10.00	0.0319
Ref Chem 5	8	3	1.00E-10	-10.00	0.0311

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	89.24	89.23	84.16
2	-5.00	99.85	100.87	98.91
3	-6.00	102.96	102.73	100.30
4	-6.30	99.75	100.89	98.45
5	-7.00	101.53	101.91	98.92
6	-8.00	102.02	102.23	98.46
7	-9.00	104.23	98.98	99.60
8	-10.00	99.99	102.68	99.96

Aromatase Assay Spreadsheet

Assay Date	9/30/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0113	23447	2074956
2	0.0112	22768	2032857
3	0.0110	23955	2177727
4			
5			

Average DPM/g soln	2095180
SD	74523
CV	3.56
µCi/g soln	0.944

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.1	10		1010.00
Dilution A			100	10.10
Dilution B			10	1.01

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0731 g
Mass of dilution B used in substrate prep	4.5525 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.569549 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01068 µg/g soln.
µg/g soln.

a. µCi/g soln 0.944
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.569549 + 0.01068
= 0.580233 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.627 µCi/µg ASDN

1034171 dpm/nmol

Assay Date	9/30/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8						
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.501	0.324	0.232	0.167	0.143	0.123	0.107	25.9	100	11540-64
	0.502	0.321	0.230	0.173	0.149	0.132	0.126			
	0.526	0.349	0.231	0.176	0.138	0.119	0.121			

Samples:	11540-6A	11540-6B	11540-64I	11345-64H
	0.313	0.147	0.191	0.197
	0.317	0.189	0.202	0.185
			0.192	0.183

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0518	0.510	0.392	0.0506
125	100	200	0.00013	200	0.0259	0.331	0.213	0.0267
50	200	1000	0.00005	200	0.0104	0.231	0.113	0.0132
25	100	1000	0.00003	200	0.0052	0.172	0.054	0.0053
10	40	1000	0.00001	200	0.0021	0.143	0.025	0.0015
5	20	1000	0.00001	200	0.0010	0.125	0.007	-0.0010

Blank	0.118	r^2=	0.992	
		m=	0.134	
		b=	-0.002	

Variables	Regression results	
m, b	0.134	-0.002
se_m, se_b	0.006	0.001
r^2, se_y	0.992	0.002
F, df	517	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.313	0.195	0.024	200	1	1	0.121	0.123	0.11
11540-6A	0.317	0.199	0.025	200	1	1	0.124		
11540-6A									
11540-6B	0.147	0.029	0.002	200	1	1	0.010	0.024	0.012
11540-6B	0.189	0.071	0.008	200	1	1	0.038		
11540-6B									
11540-64I	0.191	0.073	0.008	200	1	320	12.6	13.5	
11540-64I	0.202	0.084	0.009	200	1	320	15.0		
11540-64I	0.192	0.074	0.008	200	1	320	12.8		
11345-64H	0.197	0.079	0.009	200	1	800	34.7	30.1	
11345-64H	0.185	0.067	0.007	200	1	800	28.3		
11345-64H	0.183	0.065	0.007	200	1	800	27.2		

Assay Date	9/30/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8						
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.501	0.324	0.232	0.167	0.143	0.123	0.107	25.9	100	11540-62
	0.502	0.321	0.230	0.173	0.149	0.132	0.126			
	0.526	0.349	0.231	0.176	0.138	0.119	0.121			

Samples:	11540-6A	11540-6B	11540-64I	11345-64H
	0.313	0.147	0.191	0.197
	0.317	0.189	0.202	0.185
			0.192	0.183

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0518	0.510	0.392	0.0462
125	100	200	0.00013	200	0.0259	0.331	0.213	0.0247
50	200	1000	0.00005	200	0.0104	0.231	0.113	0.0126
25	100	1000	0.00003	200	0.0052	0.172	0.054	0.0055
10	40	1000	0.00001	200	0.0021	0.143	0.025	0.0020
5	20	1000	0.00001	200	0.0010	0.125	0.007	-0.0002

Variables		Regression results		
m, b	0.121	-0.001		
se_m, se_b	0.010	0.001		
r^2, se_y	0.980	0.002		
F, df	149	3		
ss_{reg}, ss_{resid}	0.000	0.000		

Blank 0.118

r^2= 0.980
m= 0.121
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.313	0.195	0.022	200	1	1	0.112	0.114	0.11
11540-6A	0.317	0.199	0.023	200	1	1	0.115		
11540-6A									
11540-6B	0.147	0.029	0.002	200	1	1	0.012	0.025	0.012
11540-6B	0.189	0.071	0.008	200	1	1	0.038		
11540-6B									
11540-64I	0.191	0.073	0.008	200	1	320	12.4	13.2	
11540-64I	0.202	0.084	0.009	200	1	320	14.6		
11540-64I	0.192	0.074	0.008	200	1	320	12.6		
11345-64H	0.197	0.079	0.008	200	1	800	34.0	29.8	
11345-64H	0.185	0.067	0.007	200	1	800	28.2		
11345-64H	0.183	0.065	0.007	200	1	800	27.2		

Assay Date	9/30/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	3

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	13.2
Protein Concentration (dilution added to assay, mg/mL):	0.041252

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	5.00E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/30/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4385	8770	8792	17584	0.1	209518	8.39	17357	0.0168	1	0.021	15	0.0271
			0.5	2	4407	8814											
	2	2	0.5	1	4973	9946	9808	19616	0.1	209518	9.36	19389	0.0187	1	0.021	15	0.0303
			0.5	2	4835	9670											
	3	2	0.5	1	4982	9964	9995	19990	0.1	209518	9.54	19763	0.0191	1	0.021	15	0.0309
			0.5	2	5013	10026											
	4	2	0.5	1	5067	10134	10116	20232	0.1	209518	9.66	20005	0.0193	1	0.021	15	0.0313
			0.5	2	5049	10098											
Background control	1	2	0.5	1	56	112	141	282	0.1	209518	0.13	55	0.0001	1	0.021	15	0.0001
			0.5	2	85	170											
	2	2	0.5	1	45	90	102	204	0.1	209518	0.10	-24	0.0000	1	0.021	15	0.0000
			0.5	2	57	114											
	3	2	0.5	1	50	100	94	188	0.1	209518	0.09	-40	0.0000	1	0.021	15	-0.0001
			0.5	2	44	88											
	4	2	0.5	1	58	116	118	236	0.1	209518	0.11	9	0.0000	1	0.021	15	0.0000
			0.5	2	60	120											
Positive control	1	2	0.5	1	2179	4358	4447	8894	0.1	209518	4.24	8667	0.0084	1	0.021	15	0.0135
			0.5	2	2268	4536											
	2	2	0.5	1	2259	4518	4562	9124	0.1	209518	4.35	8897	0.0086	1	0.021	15	0.0139
			0.5	2	2303	4606											
	3	2	0.5	1	2297	4594	4543	9086	0.1	209518	4.34	8859	0.0086	1	0.021	15	0.0138
			0.5	2	2246	4492											
	4	2	0.5	1	2161	4322	4295	8590	0.1	209518	4.10	8363	0.0081	1	0.021	15	0.0131
			0.5	2	2134	4268											
Negative Control	1	2	0.5	1	4985	9970	9863	19726	0.1	209518	9.41	19499	0.0189	1	0.021	15	0.0305
			0.5	2	4878	9756											
	2	2	0.5	1	5045	10090	10168	20336	0.1	209518	9.71	20109	0.0194	1	0.021	15	0.0314
			0.5	2	5123	10246											
	3	2	0.5	1	4944	9888	9912	19824	0.1	209518	9.46	19597	0.0189	1	0.021	15	0.0306
			0.5	2	4968	9936											
	4	2	0.5	1	4793	9586	9676	19352	0.1	209518	9.24	19125	0.0185	1	0.021	15	0.0299
			0.5	2	4883	9766											
Ref Chem 5	1-1	2	0.5	1	4871	9742	9691	19382	0.1	209518	9.25	19155	0.0185	1	0.021	15	0.0299
			0.5	2	4820	9640											
	1-2	2	0.5	1	4984	9968	9772	19544	0.1	209518	9.33	19317	0.0187	1	0.021	15	0.0302
			0.5	2	4788	9576											
	1-3	2	0.5	1	4732	9464	9542	19084	0.1	209518	9.11	18857	0.0182	1	0.021	15	0.0295
			0.5	2	4810	9620											
	2-1	2	0.5	1	4921	9842	9929	19858	0.1	209518	9.48	19631	0.0190	1	0.021	15	0.0307
			0.5	2	5008	10016											
	2-2	2	0.5	1	5084	10168	10274	20548	0.1	209518	9.81	20321	0.0196	1	0.021	15	0.0318
			0.5	2	5190	10380											
	2-3	2	0.5	1	4811	9622	9597	19194	0.1	209518	9.16	18967	0.0183	1	0.021	15	0.0296
			0.5	2	4786	9572											
	3-1	2	0.5	1	5017	10034	10063	20126	0.1	209518	9.61	19899	0.0192	1	0.021	15	0.0311
			0.5	2	5046	10092											
	3-2	2	0.5	1	5056	10112	10142	20284	0.1	209518	9.68	20057	0.0194	1	0.021	15	0.0313
			0.5	2	5086	10172											
	3-3	2	0.5	1	4961	9922	10045	20090	0.1	209518	9.59	19863	0.0192	1	0.021	15	0.0310
			0.5	2	5084	10168											
	4-1	2	0.5	1	5132	10264	10074	20148	0.1	209518	9.62	19921	0.0193	1	0.021	15	0.0311
			0.5	2	4942	9884											
	4-2	2	0.5	1	5033	10066	10275	20550	0.1	209518	9.81	20323	0.0197	1	0.021	15	0.0318
			0.5	2	5242	10484											
	4-3	2	0.5	1	4994	9988	10013	20026	0.1	209518	9.56	19799	0.0191	1	0.021	15	0.0309
			0.5	2	5019	10038											
	5-1	2	0.5	1	4941	9882	9947	19894	0.1	209518	9.50	19667	0.0190	1	0.021	15	0.0307
			0.5	2	5006	10012											
	5-2	2	0.5	1	5203	10406	10116	20232	0.1	209518	9.66	20005	0.0193	1	0.021	15	0.0313
			0.5	2	4913	9826											
	5-3	2	0.5	1	5043	10086	10191	20382	0.1	209518	9.73	20155	0.0195	1	0.021	15	0.0315
			0.5	2	5148	10296											
	6-1	2	0.5	1	5073	10146	10141	20282	0.1	209518	9.68	20055	0.0194	1	0.021	15	0.0313
			0.5	2	5068	10136											
	6-2	2	0.5	1	5069	10138	10224	20448	0.1	209518	9.76	20221	0.0196	1	0.021	15	0.0316
			0.5	2	5155	10310											
	6-3	2	0.5	1	5080	10160	10285	20570	0.1	209518	9.82	20343	0.0197	1	0.021	15	0.0318
			0.5	2	5205	10410											
	7-1	2	0.5	1	5046	10092	9992	19984	0.1	209518	9.54	19757	0.0191	1	0.021	15	0.0309
			0.5	2	4946	9892											
	7-2	2	0.5	1	5112	10224	10228	20456	0.1	209518	9.76	20229	0.0196	1	0.021	15	0.0316
			0.5	2	5116	10232											
	7-3	2	0.5	1	4970	9940	9974	19948	0.1	209518	9.52	19721	0.0191	1	0.021	15	0.0308
			0.5	2	5004	10008											
	8-1	2	0.5	1	3951	7902	7976	15952	0.05	104759	15.23	15725	0.0152	1	0.021	15	0.0246
			0.5	2	4025	8050											
	8-2	2	0.5	1	5051	10102	10161	20322	0.1	209518	9.70	20095	0.0194	1	0.021	15	0.0314
			0.5	2	5110	10220											
	8-3	2	0.5	1	4996	9992	9963	19926	0.1	209518	9.51	19699	0.0190	1	0.021	15	0.0308
			0.5	2	4967	9934											

Assay Date	9/30/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0287	0.0022
Full activity	End	0.0311	0.0003
Full activity	Overall	0.0299	0.0019
Background	Beginning	0.0000	8.61881E-05
Background	End	0.0000	5.30389E-05
Background	Overall	0.0000	6.47771E-05
Positive	Beginning	0.0137	0.0003
Positive	End	0.0135	0.0005
Positive	Overall	0.0136	0.0004
Negative	Beginning	0.0309	0.0007
Negative	End	0.0303	0.0005
Negative	Overall	0.0306	0.0006

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 5	1	1	1.00E-04	-4.00	0.0299
Ref Chem 5	1	2	1.00E-04	-4.00	0.0302
Ref Chem 5	1	3	1.00E-04	-4.00	0.0295
Ref Chem 5	2	1	1.00E-05	-5.00	0.0307
Ref Chem 5	2	2	1.00E-05	-5.00	0.0318
Ref Chem 5	2	3	1.00E-05	-5.00	0.0296
Ref Chem 5	3	1	1.00E-06	-6.00	0.0311
Ref Chem 5	3	2	1.00E-06	-6.00	0.0313
Ref Chem 5	3	3	1.00E-06	-6.00	0.0310
Ref Chem 5	4	1	5.00E-07	-6.30	0.0311
Ref Chem 5	4	2	5.00E-07	-6.30	0.0318
Ref Chem 5	4	3	5.00E-07	-6.30	0.0309
Ref Chem 5	5	1	1.00E-07	-7.00	0.0307
Ref Chem 5	5	2	1.00E-07	-7.00	0.0313
Ref Chem 5	5	3	1.00E-07	-7.00	0.0315
Ref Chem 5	6	1	1.00E-08	-8.00	0.0313
Ref Chem 5	6	2	1.00E-08	-8.00	0.0316
Ref Chem 5	6	3	1.00E-08	-8.00	0.0318
Ref Chem 5	7	1	1.00E-09	-9.00	0.0309
Ref Chem 5	7	2	1.00E-09	-9.00	0.0316
Ref Chem 5	7	3	1.00E-09	-9.00	0.0308
Ref Chem 5	8	1	1.00E-10	-10.00	0.0246
Ref Chem 5	8	2	1.00E-10	-10.00	0.0314
Ref Chem 5	8	3	1.00E-10	-10.00	0.0308

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	100.14	100.99	98.58
2	-5.00	102.63	106.23	99.16
3	-6.00	104.03	104.85	103.84
4	-6.30	104.14	106.24	103.51
5	-7.00	102.82	104.58	105.37
6	-8.00	104.84	105.71	106.35
7	-9.00	103.29	105.75	103.10
8	-10.00	82.21	105.05	102.98

Aromatase Assay Spreadsheet

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0210	39340	1873333	
2	0.0217	37568	1731244	
3	0.0218	38959	1787110	
4	0.0217	40419	1862627	
5	0.0214	39999	1869112	
			Average DPM/g soln	1824685
			SD	63093
			CV	3.46
			µCi/g soln	0.822

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1457 g
Mass of dilution B used in substrate prep	4.5807 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562346 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00930 µg/g soln.
µg/g soln.

a. µCi/g soln 0.822
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.562346 + 0.00930
= 0.571650 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.438 µCi/µg ASDN

914178 dpm/nmol

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.592	0.398	0.252	0.176	0.138	0.122	0.101	25.4	100	
	0.592	0.396	0.246	0.180	0.135	0.125	0.119			
	0.600	0.393	0.248	0.183	0.147	0.124	0.114			

Samples:	11540-6A	11540-6B	11540-40B	11345-15I
	0.371	0.144	0.189	0.133
	0.384	0.147	0.188	0.143
			0.190	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0508	0.595	0.483	0.0488
125	100	200	0.00013	200	0.0254	0.396	0.284	0.0279
50	200	1000	0.00005	200	0.0102	0.249	0.137	0.0124
25	100	1000	0.00003	200	0.0051	0.180	0.068	0.0052
10	40	1000	0.00001	200	0.0020	0.140	0.029	0.0010
5	20	1000	0.00001	200	0.0010	0.124	0.012	-0.0007

Variables	Regression results	
m, b	0.105	-0.002
se_m, se_b	0.005	0.001
r^2, se_y	0.990	0.002
F, df	383	4
ss_{reg}, ss_{resid}	0.002	0.000

Blank 0.111

r^2= 0.990
m= 0.105
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.371	0.260	0.025	200	1	1	0.126	0.130	0.11
11540-6A	0.384	0.273	0.027	200	1	1	0.133		
11540-6A									
11540-6B	0.144	0.033	0.001	200	1	1	0.007	0.008	0.012
11540-6B	0.147	0.036	0.002	200	1	1	0.009		
11540-6B									
11540-40B	0.189	0.078	0.006	200	1	320	9.8	9.8	
11540-40B	0.188	0.077	0.006	200	1	320	9.6		
11540-40B	0.190	0.079	0.006	200	1	320	10.0		
11345-15I	0.133	0.022	0.000	200	1	600	0.7	2.0	
11345-15I	0.143	0.032	0.001	200	1	600	3.9		
11345-15I	0.135	0.024	0.000	200	1	600	1.4		

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.592	0.398	0.252	0.176	0.138	0.122	0.101	25.4	100	
	0.592	0.396	0.246	0.180	0.135	0.125	0.119			
	0.600	0.393	0.248	0.183	0.147	0.124	0.114			

Samples:	11540-6A	11540-6B	11540-40B	11345-15I
	0.371	0.144	0.189	0.133
	0.384	0.147	0.188	0.143
			0.190	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0508	0.595	0.483	0.0426
125	100	200	0.00013	200	0.0254	0.396	0.284	0.0247
50	200	1000	0.00005	200	0.0102	0.249	0.137	0.0115
25	100	1000	0.00003	200	0.0051	0.180	0.068	0.0053
10	40	1000	0.00001	200	0.0020	0.140	0.029	0.0018
5	20	1000	0.00001	200	0.0010	0.124	0.012	0.0003

	Blank	0.111

Variables	Regression results	
m, b	0.090	-0.001
se_m, se_b	0.004	0.001
r^2, se_y	0.993	0.001
F, df	400	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2=	0.993	
m=	0.090	
b=	-0.001	

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.371	0.260	0.023	200	1	1	0.113	0.116	0.11
11540-6A	0.384	0.273	0.024	200	1	1	0.118		
11540-6A									
11540-6B	0.144	0.033	0.002	200	1	1	0.011	0.011	0.012
11540-6B	0.147	0.036	0.002	200	1	1	0.012		
11540-6B									
11540-40B	0.189	0.078	0.006	200	1	320	9.9	9.9	
11540-40B	0.188	0.077	0.006	200	1	320	9.7		
11540-40B	0.190	0.079	0.006	200	1	320	10.0		
11345-15I	0.133	0.022	0.001	200	1	600	3.4	4.5	
11345-15I	0.143	0.032	0.002	200	1	600	6.1		
11345-15I	0.135	0.024	0.001	200	1	600	4.0		

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate #	1

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	9.9
Protein Concentration (dilution added to assay, mg/mL):	0.030879

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4243	8486	8459	16918	0.1	182469	9.27	16783	0.0184	1	0.015	15	0.0396
			0.5	2	4216	8432											
	2	2	0.5	1	4362	8724	8568	17176	0.1	182469	9.41	17041	0.0186	1	0.015	15	0.0402
			0.5	2	4226	8452											
	3	2	0.5	1	3609	7218	7233	14466	0.1	182469	7.93	14331	0.0157	1	0.015	15	0.0338
			0.5	2	3624	7248											
	4	2	0.5	1	3535	7070	7131	14262	0.1	182469	7.82	14127	0.0155	1	0.015	15	0.0334
			0.5	2	3596	7192											
Background control	1	2	0.5	1	42	84	62	124	0.1	182469	0.07	-12	0.0000	1	0.015	15	0.0000
			0.5	2	20	40											
	2	2	0.5	1	40	80	68	136	0.1	182469	0.07	1	0.0000	1	0.015	15	0.0000
			0.5	2	28	56											
	3	2	0.5	1	34	68	70	140	0.1	182469	0.08	5	0.0000	1	0.015	15	0.0000
			0.5	2	36	72											
	4	2	0.5	1	36	72	71	142	0.1	182469	0.08	7	0.0000	1	0.015	15	0.0000
			0.5	2	35	70											
Positive control	1	2	0.5	1	2089	4178	4225	8450	0.1	182469	4.63	8315	0.0091	1	0.015	15	0.0196
			0.5	2	2136	4272											
	2	2	0.5	1	1987	3974	3942	7884	0.1	182469	4.32	7749	0.0085	1	0.015	15	0.0183
			0.5	2	1955	3910											
	3	2	0.5	1	1687	3374	3320	6640	0.1	182469	3.64	6505	0.0071	1	0.015	15	0.0154
			0.5	2	1633	3266											
	4	2	0.5	1	1697	3394	3359	6718	0.1	182469	3.68	6583	0.0072	1	0.015	15	0.0155
			0.5	2	1662	3324											
Negative Control	1	2	0.5	1	4239	8478	8524	17048	0.1	182469	9.34	16913	0.0185	1	0.015	15	0.0399
			0.5	2	4285	8570											
	2	2	0.5	1	4255	8510	8469	16938	0.1	182469	9.28	16803	0.0184	1	0.015	15	0.0397
			0.5	2	4214	8428											
	3	2	0.5	1	3724	7448	7353	14706	0.1	182469	8.06	14571	0.0159	1	0.015	15	0.0344
			0.5	2	3629	7258											
	4	2	0.5	1	733	1466	1477	2954	0.1	182469	1.62	2819	0.0031	1	0.015	15	0.0067
			0.5	2	744	1488											
Ref Chem 6	1-1	2	0.5	1	64	128	145	290	0.1	182469	0.16	155	0.0002	1	0.015	15	0.0004
			0.5	2	81	162											
	1-2	2	0.5	1	78	156	146	292	0.1	182469	0.16	157	0.0002	1	0.015	15	0.0004
			0.5	2	68	136											
	1-3	2	0.5	1	77	154	153	306	0.1	182469	0.17	171	0.0002	1	0.015	15	0.0004
			0.5	2	76	152											
	2-1	2	0.5	1	265	530	512	1024	0.1	182469	0.56	889	0.0010	1	0.015	15	0.0021
			0.5	2	247	494											
	2-2	2	0.5	1	285	570	536	1072	0.1	182469	0.59	937	0.0010	1	0.015	15	0.0022
			0.5	2	251	502											
	2-3	2	0.5	1	249	498	474	948	0.1	182469	0.52	813	0.0009	1	0.015	15	0.0019
			0.5	2	225	450											
	3-1	2	0.5	1	1346	2692	2709	5418	0.1	182469	2.97	5283	0.0058	1	0.015	15	0.0125
			0.5	2	1363	2726											
	3-2	2	0.5	1	1326	2652	2596	5192	0.1	182469	2.85	5057	0.0055	1	0.015	15	0.0119
			0.5	2	1270	2540											
	3-3	2	0.5	1	1287	2574	2610	5220	0.1	182469	2.86	5085	0.0056	1	0.015	15	0.0120
			0.5	2	1323	2646											
	4-1	2	0.5	1	2914	5828	5922	11844	0.1	182469	6.49	11709	0.0128	1	0.015	15	0.0277
			0.5	2	3008	6016											
	4-2	2	0.5	1	2697	5394	5528	11056	0.1	182469	6.06	10921	0.0119	1	0.015	15	0.0258
			0.5	2	2831	5662											
	4-3	2	0.5	1	2763	5526	5483	10966	0.1	182469	6.01	10831	0.0118	1	0.015	15	0.0256
			0.5	2	2720	5440											
	5-1	2	0.5	1	3442	6884	6951	13902	0.1	182469	7.62	13767	0.0151	1	0.015	15	0.0325
			0.5	2	3509	7018											
	5-2	2	0.5	1	3166	6332	6351	12702	0.1	182469	6.96	12567	0.0137	1	0.015	15	0.0297
			0.5	2	3185	6370											
	5-3	2	0.5	1	3017	6034	6086	12172	0.1	182469	6.67	12037	0.0132	1	0.015	15	0.0284
			0.5	2	3069	6138											
	6-1	2	0.5	1	3464	6928	6806	13612	0.1	182469	7.46	13477	0.0147	1	0.015	15	0.0318
			0.5	2	3342	6684											
	6-2	2	0.5	1	3193	6386	6386	12772	0.1	182469	7.00	12637	0.0138	1	0.015	15	0.0298
			0.5	2	3193	6386											
	6-3	2	0.5	1	3218	6436	6453	12906	0.1	182469	7.07	12771	0.0140	1	0.015	15	0.0302
			0.5	2	3235	6470											
	7-1	2	0.5	1	3319	6638	6649	13298	0.1	182469	7.29	13163	0.0144	1	0.015	15	0.0311
			0.5	2	3330	6660											
	7-2	2	0.5	1	3299	6598	6616	13232	0.1	182469	7.25	13097	0.0143	1	0.015	15	0.0309
			0.5	2	3317	6634											
	7-3	2	0.5	1	3227	6454	6450	12900	0.1	182469	7.07	12765	0.0140	1	0.015	15	0.0301
			0.5	2	3223	6446											
	8-1	2	0.5	1	3323	6646	6582	13164	0.1	182469	7.21	13029	0.0143	1	0.015	15	0.0308
			0.5	2	3259	6518											
	8-2	2	0.5	1	3235	6470	6443	12886	0.1	182469	7.06	12751	0.0139	1	0.015	15	0.0301
			0.5	2	3208	6416											
	8-3	2	0.5	1	3096	6192	6208	12416	0.1	182469	6.80	12281	0.0134	1	0.015	15	0.0290
			0.5	2	3112	6224											

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0399	0.0004
Full activity	End	0.0336	0.0003
Full activity	Overall	0.0368	0.0037
Background	Beginning	0.0000	2.00395E-05
Background	End	0.0000	3.33991E-06
Background	Overall	0.0000	1.90404E-05
Positive	Beginning	0.0190	0.0009
Positive	End	0.0155	0.0001
Positive	Overall	0.0172	0.0021
Negative	Beginning	0.0398	0.0002
Negative	End	0.0344	NA
Negative	Overall	0.0380	0.0031

Exclude Neg tube 4

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 6	1	1	1.00E-03	-3.00	0.0004
Ref Chem 6	1	2	1.00E-03	-3.00	0.0004
Ref Chem 6	1	3	1.00E-03	-3.00	0.0004
Ref Chem 6	2	1	1.00E-04	-4.00	0.0021
Ref Chem 6	2	2	1.00E-04	-4.00	0.0022
Ref Chem 6	2	3	1.00E-04	-4.00	0.0019
Ref Chem 6	3	1	1.00E-05	-5.00	0.0125
Ref Chem 6	3	2	1.00E-05	-5.00	0.0119
Ref Chem 6	3	3	1.00E-05	-5.00	0.0120
Ref Chem 6	4	1	1.00E-06	-6.00	0.0277
Ref Chem 6	4	2	1.00E-06	-6.00	0.0258
Ref Chem 6	4	3	1.00E-06	-6.00	0.0256
Ref Chem 6	5	1	1.00E-07	-7.00	0.0325
Ref Chem 6	5	2	1.00E-07	-7.00	0.0297
Ref Chem 6	5	3	1.00E-07	-7.00	0.0284
Ref Chem 6	6	1	1.00E-08	-8.00	0.0318
Ref Chem 6	6	2	1.00E-08	-8.00	0.0298
Ref Chem 6	6	3	1.00E-08	-8.00	0.0302
Ref Chem 6	7	1	1.00E-09	-9.00	0.0311
Ref Chem 6	7	2	1.00E-09	-9.00	0.0309
Ref Chem 6	7	3	1.00E-09	-9.00	0.0301
Ref Chem 6	8	1	1.00E-10	-10.00	0.0308
Ref Chem 6	8	2	1.00E-10	-10.00	0.0301
Ref Chem 6	8	3	1.00E-10	-10.00	0.0290

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.99	1.01	1.10
2	-4.00	5.71	6.01	5.22
3	-5.00	33.93	32.48	32.66
4	-6.00	75.20	70.14	69.56
5	-7.00	88.42	80.71	77.31
6	-8.00	86.55	81.16	82.02
7	-9.00	84.54	84.11	81.98
8	-10.00	83.68	81.89	78.87

Aromatase Assay Spreadsheet

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8		
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0201	36889	1835274		
2	0.0204	36211	1775049		
3	0.0204	38433	1883971		
4	0.0204	39411	1931912		
5	0.0203	39588	1950148		
			Average DPM/g soln		1875271
			SD		71650
			CV		3.82
			µCi/g soln		0.845

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.1	10		1010.00
Dilution A			100	10.10
Dilution B			10	1.01

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0912 g
Mass of dilution B used in substrate prep	4.5439 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.567201 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00956 µg/g soln.
µg/g soln.

a. µCi/g soln 0.845
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.567201 + 0.00956
= 0.576764 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.465 µCi/µg ASDN

931192 dpm/nmol

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8
Technician ID	1	Replicate #	2	Microsome type	Placental

Microsome ID 11540-20

Standards:	250	125	50	25	10	5	0
	0.584	0.393	0.253	0.182	0.136	0.124	0.108
	0.593	0.386	0.246	0.192	0.146	0.129	0.110
	0.593	0.392	0.251	0.177	0.142	0.126	0.120

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25.4	100	11540-42

Samples:	11540-6A	11540-6B	11540-42B	11345-16I
	0.366	0.149	0.196	0.139
	0.374	0.151	0.194	0.137
			0.196	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0508	0.590	0.477	0.0490
125	100	200	0.00013	200	0.0254	0.390	0.278	0.0275
50	200	1000	0.00005	200	0.0102	0.250	0.137	0.0125
25	100	1000	0.00003	200	0.0051	0.184	0.071	0.0054
10	40	1000	0.00001	200	0.0020	0.141	0.029	0.0009
5	20	1000	0.00001	200	0.0010	0.126	0.014	-0.0008

Variables	Regression results	
m, b	0.107	-0.002
se_m, se_b	0.005	0.001
r^2, se_y	0.990	0.002
F, df	409	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank	0.113

r^2= 0.990
m= 0.107
b= -0.002

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.366	0.253	0.025	200	1	1	0.125	0.127	0.11
11540-6A	0.374	0.261	0.026	200	1	1	0.129		
11540-6A									
11540-6B	0.149	0.036	0.002	200	1	1	0.008	0.009	0.012
11540-6B	0.151	0.038	0.002	200	1	1	0.009		
11540-6B									
11540-42B	0.196	0.083	0.007	200	1	320	10.7	10.6	
11540-42B	0.194	0.081	0.006	200	1	320	10.4		
11540-42B	0.196	0.083	0.007	200	1	320	10.7		
11345-16I	0.139	0.026	0.001	200	1	600	1.8	1.2	
11345-16I	0.137	0.024	0.000	200	1	600	1.2		
11345-16I	0.135	0.022	0.000	200	1	600	0.5		

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.584	0.393	0.253	0.182	0.136	0.124	0.108	25.4	100	11540-42
	0.593	0.386	0.246	0.192	0.146	0.129	0.110			
	0.593	0.392	0.251	0.177	0.142	0.126	0.120			

Samples:	11540-6A	11540-6B	11540-42B	11345-16I
	0.366	0.149	0.196	0.139
	0.374	0.151	0.194	0.137
			0.196	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0508	0.590	0.477	0.0431	m, b	0.092	-0.001
125	100	200	0.00013	200	0.0254	0.390	0.278	0.0246	se_m, se_b	0.005	0.001
50	200	1000	0.00005	200	0.0102	0.250	0.137	0.0117	r^2, se_y	0.990	0.001
25	100	1000	0.00003	200	0.0051	0.184	0.071	0.0055	F, df	308	3
10	40	1000	0.00001	200	0.0020	0.141	0.029	0.0016	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.126	0.014	0.0002			

Regression results are calculated using the function LINEST

Blank	0.113	r^2=	0.990		
		m=	0.092		
		b=	-0.001		

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.366	0.253	0.022	200	1	1	0.112	0.114	0.11
11540-6A	0.374	0.261	0.023	200	1	1	0.116		
11540-6A									
11540-6B	0.149	0.036	0.002	200	1	1	0.012	0.012	0.012
11540-6B	0.151	0.038	0.003	200	1	1	0.013		
11540-6B									
11540-42B	0.196	0.083	0.007	200	1	320	10.7	10.6	
11540-42B	0.194	0.081	0.006	200	1	320	10.4		
11540-42B	0.196	0.083	0.007	200	1	320	10.7		
11345-16I	0.139	0.026	0.001	200	1	600	4.2	3.7	
11345-16I	0.137	0.024	0.001	200	1	600	3.7		
11345-16I	0.135	0.022	0.001	200	1	600	3.1		

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	2

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	10.6
Protein Concentration (dilution added to assay, mg/mL):	0.033069

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	2.50E-05
4	1.00E-05
5	5.00E-06
6	1.00E-06
7	1.00E-07
8	1.00E-08

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.2	1	1929	9645	9670	19340	0.1	187527	10.31	19160	0.0206	1	0.017	15	0.0415
			0.2	2	1939	9695											
	2	2	0.1	1	990	9900	9850	19700	0.1	187527	10.51	19520	0.0210	1	0.017	15	0.0423
			0.07	2	686	9800											
	3	2	0.5	1	4450	8900	8817	17634	0.1	187527	9.40	17454	0.0187	1	0.017	15	0.0378
			0.5	2	4367	8734											
	4	2	0.5	1	4229	8458	8458	16916	0.1	187527	9.02	16736	0.0180	1	0.017	15	0.0362
			0.5	2	4229	8458											
Background control	1	2	0.5	1	51	102	105	210	0.1	187527	0.11	30	0.0000	1	0.017	15	0.0001
			0.5	2	54	108											
	2	2	0.5	1	44	88	97	194	0.1	187527	0.10	14	0.0000	1	0.017	15	0.0000
			0.5	2	53	106											
	3	2	0.5	1	40	80	72	144	0.1	187527	0.08	-37	0.0000	1	0.017	15	-0.0001
			0.5	2	32	64											
	4	2	0.5	1	39	79	87	174	0.1	187527	0.09	-7	0.0000	1	0.017	15	0.0000
			0.5	2	48	96											
Positive control	1	2	0.5	1	2332	4664	4709	9418	0.1	187527	5.02	9238	0.0099	1	0.017	15	0.0200
			0.5	2	2377	4754											
	2	2	0.5	1	2019	4038	4097	8194	0.1	187527	4.37	8014	0.0085	1	0.017	15	0.0173
			0.5	2	2078	4156											
	3	2	0.5	1	2268	4536	4499	8998	0.1	187527	4.80	8818	0.0095	1	0.017	15	0.0191
			0.5	2	2231	4462											
	4	2	0.5	1	2318	4636	4576	9152	0.1	187527	4.88	8972	0.0096	1	0.017	15	0.0194
			0.5	2	2258	4516											
Negative Control	1	2	0.5	1	4762	9524	9585	19170	0.1	187527	10.22	18990	0.0204	1	0.017	15	0.0411
			0.5	2	4823	9646											
	2	2	0.5	1	4649	9298	9196	18392	0.1	187527	9.81	18212	0.0196	1	0.017	15	0.0394
			0.5	2	4547	9094											
	3	2	0.5	1	4400	8800	8820	17640	0.1	187527	9.41	17460	0.0187	1	0.017	15	0.0378
			0.5	2	4420	8840											
	4	2	0.5	1	4048	8095	7985	15970	0.1	187527	8.52	15790	0.0170	1	0.017	15	0.0342
			0.5	2	3937	7874											
Ref Chem 6	1-1	2	0.5	1	99	198	206	412	0.1	187527	0.22	232	0.0002	1	0.017	15	0.0005
			0.5	2	107	214											
	1-2	2	0.5	1	114	228	223	446	0.1	187527	0.24	266	0.0003	1	0.017	15	0.0006
			0.5	2	109	218											
	1-3	2	0.5	1	235	470	331	662	0.1	187527	0.35	482	0.0005	1	0.017	15	0.0010
			0.5	2	96	192											
	2-1	2	0.5	1	335	670	670	1340	0.1	187527	0.71	1160	0.0012	1	0.017	15	0.0025
			0.5	2	335	670											
	2-2	2	0.5	1	336	672	665	1330	0.1	187527	0.71	1150	0.0012	1	0.017	15	0.0025
			0.5	2	329	658											
	2-3	2	0.5	1	325	650	626	1252	0.1	187527	0.67	1072	0.0012	1	0.017	15	0.0023
			0.5	2	301	602											
	3-1	2	0.5	1	933	1866	1877	3754	0.1	187527	2.00	3574	0.0038	1	0.017	15	0.0077
			0.5	2	944	1888											
	3-2	2	0.5	1	930	1860	1865	3730	0.1	187527	1.99	3550	0.0038	1	0.017	15	0.0077
			0.5	2	935	1870											
	3-3	2	0.5	1	906	1812	1796	3592	0.1	187527	1.92	3412	0.0037	1	0.017	15	0.0074
			0.5	2	890	1780											
	4-1	2	0.5	1	1621	3242	3356	6712	0.1	187527	3.58	6532	0.0070	1	0.017	15	0.0141
			0.5	2	1735	3470											
	4-2	2	0.5	1	1680	3360	3429	6858	0.1	187527	3.66	6678	0.0072	1	0.017	15	0.0145
			0.5	2	1749	3498											
	4-3	2	0.5	1	1624	3248	3253	6506	0.1	187527	3.47	6326	0.0068	1	0.017	15	0.0137
			0.5	2	1629	3258											
	5-1	2	0.5	1	2385	4770	4745	9490	0.1	187527	5.06	9310	0.0100	1	0.017	15	0.0202
			0.5	2	2360	4720											
	5-2	2	0.5	1	2320	4640	4748	9496	0.1	187527	5.06	9316	0.0100	1	0.017	15	0.0202
			0.5	2	2428	4856											
	5-3	2	0.5	1	2127	4254	4250	8500	0.1	187527	4.53	8320	0.0089	1	0.017	15	0.0180
			0.5	2	2123	4246											
	6-1	2	0.5	1	3832	7664	7648	15296	0.1	187527	8.16	15116	0.0162	1	0.017	15	0.0327
			0.5	2	3816	7632											
	6-2	2	0.5	1	3701	7402	7490	14980	0.1	187527	7.99	14800	0.0159	1	0.017	15	0.0320
			0.5	2	3789	7578											
	6-3	2	0.5	1	3577	7154	7169	14338	0.1	187527	7.65	14158	0.0152	1	0.017	15	0.0307
			0.5	2	3592	7184											
	7-1	2	0.5	1	4487	8974	8924	17848	0.1	187527	9.52	17668	0.0190	1	0.017	15	0.0382
			0.5	2	4437	8874											
	7-2	2	0.5	1	4184	8368	8362	16724	0.1	187527	8.92	16544	0.0178	1	0.017	15	0.0358
			0.5	2	4178	8356											
	7-3	2	0.5	1	4339	8678	8446	16892	0.1	187527	9.01	16712	0.0179	1	0.017	15	0.0362
			0.5	2	4107	8214											
	8-1	2	0.5	1	4441	8882	9032.25	18064.5	0.1	187527	9.63	17884	0.0192	1	0.017	15	0.0387
			0.4	2	3673	9182.5											
	8-2	2	0.5	1	4365	8730	8767	17534	0.1	187527	9.35	17354	0.0186	1	0.017	15	0.0376
			0.5	2	4402	8804											
	8-3	2	0.5	1	4421	8842	8770	17540	0.1	187527	9.35	17360	0.0186	1	0.017	15	0.0376
			0.5	2	4349	8698											

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested		Microsome 8 type	Placental	Microsome ID	11540-20	Technician ID		Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0419	0.0006
Full activity	End	0.0370	0.0011
Full activity	Overall	0.0394	0.0029
Background	Beginning	0.0000	2.44939E-05
Background	End	0.0000	4.5926E-05
Background	Overall	0.0000	6.15782E-05
Positive	Beginning	0.0187	0.0019
Positive	End	0.0193	0.0002
Positive	Overall	0.0190	0.0011
Negative	Beginning	0.0403	0.0012
Negative	End	0.0360	0.0026
Negative	Overall	0.0381	0.0030

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 6	1	1	1.00E-03	-3.00	0.0005
Ref Chem 6	1	2	1.00E-03	-3.00	0.0006
Ref Chem 6	1	3	1.00E-03	-3.00	0.0010
Ref Chem 6	2	1	1.00E-04	-4.00	0.0025
Ref Chem 6	2	2	1.00E-04	-4.00	0.0025
Ref Chem 6	2	3	1.00E-04	-4.00	0.0023
Ref Chem 6	3	1	2.50E-05	-4.60	0.0077
Ref Chem 6	3	2	2.50E-05	-4.60	0.0077
Ref Chem 6	3	3	2.50E-05	-4.60	0.0074
Ref Chem 6	4	1	1.00E-05	-5.00	0.0141
Ref Chem 6	4	2	1.00E-05	-5.00	0.0145
Ref Chem 6	4	3	1.00E-05	-5.00	0.0137
Ref Chem 6	5	1	5.00E-06	-5.30	0.0202
Ref Chem 6	5	2	5.00E-06	-5.30	0.0202
Ref Chem 6	5	3	5.00E-06	-5.30	0.0180
Ref Chem 6	6	1	1.00E-06	-6.00	0.0327
Ref Chem 6	6	2	1.00E-06	-6.00	0.0320
Ref Chem 6	6	3	1.00E-06	-6.00	0.0307
Ref Chem 6	7	1	1.00E-07	-7.00	0.0382
Ref Chem 6	7	2	1.00E-07	-7.00	0.0358
Ref Chem 6	7	3	1.00E-07	-7.00	0.0362
Ref Chem 6	8	1	1.00E-08	-8.00	0.0387
Ref Chem 6	8	2	1.00E-08	-8.00	0.0376
Ref Chem 6	8	3	1.00E-08	-8.00	0.0376

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.27	1.46	2.64
2	-4.00	6.36	6.31	5.88
3	-4.60	19.62	19.48	18.73
4	-5.00	35.85	36.66	34.72
5	-5.30	51.10	51.14	45.67
6	-6.00	82.97	81.24	77.72
7	-7.00	96.98	90.81	91.74
8	-8.00	98.17	95.26	95.29

Aromatase Assay Spreadsheet

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0218	40517	1858578	
2	0.0214	38144	1782430	
3	0.0214	40428	1889159	
4	0.0212	41160	1941509	
5	0.0212	37938	1789528	
			Average DPM/g soln	1852241
			SD	67414
			CV	3.64
			µCi/g soln	0.834

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1067 g
Mass of dilution B used in substrate prep	4.5677 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563448 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00944 µg/g soln.
µg/g soln.

a. µCi/g soln 0.834
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.563448 + 0.00944
= 0.572892 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.456 µCi/µg ASDN

925971 dpm/nmol

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8						
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.578	0.396	0.234	0.184	0.134	0.130	0.122	25.1	100	11540-43
	0.577	0.385	0.248	0.183	0.142	0.125	0.119			
	0.593	0.389	0.239	0.188	0.145	0.129	0.121			

Samples:	11540-6A	11540-6B	11540-43B	11345-17I
	0.374	0.145	0.192	0.135
	0.360	0.150	0.186	0.132
			0.188	0.134

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0502	0.583	0.462	0.0483
125	100	200	0.00013	200	0.0251	0.390	0.269	0.0276
50	200	1000	0.00005	200	0.0100	0.240	0.120	0.0115
25	100	1000	0.00003	200	0.0050	0.185	0.064	0.0056
10	40	1000	0.00001	200	0.0020	0.140	0.020	0.0008
5	20	1000	0.00001	200	0.0010	0.128	0.007	-0.0005
			Blank	0.121				

Variables	Regression results	
m, b	0.107	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.991	0.002
F, df	445	4
ss_{regr}, ss_{resid}	0.002	0.000

r^2= 0.991
m= 0.107
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.374	0.253	0.026	200	1	1	0.130	0.126	0.11
11540-6A	0.360	0.239	0.024	200	1	1	0.122		
11540-6A									
11540-6B	0.145	0.024	0.001	200	1	1	0.006	0.008	0.012
11540-6B	0.150	0.029	0.002	200	1	1	0.009		
11540-6B									
11540-43B	0.192	0.071	0.006	200	1	320	10.2	9.6	
11540-43B	0.186	0.065	0.006	200	1	320	9.1		
11540-43B	0.188	0.067	0.006	200	1	320	9.5		
11345-17I	0.135	0.014	0.000	200	1	600	0.7	0.2	
11345-17I	0.132	0.011	0.000	200	1	600	-0.3		
11345-17I	0.134	0.013	0.000	200	1	600	0.4		

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8					
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20			

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.578	0.396	0.234	0.184	0.134	0.130	0.122	25.1	100	1540-43
	0.577	0.385	0.248	0.183	0.142	0.125	0.119			
	0.593	0.389	0.239	0.188	0.145	0.129	0.121			

Samples:	11540-6A	11540-6B	11540-43B	11345-17I
	0.374	0.145	0.192	0.135
	0.360	0.150	0.186	0.132
			0.188	0.134

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0502	0.583	0.462	0.0424
125	100	200	0.00013	200	0.0251	0.390	0.269	0.0246
50	200	1000	0.00005	200	0.0100	0.240	0.120	0.0108
25	100	1000	0.00003	200	0.0050	0.185	0.064	0.0057
10	40	1000	0.00001	200	0.0020	0.140	0.020	0.0016
5	20	1000	0.00001	200	0.0010	0.128	0.007	0.0004
			Blank	0.121				

Variables	Regression results	
m, b	0.092	0.000
se_m, se_b	0.004	0.000
r^2, se_y	0.995	0.001
F, df	650	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.995
m= 0.092
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.374	0.253	0.023	200	1	1	0.116	0.113	0.11
11540-6A	0.360	0.239	0.022	200	1	1	0.109		
11540-6A									
11540-6B	0.145	0.024	0.002	200	1	1	0.010	0.011	0.012
11540-6B	0.150	0.029	0.002	200	1	1	0.012		
11540-6B									
11540-43B	0.192	0.071	0.006	200	1	320	10.2	9.7	
11540-43B	0.186	0.065	0.006	200	1	320	9.3		
11540-43B	0.188	0.067	0.006	200	1	320	9.6		
11345-17I	0.135	0.014	0.001	200	1	600	3.3	2.9	
11345-17I	0.132	0.011	0.001	200	1	600	2.4		
11345-17I	0.134	0.013	0.001	200	1	600	3.0		

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	3

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	2.50E-05
4	1.00E-05
5	5.00E-06
6	1.00E-06
7	1.00E-07
8	1.00E-08

Protein Concentration (stock microsomes, mg/mL):	9.7
Protein Concentration (dilution added to assay, mg/mL):	0.030209

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed						
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4440	8880	8867	17734	0.1	185224	9.57	17574	0.0190	1	0.015	15	0.0419
			0.5	2	4427	8854											
	2	2	0.5	1	4928	9856	9698	19396	0.1	185224	10.47	19236	0.0208	1	0.015	15	0.0458
			0.5	2	4770	9540											
	3	2	0.5	1	4189	8378	8512	17024	0.1	185224	9.19	16864	0.0182	1	0.015	15	0.0402
			0.5	2	4323	8646											
	4	2	0.5	1	4226	8452	8441	16882	0.1	185224	9.11	16722	0.0181	1	0.015	15	0.0399
			0.5	2	4215	8430											
Background control	1	2	0.5	1	35	70	80	160	0.1	185224	0.09	-1	0.0000	1	0.015	15	0.0000
			0.5	2	45	90											
	2	2	0.5	1	51	102	87	174	0.1	185224	0.09	14	0.0000	1	0.015	15	0.0000
			0.5	2	36	72											
	3	2	0.5	1	35	70	74	148	0.1	185224	0.08	-13	0.0000	1	0.015	15	0.0000
			0.5	2	39	78											
	4	2	0.5	1	33	66	80	160	0.1	185224	0.09	-1	0.0000	1	0.015	15	0.0000
			0.5	2	47	94											
Positive control	1	2	0.5	1	2359	4718	4747	9494	0.1	185224	5.13	9334	0.0101	1	0.015	15	0.0222
			0.5	2	2388	4776											
	2	2	0.5	1	2336	4672	4709	9418	0.1	185224	5.08	9258	0.0100	1	0.015	15	0.0221
			0.5	2	2373	4746											
	3	2	0.5	1	2106	4212	4250	8500	0.1	185224	4.59	8340	0.0090	1	0.015	15	0.0199
			0.5	2	2144	4288											
	4	2	0.5	1	2038	4076	4060	8120	0.1	185224	4.38	7960	0.0086	1	0.015	15	0.0190
			0.5	2	2022	4044											
Negative Control	1	2	0.5	1	4645	9290	9308	18616	0.1	185224	10.05	18456	0.0199	1	0.015	15	0.0440
			0.5	2	4663	9326											
	2	2	0.5	1	4753	9506	9421	18842	0.1	185224	10.17	18682	0.0202	1	0.015	15	0.0445
			0.5	2	4668	9336											
	3	2	0.5	1	4098	8196	8258	16516	0.1	185224	8.92	16356	0.0177	1	0.015	15	0.0390
			0.5	2	4160	8320											
	4	2	0.5	1	3877	7754	7897	15794	0.1	185224	8.53	15634	0.0169	1	0.015	15	0.0373
			0.5	2	4020	8040											
Ref Chem 6	1-1	2	0.5	1	84	168	168	336	0.1	185224	0.18	176	0.0002	1	0.015	15	0.0004
			0.5	2	84	168											
	1-2	2	0.5	1	74	148	156	312	0.1	185224	0.17	152	0.0002	1	0.015	15	0.0004
			0.5	2	82	164											
	1-3	2	0.5	1	64	128	145	290	0.1	185224	0.16	130	0.0001	1	0.015	15	0.0003
			0.5	2	81	162											
	2-1	2	0.5	1	325	650	653	1306	0.1	185224	0.71	1146	0.0012	1	0.015	15	0.0027
			0.5	2	328	656											
	2-2	2	0.5	1	304	608	638	1276	0.1	185224	0.69	1116	0.0012	1	0.015	15	0.0027
			0.5	2	334	668											
	2-3	2	0.5	1	326	652	648	1296	0.1	185224	0.70	1136	0.0012	1	0.015	15	0.0027
			0.5	2	322	644											
	3-1	2	0.5	1	980	1960	1933	3866	0.1	185224	2.09	3706	0.0040	1	0.015	15	0.0088
			0.5	2	953	1906											
	3-2	2	0.5	1	1024	2048	2027	4054	0.1	185224	2.19	3894	0.0042	1	0.015	15	0.0093
			0.5	2	1003	2006											
	3-3	2	0.5	1	931	1862	1888	3776	0.1	185224	2.04	3616	0.0039	1	0.015	15	0.0086
			0.5	2	957	1914											
	4-1	2	0.5	1	1791	3582	3520	7040	0.1	185224	3.80	6880	0.0074	1	0.015	15	0.0164
			0.5	2	1729	3458											
	4-2	2	0.5	1	1673	3346	3346	6692	0.1	185224	3.61	6532	0.0071	1	0.015	15	0.0156
			0.5	2	1673	3346											
	4-3	2	0.5	1	1698	3396	3420	6840	0.1	185224	3.69	6680	0.0072	1	0.015	15	0.0159
			0.5	2	1722	3444											
	5-1	2	0.5	1	2537	5074	5025	10050	0.1	185224	5.43	9890	0.0107	1	0.015	15	0.0236
			0.5	2	2488	4976											
	5-2	2	0.5	1	2510	5020	5010	10020	0.1	185224	5.41	9860	0.0106	1	0.015	15	0.0235
			0.5	2	2500	5000											
	5-3	2	0.5	1	2501	5002	4978	9956	0.1	185224	5.38	9796	0.0106	1	0.015	15	0.0233
			0.5	2	2477	4954											
	6-1	2	0.5	1	3493	6986	7053	14106	0.1	185224	7.62	13946	0.0151	1	0.015	15	0.0332
			0.5	2	3560	7120											
	6-2	2	0.5	1	3789	7578	7556	15112	0.1	185224	8.16	14952	0.0161	1	0.015	15	0.0356
			0.5	2	3767	7534											
	6-3	2	0.5	1	3725	7450	7411	14822	0.1	185224	8.00	14662	0.0158	1	0.015	15	0.0349
			0.5	2	3686	7372											
	7-1	2	0.5	1	4564	9128	9055	18110	0.1	185224	9.78	17950	0.0194	1	0.015	15	0.0428
			0.5	2	4491	8982											
	7-2	2	0.5	1	4356	8712	8714	17428	0.1	185224	9.41	17268	0.0186	1	0.015	15	0.0412
			0.5	2	4358	8716											
	7-3	2	0.5	1	4332	8664	8628	17256	0.1	185224	9.32	17096	0.0185	1	0.015	15	0.0407
			0.5	2	4296	8592											
	8-1	2	0.5	1	4561	9122	9006	18012	0.1	185224	9.72	17852	0.0193	1	0.015	15	0.0425
			0.5	2	4445	8890											
	8-2	2	0.5	1	4446	8892	8835	17670	0.1	185224	9.54	17510	0.0189	1	0.015	15	0.0417
			0.5	2	4389	8778											
	8-3	2	0.5	1	4259	8518	8639	17278	0.1	185224	9.33	17118	0.0185	1	0.015	15	0.0408
			0.5	2	4380	8760											

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0439	0.0028
Full activity	End	0.0400	0.0002
Full activity	Overall	0.0419	0.0027
Background	Beginning	0.0000	2.35933E-05
Background	End	0.0000	2.02228E-05
Background	Overall	0.0000	2.53347E-05
Positive	Beginning	0.0222	0.0001
Positive	End	0.0194	0.0006
Positive	Overall	0.0208	0.0016
Negative	Beginning	0.0443	0.0004
Negative	End	0.0381	0.0012
Negative	Overall	0.0412	0.0036

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 6	1	1	1.00E-03	-3.00	0.0004
Ref Chem 6	1	2	1.00E-03	-3.00	0.0004
Ref Chem 6	1	3	1.00E-03	-3.00	0.0003
Ref Chem 6	2	1	1.00E-04	-4.00	0.0027
Ref Chem 6	2	2	1.00E-04	-4.00	0.0027
Ref Chem 6	2	3	1.00E-04	-4.00	0.0027
Ref Chem 6	3	1	2.50E-05	-4.60	0.0088
Ref Chem 6	3	2	2.50E-05	-4.60	0.0093
Ref Chem 6	3	3	2.50E-05	-4.60	0.0086
Ref Chem 6	4	1	1.00E-05	-5.00	0.0164
Ref Chem 6	4	2	1.00E-05	-5.00	0.0156
Ref Chem 6	4	3	1.00E-05	-5.00	0.0159
Ref Chem 6	5	1	5.00E-06	-5.30	0.0236
Ref Chem 6	5	2	5.00E-06	-5.30	0.0235
Ref Chem 6	5	3	5.00E-06	-5.30	0.0233
Ref Chem 6	6	1	1.00E-06	-6.00	0.0332
Ref Chem 6	6	2	1.00E-06	-6.00	0.0356
Ref Chem 6	6	3	1.00E-06	-6.00	0.0349
Ref Chem 6	7	1	1.00E-07	-7.00	0.0428
Ref Chem 6	7	2	1.00E-07	-7.00	0.0412
Ref Chem 6	7	3	1.00E-07	-7.00	0.0407
Ref Chem 6	8	1	1.00E-08	-8.00	0.0425
Ref Chem 6	8	2	1.00E-08	-8.00	0.0417
Ref Chem 6	8	3	1.00E-08	-8.00	0.0408

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.00	0.86	0.74
2	-4.00	6.51	6.34	6.45
3	-4.60	21.06	22.12	20.54
4	-5.00	39.09	37.11	37.95
5	-5.30	56.20	56.02	55.66
6	-6.00	79.24	84.96	83.31
7	-7.00	101.99	98.12	97.14
8	-8.00	101.44	99.49	97.27

Aromatase Assay Spreadsheet

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0203	34248	1687094	
2	0.0196	31546	1609490	
3	0.0194	33467	1725103	
4	0.0194	33734	1738866	
5	0.0193	33936	1758342	
			Average DPM/g soln	1703779
			SD	58804
			CV	3.45
			µCi/g soln	0.767

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.2	10		1020.00
Dilution A			100	10.20
Dilution B			10	1.02

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1243 g
Mass of dilution B used in substrate prep	4.5674 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.573434 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00869 µg/g soln.
µg/g soln.

a. µCi/g soln 0.767
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.573434 + 0.00869
= 0.582122 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.318 µCi/µg ASDN

838248 dpm/nmol

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.1	100	11540-45
	0.547	0.375	0.230	0.174	0.123	0.112	0.104			
	0.572	0.373	0.236	0.180	0.133	0.119	0.106			
	0.574	0.379	0.238	0.172	0.128	0.126	0.112			

Samples:	11540-6A	11540-6B	11540-45C	11345-18I
	0.368	0.121	0.186	0.121
	0.341	0.147	0.186	0.139
			0.180	0.138

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0502	0.564	0.457	0.0482
125	100	200	0.00013	200	0.0251	0.376	0.268	0.0276
50	200	1000	0.00005	200	0.0100	0.235	0.127	0.0121
25	100	1000	0.00003	200	0.0050	0.175	0.068	0.0056
10	40	1000	0.00001	200	0.0020	0.128	0.021	0.0004
5	20	1000	0.00001	200	0.0010	0.119	0.012	-0.0005

Variables	Regression results			
m, b	0.109	-0.002		
se_m, se_b	0.006	0.001		
r^2, se_y	0.989	0.002		
F, df	371	4		
ss_{reg}, ss_{resid}	0.002	0.000		

Regression results are calculated using the function LINEST

Blank	0.107	r^2=	0.989	
		m=	0.109	
		b=	-0.002	

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.368	0.261	0.027	200	1	1	0.134	0.126	0.11
11540-6A	0.341	0.234	0.024	200	1	1	0.119		
11540-6A									
11540-6B	0.121	0.014	0.000	200	1	1	-0.002	0.005	0.012
11540-6B	0.147	0.040	0.003	200	1	1	0.013		
11540-6B									
11540-45C	0.186	0.079	0.007	200	1	800	27.1	26.3	
11540-45C	0.186	0.079	0.007	200	1	800	27.1		
11540-45C	0.180	0.073	0.006	200	1	800	24.5		
11345-18I	0.121	0.014	0.000	200	1	600	-1.0	2.8	
11345-18I	0.139	0.032	0.002	200	1	600	4.9		
11345-18I	0.138	0.031	0.002	200	1	600	4.6		

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested		8					
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.547	0.375	0.230	0.174	0.123	0.112	0.104	25.1	100	11540-45
	0.572	0.373	0.236	0.180	0.133	0.119	0.106			
	0.574	0.379	0.238	0.172	0.128	0.126	0.112			

Samples:	11540-6A	11540-6B	11540-45C	11345-18I
	0.368	0.121	0.186	0.121
	0.341	0.147	0.186	0.139
			0.180	0.138

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0502	0.564	0.457	0.0420
125	100	200	0.00013	200	0.0251	0.376	0.268	0.0244
50	200	1000	0.00005	200	0.0100	0.235	0.127	0.0113
25	100	1000	0.00003	200	0.0050	0.175	0.068	0.0057
10	40	1000	0.00001	200	0.0020	0.128	0.021	0.0013
5	20	1000	0.00001	200	0.0010	0.119	0.012	0.0005

Variables	Regression results	
m, b	0.093	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.992	0.001
F, df	358	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

Blank	0.107	r^2=	0.992		
		m=	0.093		
		b=	-0.001		

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.368	0.261	0.024	200	1	1	0.119	0.112	0.11
11540-6A	0.341	0.234	0.021	200	1	1	0.106		
11540-6A									
11540-6B	0.121	0.014	0.001	200	1	1	0.003	0.009	0.012
11540-6B	0.147	0.040	0.003	200	1	1	0.015		
11540-6B									
11540-45C	0.186	0.079	0.007	200	1	800	26.9	26.1	
11540-45C	0.186	0.079	0.007	200	1	800	26.9		
11540-45C	0.180	0.073	0.006	200	1	800	24.6		
11345-18I	0.121	0.014	0.001	200	1	600	2.0	5.2	
11345-18I	0.139	0.032	0.002	200	1	600	7.0		
11345-18I	0.138	0.031	0.002	200	1	600	6.7		

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	1

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	26.1
Protein Concentration (dilution added to assay, mg/mL):	0.032656

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5880	11760	11860	23720	0.1	170378	13.92	23555	0.0281	1	0.016	15	0.0574
			0.5	2	5980	11960											
	2	2	0.1	1	1184	11840	12205	24410	0.1	170378	14.33	24245	0.0289	1	0.016	15	0.0590
			0.1	2	1257	12570											
	3	2	0.5	1	5324	10648	10671	21342	0.1	170378	12.53	21177	0.0253	1	0.016	15	0.0516
			0.5	2	5347	10694											
	4	2	0.5	1	5312	10624	10830	21660	0.1	170378	12.71	21495	0.0256	1	0.016	15	0.0523
			0.5	2	5518	11036											
Background control	1	2	0.5	1	41	82	87	174	0.1	170378	0.10	9	0.0000	1	0.016	15	0.0000
			0.5	2	46	92											
	2	2	0.5	1	35	70	79	158	0.1	170378	0.09	-7	0.0000	1	0.016	15	0.0000
			0.5	2	44	88											
	3	2	0.5	1	29	58	65	130	0.1	170378	0.08	-35	0.0000	1	0.016	15	-0.0001
			0.5	2	36	72											
	4	2	0.5	1	54	108	99	198	0.1	170378	0.12	33	0.0000	1	0.016	15	0.0001
			0.5	2	45	90											
Positive control	1	2	0.5	1	2942	5884	5884	11768	0.1	170378	6.91	11603	0.0136	1	0.016	15	0.0283
			0.5	2	2942	5884											
	2	2	0.5	1	2794	5588	5642	11284	0.1	170378	6.62	11119	0.0133	1	0.016	15	0.0271
			0.5	2	2848	5696											
	3	2	0.5	1	2575	5150	5244	10488	0.1	170378	6.16	10323	0.0123	1	0.016	15	0.0251
			0.5	2	2669	5338											
	4	2	0.5	1	2652	5304	5244	10488	0.1	170378	6.16	10323	0.0123	1	0.016	15	0.0251
			0.5	2	2592	5184											
Negative Control	1	2	0.5	1	5901	11802	11728	23456	0.1	170378	13.77	23291	0.0278	1	0.016	15	0.0567
			0.5	2	5827	11654											
	2	2	0.5	1	5757	11514	11530	23060	0.1	170378	13.53	22895	0.0273	1	0.016	15	0.0558
			0.5	2	5773	11546											
	3	2	0.5	1	5200	10400	10421	20842	0.1	170378	12.23	20677	0.0247	1	0.016	15	0.0504
			0.5	2	5221	10442											
	4	2	0.5	1	5029	10058	10080	20160	0.1	170378	11.83	19995	0.0239	1	0.016	15	0.0487
			0.5	2	5051	10102											
Ref Chem 7	1-1	2	0.5	1	42	84	89	178	0.1	170378	0.10	13	0.0000	1	0.016	15	0.0000
			0.5	2	47	94											
	1-2	2	0.5	1	41	82	96	192	0.1	170378	0.11	27	0.0000	1	0.016	15	0.0001
			0.5	2	55	110											
	1-3	2	0.5	1	45	90	93	186	0.1	170378	0.11	21	0.0000	1	0.016	15	0.0001
			0.5	2	48	96											
	2-1	2	0.5	1	52	104	118	236	0.1	170378	0.14	71	0.0001	1	0.016	15	0.0002
			0.5	2	66	132											
	2-2	2	0.5	1	42	84	79	158	0.1	170378	0.09	-7	0.0000	1	0.016	15	0.0000
			0.5	2	37	74											
	2-3	2	0.5	1	45	90	72	144	0.1	170378	0.08	-21	0.0000	1	0.016	15	-0.0001
			0.5	2	27	54											
	3-1	2	0.5	1	45	90	78	156	0.1	170378	0.09	-9	0.0000	1	0.016	15	0.0000
			0.5	2	33	66											
	3-2	2	0.5	1	46	92	93	186	0.1	170378	0.11	21	0.0000	1	0.016	15	0.0001
			0.5	2	47	94											
	3-3	2	0.5	1	52	104	80	160	0.1	170378	0.09	-5	0.0000	1	0.016	15	0.0000
			0.5	2	28	56											
	4-1	2	0.5	1	52	104	101	202	0.1	170378	0.12	37	0.0000	1	0.016	15	0.0001
			0.5	2	49	98											
	4-2	2	0.5	1	48	96	97	194	0.1	170378	0.11	29	0.0000	1	0.016	15	0.0001
			0.5	2	49	98											
	4-3	2	0.5	1	57	114	109	218	0.1	170378	0.13	53	0.0001	1	0.016	15	0.0001
			0.5	2	52	104											
	5-1	2	0.5	1	114	228	218	436	0.1	170378	0.26	271	0.0003	1	0.016	15	0.0007
			0.5	2	104	208											
	5-2	2	0.5	1	110	220	223	446	0.1	170378	0.26	281	0.0003	1	0.016	15	0.0007
			0.5	2	113	226											
	5-3	2	0.5	1	131	262	226	452	0.1	170378	0.27	287	0.0003	1	0.016	15	0.0007
			0.5	2	95	190											
	6-1	2	0.5	1	675	1350	1359	2718	0.1	170378	1.60	2553	0.0030	1	0.016	15	0.0062
			0.5	2	684	1368											
	6-2	2	0.5	1	681	1362	1337	2674	0.1	170378	1.57	2509	0.0030	1	0.016	15	0.0061
			0.5	2	656	1312											
	6-3	2	0.5	1	673	1346	1325	2650	0.1	170378	1.56	2485	0.0030	1	0.016	15	0.0061
			0.5	2	652	1304											
	7-1	2	0.5	1	3420	6840	6751	13502	0.1	170378	7.92	13337	0.0159	1	0.016	15	0.0325
			0.5	2	3331	6662											
	7-2	2	0.5	1	528	1056	1076	2152	0.1	170378	1.26	1987	0.0024	1	0.016	15	0.0048
			0.5	2	548	1096											
	7-3	2	0.5	1	3208	6416	6532	13064	0.1	170378	7.67	12899	0.0154	1	0.016	15	0.0314
			0.5	2	3324	6648											
	8-1	2	0.5	1	5486	10972	11042	22084	0.1	170378	12.96	21919	0.0261	1	0.016	15	0.0534
			0.5	2	5556	11112											
	8-2	2	0.5	1	5186	10372	10427	20854	0.1	170378	12.24	20689	0.0247	1	0.016	15	0.0504
			0.5	2	5241	10482											
	8-3	2	0.5	1	5146	10292	10432	20864	0.1	170378	12.25	20699	0.0247	1	0.016	15	0.0504
			0.5	2	5286	10572											

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0582	0.0012
Full activity	End	0.0520	0.0005
Full activity	Overall	0.0551	0.0037
Background	Beginning	0.0000	2.75535E-05
Background	End	0.0000	0.000117102
Background	Overall	0.0000	6.95122E-05
Positive	Beginning	0.0277	0.0008
Positive	End	0.0251	0.0000
Positive	Overall	0.0264	0.0015
Negative	Beginning	0.0562	0.0007
Negative	End	0.0495	0.0012
Negative	Overall	0.0529	0.0040

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 7	1	1	1.00E-03	-3.00	0.0000
Ref Chem 7	1	2	1.00E-03	-3.00	0.0001
Ref Chem 7	1	3	1.00E-03	-3.00	0.0001
Ref Chem 7	2	1	1.00E-04	-4.00	0.0002
Ref Chem 7	2	2	1.00E-04	-4.00	0.0000
Ref Chem 7	2	3	1.00E-04	-4.00	-0.0001
Ref Chem 7	3	1	1.00E-05	-5.00	0.0000
Ref Chem 7	3	2	1.00E-05	-5.00	0.0001
Ref Chem 7	3	3	1.00E-05	-5.00	0.0000
Ref Chem 7	4	1	1.00E-06	-6.00	0.0001
Ref Chem 7	4	2	1.00E-06	-6.00	0.0001
Ref Chem 7	4	3	1.00E-06	-6.00	0.0001
Ref Chem 7	5	1	1.00E-07	-7.00	0.0007
Ref Chem 7	5	2	1.00E-07	-7.00	0.0007
Ref Chem 7	5	3	1.00E-07	-7.00	0.0007
Ref Chem 7	6	1	1.00E-08	-8.00	0.0062
Ref Chem 7	6	2	1.00E-08	-8.00	0.0061
Ref Chem 7	6	3	1.00E-08	-8.00	0.0061
Ref Chem 7	7	1	1.00E-09	-9.00	0.0325
Ref Chem 7	7	2	1.00E-09	-9.00	0.0048
Ref Chem 7	7	3	1.00E-09	-9.00	0.0314
Ref Chem 7	8	1	1.00E-10	-10.00	0.0534
Ref Chem 7	8	2	1.00E-10	-10.00	0.0504
Ref Chem 7	8	3	1.00E-10	-10.00	0.0504

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.06	0.12	0.09
2	-4.00	0.31	-0.03	-0.09
3	-5.00	-0.04	0.09	-0.02
4	-6.00	0.16	0.13	0.23
5	-7.00	1.20	1.24	1.27
6	-8.00	11.29	11.09	10.99
7	-9.00	58.97	8.79	57.03
8	-10.00	96.91	91.47	91.52

Aromatase Assay Spreadsheet

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8		
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	36534	1835879	
2	0.0196	34683	1769541	
3	0.0196	36022	1837857	
4	0.0197	35995	1827157	
5	0.0196	36641	1869439	
			Average DPM/g soln	1827975
			SD	36382
			CV	1.99
			µCi/g soln	0.823

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.3	10		1030.00
Dilution A			100	10.30
Dilution B			10	1.03

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0964 g
Mass of dilution B used in substrate prep	4.5643 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.580657 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00932 µg/g soln.
µg/g soln.

a. µCi/g soln 0.823
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.580657 + 0.00932
= 0.589978 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.396 µCi/µg ASDN

887376 dpm/nmol

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.1	100	11540-47
	0.559	0.399	0.239	0.176	0.130	0.127	0.108			
	0.567	0.397	0.236	0.186	0.140	0.122	0.112			
	0.552	0.386	0.241	0.175	0.146	0.128	0.124			

Samples:	11540-6A	11540-6B	11540-47C	11345-19I
	0.364	0.149	0.190	0.141
	0.362	0.143	0.189	0.144
			0.193	0.143

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0502	0.559	0.445	0.0473
125	100	200	0.00013	200	0.0251	0.394	0.279	0.0290
50	200	1000	0.00005	200	0.0100	0.239	0.124	0.0118
25	100	1000	0.00003	200	0.0050	0.179	0.064	0.0052
10	40	1000	0.00001	200	0.0020	0.139	0.024	0.0007
5	20	1000	0.00001	200	0.0010	0.126	0.011	-0.0007

Variables	Regression results	
m, b	0.111	-0.002
se_m, se_b	0.007	0.002
r^2, se_y	0.983	0.003
F, df	230	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank	0.115	r^2=	0.983	
		m=	0.111	
		b=	-0.002	

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.364	0.249	0.026	200	1	1	0.128	0.128	0.11
11540-6A	0.362	0.247	0.025	200	1	1	0.127		
11540-6A									
11540-6B	0.149	0.034	0.002	200	1	1	0.009	0.008	0.012
11540-6B	0.143	0.028	0.001	200	1	1	0.006		
11540-6B									
11540-47C	0.190	0.075	0.006	200	1	800	25.7	26.0	
11540-47C	0.189	0.074	0.006	200	1	800	25.2		
11540-47C	0.193	0.078	0.007	200	1	800	27.0		
11345-19I	0.141	0.026	0.001	200	1	600	3.0	3.5	
11345-19I	0.144	0.029	0.001	200	1	600	4.0		
11345-19I	0.143	0.028	0.001	200	1	600	3.6		

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.1	100	11540-47
	0.559	0.399	0.239	0.176	0.130	0.127	0.108			
	0.567	0.397	0.236	0.186	0.140	0.122	0.112			
	0.552	0.386	0.241	0.175	0.146	0.128	0.124			

Samples:	11540-6A	11540-6B	11540-47C	11345-19I
	0.364	0.149	0.190	0.141
	0.362	0.143	0.189	0.144
			0.193	0.143

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0502	0.559	0.445	0.0396	m, b	0.090	0.000
125	100	200	0.00013	200	0.0251	0.394	0.279	0.0247	se_m, se_b	0.003	0.000
50	200	1000	0.00005	200	0.0100	0.239	0.124	0.0107	r^2, se_y	0.997	0.001
25	100	1000	0.00003	200	0.0050	0.179	0.064	0.0054	F, df	1143	3
10	40	1000	0.00001	200	0.0020	0.139	0.024	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.126	0.011	0.0006			
			Blank	0.115	r^2=	0.997					
					m=	0.090					
					b=	0.000					

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.364	0.249	0.022	200	1	1	0.110	0.110	0.11
11540-6A	0.362	0.247	0.022	200	1	1	0.109		
11540-6A									
11540-6B	0.149	0.034	0.003	200	1	1	0.013	0.012	0.012
11540-6B	0.143	0.028	0.002	200	1	1	0.011		
11540-6B									
11540-47C	0.190	0.075	0.006	200	1	800	25.5	25.7	
11540-47C	0.189	0.074	0.006	200	1	800	25.1		
11540-47C	0.193	0.078	0.007	200	1	800	26.5		
11345-19I	0.141	0.026	0.002	200	1	600	5.8	6.3	
11345-19I	0.144	0.029	0.002	200	1	600	6.7		
11345-19I	0.143	0.028	0.002	200	1	600	6.4		

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	2

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-06
2	1.00E-07
3	1.00E-08
4	5.00E-09
5	1.00E-09
6	5.00E-10
7	2.50E-10
8	1.00E-10

Protein Concentration (stock microsomes, mg/mL):	25.7
Protein Concentration (dilution added to assay, mg/mL):	0.03212

Assay Date	9/24/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5412	10824	10977	21954	0.1	182797	12.01	21802	0.0246	1	0.016	15	0.0510
			0.5	2	5565	11130											
	2	2	0.5	1	5834	11668	11686	23372	0.1	182797	12.79	23220	0.0262	1	0.016	15	0.0543
			0.5	2	5852	11704											
	3	2	0.5	1	4686	9372	9542	19084	0.1	182797	10.44	18932	0.0213	1	0.016	15	0.0443
			0.5	2	4856	9712											
	4	2	0.5	1	4815	9630	9867	19734	0.1	182797	10.78	19582	0.0220	1	0.016	15	0.0456
			0.5	2	5042	10084											
Background control	1	2	0.5	1	49	98	88	176	0.1	182797	0.10	24	0.0000	1	0.016	15	0.0001
			0.5	2	39	78											
	2	2	0.5	1	21	42	47	94	0.1	182797	0.05	-58	-0.0001	1	0.016	15	-0.0001
			0.5	2	26	52											
	3	2	0.5	1	39	78	73	146	0.1	182797	0.08	-6	0.0000	1	0.016	15	0.0000
			0.5	2	34	68											
	4	2	0.5	1	46	92	96	192	0.1	182797	0.11	40	0.0000	1	0.016	15	0.0001
			0.5	2	50	100											
Positive control	1	2	0.5	1	3103	6206	6199	12398	0.1	182797	6.78	12246	0.0138	1	0.016	15	0.0286
			0.5	2	3096	6192											
	2	2	0.5	1	2955	5910	5856	11712	0.1	182797	6.41	11560	0.0130	1	0.016	15	0.0270
			0.5	2	2901	5802											
	3	2	0.5	1	2275	4550	4685	9370	0.1	182797	5.13	9218	0.0104	1	0.016	15	0.0215
			0.5	2	2410	4820											
	4	2	0.5	1	2540	5080	5088	10176	0.1	182797	5.57	10024	0.0113	1	0.016	15	0.0234
			0.5	2	2548	5096											
Negative Control	1	2	0.5	1	5449	10898	10987	21974	0.1	182797	12.02	21822	0.0246	1	0.016	15	0.0510
			0.5	2	5538	11076											
	2	2	0.5	1	5413	10826	10858	21716	0.1	182797	11.86	21564	0.0243	1	0.016	15	0.0504
			0.5	2	5445	10890											
	3	2	0.5	1	4250	8500	8613	17226	0.1	182797	9.42	17074	0.0192	1	0.016	15	0.0399
			0.5	2	4363	8726											
	4	2	0.5	1	4853	9706	9690	19380	0.1	182797	10.60	19228	0.0217	1	0.016	15	0.0450
			0.5	2	4837	9674											
Ref Chem 7	1-1	2	0.5	1	48	96	86	172	0.1	182797	0.09	20	0.0000	1	0.016	15	0.0000
			0.5	2	38	76											
	1-2	2	0.5	1	41	82	86	172	0.1	182797	0.09	20	0.0000	1	0.016	15	0.0000
			0.5	2	45	90											
	1-3	2	0.5	1	53	106	89	178	0.1	182797	0.10	26	0.0000	1	0.016	15	0.0001
			0.5	2	36	72											
	2-1	2	0.5	1	96	192	225	450	0.1	182797	0.25	298	0.0003	1	0.016	15	0.0007
			0.5	2	129	258											
	2-2	2	0.5	1	110	220	225	450	0.1	182797	0.25	298	0.0003	1	0.016	15	0.0007
			0.5	2	115	230											
	2-3	2	0.5	1	110	220	224	448	0.1	182797	0.25	296	0.0003	1	0.016	15	0.0007
			0.5	2	114	228											
	3-1	2	0.5	1	704	1408	1420	2840	0.1	182797	1.55	2688	0.0030	1	0.016	15	0.0063
			0.5	2	716	1432											
	3-2	2	0.5	1	729	1458	1505	3010	0.1	182797	1.65	2858	0.0032	1	0.016	15	0.0067
			0.5	2	776	1552											
	3-3	2	0.5	1	716	1432	1419	2838	0.1	182797	1.55	2686	0.0030	1	0.016	15	0.0063
			0.5	2	703	1406											
	4-1	2	0.5	1	1296	2592	2520	5040	0.1	182797	2.76	4888	0.0055	1	0.016	15	0.0114
			0.5	2	1224	2448											
	4-2	2	0.5	1	1243	2486	2536	5072	0.1	182797	2.77	4920	0.0055	1	0.016	15	0.0115
			0.5	2	1293	2586											
	4-3	2	0.5	1	1246	2492	2486	4972	0.1	182797	2.72	4820	0.0054	1	0.016	15	0.0113
			0.5	2	1240	2480											
	5-1	2	0.5	1	3280	6560	6713	13426	0.1	182797	7.34	13274	0.0150	1	0.016	15	0.0310
			0.5	2	3433	6866											
	5-2	2	0.5	1	3199	6398	6214	12428	0.1	182797	6.80	12276	0.0138	1	0.016	15	0.0287
			0.5	2	3015	6030											
	5-3	2	0.5	1	3164	6328	6319	12638	0.1	182797	6.91	12486	0.0141	1	0.016	15	0.0292
			0.5	2	3155	6310											
	6-1	2	0.5	1	4180	8360	8240	16480	0.1	182797	9.02	16328	0.0184	1	0.016	15	0.0382
			0.5	2	4060	8120											
	6-2	2	0.5	1	4119	8238	8212	16424	0.1	182797	8.98	16272	0.0183	1	0.016	15	0.0381
			0.5	2	4093	8186											
	6-3	2	0.5	1	3935	7870	7892	15784	0.1	182797	8.63	15632	0.0176	1	0.016	15	0.0366
			0.5	2	3957	7914											
	7-1	2	0.5	1	4644	9288	9420	18840	0.1	182797	10.31	18688	0.0211	1	0.016	15	0.0437
			0.5	2	4776	9552											
	7-2	2	0.5	1	4537	9074	9190	18380	0.1	182797	10.05	18228	0.0205	1	0.016	15	0.0426
			0.5	2	4653	9306											
	7-3	2	0.5	1	4612	9224	9196	18392	0.1	182797	10.06	18240	0.0205	1	0.016	15	0.0427
			0.5	2	4584	9168											
	8-1	2	0.5	1	5005	10010	10133	20266	0.1	182797	11.09	20114	0.0227	1	0.016	15	0.0470
			0.5	2	5128	10256											
	8-2	2	0.5	1	4915	9830	9828	19656	0.1	182797	10.75	19504	0.0220	1	0.016	15	0.0456
			0.5	2	4913	9826											
	8-3	2	0.5	1	4840	9680	9828	19656	0.1	182797	10.75	19504	0.0220	1	0.016	15	0.0456
			0.5	2	4988	9976											

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0527	0.0023
Full activity	End	0.0450	0.0010
Full activity	Overall	0.0488	0.0047
Background	Beginning	0.0000	0.000135622
Background	End	0.0000	7.60806E-05
Background	Overall	0.0000	0.00010084
Positive	Beginning	0.0278	0.0011
Positive	End	0.0225	0.0013
Positive	Overall	0.0252	0.0032
Negative	Beginning	0.0507	0.0004
Negative	End	0.0425	0.0036
Negative	Overall	0.0466	0.0052

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 7	1	1	1.00E-06	-6.00	0.0000
Ref Chem 7	1	2	1.00E-06	-6.00	0.0000
Ref Chem 7	1	3	1.00E-06	-6.00	0.0001
Ref Chem 7	2	1	1.00E-07	-7.00	0.0007
Ref Chem 7	2	2	1.00E-07	-7.00	0.0007
Ref Chem 7	2	3	1.00E-07	-7.00	0.0007
Ref Chem 7	3	1	1.00E-08	-8.00	0.0063
Ref Chem 7	3	2	1.00E-08	-8.00	0.0067
Ref Chem 7	3	3	1.00E-08	-8.00	0.0063
Ref Chem 7	4	1	5.00E-09	-8.30	0.0114
Ref Chem 7	4	2	5.00E-09	-8.30	0.0115
Ref Chem 7	4	3	5.00E-09	-8.30	0.0113
Ref Chem 7	5	1	1.00E-09	-9.00	0.0310
Ref Chem 7	5	2	1.00E-09	-9.00	0.0287
Ref Chem 7	5	3	1.00E-09	-9.00	0.0292
Ref Chem 7	6	1	5.00E-10	-9.30	0.0382
Ref Chem 7	6	2	5.00E-10	-9.30	0.0381
Ref Chem 7	6	3	5.00E-10	-9.30	0.0366
Ref Chem 7	7	1	2.50E-10	-9.60	0.0437
Ref Chem 7	7	2	2.50E-10	-9.60	0.0426
Ref Chem 7	7	3	2.50E-10	-9.60	0.0427
Ref Chem 7	8	1	1.00E-10	-10.00	0.0470
Ref Chem 7	8	2	1.00E-10	-10.00	0.0456
Ref Chem 7	8	3	1.00E-10	-10.00	0.0456

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-6.00	0.10	0.10	0.12
2	-7.00	1.43	1.43	1.42
3	-8.00	12.87	13.69	12.86
4	-8.30	23.41	23.56	23.09
5	-9.00	63.58	58.80	59.80
6	-9.30	78.20	77.93	74.87
7	-9.60	89.51	87.30	87.36
8	-10.00	96.34	93.41	93.41

Aromatase Assay Spreadsheet

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	36222	1829394	
2	0.0196	33945	1731888	
3	0.0194	34546	1780722	
4	0.0194	35404	1824948	
5	0.0196	36543	1864439	
			Average DPM/g soln	1806278
			SD	51119
			CV	2.83
			µCi/g soln	0.814

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0578 g
Mass of dilution B used in substrate prep	4.5227 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.561282 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00921 µg/g soln.
µg/g soln.

a. µCi/g soln 0.814
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.561282 + 0.00921
= 0.570493 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.426 µCi/µg ASDN

906792 dpm/nmol

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested		8					
Technician ID	1	Replicate #	3	Microsome type	Placental		Microsome ID	11540-22			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.2	100	11540-48
	0.505	0.339	0.222	0.172	0.133	0.133	0.108			
	0.503	0.347	0.243	0.180	0.147	0.130	0.112			
	0.505	0.328	0.239	0.182	0.145	0.122	0.120			

Samples:	11540-6A	11540-6B	11540-48C	11345-20I
	0.358	0.138	0.187	0.141
	0.338	0.147	0.185	0.139
			0.186	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0504	0.504	0.391	0.0487
125	100	200	0.00013	200	0.0252	0.338	0.225	0.0267
50	200	1000	0.00005	200	0.0101	0.235	0.121	0.0130
25	100	1000	0.00003	200	0.0050	0.178	0.065	0.0056
10	40	1000	0.00001	200	0.0020	0.142	0.028	0.0008
5	20	1000	0.00001	200	0.0010	0.128	0.015	-0.0010
			Blank	0.113				

Variables	Regression results	
m, b	0.132	-0.003
se_m, se_b	0.007	0.001
r^2, se_y	0.989	0.002
F, df	366	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.989
m= 0.132
b= -0.003

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.358	0.245	0.029	200	1	1	0.147	0.140	0.11
11540-6A	0.338	0.225	0.027	200	1	1	0.134		
11540-6A									
11540-6B	0.138	0.025	0.000	200	1	1	0.001	0.004	0.012
11540-6B	0.147	0.034	0.001	200	1	1	0.007		
11540-6B									
11540-48C	0.187	0.074	0.007	200	1	800	27.0	26.4	
11540-48C	0.185	0.072	0.006	200	1	800	25.9		
11540-48C	0.186	0.073	0.007	200	1	800	26.4		
11345-20I	0.141	0.028	0.001	200	1	600	2.0	0.9	
11345-20I	0.139	0.026	0.000	200	1	600	1.2		
11345-20I	0.135	0.022	0.000	200	1	600	-0.4		

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8						
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.505	0.339	0.222	0.172	0.133	0.133	0.108	25.2	100	11540-48
	0.503	0.347	0.243	0.180	0.147	0.130	0.112			
	0.505	0.328	0.239	0.182	0.145	0.122	0.120			

Samples:	11540-6A	11540-6B	11540-48C	11345-20I
	0.358	0.138	0.187	0.141
	0.338	0.147	0.185	0.139
			0.186	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0504	0.504	0.391	0.0431
125	100	200	0.00013	200	0.0252	0.338	0.225	0.0240
50	200	1000	0.00005	200	0.0101	0.235	0.121	0.0122
25	100	1000	0.00003	200	0.0050	0.178	0.065	0.0057
10	40	1000	0.00001	200	0.0020	0.142	0.028	0.0015
5	20	1000	0.00001	200	0.0010	0.128	0.015	0.0000
			Blank	0.113				

Variables	Regression results	
m, b	0.115	-0.002
se_m, se_b	0.009	0.001
r^2, se_y	0.981	0.002
F, df	154	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.981
m= 0.115
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.358	0.245	0.026	200	1	1	0.132	0.126	0.11
11540-6A	0.338	0.225	0.024	200	1	1	0.120		
11540-6A									
11540-6B	0.138	0.025	0.001	200	1	1	0.005	0.008	0.012
11540-6B	0.147	0.034	0.002	200	1	1	0.011		
11540-6B									
11540-48C	0.187	0.074	0.007	200	1	800	26.8	26.4	
11540-48C	0.185	0.072	0.006	200	1	800	25.9		
11540-48C	0.186	0.073	0.007	200	1	800	26.4		
11345-20I	0.141	0.028	0.001	200	1	600	4.3	3.4	
11345-20I	0.139	0.026	0.001	200	1	600	3.6		
11345-20I	0.135	0.022	0.001	200	1	600	2.2		

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate #	3

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	26.4
Protein Concentration (dilution added to assay, mg/mL):	0.032945

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-06
2	1.00E-07
3	1.00E-08
4	5.00E-09
5	1.00E-09
6	5.00E-10
7	2.50E-10
8	1.00E-10

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5771	11542	11672	23344	0.1	180628	12.92	23222	0.0256	1	0.016	15	0.0518
			0.5	2	5901	11802											
	2	2	0.5	1	5762	11524	11474	22948	0.1	180628	12.70	22826	0.0252	1	0.016	15	0.0509
			0.5	2	5712	11424											
	3	2	0.5	1	5239	10478	10398	20796	0.1	180628	11.51	20674	0.0228	1	0.016	15	0.0451
			0.5	2	5159	10318											
	4	2	0.5	1	5020	10040	10279	20558	0.1	180628	11.38	20436	0.0225	1	0.016	15	0.0456
			0.5	2	5259	10518											
Background control	1	2	0.5	1	22	44	58	116	0.1	180628	0.06	-6	0.0000	1	0.016	15	0.0000
			0.5	2	36	72											
	2	2	0.5	1	30	60	70	140	0.1	180628	0.08	18	0.0000	1	0.016	15	0.0000
			0.5	2	40	80											
	3	2	0.5	1	34	68	56	116	0.1	180628	0.06	-6	0.0000	1	0.016	15	0.0000
			0.5	2	24	48											
	4	2	0.5	1	31	62	58	116	0.1	180628	0.06	-6	0.0000	1	0.016	15	0.0000
			0.5	2	27	54											
Positive control	1	2	0.5	1	2853	5706	5717	11434	0.1	180628	6.33	11312	0.0125	1	0.016	15	0.0252
			0.5	2	2864	5728											
	2	2	0.5	1	2640	5280	5387	10774	0.1	180628	5.96	10652	0.0117	1	0.016	15	0.0238
			0.5	2	2747	5494											
	3	2	0.5	1	2528	5056	4994	9988	0.1	180628	5.53	9866	0.0109	1	0.016	15	0.0220
			0.5	2	2466	4932											
	4	2	0.5	1	2499	4998	5061	10122	0.1	180628	5.60	10000	0.0110	1	0.016	15	0.0223
			0.5	2	2562	5124											
Negative Control	1	2	0.5	1	5475	10950	10993	21986	0.1	180628	12.17	21864	0.0241	1	0.016	15	0.0488
			0.5	2	5518	11036											
	2	2	0.5	1	5439	10878	10943	21886	0.1	180628	12.12	21764	0.0240	1	0.016	15	0.0486
			0.5	2	5504	11008											
	3	2	0.5	1	4916	9832	9938	19876	0.1	180628	11.00	19754	0.0218	1	0.016	15	0.0441
			0.5	2	5022	10044											
	4	2	0.5	1	4780	9560	9520	19040	0.1	180628	10.54	18918	0.0209	1	0.016	15	0.0422
			0.5	2	4740	9480											
Ref Chem 7	1-1	2	0.5	1	39	78	42	84	0.1	180628	0.05	-38	0.0000	1	0.016	15	-0.0001
			0.5	2	3	6											
	1-2	2	0.5	1	39	78	64	128	0.1	180628	0.07	6	0.0000	1	0.016	15	0.0000
			0.5	2	25	50											
	1-3	2	0.5	1	43	86	81	162	0.1	180628	0.09	40	0.0000	1	0.016	15	0.0001
			0.5	2	38	76											
	2-1	2	0.5	1	97	194	198	396	0.1	180628	0.22	274	0.0003	1	0.016	15	0.0006
			0.5	2	101	202											
	2-2	2	0.5	1	99	198	202	404	0.1	180628	0.22	282	0.0003	1	0.016	15	0.0006
			0.5	2	103	206											
	2-3	2	0.5	1	118	236	225	450	0.1	180628	0.25	328	0.0004	1	0.016	15	0.0007
			0.5	2	107	214											
	3-1	2	0.5	1	700	1400	1395	2790	0.1	180628	1.54	2668	0.0029	1	0.016	15	0.0060
			0.5	2	695	1390											
	3-2	2	0.5	1	722	1444	1427	2854	0.1	180628	1.58	2732	0.0030	1	0.016	15	0.0061
			0.5	2	705	1410											
	3-3	2	0.5	1	676	1352	1389	2778	0.1	180628	1.54	2656	0.0029	1	0.016	15	0.0059
			0.5	2	713	1426											
	4-1	2	0.5	1	1220	2440	2432	4864	0.1	180628	2.69	4742	0.0052	1	0.016	15	0.0106
			0.5	2	1212	2424											
	4-2	2	0.5	1	1192	2384	2335	4670	0.1	180628	2.59	4548	0.0050	1	0.016	15	0.0101
			0.5	2	1143	2286											
	4-3	2	0.5	1	1175	2350	2376	4752	0.1	180628	2.63	4630	0.0051	1	0.016	15	0.0103
			0.5	2	1201	2402											
	5-1	2	0.5	1	3356	6712	6632	13264	0.1	180628	7.34	13142	0.0145	1	0.016	15	0.0293
			0.5	2	3276	6552											
	5-2	2	0.5	1	3348	6696	6706	13412	0.1	180628	7.43	13290	0.0147	1	0.016	15	0.0297
			0.5	2	3358	6716											
	5-3	2	0.5	1	3199	6398	6481	12962	0.1	180628	7.18	12840	0.0142	1	0.016	15	0.0287
			0.5	2	3282	6564											
	6-1	2	0.5	1	4198	8396	8359	16718	0.1	180628	9.26	16596	0.0183	1	0.016	15	0.0370
			0.5	2	4161	8322											
	6-2	2	0.5	1	4088	8176	8139	16278	0.1	180628	9.01	16156	0.0178	1	0.016	15	0.0361
			0.5	2	4051	8102											
	6-3	2	0.5	1	4104	8208	8231	16462	0.1	180628	9.11	16340	0.0180	1	0.016	15	0.0365
			0.5	2	4127	8254											
	7-1	2	0.5	1	4781	9562	9522	19044	0.1	180628	10.54	18922	0.0209	1	0.016	15	0.0422
			0.5	2	4741	9482											
	7-2	2	0.5	1	4603	9206	9307	18614	0.1	180628	10.31	18492	0.0204	1	0.016	15	0.0413
			0.5	2	4704	9408											
	7-3	2	0.5	1	4507	9014	9070	18140	0.1	180628	10.04	18018	0.0199	1	0.016	15	0.0402
			0.5	2	4563	9126											
	8-1	2	0.5	1	5149	10298	10304	20608	0.1	180628	11.41	20486	0.0226	1	0.016	15	0.0457
			0.5	2	5155	10310											
	8-2	2	0.5	1	4940	9880	10006	20012	0.1	180628	11.08	19890	0.0219	1	0.016	15	0.0444
			0.5	2	5066	10132											
	8-3	2	0.5	1	4942	9884	9988	19976	0.1	180628	11.06	19854	0.0219	1	0.016	15	0.0443
			0.5	2	5046	10092											

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0514	0.0006
Full activity	End	0.0459	0.0004
Full activity	Overall	0.0486	0.0032
Background	Beginning	0.0000	3.78711E-05
Background	End	0.0000	0
Background	Overall	0.0000	2.67789E-05
Positive	Beginning	0.0245	0.0010
Positive	End	0.0222	0.0002
Positive	Overall	0.0233	0.0015
Negative	Beginning	0.0487	0.0002
Negative	End	0.0431	0.0013
Negative	Overall	0.0459	0.0033

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 7	1	1	1.00E-06	-6.00	-0.0001
Ref Chem 7	1	2	1.00E-06	-6.00	0.0000
Ref Chem 7	1	3	1.00E-06	-6.00	0.0001
Ref Chem 7	2	1	1.00E-07	-7.00	0.0006
Ref Chem 7	2	2	1.00E-07	-7.00	0.0006
Ref Chem 7	2	3	1.00E-07	-7.00	0.0007
Ref Chem 7	3	1	1.00E-08	-8.00	0.0060
Ref Chem 7	3	2	1.00E-08	-8.00	0.0061
Ref Chem 7	3	3	1.00E-08	-8.00	0.0059
Ref Chem 7	4	1	5.00E-09	-8.30	0.0106
Ref Chem 7	4	2	5.00E-09	-8.30	0.0101
Ref Chem 7	4	3	5.00E-09	-8.30	0.0103
Ref Chem 7	5	1	1.00E-09	-9.00	0.0293
Ref Chem 7	5	2	1.00E-09	-9.00	0.0297
Ref Chem 7	5	3	1.00E-09	-9.00	0.0287
Ref Chem 7	6	1	5.00E-10	-9.30	0.0370
Ref Chem 7	6	2	5.00E-10	-9.30	0.0361
Ref Chem 7	6	3	5.00E-10	-9.30	0.0365
Ref Chem 7	7	1	2.50E-10	-9.60	0.0422
Ref Chem 7	7	2	2.50E-10	-9.60	0.0413
Ref Chem 7	7	3	2.50E-10	-9.60	0.0402
Ref Chem 7	8	1	1.00E-10	-10.00	0.0457
Ref Chem 7	8	2	1.00E-10	-10.00	0.0444
Ref Chem 7	8	3	1.00E-10	-10.00	0.0443

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-6.00	-0.17	0.03	0.18
2	-7.00	1.26	1.29	1.51
3	-8.00	12.24	12.54	12.19
4	-8.30	21.76	20.87	21.25
5	-9.00	60.31	60.99	58.93
6	-9.30	76.17	74.15	74.99
7	-9.60	86.84	84.87	82.69
8	-10.00	94.02	91.28	91.12

Aromatase Assay Spreadsheet

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0203	34248	1687094	
2	0.0196	31546	1609490	
3	0.0194	33467	1725103	
4	0.0194	33734	1738866	
5	0.0193	33936	1758342	
			Average DPM/g soln	1703779
			SD	58804
			CV	3.45
			µCi/g soln	0.767

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.2	10		1020.00
Dilution A			100	10.20
Dilution B			10	1.02

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1243 g
Mass of dilution B used in substrate prep	4.5674 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.573434 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00869 µg/g soln.
µg/g soln.

a. µCi/g soln	0.767
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.573434 + 0.00869
= 0.582122 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.318 µCi/µg ASDN

838248 dpm/nmol

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested		8					
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.547	0.375	0.230	0.174	0.123	0.112	0.104	25.1	100	11540-45
	0.572	0.373	0.236	0.180	0.133	0.119	0.106			
	0.574	0.379	0.238	0.172	0.128	0.126	0.112			

Samples:	11540-6A	11540-6B	11540-45C	11345-18I
	0.368	0.121	0.186	0.121
	0.341	0.147	0.186	0.139
			0.180	0.138

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0502	0.564	0.457	0.0482
125	100	200	0.00013	200	0.0251	0.376	0.268	0.0276
50	200	1000	0.00005	200	0.0100	0.235	0.127	0.0121
25	100	1000	0.00003	200	0.0050	0.175	0.068	0.0056
10	40	1000	0.00001	200	0.0020	0.128	0.021	0.0004
5	20	1000	0.00001	200	0.0010	0.119	0.012	-0.0005
			Blank	0.107				

Variables	Regression results	
m, b	0.109	-0.002
se_m, se_b	0.006	0.001
r^2, se_y	0.989	0.002
F, df	371	4
ss_{regr}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

r^2= 0.989
m= 0.109
b= -0.002

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.368	0.261	0.027	200	1	1	0.134	0.126	0.11
11540-6A	0.341	0.234	0.024	200	1	1	0.119		
11540-6A									
11540-6B	0.121	0.014	0.000	200	1	1	-0.002	0.005	0.012
11540-6B	0.147	0.040	0.003	200	1	1	0.013		
11540-6B									
11540-45C	0.186	0.079	0.007	200	1	800	27.1	26.3	
11540-45C	0.186	0.079	0.007	200	1	800	27.1		
11540-45C	0.180	0.073	0.006	200	1	800	24.5		
11345-18I	0.121	0.014	0.000	200	1	600	-1.0	2.8	
11345-18I	0.139	0.032	0.002	200	1	600	4.9		
11345-18I	0.138	0.031	0.002	200	1	600	4.6		

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-22				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.547	0.375	0.230	0.174	0.123	0.112	0.104	25.1	100	11540-45
	0.572	0.373	0.236	0.180	0.133	0.119	0.106			
	0.574	0.379	0.238	0.172	0.128	0.126	0.112			

Samples:	11540-6A	11540-6B	11540-45C	11345-18I
	0.368	0.121	0.186	0.121
	0.341	0.147	0.186	0.139
			0.180	0.138

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0502	0.564	0.457	0.0420
125	100	200	0.00013	200	0.0251	0.376	0.268	0.0244
50	200	1000	0.00005	200	0.0100	0.235	0.127	0.0113
25	100	1000	0.00003	200	0.0050	0.175	0.068	0.0057
10	40	1000	0.00001	200	0.0020	0.128	0.021	0.0013
5	20	1000	0.00001	200	0.0010	0.119	0.012	0.0005

Variables	Regression results	
m, b	0.093	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.992	0.001
F, df	358	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

		r^2=	0.992
Blank	0.107	m=	0.093
		b=	-0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.368	0.261	0.024	200	1	1	0.119	0.112	0.11
11540-6A	0.341	0.234	0.021	200	1	1	0.106		
11540-6A									
11540-6B	0.121	0.014	0.001	200	1	1	0.003	0.009	0.012
11540-6B	0.147	0.040	0.003	200	1	1	0.015		
11540-6B									
11540-45C	0.186	0.079	0.007	200	1	800	26.9	26.1	
11540-45C	0.186	0.079	0.007	200	1	800	26.9		
11540-45C	0.180	0.073	0.006	200	1	800	24.6		
11345-18I	0.121	0.014	0.001	200	1	600	2.0	5.2	
11345-18I	0.139	0.032	0.002	200	1	600	7.0		
11345-18I	0.138	0.031	0.002	200	1	600	6.7		

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	1

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	26.1
Protein Concentration (dilution added to assay, mg/mL):	0.032656

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	5.00E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5880	11760	11860	23720	0.1	170378	13.92	23555	0.0261	1	0.016	15	0.0574
			0.5	2	5960	11960											
	2	2	0.1	1	1184	11840	12205	24410	0.1	170378	14.33	24245	0.0269	1	0.016	15	0.0590
			0.1	2	1257	12570											
	3	2	0.5	1	5324	10648	10671	21342	0.1	170378	12.53	21177	0.0253	1	0.016	15	0.0516
			0.5	2	5347	10694											
	4	2	0.5	1	5312	10624	10830	21660	0.1	170378	12.71	21495	0.0256	1	0.016	15	0.0523
			0.5	2	5518	11036											
Background control	1	2	0.5	1	41	82	87	174	0.1	170378	0.10	9	0.0000	1	0.016	15	0.0000
			0.5	2	46	92											
	2	2	0.5	1	35	70	79	158	0.1	170378	0.09	-7	0.0000	1	0.016	15	0.0000
			0.5	2	44	88											
	3	2	0.5	1	29	58	65	130	0.1	170378	0.08	-35	0.0000	1	0.016	15	-0.0001
			0.5	2	36	72											
	4	2	0.5	1	54	108	99	198	0.1	170378	0.12	33	0.0000	1	0.016	15	0.0001
			0.5	2	45	90											
Positive control	1	2	0.5	1	2942	5884	5884	11768	0.1	170378	6.91	11603	0.0138	1	0.016	15	0.0283
			0.5	2	2942	5884											
	2	2	0.5	1	2794	5588	5642	11284	0.1	170378	6.62	11119	0.0133	1	0.016	15	0.0271
			0.5	2	2848	5696											
	3	2	0.5	1	2575	5150	5244	10488	0.1	170378	6.16	10323	0.0123	1	0.016	15	0.0251
			0.5	2	2669	5338											
	4	2	0.5	1	2652	5304	5244	10488	0.1	170378	6.16	10323	0.0123	1	0.016	15	0.0251
			0.5	2	2592	5184											
Negative Control	1	2	0.5	1	5901	11802	11728	23456	0.1	170378	13.77	23291	0.0278	1	0.016	15	0.0567
			0.5	2	5827	11654											
	2	2	0.5	1	5757	11514	11530	23060	0.1	170378	13.53	22895	0.0273	1	0.016	15	0.0558
			0.5	2	5773	11546											
	3	2	0.5	1	5200	10400	10421	20842	0.1	170378	12.23	20677	0.0247	1	0.016	15	0.0504
			0.5	2	5221	10442											
	4	2	0.5	1	5029	10058	10080	20160	0.1	170378	11.83	19995	0.0239	1	0.016	15	0.0487
			0.5	2	5051	10102											
Ref Chem 8	1-1	2	0.5	1	1300	2600	2680	5360	0.1	170378	3.15	5195	0.0062	1	0.016	15	0.0127
			0.5	2	1380	2760											
	1-2	2	0.5	1	1270	2540	2687	5374	0.1	170378	3.15	5209	0.0062	1	0.016	15	0.0127
			0.5	2	1417	2834											
	1-3	2	0.5	1	1348	2696	2754	5508	0.1	170378	3.23	5343	0.0064	1	0.016	15	0.0130
			0.5	2	1406	2812											
	2-1	2	0.5	1	1344	2688	2660	5320	0.1	170378	3.12	5155	0.0061	1	0.016	15	0.0126
			0.5	2	1316	2632											
	2-2	2	0.5	1	1347	2694	2637	5274	0.1	170378	3.10	5109	0.0061	1	0.016	15	0.0124
			0.5	2	1290	2580											
	2-3	2	0.5	1	1308	2616	2673	5346	0.1	170378	3.14	5181	0.0062	1	0.016	15	0.0126
			0.5	2	1365	2730											
	3-1	2	0.5	1	3914	7828	7992	15984	0.1	170378	9.38	15819	0.0189	1	0.016	15	0.0385
			0.5	2	4078	8156											
	3-2	2	0.5	1	4024	8048	8110	16220	0.1	170378	9.52	16055	0.0192	1	0.016	15	0.0391
			0.5	2	4086	8172											
	3-3	2	0.5	1	3933	7866	7899	15798	0.1	170378	9.27	15633	0.0186	1	0.016	15	0.0381
			0.5	2	3966	7932											
	4-1	2	0.5	1	4475	8950	8991	17982	0.1	170378	10.55	17817	0.0213	1	0.016	15	0.0434
			0.5	2	4516	9032											
	4-2	2	0.5	1	4624	9248	9246	18492	0.1	170378	10.85	18327	0.0219	1	0.016	15	0.0446
			0.5	2	4622	9244											
	4-3	2	0.5	1	4631	9262	9158	18316	0.1	170378	10.75	18151	0.0217	1	0.016	15	0.0442
			0.5	2	4527	9054											
	5-1	2	0.5	1	5231	10462	10464	20928	0.1	170378	12.28	20763	0.0248	1	0.016	15	0.0506
			0.5	2	5233	10466											
	5-2	2	0.5	1	5129	10258	10495	20990	0.1	170378	12.32	20825	0.0248	1	0.016	15	0.0507
			0.5	2	5366	10732											
	5-3	2	0.5	1	5329	10658	10597	21194	0.1	170378	12.44	21029	0.0251	1	0.016	15	0.0512
			0.5	2	5268	10536											
	6-1	2	0.5	1	5260	10520	10597	21194	0.1	170378	12.44	21029	0.0251	1	0.016	15	0.0512
			0.5	2	5337	10674											
	6-2	2	0.5	1	5291	10582	10811	21622	0.1	170378	12.69	21457	0.0256	1	0.016	15	0.0523
			0.5	2	5520	11040											
	6-3	2	0.5	1	5470	10940	10981	21962	0.1	170378	12.89	21797	0.0260	1	0.016	15	0.0531
			0.5	2	5511	11022											
	7-1	2	0.5	1	5368	10736	10793	21586	0.1	170378	12.67	21421	0.0256	1	0.016	15	0.0522
			0.5	2	5425	10850											
	7-2	2	0.5	1	5453	10906	10913	21826	0.1	170378	12.81	21661	0.0258	1	0.016	15	0.0528
			0.5	2	5460	10920											
	7-3	2	0.5	1	5455	10910	10910	21820	0.1	170378	12.81	21655	0.0258	1	0.016	15	0.0527
			0.5	2	5455	10910											
	8-1	2	0.5	1	5170	10340	10386	20772	0.1	170378	12.19	20607	0.0246	1	0.016	15	0.0502
			0.5	2	5216	10432											
	8-2	2	0.5	1	5332	10664	10651	21302	0.1	170378	12.50	21137	0.0252	1	0.016	15	0.0515
			0.5	2	5319	10638											
	8-3	2	0.5	1	5378	10756	10864	21728	0.1	170378	12.75	21563	0.0257	1	0.016	15	0.0525
			0.5	2	5486	10972											

Assay Date	8/22/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested		Microsome 8 type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0582	0.0012
Full activity	End	0.0520	0.0005
Full activity	Overall	0.0551	0.0037
Background	Beginning	0.0000	2.75535E-05
Background	End	0.0000	0.000117102
Background	Overall	0.0000	6.95122E-05
Positive	Beginning	0.0277	0.0008
Positive	End	0.0251	0.0000
Positive	Overall	0.0264	0.0015
Negative	Beginning	0.0562	0.0007
Negative	End	0.0495	0.0012
Negative	Overall	0.0529	0.0040

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 8	1	1	1.00E-04	-4.00	0.0127
Ref Chem 8	1	2	1.00E-04	-4.00	0.0127
Ref Chem 8	1	3	1.00E-04	-4.00	0.0130
Ref Chem 8	2	1	1.00E-05	-5.00	0.0126
Ref Chem 8	2	2	1.00E-05	-5.00	0.0124
Ref Chem 8	2	3	1.00E-05	-5.00	0.0126
Ref Chem 8	3	1	1.00E-06	-6.00	0.0385
Ref Chem 8	3	2	1.00E-06	-6.00	0.0391
Ref Chem 8	3	3	1.00E-06	-6.00	0.0381
Ref Chem 8	4	1	5.00E-07	-6.30	0.0434
Ref Chem 8	4	2	5.00E-07	-6.30	0.0446
Ref Chem 8	4	3	5.00E-07	-6.30	0.0442
Ref Chem 8	5	1	1.00E-07	-7.00	0.0506
Ref Chem 8	5	2	1.00E-07	-7.00	0.0507
Ref Chem 8	5	3	1.00E-07	-7.00	0.0512
Ref Chem 8	6	1	1.00E-08	-8.00	0.0512
Ref Chem 8	6	2	1.00E-08	-8.00	0.0523
Ref Chem 8	6	3	1.00E-08	-8.00	0.0531
Ref Chem 8	7	1	1.00E-09	-9.00	0.0522
Ref Chem 8	7	2	1.00E-09	-9.00	0.0528
Ref Chem 8	7	3	1.00E-09	-9.00	0.0527
Ref Chem 8	8	1	1.00E-10	-10.00	0.0502
Ref Chem 8	8	2	1.00E-10	-10.00	0.0515
Ref Chem 8	8	3	1.00E-10	-10.00	0.0525

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	22.97	23.03	23.62
2	-5.00	22.79	22.59	22.91
3	-6.00	69.94	70.98	69.12
4	-6.30	78.77	81.03	80.25
5	-7.00	91.80	92.07	92.97
6	-8.00	92.97	94.87	96.37
7	-9.00	94.71	95.77	95.74
8	-10.00	91.11	93.45	95.34

Aromatase Assay Spreadsheet

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8		
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	36534	1835879	
2	0.0196	34683	1769541	
3	0.0196	36022	1837857	
4	0.0197	35995	1827157	
5	0.0196	36641	1869439	
			Average DPM/g soln	1827975
			SD	36382
			CV	1.99
			µCi/g soln	0.823

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.3	10		1030.00
Dilution A			100	10.30
Dilution B			10	1.03

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0964 g
Mass of dilution B used in substrate prep	4.5643 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.580657 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00932 µg/g soln.
µg/g soln.

a. µCi/g soln 0.823
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.580657 + 0.00932
= 0.589978 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.396 µCi/µg ASDN

887376 dpm/nmol

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested		8					
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.1	100	11540-47
	0.559	0.399	0.239	0.176	0.130	0.127	0.108			
	0.567	0.397	0.236	0.186	0.140	0.122	0.112			
	0.552	0.386	0.241	0.175	0.146	0.128	0.124			

Samples:	11540-6A	11540-6B	11540-47C	11345-19I
	0.364	0.149	0.190	0.141
	0.362	0.143	0.189	0.144
			0.193	0.143

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0502	0.559	0.445	0.0473
125	100	200	0.00013	200	0.0251	0.394	0.279	0.0290
50	200	1000	0.00005	200	0.0100	0.239	0.124	0.0118
25	100	1000	0.00003	200	0.0050	0.179	0.064	0.0052
10	40	1000	0.00001	200	0.0020	0.139	0.024	0.0007
5	20	1000	0.00001	200	0.0010	0.126	0.011	-0.0007

Variables	Regression results			
m, b	0.111	-0.002		
se_m, se_b	0.007	0.002		
r^2, se_y	0.983	0.003		
F, df	230	4		
ss_{reg}, ss_{resid}	0.002	0.000		

Regression results are calculated using the function LINEST

Blank	0.115	r^2=	0.983	
		m=	0.111	
		b=	-0.002	

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.364	0.249	0.026	200	1	1	0.128	0.128	0.11
11540-6A	0.362	0.247	0.025	200	1	1	0.127		
11540-6A									
11540-6B	0.149	0.034	0.002	200	1	1	0.009	0.008	0.012
11540-6B	0.143	0.028	0.001	200	1	1	0.006		
11540-6B									
11540-47C	0.190	0.075	0.006	200	1	800	25.7	26.0	
11540-47C	0.189	0.074	0.006	200	1	800	25.2		
11540-47C	0.193	0.078	0.007	200	1	800	27.0		
11345-19I	0.141	0.026	0.001	200	1	600	3.0	3.5	
11345-19I	0.144	0.029	0.001	200	1	600	4.0		
11345-19I	0.143	0.028	0.001	200	1	600	3.6		

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.1	100	11540-47
	0.559	0.399	0.239	0.176	0.130	0.127	0.108			
	0.567	0.397	0.236	0.186	0.140	0.122	0.112			
	0.552	0.386	0.241	0.175	0.146	0.128	0.124			

Samples:	11540-6A	11540-6B	11540-47C	11345-19I
	0.364	0.149	0.190	0.141
	0.362	0.143	0.189	0.144
			0.193	0.143

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0502	0.559	0.445	0.0396	m, b	0.090	0.000
125	100	200	0.00013	200	0.0251	0.394	0.279	0.0247	se_m, se_b	0.003	0.000
50	200	1000	0.00005	200	0.0100	0.239	0.124	0.0107	r^2, se_y	0.997	0.001
25	100	1000	0.00003	200	0.0050	0.179	0.064	0.0054	F, df	1143	3
10	40	1000	0.00001	200	0.0020	0.139	0.024	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.126	0.011	0.0006			
			Blank	0.115	r^2=	0.997					
					m=	0.090					
					b=	0.000					

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.364	0.249	0.022	200	1	1	0.110	0.110	0.11
11540-6A	0.362	0.247	0.022	200	1	1	0.109		
11540-6A									
11540-6B	0.149	0.034	0.003	200	1	1	0.013	0.012	0.012
11540-6B	0.143	0.028	0.002	200	1	1	0.011		
11540-6B									
11540-47C	0.190	0.075	0.006	200	1	800	25.5	25.7	
11540-47C	0.189	0.074	0.006	200	1	800	25.1		
11540-47C	0.193	0.078	0.007	200	1	800	26.5		
11345-19I	0.141	0.026	0.002	200	1	600	5.8	6.3	
11345-19I	0.144	0.029	0.002	200	1	600	6.7		
11345-19I	0.143	0.028	0.002	200	1	600	6.4		

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	2

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	25.7
Protein Concentration (dilution added to assay, mg/mL):	0.03212

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	2.50E-06
5	1.00E-06
6	5.00E-07
7	1.00E-07
8	1.00E-08

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5412	10824	10977	21954	0.1	182797	12.01	21802	0.0246	1	0.016	15	0.0510
			0.5	2	5565	11130											
	2	2	0.5	1	5834	11668	11686	23372	0.1	182797	12.79	23220	0.0262	1	0.016	15	0.0543
			0.5	2	5852	11704											
	3	2	0.5	1	4686	9372	9542	19084	0.1	182797	10.44	18932	0.0213	1	0.016	15	0.0443
			0.5	2	4856	9712											
	4	2	0.5	1	4815	9630	9857	19714	0.1	182797	10.78	19562	0.0220	1	0.016	15	0.0458
			0.5	2	5042	10084											
Background control	1	2	0.5	1	49	98	88	176	0.1	182797	0.10	24	0.0000	1	0.016	15	0.0001
			0.5	2	39	78											
	2	2	0.5	1	21	42	47	94	0.1	182797	0.05	-58	-0.0001	1	0.016	15	-0.0001
			0.5	2	26	52											
	3	2	0.5	1	39	78	73	146	0.1	182797	0.08	-6	0.0000	1	0.016	15	0.0000
			0.5	2	34	68											
	4	2	0.5	1	46	92	96	192	0.1	182797	0.11	40	0.0000	1	0.016	15	0.0001
			0.5	2	50	100											
Positive control	1	2	0.5	1	3103	6206	6199	12398	0.1	182797	6.78	12246	0.0138	1	0.016	15	0.0286
			0.5	2	3096	6192											
	2	2	0.5	1	2955	5910	5856	11712	0.1	182797	6.41	11560	0.0130	1	0.016	15	0.0270
			0.5	2	2901	5802											
	3	2	0.5	1	2275	4550	4685	9370	0.1	182797	5.13	9218	0.0104	1	0.016	15	0.0216
			0.5	2	2410	4820											
	4	2	0.5	1	2540	5080	5088	10176	0.1	182797	5.57	10024	0.0113	1	0.016	15	0.0234
			0.5	2	2548	5096											
Negative Control	1	2	0.5	1	5449	10898	10987	21974	0.1	182797	12.02	21822	0.0246	1	0.016	15	0.0510
			0.5	2	5538	11076											
	2	2	0.5	1	5413	10826	10858	21716	0.1	182797	11.88	21564	0.0243	1	0.016	15	0.0504
			0.5	2	5445	10890											
	3	2	0.5	1	4250	8500	8613	17226	0.1	182797	9.42	17074	0.0192	1	0.016	15	0.0399
			0.5	2	4363	8726											
	4	2	0.5	1	4853	9706	9690	19380	0.1	182797	10.60	19228	0.0217	1	0.016	15	0.0450
			0.5	2	4837	9674											
Ref Chem 8	1-1	2	0.5	1	1154	2308	2385	4770	0.1	182797	2.61	4618	0.0052	1	0.016	15	0.0108
			0.5	2	1231	2462											
	1-2	2	0.5	1	1247	2494	2554	5108	0.1	182797	2.79	4956	0.0056	1	0.016	15	0.0116
			0.5	2	1307	2614											
	1-3	2	0.5	1	1151	2302	2320	4640	0.1	182797	2.54	4488	0.0051	1	0.016	15	0.0105
			0.5	2	1169	2338											
	2-1	2	0.5	1	1259	2518	2534	5068	0.1	182797	2.77	4916	0.0055	1	0.016	15	0.0115
			0.5	2	1275	2550											
	2-2	2	0.5	1	1185	2370	2422	4844	0.1	182797	2.65	4692	0.0053	1	0.016	15	0.0110
			0.5	2	1237	2474											
	2-3	2	0.5	1	1218	2436	2425	4850	0.1	182797	2.65	4698	0.0053	1	0.016	15	0.0110
			0.5	2	1207	2414											
	3-1	2	0.5	1	1987	3974	3922	7844	0.1	182797	4.29	7692	0.0087	1	0.016	15	0.0180
			0.5	2	1935	3870											
	3-2	2	0.5	1	1876	3752	3688	7376	0.1	182797	4.04	7224	0.0081	1	0.016	15	0.0169
			0.5	2	1812	3624											
	3-3	2	0.5	1	1802	3604	3650	7300	0.1	182797	3.99	7148	0.0081	1	0.016	15	0.0167
			0.5	2	1848	3696											
	4-1	2	0.5	1	2703	5406	5448	10896	0.1	182797	5.96	10744	0.0121	1	0.016	15	0.0251
			0.5	2	2745	5490											
	4-2	2	0.5	1	2575	5150	5337	10674	0.1	182797	5.84	10522	0.0119	1	0.016	15	0.0246
			0.5	2	2762	5524											
	4-3	2	0.5	1	2586	5172	5102	10204	0.1	182797	5.58	10052	0.0113	1	0.016	15	0.0235
			0.5	2	2516	5032											
	5-1	2	0.5	1	3845	7690	7633	15266	0.1	182797	8.35	15114	0.0170	1	0.016	15	0.0354
			0.5	2	3788	7576											
	5-2	2	0.5	1	3619	7238	7434	14868	0.1	182797	8.13	14716	0.0166	1	0.016	15	0.0344
			0.5	2	3815	7630											
	5-3	2	0.5	1	3530	7060	7053	14106	0.1	182797	7.72	13954	0.0157	1	0.016	15	0.0326
			0.5	2	3523	7046											
	6-1	2	0.5	1	4204	8408	8479	16956	0.1	182797	9.28	16806	0.0189	1	0.016	15	0.0393
			0.5	2	4275	8550											
	6-2	2	0.5	1	4203	8406	8408	16816	0.1	182797	9.20	16664	0.0186	1	0.016	15	0.0390
			0.5	2	4205	8410											
	6-3	2	0.5	1	4143	8286	8384	16768	0.1	182797	9.17	16616	0.0187	1	0.016	15	0.0389
			0.5	2	4241	8482											
	7-1	2	0.5	1	4979	9958	10016	20032	0.1	182797	10.96	19880	0.0224	1	0.016	15	0.0465
			0.5	2	5037	10074											
	7-2	2	0.5	1	4603	9206	9349	18698	0.1	182797	10.23	18546	0.0209	1	0.016	15	0.0434
			0.5	2	4746	9492											
	7-3	2	0.5	1	4366	8732	8745	17490	0.1	182797	9.57	17338	0.0195	1	0.016	15	0.0406
			0.5	2	4379	8758											
	8-1	2	0.5	1	4723	9446	9548	19096	0.1	182797	10.45	18944	0.0213	1	0.016	15	0.0443
			0.5	2	4825	9650											
	8-2	2	0.5	1	4767	9534	9560	19120	0.1	182797	10.46	18968	0.0214	1	0.016	15	0.0444
			0.5	2	4793	9586											
	8-3	2	0.5	1	4753	9506	9441	18882	0.1	182797	10.33	18730	0.0211	1	0.016	15	0.0438
			0.5	2	4688	9376											

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	Microsome 8 type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0527	0.0023
Full activity	End	0.0450	0.0010
Full activity	Overall	0.0488	0.0047
Background	Beginning	0.0000	0.000135622
Background	End	0.0000	7.60806E-05
Background	Overall	0.0000	0.00010084
Positive	Beginning	0.0278	0.0011
Positive	End	0.0225	0.0013
Positive	Overall	0.0252	0.0032
Negative	Beginning	0.0507	0.0004
Negative	End	0.0425	0.0036
Negative	Overall	0.0466	0.0052

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 8	1	1	1.00E-04	-4.00	0.0108
Ref Chem 8	1	2	1.00E-04	-4.00	0.0116
Ref Chem 8	1	3	1.00E-04	-4.00	0.0105
Ref Chem 8	2	1	1.00E-05	-5.00	0.0115
Ref Chem 8	2	2	1.00E-05	-5.00	0.0110
Ref Chem 8	2	3	1.00E-05	-5.00	0.0110
Ref Chem 8	3	1	5.00E-06	-5.30	0.0180
Ref Chem 8	3	2	5.00E-06	-5.30	0.0169
Ref Chem 8	3	3	5.00E-06	-5.30	0.0167
Ref Chem 8	4	1	2.50E-06	-5.60	0.0251
Ref Chem 8	4	2	2.50E-06	-5.60	0.0246
Ref Chem 8	4	3	2.50E-06	-5.60	0.0235
Ref Chem 8	5	1	1.00E-06	-6.00	0.0354
Ref Chem 8	5	2	1.00E-06	-6.00	0.0344
Ref Chem 8	5	3	1.00E-06	-6.00	0.0326
Ref Chem 8	6	1	5.00E-07	-6.30	0.0393
Ref Chem 8	6	2	5.00E-07	-6.30	0.0390
Ref Chem 8	6	3	5.00E-07	-6.30	0.0389
Ref Chem 8	7	1	1.00E-07	-7.00	0.0465
Ref Chem 8	7	2	1.00E-07	-7.00	0.0434
Ref Chem 8	7	3	1.00E-07	-7.00	0.0406
Ref Chem 8	8	1	1.00E-08	-8.00	0.0443
Ref Chem 8	8	2	1.00E-08	-8.00	0.0444
Ref Chem 8	8	3	1.00E-08	-8.00	0.0438

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	22.12	23.74	21.50
2	-5.00	23.55	22.47	22.50
3	-5.30	36.84	34.60	34.24
4	-5.60	51.46	50.40	48.14
5	-6.00	72.39	70.48	66.83
6	-6.30	80.49	79.81	79.58
7	-7.00	95.22	88.83	83.04
8	-8.00	90.73	90.85	89.71

Aromatase Assay Spreadsheet

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	36222	1829394	
2	0.0196	33945	1731888	
3	0.0194	34546	1780722	
4	0.0194	35404	1824948	
5	0.0196	36543	1864439	
			Average DPM/g soln	1806278
			SD	51119
			CV	2.83
			µCi/g soln	0.814

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0578 g
Mass of dilution B used in substrate prep	4.5227 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.561282 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00921 µg/g soln.
µg/g soln.

a. µCi/g soln 0.814
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.561282 + 0.00921
= 0.570493 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.426 µCi/µg ASDN

906792 dpm/nmol

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8
Technician ID	1	Replicate #	3	Microsome type	Placental

Microsome ID 11540-22

	250	125	50	25	10	5	0
Standards:	0.505	0.339	0.222	0.172	0.133	0.133	0 108
	0.503	0.347	0.243	0.180	0.147	0.130	0.112
	0.505	0.328	0.239	0.182	0.145	0.122	0.120

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25.2	100	11540-48

	11540-6A	11540-6B	11540-48C	11345-20I
Samples:	0.358	0.138	0.187	0.141
	0.338	0.147	0.185	0.139
			0.186	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0504	0.504	0.391	0.0487
125	100	200	0.00013	200	0.0252	0.338	0.225	0.0267
50	200	1000	0.00005	200	0.0101	0.235	0.121	0.0130
25	100	1000	0.00003	200	0 0050	0.178	0.065	0.0056
10	40	1000	0.00001	200	0.0020	0.142	0.028	0.0008
5	20	1000	0.00001	200	0.0010	0.128	0.015	-0.0010
			Blank	0.113				

Variables	Regression results	
m, b	0.132	-0.003
se_m, se_b	0.007	0.001
r^2, se_y	0.989	0.002
F, df	366	4
ss_{regr}, ss_{resid}	0.002	0.000

r^2= 0.989
m= 0.132
b= -0.003

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.358	0.245	0.029	200	1	1	0.147	0.140	0.11
11540-6A	0.338	0.225	0.027	200	1	1	0.134		
11540-6A									
11540-6B	0.138	0.025	0.000	200	1	1	0.001	0.004	0.012
11540-6B	0.147	0.034	0.001	200	1	1	0.007		
11540-6B									
11540-48C	0.187	0.074	0.007	200	1	800	27.0	26.4	
11540-48C	0.185	0.072	0.006	200	1	800	25.9		
11540-48C	0.186	0.073	0.007	200	1	800	26.4		
11345-20I	0.141	0.028	0.001	200	1	600	2.0	0.9	
11345-20I	0.139	0.026	0.000	200	1	600	1.2		
11345-20I	0.135	0.022	0.000	200	1	600	-0.4		

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8						
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-22				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.2	100	11540-48
	0.505	0.339	0.222	0.172	0.133	0.133	0.108			
	0.503	0.347	0.243	0.180	0.147	0.130	0.112			
	0.505	0.328	0.239	0.182	0.145	0.122	0.120			

Samples:	11540-6A	11540-6B	11540-48C	11345-20I
	0.358	0.138	0.187	0.141
	0.338	0.147	0.185	0.139
			0.186	0.135

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0504	0.504	0.391	0.0431	m, b	0.115	-0.002
125	100	200	0.00013	200	0.0252	0.338	0.225	0.0240	se_m, se_b	0.009	0.001
50	200	1000	0.00005	200	0.0101	0.235	0.121	0.0122	r^2, se_y	0.981	0.002
25	100	1000	0.00003	200	0.0050	0.178	0.065	0.0057	F, df	154	3
10	40	1000	0.00001	200	0.0020	0.142	0.028	0.0015	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.128	0.015	0.0000			
			Blank	0.113	r^2=	0.981					
					m=	0.115					
					b=	-0.002					

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.358	0.245	0.026	200	1	1	0.132	0.126	0.11
11540-6A	0.338	0.225	0.024	200	1	1	0.120		
11540-6A									
11540-6B	0.138	0.025	0.001	200	1	1	0.005	0.008	0.012
11540-6B	0.147	0.034	0.002	200	1	1	0.011		
11540-6B									
11540-48C	0.187	0.074	0.007	200	1	800	26.8	26.4	
11540-48C	0.185	0.072	0.006	200	1	800	25.9		
11540-48C	0.186	0.073	0.007	200	1	800	26.4		
11345-20I	0.141	0.028	0.001	200	1	600	4.3	3.4	
11345-20I	0.139	0.026	0.001	200	1	600	3.6		
11345-20I	0.135	0.022	0.001	200	1	600	2.2		

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	3

Microsome Dilution Details		
Dilution A	0.125	mL microsome Stock used
	10	mL total volume
	80	dilution factor
Dilution B	8	mL microsome Dilution A used
	80	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	26.4
Protein Concentration (dilution added to assay, mg/mL):	0.032945

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	2.50E-06
5	1.00E-06
6	5.00E-07
7	1.00E-07
8	1.00E-08

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	1	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5771	11542	11672	23344	0.1	180628	12.92	23222	0.0256	1	0.016	15	0.0518
			0.5	2	5901	11802											
	2	2	0.5	1	5762	11524	11474	22948	0.1	180628	12.70	22826	0.0252	1	0.016	15	0.0509
			0.5	2	5712	11424											
	3	2	0.5	1	5239	10478	10398	20796	0.1	180628	11.51	20674	0.0228	1	0.016	15	0.0461
			0.5	2	5159	10318											
	4	2	0.5	1	5020	10040	10279	20558	0.1	180628	11.38	20436	0.0225	1	0.016	15	0.0456
			0.5	2	5259	10518											
Background control	1	2	0.5	1	22	44	58	116	0.1	180628	0.06	-6	0.0000	1	0.016	15	0.0000
			0.5	2	36	72											
	2	2	0.5	1	30	60	70	140	0.1	180628	0.08	18	0.0000	1	0.016	15	0.0000
			0.5	2	40	80											
	3	2	0.5	1	34	68	58	116	0.1	180628	0.06	-6	0.0000	1	0.016	15	0.0000
			0.5	2	24	48											
	4	2	0.5	1	31	62	58	116	0.1	180628	0.06	-6	0.0000	1	0.016	15	0.0000
			0.5	2	27	54											
Positive control	1	2	0.5	1	2853	5706	5717	11434	0.1	180628	6.33	11312	0.0125	1	0.016	15	0.0252
			0.5	2	2864	5728											
	2	2	0.5	1	2640	5280	5387	10774	0.1	180628	5.96	10652	0.0117	1	0.016	15	0.0238
			0.5	2	2747	5494											
	3	2	0.5	1	2528	5056	4994	9988	0.1	180628	5.53	9866	0.0109	1	0.016	15	0.0220
			0.5	2	2466	4932											
	4	2	0.5	1	2499	4998	5061	10122	0.1	180628	5.60	10000	0.0110	1	0.016	15	0.0223
			0.5	2	2562	5124											
Negative Control	1	2	0.5	1	5475	10950	10993	21986	0.1	180628	12.17	21864	0.0241	1	0.016	15	0.0488
			0.5	2	5518	11036											
	2	2	0.5	1	5439	10878	10943	21886	0.1	180628	12.12	21764	0.0240	1	0.016	15	0.0486
			0.5	2	5504	11008											
	3	2	0.5	1	4916	9832	9938	19876	0.1	180628	11.00	19754	0.0218	1	0.016	15	0.0441
			0.5	2	5022	10044											
	4	2	0.5	1	4780	9560	9520	19040	0.1	180628	10.54	18918	0.0209	1	0.016	15	0.0422
			0.5	2	4740	9480											
Ref Chem 8	1-1	2	0.5	1	1301	2602	2589	5178	0.1	180628	2.87	5056	0.0056	1	0.016	15	0.0113
			0.5	2	1288	2576											
	1-2	2	0.5	1	1233	2466	2428	4856	0.1	180628	2.69	4734	0.0052	1	0.016	15	0.0106
			0.5	2	1195	2390											
	1-3	2	0.5	1	1225	2450	2437	4874	0.1	180628	2.70	4752	0.0052	1	0.016	15	0.0106
			0.5	2	1212	2424											
	2-1	2	0.5	1	1269	2538	2536	5072	0.1	180628	2.81	4950	0.0055	1	0.016	15	0.0110
			0.5	2	1267	2534											
	2-2	2	0.5	1	1288	2576	2583	5166	0.1	180628	2.86	5044	0.0056	1	0.016	15	0.0113
			0.5	2	1295	2590											
	2-3	2	0.5	1	1257	2514	2505	5010	0.1	180628	2.77	4888	0.0054	1	0.016	15	0.0109
			0.5	2	1248	2496											
	3-1	2	0.5	1	2018	4036	4092	8184	0.1	180628	4.53	8062	0.0089	1	0.016	15	0.0180
			0.5	2	2074	4148											
	3-2	2	0.5	1	1997	3994	3945	7890	0.1	180628	4.37	7768	0.0086	1	0.016	15	0.0173
			0.5	2	1948	3896											
	3-3	2	0.5	1	1831	3662	3808	7616	0.1	180628	4.22	7494	0.0083	1	0.016	15	0.0167
			0.5	2	1977	3954											
	4-1	2	0.5	1	2828	5656	5600	11200	0.1	180628	6.20	11078	0.0122	1	0.016	15	0.0247
			0.5	2	2772	5544											
	4-2	2	0.5	1	2858	5716	5765	11530	0.1	180628	6.38	11408	0.0126	1	0.016	15	0.0255
			0.5	2	2907	5814											
	4-3	2	0.5	1	2727	5454	5477	10954	0.1	180628	6.06	10832	0.0119	1	0.016	15	0.0242
			0.5	2	2750	5500											
	5-1	2	0.5	1	3876	7752	7820	15640	0.1	180628	8.66	15518	0.0171	1	0.016	15	0.0346
			0.5	2	3944	7888											
	5-2	2	0.5	1	3963	7966	7910	15820	0.1	180628	8.76	15698	0.0173	1	0.016	15	0.0350
			0.5	2	3927	7854											
	5-3	2	0.5	1	3824	7648	7699	15398	0.1	180628	8.52	15276	0.0168	1	0.016	15	0.0341
			0.5	2	3875	7750											
	6-1	2	0.5	1	4448	8896	8956	17912	0.1	180628	9.92	17790	0.0196	1	0.016	15	0.0397
			0.5	2	4508	9016											
	6-2	2	0.5	1	4625	9250	9138	18276	0.1	180628	10.12	18154	0.0200	1	0.016	15	0.0405
			0.5	2	4513	9026											
	6-3	2	0.5	1	4323	8646	8932	17864	0.1	180628	9.89	17742	0.0196	1	0.016	15	0.0396
			0.5	2	4609	9218											
	7-1	2	0.5	1	4977	9954	10052	20104	0.1	180628	11.13	19982	0.0220	1	0.016	15	0.0446
			0.5	2	5075	10150											
	7-2	2	0.5	1	5197	10394	10436	20872	0.1	180628	11.56	20750	0.0229	1	0.016	15	0.0463
			0.5	2	5239	10478											
	7-3	2	0.5	1	5166	10332	10360	20720	0.1	180628	11.47	20598	0.0227	1	0.016	15	0.0460
			0.5	2	5194	10388											
	8-1	2	0.5	1	5597	11194	11368	22736	0.1	180628	12.59	22614	0.0249	1	0.016	15	0.0505
			0.5	2	5771	11542											
	8-2	2	0.5	1	5135	10270	10323	20646	0.1	180628	11.43	20524	0.0226	1	0.016	15	0.0458
			0.5	2	5188	10376											
	8-3	2	0.5	1	5105	10210	10275	20550	0.1	180628	11.38	20428	0.0225	1	0.016	15	0.0456
			0.5	2	5170	10340											

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-22	Technician ID	Replicate # 1	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0514	0.0006
Full activity	End	0.0459	0.0004
Full activity	Overall	0.0486	0.0032
Background	Beginning	0.0000	3.78711E-05
Background	End	0.0000	0
Background	Overall	0.0000	2.67789E-05
Positive	Beginning	0.0245	0.0010
Positive	End	0.0222	0.0002
Positive	Overall	0.0233	0.0015
Negative	Beginning	0.0487	0.0002
Negative	End	0.0431	0.0013
Negative	Overall	0.0459	0.0033

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 8	1	1	1.00E-04	-4.00	0.0113
Ref Chem 8	1	2	1.00E-04	-4.00	0.0106
Ref Chem 8	1	3	1.00E-04	-4.00	0.0106
Ref Chem 8	2	1	1.00E-05	-5.00	0.0110
Ref Chem 8	2	2	1.00E-05	-5.00	0.0113
Ref Chem 8	2	3	1.00E-05	-5.00	0.0109
Ref Chem 8	3	1	5.00E-06	-5.30	0.0180
Ref Chem 8	3	2	5.00E-06	-5.30	0.0173
Ref Chem 8	3	3	5.00E-06	-5.30	0.0167
Ref Chem 8	4	1	2.50E-06	-5.60	0.0247
Ref Chem 8	4	2	2.50E-06	-5.60	0.0255
Ref Chem 8	4	3	2.50E-06	-5.60	0.0242
Ref Chem 8	5	1	1.00E-06	-6.00	0.0346
Ref Chem 8	5	2	1.00E-06	-6.00	0.0350
Ref Chem 8	5	3	1.00E-06	-6.00	0.0341
Ref Chem 8	6	1	5.00E-07	-6.30	0.0397
Ref Chem 8	6	2	5.00E-07	-6.30	0.0405
Ref Chem 8	6	3	5.00E-07	-6.30	0.0396
Ref Chem 8	7	1	1.00E-07	-7.00	0.0446
Ref Chem 8	7	2	1.00E-07	-7.00	0.0463
Ref Chem 8	7	3	1.00E-07	-7.00	0.0460
Ref Chem 8	8	1	1.00E-08	-8.00	0.0505
Ref Chem 8	8	2	1.00E-08	-8.00	0.0458
Ref Chem 8	8	3	1.00E-08	-8.00	0.0456

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	23.20	21.73	21.81
2	-5.00	22.72	23.15	22.43
3	-5.30	37.00	35.65	34.39
4	-5.60	50.84	52.36	49.71
5	-6.00	71.22	72.04	70.11
6	-6.30	81.64	83.32	81.42
7	-7.00	91.70	95.23	94.53
8	-8.00	103.78	94.19	93.75

Aromatase Assay Spreadsheet

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0202	34199	1693020		
2	0.0194	31900	1644330		
3	0.0194	32329	1666443		
4	0.0193	33162	1718238		
5	0.0194	33996	1752371		
			Average DPM/g soln		1694880
			SD		42480
			CV		2.51
			µCi/g soln		0.763

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0521 g
Mass of dilution B used in substrate prep	4.5126 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.560425 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00864 µg/g soln.
µg/g soln.

a. µCi/g soln 0.763
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.560425 + 0.00864
= 0.569068 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.342 µCi/µg ASDN

852998 dpm/nmol

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.584	0.393	0.254	0.193	0.142	0.138	0.121	25.8	100	11540-51
	0.571	0.388	0.245	0.186	0.145	0.127	0.108			
	0.566	0.384	0.250	0.180	0.153	0.125	0.121			

Samples:	11540-6A	11540-6B	11540-51B	11345-22H	11345-22I
	0.369	0.144	0.190	0.363	0.149
	0.371	0.145	0.192	0.366	0.145
			0.198	0.370	0.156

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0516	0.574	0.457	0.0495
125	100	200	0.00013	200	0.0258	0.388	0.272	0.0284
50	200	1000	0.00005	200	0.0103	0.250	0.133	0.0127
25	100	1000	0.00003	200	0.0052	0.186	0.070	0.0055
10	40	1000	0.00001	200	0.0021	0.147	0.030	0.0009
5	20	1000	0.00001	200	0.0010	0.130	0.013	-0.0009

Variables	Regression results	
m, b	0.114	-0.002
se_m, se_b	0.006	0.001
r^2, se_y	0.989	0.002
F, df	347	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

		r^2=	0.989
Blank	0.117	m=	0.114
		b=	-0.002

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.369	0.252	0.026	200	1	1	0.131	0.132	0.11
11540-6A	0.371	0.254	0.026	200	1	1	0.132		
11540-6A									
11540-6B	0.144	0.027	0.001	200	1	1	0.003	0.004	0.012
11540-6B	0.145	0.028	0.001	200	1	1	0.004		
11540-6B									
11540-51B	0.190	0.073	0.006	200	1	320	9.4	10.0	
11540-51B	0.192	0.075	0.006	200	1	320	9.8		
11540-51B	0.198	0.081	0.007	200	1	320	10.9		
11345-22H	0.363	0.246	0.026	200	1	50	6.4	6.5	
11345-22H	0.366	0.249	0.026	200	1	50	6.5		
11345-22H	0.370	0.253	0.026	200	1	50	6.6		
11345-22I	0.149	0.032	0.001	200	1	600	3.6	4.0	
11345-22I	0.145	0.028	0.001	200	1	600	2.3		
11345-22I	0.156	0.039	0.002	200	1	600	6.0		

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8						
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.8	100	11540-51
	0.584	0.393	0.254	0.193	0.142	0.138	0.121			
	0.571	0.388	0.245	0.186	0.145	0.127	0.108			
	0.566	0.384	0.250	0.180	0.153	0.125	0.121			

Samples:	11540-6A	11540-6B	11540-51B	11345-22H	11345-22I
	0.369	0.144	0.190	0.363	0.149
	0.371	0.145	0.192	0.366	0.145
			0.198	0.370	0.156

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0516	0.574	0.457	0.0429
125	100	200	0.00013	200	0.0258	0.388	0.272	0.0251
50	200	1000	0.00005	200	0.0103	0.250	0.133	0.0117
25	100	1000	0.00003	200	0.0052	0.186	0.070	0.0056
10	40	1000	0.00001	200	0.0021	0.147	0.030	0.0018
5	20	1000	0.00001	200	0.0010	0.130	0.013	0.0002

Blank	0.117	r^2=	0.992		
		m=	0.096		
		b=	-0.001		

Variables	Regression results	
m, b	0.096	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.992	0.001
F, df	352	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.369	0.252	0.023	200	1	1	0.116	0.117	0.11
11540-6A	0.371	0.254	0.023	200	1	1	0.117		
11540-6A									
11540-6B	0.144	0.027	0.002	200	1	1	0.008	0.008	0.012
11540-6B	0.145	0.028	0.002	200	1	1	0.008		
11540-6B									
11540-51B	0.190	0.073	0.006	200	1	320	9.5	10.1	
11540-51B	0.192	0.075	0.006	200	1	320	9.9		
11540-51B	0.198	0.081	0.007	200	1	320	10.8		
11345-22H	0.363	0.246	0.023	200	1	50	5.7	5.7	
11345-22H	0.366	0.249	0.023	200	1	50	5.7		
11345-22H	0.370	0.253	0.023	200	1	50	5.8		
11345-22I	0.149	0.032	0.002	200	1	600	6.0	6.3	
11345-22I	0.145	0.028	0.002	200	1	600	4.9		
11345-22I	0.156	0.039	0.003	200	1	600	8.1		

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	1

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	10.1
Protein Concentration (dilution added to assay, mg/mL):	0.031434

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4016	8032	8112	16224	0.1	169488	9.57	16049	0.0188	1	0.016	15	0.0399
			0.5	2	4096	8192											
	2	2	0.5	1	3970	7940	8128	16256	0.1	169488	9.59	16081	0.0189	1	0.016	15	0.0400
			0.5	2	4158	8316											
	3	2	0.5	1	3596	7192	7119	14238	0.1	169488	8.40	14063	0.0165	1	0.016	15	0.0350
			0.5	2	3523	7046											
	4	2	0.5	1	3601	7202	7171	14342	0.1	169488	8.46	14167	0.0166	1	0.016	15	0.0352
			0.5	2	3570	7140											
Background control	1	2	0.5	1	51	102	86	172	0.1	169488	0.10	-4	0.0000	1	0.016	15	0.0000
			0.5	2	35	70											
	2	2	0.5	1	44	88	88	176	0.1	169488	0.10	1	0.0000	1	0.016	15	0.0000
			0.5	2	44	88											
	3	2	0.5	1	46	92	86	172	0.1	169488	0.10	-4	0.0000	1	0.016	15	0.0000
			0.5	2	40	80											
	4	2	0.5	1	42	84	91	182	0.1	169488	0.11	7	0.0000	1	0.016	15	0.0000
			0.5	2	49	98											
Positive control	1	2	0.5	1	2203	4406	4424	8848	0.1	169488	5.22	8673	0.0102	1	0.016	15	0.0216
			0.5	2	2221	4442											
	2	2	0.5	1	1974	3948	3951	7902	0.1	169488	4.66	7727	0.0091	1	0.016	15	0.0192
			0.5	2	1977	3954											
	3	2	0.5	1	1848	3696	3735	7470	0.1	169488	4.41	7295	0.0086	1	0.016	15	0.0181
			0.5	2	1887	3774											
	4	2	0.5	1	1856	3712	3739	7478	0.1	169488	4.41	7303	0.0086	1	0.016	15	0.0182
			0.5	2	1883	3766											
Negative Control	1	2	0.5	1	4051	8102	8098	16196	0.1	169488	9.56	16021	0.0188	1	0.016	15	0.0398
			0.5	2	4047	8094											
	2	2	0.5	1	3926	7852	8021	16042	0.1	169488	9.46	15867	0.0186	1	0.016	15	0.0394
			0.5	2	4095	8190											
	3	2	0.5	1	3503	7006	7137	14274	0.1	169488	8.42	14099	0.0165	1	0.016	15	0.0351
			0.5	2	3634	7268											
	4	2	0.5	1	3227	6454	6467	12934	0.1	169488	7.63	12759	0.0150	1	0.016	15	0.0317
			0.5	2	3240	6480											
Ref Chem 9	1-1	2	0.5	1	154	308	310	620	0.1	169488	0.37	445	0.0005	1	0.016	15	0.0011
			0.5	2	156	312											
	1-2	2	0.5	1	198	396	411	822	0.1	169488	0.48	647	0.0008	1	0.016	15	0.0016
			0.5	2	213	426											
	1-3	2	0.5	1	219	438	443	886	0.1	169488	0.52	711	0.0008	1	0.016	15	0.0018
			0.5	2	224	448											
	2-1	2	0.5	1	1337	2674	2763	5526	0.1	169488	3.26	5351	0.0063	1	0.016	15	0.0133
			0.5	2	1426	2852											
	2-2	2	0.5	1	1695	3390	3484	6968	0.1	169488	4.11	6793	0.0080	1	0.016	15	0.0169
			0.5	2	1789	3578											
	2-3	2	0.5	1	1488	2976	3019	6038	0.1	169488	3.56	5863	0.0069	1	0.016	15	0.0146
			0.5	2	1531	3062											
	3-1	2	0.5	1	2697	5394	5422	10844	0.1	169488	6.40	10669	0.0125	1	0.016	15	0.0265
			0.5	2	2725	5450											
	3-2	2	0.5	1	2611	5222	5175	10350	0.1	169488	6.11	10175	0.0119	1	0.016	15	0.0253
			0.5	2	2564	5128											
	3-3	2	0.5	1	2545	5090	5128	10256	0.1	169488	6.05	10081	0.0118	1	0.016	15	0.0251
			0.5	2	2583	5166											
	4-1	2	0.5	1	3513	7026	6970	13940	0.1	169488	8.22	13765	0.0161	1	0.016	15	0.0342
			0.5	2	3457	6914											
	4-2	2	0.5	1	3425	6850	6976	13952	0.1	169488	8.23	13777	0.0162	1	0.016	15	0.0343
			0.5	2	3551	7102											
	4-3	2	0.5	1	3404	6808	6775	13550	0.1	169488	7.99	13375	0.0157	1	0.016	15	0.0333
			0.5	2	3371	6742											
	5-1	2	0.5	1	3842	7684	7609	15218	0.1	169488	8.98	15043	0.0176	1	0.016	15	0.0374
			0.5	2	3767	7534											
	5-2	2	0.5	1	3807	7614	7593	15186	0.1	169488	8.96	15011	0.0176	1	0.016	15	0.0373
			0.5	2	3786	7572											
	5-3	2	0.5	1	3654	7308	7245	14490	0.1	169488	8.55	14315	0.0168	1	0.016	15	0.0356
			0.5	2	3591	7182											
	6-1	2	0.5	1	4040	8080	8027	16054	0.1	169488	9.47	15879	0.0186	1	0.016	15	0.0395
			0.5	2	3987	7974											
	6-2	2	0.5	1	3834	7668	7620	15240	0.1	169488	8.99	15065	0.0177	1	0.016	15	0.0375
			0.5	2	3786	7572											
	6-3	2	0.5	1	3692	7384	7499	14998	0.1	169488	8.85	14823	0.0174	1	0.016	15	0.0369
			0.5	2	3807	7614											
	7-1	2	0.5	1	3871	7742	7817	15634	0.1	169488	9.22	15459	0.0181	1	0.016	15	0.0384
			0.5	2	3946	7892											
	7-2	2	0.5	1	3916	7832	7800	15600	0.1	169488	9.20	15425	0.0181	1	0.016	15	0.0384
			0.5	2	3884	7768											
	7-3	2	0.5	1	3763	7526	7482	14964	0.1	169488	8.83	14789	0.0173	1	0.016	15	0.0368
			0.5	2	3719	7438											
	8-1	2	0.5	1	3913	7826	7881	15762	0.1	169488	9.30	15587	0.0183	1	0.016	15	0.0388
			0.5	2	3968	7936											
	8-2	2	0.5	1	3801	7602	7684	15366	0.1	169488	9.07	15193	0.0178	1	0.016	15	0.0378
			0.5	2	3883	7766											
	8-3	2	0.5	1	3672	7344	7361	14722	0.1	169488	8.69	14547	0.0171	1	0.016	15	0.0362
			0.5	2	3689	7378											

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0399	0.0001
Full activity	End	0.0351	0.0002
Full activity	Overall	0.0375	0.0028
Background	Beginning	0.0000	7.03237E-06
Background	End	0.0000	1.75809E-05
Background	Overall	0.0000	1.17499E-05
Positive	Beginning	0.0204	0.0017
Positive	End	0.0181	0.0000
Positive	Overall	0.0193	0.0016
Negative	Beginning	0.0396	0.0003
Negative	End	0.0334	0.0024
Negative	Overall	0.0365	0.0039

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 9	1	1	1.00E-03	-3.00	0.0011
Ref Chem 9	1	2	1.00E-03	-3.00	0.0016
Ref Chem 9	1	3	1.00E-03	-3.00	0.0018
Ref Chem 9	2	1	1.00E-04	-4.00	0.0133
Ref Chem 9	2	2	1.00E-04	-4.00	0.0169
Ref Chem 9	2	3	1.00E-04	-4.00	0.0146
Ref Chem 9	3	1	1.00E-05	-5.00	0.0265
Ref Chem 9	3	2	1.00E-05	-5.00	0.0253
Ref Chem 9	3	3	1.00E-05	-5.00	0.0251
Ref Chem 9	4	1	1.00E-06	-6.00	0.0342
Ref Chem 9	4	2	1.00E-06	-6.00	0.0343
Ref Chem 9	4	3	1.00E-06	-6.00	0.0333
Ref Chem 9	5	1	1.00E-07	-7.00	0.0374
Ref Chem 9	5	2	1.00E-07	-7.00	0.0373
Ref Chem 9	5	3	1.00E-07	-7.00	0.0356
Ref Chem 9	6	1	1.00E-08	-8.00	0.0395
Ref Chem 9	6	2	1.00E-08	-8.00	0.0375
Ref Chem 9	6	3	1.00E-08	-8.00	0.0369
Ref Chem 9	7	1	1.00E-09	-9.00	0.0384
Ref Chem 9	7	2	1.00E-09	-9.00	0.0384
Ref Chem 9	7	3	1.00E-09	-9.00	0.0368
Ref Chem 9	8	1	1.00E-10	-10.00	0.0388
Ref Chem 9	8	2	1.00E-10	-10.00	0.0378
Ref Chem 9	8	3	1.00E-10	-10.00	0.0362

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.95	4.28	4.71
2	-4.00	35.46	45.01	38.85
3	-5.00	70.70	67.43	66.80
4	-6.00	91.22	91.30	88.63
5	-7.00	99.69	99.48	94.86
6	-8.00	105.23	99.83	98.23
7	-9.00	102.45	102.22	98.01
8	-10.00	103.29	100.68	96.40

Aromatase Assay Spreadsheet

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8		
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	34622	1739799	
2	0.0195	32944	1689436	
3	0.0195	34328	1760410	
4	0.0194	34591	1783041	
5	0.0192	36152	1882917	
			Average DPM/g soln	1771121
			SD	71429
			CV	4.03
			µCi/g soln	0.798

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.1	10		1010.00
Dilution A			100	10.10
Dilution B			10	1.01

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0792 g
Mass of dilution B used in substrate prep	4.5326 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.566631 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00903 µg/g soln.
µg/g soln.

a. µCi/g soln	0.798
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.566631 + 0.00903
= 0.575662 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.386 µCi/µg ASDN

881157 dpm/nmol

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.9	100	11540-53
	0.522	0.381	0.237	0.181	0.145	0.132	0.122			
	0.568	0.377	0.254	0.180	0.131	0.126	0.119			
	0.543	0.395	0.235	0.181	0.143	0.130	0.115			

Samples:	11540-6A	11540-6B	11540-53B	11345-23H	11345-23I
	0.354	0.129	0.194	0.346	0.188
	0.342	0.142	0.205	0.360	0.185
			0.190	0.354	0.189

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0518	0.544	0.426	0.0488
125	100	200	0.00013	200	0.0259	0.384	0.266	0.0297
50	200	1000	0.00005	200	0.0104	0.242	0.123	0.0127
25	100	1000	0.00003	200	0.0052	0.181	0.062	0.0054
10	40	1000	0.00001	200	0.0021	0.140	0.021	0.0005
5	20	1000	0.00001	200	0.0010	0.129	0.011	-0.0008
			Blank	0.119				

Variables	Regression results	
m, b	0.120	-0.002
se_m, se_b	0.008	0.002
r^2, se_y	0.982	0.003
F, df	221	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.982
m= 0.120
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.354	0.235	0.026	200	1	1	0.130	0.127	0.11
11540-6A	0.342	0.223	0.025	200	1	1	0.123		
11540-6A									
11540-6B	0.129	0.010	-0.001	200	1	1	-0.004	0.000	0.012
11540-6B	0.142	0.023	0.001	200	1	1	0.004		
11540-6B									
11540-53B	0.194	0.075	0.007	200	1	320	11.2	11.6	
11540-53B	0.205	0.086	0.008	200	1	320	13.3		
11540-53B	0.190	0.071	0.006	200	1	320	10.4		
11345-23H	0.346	0.227	0.025	200	1	50	6.3	6.5	
11345-23H	0.360	0.241	0.027	200	1	50	6.7		
11345-23H	0.354	0.235	0.026	200	1	50	6.5		
11345-23I	0.188	0.069	0.006	200	1	600	18.8	18.5	
11345-23I	0.185	0.066	0.006	200	1	600	17.7		
11345-23I	0.189	0.070	0.006	200	1	600	19.1		

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.522	0.381	0.237	0.181	0.145	0.132	0.122	25.9	100	11540-53
	0.568	0.377	0.254	0.180	0.131	0.126	0.119			
	0.543	0.395	0.235	0.181	0.143	0.130	0.115			

Samples:	11540-6A	11540-6B	11540-53B	11345-23H	11345-23I
	0.354	0.129	0.194	0.346	0.188
	0.342	0.142	0.205	0.360	0.185
			0.190	0.354	0.189

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0518	0.544	0.426	0.0409
125	100	200	0.00013	200	0.0259	0.384	0.266	0.0253
50	200	1000	0.00005	200	0.0104	0.242	0.123	0.0115
25	100	1000	0.00003	200	0.0052	0.181	0.062	0.0056
10	40	1000	0.00001	200	0.0021	0.140	0.021	0.0016
5	20	1000	0.00001	200	0.0010	0.129	0.011	0.0006

	Blank	0.119
r^2=		0.995
m=		0.097
b=		0.000

Variables	Regression results	
m, b	0.097	0.000
se_m, se_b	0.004	0.001
r^2, se_y	0.995	0.001
F, df	548	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.354	0.235	0.022	200	1	1	0.112	0.109	0.11
11540-6A	0.342	0.223	0.021	200	1	1	0.106		
11540-6A									
11540-6B	0.129	0.010	0.001	200	1	1	0.003	0.006	0.012
11540-6B	0.142	0.023	0.002	200	1	1	0.009		
11540-6B									
11540-53B	0.194	0.075	0.007	200	1	320	11.0	11.3	
11540-53B	0.205	0.086	0.008	200	1	320	12.7		
11540-53B	0.190	0.071	0.006	200	1	320	10.3		
11345-23H	0.346	0.227	0.022	200	1	50	5.4	5.6	
11345-23H	0.360	0.241	0.023	200	1	50	5.7		
11345-23H	0.354	0.235	0.022	200	1	50	5.6		
11345-23I	0.188	0.069	0.006	200	1	600	18.8	18.6	
11345-23I	0.185	0.066	0.006	200	1	600	17.9		
11345-23I	0.189	0.070	0.006	200	1	600	19.1		

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1	2

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	11.3
Protein Concentration (dilution added to assay, mg/mL):	0.035387

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	5.00E-04
3	2.50E-04
4	1.00E-04
5	1.00E-05
6	1.00E-06
7	1.00E-07
8	1.00E-08

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5387	10774	10795	21590	0.1	177112	12.19	21399	0.0243	1	0.018	15	0.0486
			0.5	2	5408	10816											
	2	2	0.5	1	5326	10652	10695	21390	0.1	177112	12.08	21199	0.0241	1	0.018	15	0.0483
			0.5	2	5369	10738											
	3	2	0.5	1	4566	9132	9243	18486	0.1	177112	10.44	18295	0.0208	1	0.018	15	0.0391
			0.5	2	4677	9354											
	4	2	0.5	1	4679	9358	9467	18934	0.1	177112	10.69	18743	0.0213	1	0.018	15	0.0401
			0.5	2	4788	9576											
Background control	1	2	0.5	1	36	72	78	156	0.1	177112	0.09	-36	0.0000	1	0.018	15	-0.0001
			0.5	2	42	84											
	2	2	0.5	1	66	132	131	262	0.1	177112	0.15	71	0.0001	1	0.018	15	0.0002
			0.5	2	65	130											
	3	2	0.5	1	41	82	81	162	0.1	177112	0.09	-30	0.0000	1	0.018	15	-0.0001
			0.5	2	40	80											
	4	2	0.5	1	41	82	93	186	0.1	177112	0.11	-6	0.0000	1	0.018	15	0.0000
			0.5	2	52	104											
Positive control	1	2	0.5	1	2208	4416	4510	9020	0.1	177112	5.09	8829	0.0100	1	0.018	15	0.0189
			0.5	2	2302	4604											
	2	2	0.5	1	2161	4322	4283	8566	0.1	177112	4.84	8375	0.0095	1	0.018	15	0.0179
			0.5	2	2122	4244											
	3	2	0.5	1	2042	4084	4082	8164	0.1	177112	4.61	7973	0.0090	1	0.018	15	0.0170
			0.5	2	2040	4080											
	4	2	0.5	1	2017	4034	3996	7992	0.1	177112	4.51	7801	0.0089	1	0.018	15	0.0167
			0.5	2	1979	3958											
Negative Control	1	2	0.5	1	5034	10068	10094	20188	0.1	177112	11.40	19997	0.0227	1	0.018	15	0.0426
			0.5	2	5060	10120											
	2	2	0.5	1	4846	9692	9700	19400	0.1	177112	10.95	19209	0.0218	1	0.018	15	0.0411
			0.5	2	4854	9708											
	3	2	0.5	1	4639	9278	9086	18172	0.1	177112	10.26	17981	0.0204	1	0.018	15	0.0384
			0.5	2	4447	8894											
	4	2	0.5	1	4503	9006	9028	18056	0.1	177112	10.19	17865	0.0203	1	0.018	15	0.0382
			0.5	2	4525	9050											
Ref Chem 9	1-1	2	0.5	1	138	276	283	566	0.1	177112	0.32	375	0.0004	1	0.018	15	0.0008
			0.5	2	145	290											
	1-2	2	0.5	1	132	264	294	588	0.1	177112	0.33	397	0.0004	1	0.018	15	0.0008
			0.5	2	162	324											
	1-3	2	0.5	1	163	326	299	598	0.1	177112	0.34	407	0.0005	1	0.018	15	0.0009
			0.5	2	136	272											
	2-1	2	0.5	1	244	488	485	970	0.1	177112	0.55	779	0.0009	1	0.018	15	0.0017
			0.5	2	241	482											
	2-2	2	0.5	1	295	590	577	1154	0.1	177112	0.65	963	0.0011	1	0.018	15	0.0021
			0.5	2	282	564											
	2-3	2	0.5	1	319	638	657	1314	0.1	177112	0.74	1123	0.0013	1	0.018	15	0.0024
			0.5	2	338	676											
	3-1	2	0.5	1	436	872	875	1750	0.1	177112	0.99	1559	0.0018	1	0.018	15	0.0033
			0.5	2	439	878											
	3-2	2	0.5	1	657	1314	1257	2514	0.1	177112	1.42	2323	0.0026	1	0.018	15	0.0050
			0.5	2	600	1200											
	3-3	2	0.5	1	619	1236	1221	2442	0.1	177112	1.38	2251	0.0026	1	0.018	15	0.0048
			0.5	2	602	1204											
	4-1	2	0.5	1	1025	2050	1998	3996	0.1	177112	2.26	3805	0.0043	1	0.018	15	0.0081
			0.5	2	973	1946											
	4-2	2	0.5	1	1381	2762	2673	5346	0.1	177112	3.02	5155	0.0058	1	0.018	15	0.0110
			0.5	2	1292	2584											
	4-3	2	0.5	1	1071	2142	2174	4348	0.1	177112	2.45	4157	0.0047	1	0.018	15	0.0089
			0.5	2	1103	2206											
	5-1	2	0.5	1	3669	7338	7482	14964	0.1	177112	8.45	14773	0.0168	1	0.018	15	0.0316
			0.5	2	3813	7626											
	5-2	2	0.5	1	3442	6884	6900	13800	0.1	177112	7.79	13609	0.0154	1	0.018	15	0.0291
			0.5	2	3458	6916											
	5-3	2	0.5	1	3047	6094	6124	12248	0.1	177112	6.92	12057	0.0137	1	0.018	15	0.0258
			0.5	2	3077	6154											
	6-1	2	0.5	1	4329	8658	8733	17466	0.1	177112	9.86	17276	0.0196	1	0.018	15	0.0369
			0.5	2	4404	8808											
	6-2	2	0.5	1	4431	8862	8802	17604	0.1	177112	9.94	17413	0.0198	1	0.018	15	0.0372
			0.5	2	4371	8742											
	6-3	2	0.5	1	4363	8726	8797	17594	0.1	177112	9.93	17403	0.0197	1	0.018	15	0.0372
			0.5	2	4434	8868											
	7-1	2	0.5	1	4624	9248	9398	18796	0.1	177112	10.61	18605	0.0211	1	0.018	15	0.0398
			0.5	2	4774	9548											
	7-2	2	0.5	1	4895	9790	9877	19754	0.1	177112	11.15	19563	0.0222	1	0.018	15	0.0418
			0.5	2	4982	9964											
	7-3	2	0.5	1	4480	8960	9073	18146	0.1	177112	10.25	17955	0.0204	1	0.018	15	0.0384
			0.5	2	4593	9186											
	8-1	2	0.5	1	5156	10312	10343	20686	0.1	177112	11.68	20495	0.0233	1	0.018	15	0.0438
			0.5	2	5187	10374											
	8-2	2	0.5	1	5009	10018	10157	20314	0.1	177112	11.47	20123	0.0228	1	0.018	15	0.0430
			0.5	2	5148	10296											
	8-3	2	0.5	1	4368	8736	8732	17464	0.1	177112	9.86	17273	0.0196	1	0.018	15	0.0369
			0.5	2	4364	8728											

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0455	0.0003
Full activity	End	0.0396	0.0007
Full activity	Overall	0.0426	0.0035
Background	Beginning	0.0000	0.000160253
Background	End	0.0000	3.62838E-05
Background	Overall	0.0000	0.000104239
Positive	Beginning	0.0184	0.0007
Positive	End	0.0169	0.0003
Positive	Overall	0.0176	0.0010
Negative	Beginning	0.0419	0.0012
Negative	End	0.0383	0.0002
Negative	Overall	0.0401	0.0022

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 9	1	1	1.00E-03	-3.00	0.0008
Ref Chem 9	1	2	1.00E-03	-3.00	0.0008
Ref Chem 9	1	3	1.00E-03	-3.00	0.0009
Ref Chem 9	2	1	5.00E-04	-3.30	0.0017
Ref Chem 9	2	2	5.00E-04	-3.30	0.0021
Ref Chem 9	2	3	5.00E-04	-3.30	0.0024
Ref Chem 9	3	1	2.50E-04	-3.60	0.0033
Ref Chem 9	3	2	2.50E-04	-3.60	0.0050
Ref Chem 9	3	3	2.50E-04	-3.60	0.0048
Ref Chem 9	4	1	1.00E-04	-4.00	0.0081
Ref Chem 9	4	2	1.00E-04	-4.00	0.0110
Ref Chem 9	4	3	1.00E-04	-4.00	0.0089
Ref Chem 9	5	1	1.00E-05	-5.00	0.0316
Ref Chem 9	5	2	1.00E-05	-5.00	0.0291
Ref Chem 9	5	3	1.00E-05	-5.00	0.0258
Ref Chem 9	6	1	1.00E-06	-6.00	0.0369
Ref Chem 9	6	2	1.00E-06	-6.00	0.0372
Ref Chem 9	6	3	1.00E-06	-6.00	0.0372
Ref Chem 9	7	1	1.00E-07	-7.00	0.0398
Ref Chem 9	7	2	1.00E-07	-7.00	0.0418
Ref Chem 9	7	3	1.00E-07	-7.00	0.0384
Ref Chem 9	8	1	1.00E-08	-8.00	0.0438
Ref Chem 9	8	2	1.00E-08	-8.00	0.0430
Ref Chem 9	8	3	1.00E-08	-8.00	0.0369

Percent of control values				
		Replicate		
Level	Log[test substance]	1	2	3
1	-3.00	1.88	1.99	2.04
2	-3.30	3.91	4.83	5.64
3	-3.60	7.83	11.67	11.30
4	-4.00	19.11	25.89	20.88
5	-5.00	74.20	68.36	60.56
6	-6.00	86.77	87.46	87.41
7	-7.00	93.45	98.26	90.19
8	-8.00	102.94	101.07	86.76

Aromatase Assay Spreadsheet

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	35194	1768543	
2	0.0196	32199	1642806	
3	0.0197	35939	1824315	
4	0.0195	33593	1722718	
5	0.0194	34266	1766289	
			Average DPM/g soln	1744934
			SD	67525
			CV	3.87
			µCi/g soln	0.786

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.2	10		1020.00
Dilution A			100	10.20
Dilution B			10	1.02

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.5236 g
Mass of dilution B used in substrate prep	4.8759 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.583488 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00890 µg/g soln.
µg/g soln.

a. µCi/g soln 0.786
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.583488 + 0.00890
= 0.592386 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.327 µCi/µg ASDN

843621 dpm/nmol

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8					
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20			

Standards:	250	125	50	25	10	5	0
	0.530	0.371	0.240	0.191	0.146	0.122	0.114
	0.555	0.379	0.246	0.180	0.146	0.127	0.115
	0.562	0.377	0.248	0.189	0.146	0.120	0.118

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25.5	100	11540-54

Samples:	11540-6A	11540-6B	11540-54B	11345-24H	11345-24I
	0.359	0.145	0.190	0.362	0.140
	0.352	0.153	0.187	0.378	0.138
			0.195	0.359	0.140

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0510	0.549	0.433	0.0486
125	100	200	0.00013	200	0.0255	0.376	0.260	0.0282
50	200	1000	0.00005	200	0.0102	0.245	0.129	0.0127
25	100	1000	0.00003	200	0.0051	0.187	0.071	0.0059
10	40	1000	0.00001	200	0.0020	0.146	0.030	0.0011
5	20	1000	0.00001	200	0.0010	0.123	0.007	-0.0016

		Variables	Regression results	
Blank	0.116	m, b	0.118	-0.002
		se_m, se_b	0.007	0.002
r^2=	0.985	r^2, se_y	0.985	0.003
m=	0.118	F, df	268	4
b=	-0.002	ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.359	0.243	0.026	200	1	1	0.131	0.129	0.11
11540-6A	0.352	0.236	0.025	200	1	1	0.127		
11540-6A									
11540-6B	0.145	0.029	0.001	200	1	1	0.005	0.007	0.012
11540-6B	0.153	0.037	0.002	200	1	1	0.010		
11540-6B									
11540-54B	0.190	0.074	0.006	200	1	320	10.0	10.2	
11540-54B	0.187	0.071	0.006	200	1	320	9.5		
11540-54B	0.195	0.079	0.007	200	1	320	11.0		
11345-24H	0.362	0.246	0.027	200	1	50	6.6	6.8	
11345-24H	0.378	0.262	0.028	200	1	50	7.1		
11345-24H	0.359	0.243	0.026	200	1	50	6.6		
11345-24I	0.140	0.024	0.000	200	1	600	1.1	0.9	
11345-24I	0.138	0.022	0.000	200	1	600	0.4		
11345-24I	0.140	0.024	0.000	200	1	600	1.1		

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested		8					
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.530	0.371	0.240	0.191	0.146	0.122	0.114	25.5	100	11540-54
	0.555	0.379	0.246	0.180	0.146	0.127	0.115			
	0.562	0.377	0.248	0.189	0.146	0.120	0.118			

Samples:	11540-6A	11540-6B	11540-54B	11345-24H	11345-24I
	0.359	0.145	0.190	0.362	0.140
	0.352	0.153	0.187	0.378	0.138
			0.195	0.359	0.140

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0510	0.549	0.433	0.0417
125	100	200	0.00013	200	0.0255	0.376	0.260	0.0246
50	200	1000	0.00005	200	0.0102	0.245	0.129	0.0117
25	100	1000	0.00003	200	0.0051	0.187	0.071	0.0060
10	40	1000	0.00001	200	0.0020	0.146	0.030	0.0020
5	20	1000	0.00001	200	0.0010	0.123	0.007	-0.0003

Variables	Regression results	
m, b	0.099	-0.001
se_m, se_b	0.007	0.001
r^2, se_y	0.986	0.001
F, df	214	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

Blank	0.116	r^2=	0.986
		m=	0.099
		b=	-0.001

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.359	0.243	0.023	200	1	1	0.115	0.113	0.11
11540-6A	0.352	0.236	0.022	200	1	1	0.111		
11540-6A									
11540-6B	0.145	0.029	0.002	200	1	1	0.009	0.011	0.012
11540-6B	0.153	0.037	0.003	200	1	1	0.013		
11540-6B									
11540-54B	0.190	0.074	0.006	200	1	320	10.1	10.2	
11540-54B	0.187	0.071	0.006	200	1	320	9.6		
11540-54B	0.195	0.079	0.007	200	1	320	10.9		
11345-24H	0.362	0.246	0.023	200	1	50	5.8	5.9	
11345-24H	0.378	0.262	0.025	200	1	50	6.2		
11345-24H	0.359	0.243	0.023	200	1	50	5.7		
11345-24I	0.140	0.024	0.001	200	1	600	4.1	3.9	
11345-24I	0.138	0.022	0.001	200	1	600	3.5		
11345-24I	0.140	0.024	0.001	200	1	600	4.1		

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1	3

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	10.2
Protein Concentration (dilution added to assay, mg/mL):	0.031775

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	5.00E-04
3	2.50E-04
4	1.00E-04
5	1.00E-05
6	1.00E-06
7	1.00E-07
8	1.00E-08

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4119	8238	8240	16480	0.1	174493	9.44	16318	0.0193	1	0.016	15	0.0406
			0.5	2	4121	8242											
	2	2	0.5	1	4045	8090	8181	16362	0.1	174493	9.38	16200	0.0192	1	0.016	15	0.0403
			0.5	2	4136	8272											
	3	2	0.5	1	3904	7808	7847	15694	0.1	174493	8.99	15532	0.0184	1	0.016	15	0.0386
			0.5	2	3943	7886											
	4	2	0.5	1	3917	7834	7755	15510	0.1	174493	8.89	15348	0.0182	1	0.016	15	0.0382
			0.5	2	3838	7676											
Background control	1	2	0.5	1	51	102	94	188	0.1	174493	0.11	26	0.0000	1	0.016	15	0.0001
			0.5	2	43	86											
	2	2	0.5	1	39	78	85	170	0.1	174493	0.10	8	0.0000	1	0.016	15	0.0000
			0.5	2	46	92											
	3	2	0.5	1	38	76	78	156	0.1	174493	0.09	-6	0.0000	1	0.016	15	0.0000
			0.5	2	40	80											
	4	2	0.5	1	30	60	67	134	0.1	174493	0.08	-28	0.0000	1	0.016	15	-0.0001
			0.5	2	37	74											
Positive control	1	2	0.5	1	2093	4186	4274	8548	0.1	174493	4.90	8386	0.0099	1	0.016	15	0.0209
			0.5	2	2181	4362											
	2	2	0.5	1	1976	3952	4010	8020	0.1	174493	4.60	7858	0.0093	1	0.016	15	0.0195
			0.5	2	2034	4068											
	3	2	0.5	1	1885	3770	3800	7600	0.1	174493	4.36	7438	0.0088	1	0.016	15	0.0185
			0.5	2	1915	3830											
	4	2	0.5	1	1905	3810	3797	7594	0.1	174493	4.35	7432	0.0088	1	0.016	15	0.0185
			0.5	2	1892	3784											
Negative Control	1	2	0.5	1	4086	8172	8141	16282	0.1	174493	9.33	16120	0.0191	1	0.016	15	0.0401
			0.5	2	4055	8110											
	2	2	0.5	1	4047	8094	8046	16092	0.1	174493	9.22	15930	0.0189	1	0.016	15	0.0396
			0.5	2	3999	7998											
	3	2	0.5	1	3881	7762	7783	15566	0.1	174493	8.92	15404	0.0183	1	0.016	15	0.0383
			0.5	2	3902	7804											
	4	2	0.5	1	3724	7448	7387	14774	0.1	174493	8.47	14812	0.0173	1	0.016	15	0.0363
			0.5	2	3663	7326											
Ref Chem 9	1-1	2	0.5	1	145	290	276	552	0.1	174493	0.32	390	0.0005	1	0.016	15	0.0010
			0.5	2	131	262											
	1-2	2	0.5	1	147	294	284	568	0.1	174493	0.33	406	0.0005	1	0.016	15	0.0010
			0.5	2	137	274											
	1-3	2	0.5	1	129	258	254	508	0.1	174493	0.29	346	0.0004	1	0.016	15	0.0009
			0.5	2	125	250											
	2-1	2	0.5	1	302	604	597	1194	0.1	174493	0.68	1032	0.0012	1	0.016	15	0.0026
			0.5	2	295	590											
	2-2	2	0.5	1	260	520	527	1054	0.1	174493	0.60	892	0.0011	1	0.016	15	0.0022
			0.5	2	267	534											
	2-3	2	0.5	1	324	648	637	1274	0.1	174493	0.73	1112	0.0013	1	0.016	15	0.0028
			0.5	2	313	626											
	3-1	2	0.5	1	636	1272	1242	2484	0.1	174493	1.42	2322	0.0026	1	0.016	15	0.0058
			0.5	2	606	1212											
	3-2	2	0.5	1	494	988	958	1916	0.1	174493	1.10	1754	0.0021	1	0.016	15	0.0044
			0.5	2	464	928											
	3-3	2	0.5	1	681	1362	1334	2668	0.1	174493	1.53	2606	0.0030	1	0.016	15	0.0062
			0.5	2	653	1306											
	4-1	2	0.5	1	1071	2142	2111	4222	0.1	174493	2.42	4060	0.0048	1	0.016	15	0.0101
			0.5	2	1040	2080											
	4-2	2	0.5	1	1041	2082	2072	4144	0.1	174493	2.37	3982	0.0047	1	0.016	15	0.0099
			0.5	2	1031	2062											
	4-3	2	0.5	1	908	1816	1824	3648	0.1	174493	2.09	3486	0.0041	1	0.016	15	0.0087
			0.5	2	916	1832											
	5-1	2	0.5	1	2717	5434	5498	10996	0.1	174493	6.30	10834	0.0128	1	0.016	15	0.0269
			0.5	2	2781	5562											
	5-2	2	0.5	1	2630	5260	5190	10380	0.1	174493	5.95	10218	0.0121	1	0.016	15	0.0254
			0.5	2	2560	5120											
	5-3	2	0.5	1	2457	4914	4852	9704	0.1	174493	5.56	9542	0.0113	1	0.016	15	0.0237
			0.5	2	2395	4790											
	6-1	2	0.5	1	3700	7400	7432	14864	0.1	174493	8.52	14702	0.0174	1	0.016	15	0.0366
			0.5	2	3732	7464											
	6-2	2	0.5	1	3755	7510	7465	14930	0.1	174493	8.56	14768	0.0175	1	0.016	15	0.0367
			0.5	2	3710	7420											
	6-3	2	0.5	1	3706	7412	7398	14796	0.1	174493	8.48	14634	0.0173	1	0.016	15	0.0364
			0.5	2	3692	7384											
	7-1	2	0.5	1	3882	7764	7880	15760	0.1	174493	9.03	15598	0.0185	1	0.016	15	0.0388
			0.5	2	3998	7996											
	7-2	2	0.5	1	3943	7886	7935	15870	0.1	174493	9.09	15708	0.0186	1	0.016	15	0.0391
			0.5	2	3992	7984											
	7-3	2	0.5	1	4007	8014	7905	15810	0.1	174493	9.06	15648	0.0185	1	0.016	15	0.0389
			0.5	2	3898	7796											
	8-1	2	0.5	1	4022	8044	8117	16234	0.1	174493	9.30	16072	0.0191	1	0.016	15	0.0400
			0.5	2	4095	8190											
	8-2	2	0.5	1	4084	8168	8212	16424	0.1	174493	9.41	16262	0.0193	1	0.016	15	0.0404
			0.5	2	4128	8256											
	8-3	2	0.5	1	4001	8002	7934	15868	0.1	174493	9.09	15706	0.0186	1	0.016	15	0.0391
			0.5	2	3933	7866											

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0404	0.0002
Full activity	End	0.0384	0.0003
Full activity	Overall	0.0394	0.0012
Background	Beginning	0.0000	3.1654E-05
Background	End	0.0000	3.86882E-05
Background	Overall	0.0000	5.67117E-05
Positive	Beginning	0.0202	0.0009
Positive	End	0.0185	0.0000
Positive	Overall	0.0193	0.0011
Negative	Beginning	0.0399	0.0003
Negative	End	0.0373	0.0014
Negative	Overall	0.0386	0.0017

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 9	1	1	1.00E-03	-3.00	0.0010
Ref Chem 9	1	2	1.00E-03	-3.00	0.0010
Ref Chem 9	1	3	1.00E-03	-3.00	0.0009
Ref Chem 9	2	1	5.00E-04	-3.30	0.0026
Ref Chem 9	2	2	5.00E-04	-3.30	0.0022
Ref Chem 9	2	3	5.00E-04	-3.30	0.0028
Ref Chem 9	3	1	2.50E-04	-3.60	0.0058
Ref Chem 9	3	2	2.50E-04	-3.60	0.0044
Ref Chem 9	3	3	2.50E-04	-3.60	0.0062
Ref Chem 9	4	1	1.00E-04	-4.00	0.0101
Ref Chem 9	4	2	1.00E-04	-4.00	0.0099
Ref Chem 9	4	3	1.00E-04	-4.00	0.0087
Ref Chem 9	5	1	1.00E-05	-5.00	0.0269
Ref Chem 9	5	2	1.00E-05	-5.00	0.0254
Ref Chem 9	5	3	1.00E-05	-5.00	0.0237
Ref Chem 9	6	1	1.00E-06	-6.00	0.0366
Ref Chem 9	6	2	1.00E-06	-6.00	0.0367
Ref Chem 9	6	3	1.00E-06	-6.00	0.0364
Ref Chem 9	7	1	1.00E-07	-7.00	0.0388
Ref Chem 9	7	2	1.00E-07	-7.00	0.0391
Ref Chem 9	7	3	1.00E-07	-7.00	0.0389
Ref Chem 9	8	1	1.00E-08	-8.00	0.0400
Ref Chem 9	8	2	1.00E-08	-8.00	0.0404
Ref Chem 9	8	3	1.00E-08	-8.00	0.0391

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.46	2.56	2.18
2	-3.30	6.51	5.63	7.02
3	-3.60	14.65	11.07	15.81
4	-4.00	25.62	25.12	21.99
5	-5.00	68.36	64.47	60.20
6	-6.00	92.76	93.18	92.33
7	-7.00	98.41	99.11	98.73
8	-8.00	101.40	102.60	99.09

Aromatase Assay Spreadsheet

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8		
Technician ID	1	Replicate #	1	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0202	34199	1693020	
2	0.0194	31900	1644330	
3	0.0194	32329	1666443	
4	0.0193	33162	1718238	
5	0.0194	33996	1752371	
			Average DPM/g soln	1694880
			SD	42480
			CV	2.51
			µCi/g soln	0.763

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0521 g
Mass of dilution B used in substrate prep	4.5126 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.560425 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00864 µg/g soln.
µg/g soln.

a. µCi/g soln 0.763
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.560425 + 0.00864
= 0.569068 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.342 µCi/µg ASDN

852998 dpm/nmol

Assay Date 9/8/2005 | Test Chemical ID Ref Chem 10 | # Concentrations tested 8

Technician ID 1 | Replicate # 1 | Microsome type Placental | Microsome ID 11540-20

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.8	100	11540-51
	0.584	0.393	0.254	0.193	0.142	0.138	0.121			
	0.571	0.388	0.245	0.186	0.145	0.127	0.108			
	0.566	0.384	0.250	0.180	0.153	0.125	0.121			

Samples:	11540-6A	11540-6B	11540-51B	11345-22H	11345-22I
	0.369	0.144	0.190	0.363	0.149
	0.371	0.145	0.192	0.366	0.145
			0.198	0.370	0.156

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0516	0.574	0.457	0.0495
125	100	200	0.00013	200	0.0258	0.388	0.272	0.0284
50	200	1000	0.00005	200	0.0103	0.250	0.133	0.0127
25	100	1000	0.00003	200	0.0052	0.186	0.070	0.0055
10	40	1000	0.00001	200	0.0021	0.147	0.030	0.0009
5	20	1000	0.00001	200	0.0010	0.130	0.013	-0.0009

Blank 0.117

r^2= 0.989
m= 0.114
b= -0.002

Variables	Regression results	
m, b	0.114	-0.002
se_m, se_b	0.006	0.001
r^2, se_y	0.989	0.002
F, df	347	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.369	0.252	0.026	200	1	1	0.131	0.132	0.11
11540-6A	0.371	0.254	0.026	200	1	1	0.132		
11540-6A									
11540-6B	0.144	0.027	0.001	200	1	1	0.003	0.004	0.012
11540-6B	0.145	0.028	0.001	200	1	1	0.004		
11540-6B									
11540-51B	0.190	0.073	0.006	200	1	320	9.4	10.0	
11540-51B	0.192	0.075	0.006	200	1	320	9.8		
11540-51B	0.198	0.081	0.007	200	1	320	10.9		
11345-22H	0.363	0.246	0.026	200	1	50	6.4	6.5	
11345-22H	0.366	0.249	0.026	200	1	50	6.5		
11345-22H	0.370	0.253	0.026	200	1	50	6.6		
11345-22I	0.149	0.032	0.001	200	1	600	3.6	4.0	
11345-22I	0.145	0.028	0.001	200	1	600	2.3		
11345-22I	0.156	0.039	0.002	200	1	600	6.0		

Assay Date 9/8/2005 | Test Chemical ID Ref Chem 10 | # Concentrations tested 8

Technician ID 1 | Replicate # 1 | Microsome type Placental | Microsome ID 11540-20

	250	125	50	25	10	5	0
Standards:	0.584	0.393	0.254	0.193	0.142	0.138	0.121
	0.571	0.388	0.245	0.186	0.145	0.127	0.108
	0.566	0.384	0.250	0.180	0.153	0.125	0.121

Protein stock (mg BSA) 25.8 | Total volume of stock (mL) 100 | Protein stock ID 11540-51

	11540-6A	11540-6B	11540-51B	11345-22H	11345-22I
Samples:	0.369	0.144	0.190	0.363	0.149
	0.371	0.145	0.192	0.366	0.145
			0.198	0.370	0.156

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0516	0.574	0.457	0.0429
125	100	200	0.00013	200	0.0258	0.388	0.272	0.0251
50	200	1000	0.00005	200	0.0103	0.250	0.133	0.0117
25	100	1000	0.00003	200	0.0052	0.186	0.070	0.0056
10	40	1000	0.00001	200	0.0021	0.147	0.030	0.0018
5	20	1000	0.00001	200	0.0010	0.130	0.013	0.0002
			Blank	0.117				

Variables	Regression results	
m, b	0.096	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.992	0.001
F, df	352	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.992
m= 0.096
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.369	0.252	0.023	200	1	1	0.116	0.117	0.11
11540-6A	0.371	0.254	0.023	200	1	1	0.117		
11540-6A									
11540-6B	0.144	0.027	0.002	200	1	1	0.008	0.008	0.012
11540-6B	0.145	0.028	0.002	200	1	1	0.008		
11540-6B									
11540-51B	0.190	0.073	0.006	200	1	320	9.5	10.1	
11540-51B	0.192	0.075	0.006	200	1	320	9.9		
11540-51B	0.198	0.081	0.007	200	1	320	10.8		
11345-22H	0.363	0.246	0.023	200	1	50	5.7	5.7	
11345-22H	0.366	0.249	0.023	200	1	50	5.7		
11345-22H	0.370	0.253	0.023	200	1	50	5.8		
11345-22I	0.149	0.032	0.002	200	1	600	6.0	6.3	
11345-22I	0.145	0.028	0.002	200	1	600	4.9		
11345-22I	0.156	0.039	0.003	200	1	600	8.1		

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	1

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	10.1
Protein Concentration (dilution added to assay, mg/mL):	0.031434

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4016	8032	8112	16224	0.1	169488	9.57	16049	0.0188	1	0.016	15	0.0399
			0.5	2	4096	8192											
	2	2	0.5	1	3970	7940	8128	16256	0.1	169488	9.59	16081	0.0189	1	0.016	15	0.0400
			0.5	2	4158	8316											
	3	2	0.5	1	3596	7192	7119	14238	0.1	169488	8.40	14063	0.0165	1	0.016	15	0.0350
			0.5	2	3523	7046											
	4	2	0.5	1	3601	7202	7171	14342	0.1	169488	8.46	14167	0.0168	1	0.016	15	0.0352
			0.5	2	3570	7140											
Background control	1	2	0.5	1	51	102	86	172	0.1	169488	0.10	-4	0.0000	1	0.016	15	0.0000
			0.5	2	35	70											
	2	2	0.5	1	44	88	88	176	0.1	169488	0.10	1	0.0000	1	0.016	15	0.0000
			0.5	2	44	88											
	3	2	0.5	1	46	92	86	172	0.1	169488	0.10	-4	0.0000	1	0.016	15	0.0000
			0.5	2	40	80											
	4	2	0.5	1	42	84	91	182	0.1	169488	0.11	7	0.0000	1	0.016	15	0.0000
			0.5	2	49	98											
Positive control	1	2	0.5	1	2203	4406	4424	8848	0.1	169488	5.22	8673	0.0102	1	0.016	15	0.0218
			0.5	2	2221	4442											
	2	2	0.5	1	1974	3948	3951	7902	0.1	169488	4.66	7727	0.0091	1	0.016	15	0.0192
			0.5	2	1977	3954											
	3	2	0.5	1	1848	3696	3735	7470	0.1	169488	4.41	7295	0.0086	1	0.016	15	0.0181
			0.5	2	1887	3774											
	4	2	0.5	1	1856	3712	3739	7478	0.1	169488	4.41	7303	0.0086	1	0.016	15	0.0182
			0.5	2	1883	3766											
Negative Control	1	2	0.5	1	4051	8102	8098	16196	0.1	169488	9.56	16021	0.0188	1	0.016	15	0.0398
			0.5	2	4047	8094											
	2	2	0.5	1	3926	7852	8021	16042	0.1	169488	9.46	15867	0.0186	1	0.016	15	0.0394
			0.5	2	4095	8190											
	3	2	0.5	1	3503	7006	7137	14274	0.1	169488	8.42	14099	0.0165	1	0.016	15	0.0351
			0.5	2	3634	7269											
	4	2	0.5	1	3227	6454	6467	12934	0.1	169488	7.63	12759	0.0150	1	0.016	15	0.0317
			0.5	2	3240	6480											
Ref Chem 10	1-1	2	0.5	1	3065	6130	6217	12434	0.1	169488	7.34	12259	0.0144	1	0.016	15	0.0305
			0.5	2	3152	6304											
	1-2	2	0.5	1	3021	6042	6172	12344	0.1	169488	7.28	12169	0.0143	1	0.016	15	0.0303
			0.5	2	3151	6302											
	1-3	2	0.5	1	2990	5980	6112	12224	0.1	169488	7.21	12049	0.0141	1	0.016	15	0.0300
			0.5	2	3122	6244											
	2-1	2	0.5	1	3466	6932	6877	13754	0.1	169488	8.12	13579	0.0159	1	0.016	15	0.0338
			0.5	2	3411	6822											
	2-2	2	0.5	1	3429	6858	6937	13874	0.1	169488	8.19	13699	0.0161	1	0.016	15	0.0341
			0.5	2	3508	7016											
	2-3	2	0.5	1	3512	7024	7005	14010	0.1	169488	8.27	13835	0.0162	1	0.016	15	0.0344
			0.5	2	3493	6986											
	3-1	2	0.5	1	3691	7382	7485	14970	0.1	169488	8.83	14795	0.0173	1	0.016	15	0.0368
			0.5	2	3794	7588											
	3-2	2	0.5	1	3688	7336	7425	14850	0.1	169488	8.76	14675	0.0172	1	0.016	15	0.0365
			0.5	2	3757	7514											
	3-3	2	0.5	1	3466	6932	7118	14236	0.1	169488	8.40	14061	0.0165	1	0.016	15	0.0350
			0.5	2	3652	7304											
	4-1	2	0.5	1	3545	7090	7127	14254	0.1	169488	8.41	14079	0.0165	1	0.016	15	0.0350
			0.5	2	3582	7164											
	4-2	2	0.5	1	3660	7320	7336	14672	0.1	169488	8.66	14497	0.0170	1	0.016	15	0.0360
			0.5	2	3676	7352											
	4-3	2	0.5	1	3682	7364	7222	14444	0.1	169488	8.52	14269	0.0167	1	0.016	15	0.0355
			0.5	2	3540	7080											
	5-1	2	0.5	1	3859	7718	7598	15196	0.1	169488	8.97	15021	0.0176	1	0.016	15	0.0373
			0.5	2	3739	7478											
	5-2	2	0.5	1	3733	7466	7495	14990	0.1	169488	8.84	14815	0.0174	1	0.016	15	0.0368
			0.5	2	3762	7524											
	5-3	2	0.5	1	3697	7394	7394	14788	0.1	169488	8.73	14613	0.0171	1	0.016	15	0.0363
			0.5	2	3697	7394											
	6-1	2	0.5	1	3657	7314	7424	14848	0.1	169488	8.76	14673	0.0172	1	0.016	15	0.0365
			0.5	2	3767	7534											
	6-2	2	0.5	1	3481	6962	6896	13792	0.1	169488	8.14	13617	0.0160	1	0.016	15	0.0339
			0.5	2	3415	6830											
	6-3	2	0.5	1	3543	7086	7251	14502	0.1	169488	8.56	14327	0.0168	1	0.016	15	0.0356
			0.5	2	3708	7416											
	7-1	2	0.5	1	3633	7266	7303	14606	0.1	169488	8.62	14431	0.0169	1	0.016	15	0.0359
			0.5	2	3670	7340											
	7-2	2	0.5	1	3613	7226	7403	14806	0.1	169488	8.74	14631	0.0172	1	0.016	15	0.0364
			0.5	2	3790	7580											
	7-3	2	0.5	1	3441	6882	6941	13882	0.1	169488	8.19	13707	0.0161	1	0.016	15	0.0341
			0.5	2	3500	7000											
	8-1	2	0.5	1	3535	7070	7129	14258	0.1	169488	8.41	14083	0.0165	1	0.016	15	0.0350
			0.5	2	3594	7188											
	8-2	2	0.5	1	3584	7168	7208	14416	0.1	169488	8.51	14241	0.0167	1	0.016	15	0.0354
			0.5	2	3624	7248											
	8-3	2	0.5	1	3474	6948	7004	14008	0.1	169488	8.26	13833	0.0162	1	0.016	15	0.0344
			0.5	2	3530	7060											

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0399	0.0001
Full activity	End	0.0351	0.0002
Full activity	Overall	0.0375	0.0028
Background	Beginning	0.0000	7.03237E-06
Background	End	0.0000	1.75809E-05
Background	Overall	0.0000	1.17499E-05
Positive	Beginning	0.0204	0.0017
Positive	End	0.0181	0.0000
Positive	Overall	0.0193	0.0016
Negative	Beginning	0.0396	0.0003
Negative	End	0.0334	0.0024
Negative	Overall	0.0365	0.0039

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 10	1	1	1.00E-03	-3.00	0.0305
Ref Chem 10	1	2	1.00E-03	-3.00	0.0303
Ref Chem 10	1	3	1.00E-03	-3.00	0.0300
Ref Chem 10	2	1	1.00E-04	-4.00	0.0338
Ref Chem 10	2	2	1.00E-04	-4.00	0.0341
Ref Chem 10	2	3	1.00E-04	-4.00	0.0344
Ref Chem 10	3	1	1.00E-05	-5.00	0.0368
Ref Chem 10	3	2	1.00E-05	-5.00	0.0365
Ref Chem 10	3	3	1.00E-05	-5.00	0.0350
Ref Chem 10	4	1	1.00E-06	-6.00	0.0350
Ref Chem 10	4	2	1.00E-06	-6.00	0.0360
Ref Chem 10	4	3	1.00E-06	-6.00	0.0355
Ref Chem 10	5	1	1.00E-07	-7.00	0.0373
Ref Chem 10	5	2	1.00E-07	-7.00	0.0368
Ref Chem 10	5	3	1.00E-07	-7.00	0.0363
Ref Chem 10	6	1	1.00E-08	-8.00	0.0365
Ref Chem 10	6	2	1.00E-08	-8.00	0.0339
Ref Chem 10	6	3	1.00E-08	-8.00	0.0356
Ref Chem 10	7	1	1.00E-09	-9.00	0.0359
Ref Chem 10	7	2	1.00E-09	-9.00	0.0364
Ref Chem 10	7	3	1.00E-09	-9.00	0.0341
Ref Chem 10	8	1	1.00E-10	-10.00	0.0350
Ref Chem 10	8	2	1.00E-10	-10.00	0.0354
Ref Chem 10	8	3	1.00E-10	-10.00	0.0344

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	81.24	80.64	79.85
2	-4.00	89.99	90.78	91.68
3	-5.00	98.04	97.25	93.18
4	-6.00	93.30	96.07	94.56
5	-7.00	99.54	98.18	96.84
6	-8.00	97.24	90.24	94.94
7	-9.00	95.63	96.96	90.83
8	-10.00	93.33	94.37	91.67

Aromatase Assay Spreadsheet

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8		
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	34622	1739799		
2	0.0195	32944	1689436		
3	0.0195	34328	1760410		
4	0.0194	34591	1783041		
5	0.0192	36152	1882917		
			Average DPM/g soln		1771121
			SD		71429
			CV		4.03
			µCi/g soln		0.798

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.1	10		1010.00
Dilution A			100	10.10
Dilution B			10	1.01

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0792 g
Mass of dilution B used in substrate prep	4.5326 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.566631 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00903 µg/g soln.
µg/g soln.

a. µCi/g soln 0.798
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.566631 + 0.00903
= 0.575662 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.386 µCi/µg ASDN

881157 dpm/nmol

Assay Date 9/13/2005 | Test Chemical ID Ref Chem 10 | # Concentrations tested 8

Technician ID 1 | Replicate # 2 | Microsome type Placental | Microsome ID 11540-20

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.9	100	11540-53
	0.522	0.381	0.237	0.181	0.145	0.132	0.122			
	0.568	0.377	0.254	0.180	0.131	0.126	0.119			
	0.543	0.395	0.235	0.181	0.143	0.130	0.115			

Samples:	11540-6A	11540-6B	11540-53B	11345-23H	11345-23I
	0.354	0.129	0.194	0.346	0.188
	0.342	0.142	0.205	0.360	0.185
			0.190	0.354	0.189

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0518	0.544	0.426	0.0488
125	100	200	0.00013	200	0.0259	0.384	0.266	0.0297
50	200	1000	0.00005	200	0.0104	0.242	0.123	0.0127
25	100	1000	0.00003	200	0.0052	0.181	0.062	0.0054
10	40	1000	0.00001	200	0.0021	0.140	0.021	0.0005
5	20	1000	0.00001	200	0.0010	0.129	0.011	-0.0008

Variables		Regression results		
m, b	0.120	-0.002		
se_m, se_b	0.008	0.002		
r^2, se_y	0.982	0.003		
F, df	221	4		
ss_{reg}, ss_{resid}	0.002	0.000		

Blank 0.119

r^2= 0.982
m= 0.120
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.354	0.235	0.026	200	1	1	0.130	0.127	0.11
11540-6A	0.342	0.223	0.025	200	1	1	0.123		
11540-6A									
11540-6B	0.129	0.010	-0.001	200	1	1	-0.004	0.000	0.012
11540-6B	0.142	0.023	0.001	200	1	1	0.004		
11540-6B									
11540-53B	0.194	0.075	0.007	200	1	320	11.2	11.6	
11540-53B	0.205	0.086	0.008	200	1	320	13.3		
11540-53B	0.190	0.071	0.006	200	1	320	10.4		
11345-23H	0.346	0.227	0.025	200	1	50	6.3	6.5	
11345-23H	0.360	0.241	0.027	200	1	50	6.7		
11345-23H	0.354	0.235	0.026	200	1	50	6.5		
11345-23I	0.188	0.069	0.006	200	1	600	18.8	18.5	
11345-23I	0.185	0.066	0.006	200	1	600	17.7		
11345-23I	0.189	0.070	0.006	200	1	600	19.1		

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8						
Technician ID	1	Replicate #	2	Microsome type	Placental	Microsome ID	11540-20				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.9	100	11540-53
	0.522	0.381	0.237	0.181	0.145	0.132	0.122			
	0.568	0.377	0.254	0.180	0.131	0.126	0.119			
	0.543	0.395	0.235	0.181	0.143	0.130	0.115			

Samples:	11540-6A	11540-6B	11540-53B	11345-23H	11345-23I
	0.354	0.129	0.194	0.346	0.188
	0.342	0.142	0.205	0.360	0.185
			0.190	0.354	0.189

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0518	0.544	0.426	0.0409
125	100	200	0.00013	200	0.0259	0.384	0.266	0.0253
50	200	1000	0.00005	200	0.0104	0.242	0.123	0.0115
25	100	1000	0.00003	200	0.0052	0.181	0.062	0.0056
10	40	1000	0.00001	200	0.0021	0.140	0.021	0.0016
5	20	1000	0.00001	200	0.0010	0.129	0.011	0.0006

Blank	0.119	r^2=	0.995		
		m=	0.097		
		b=	0.000		

Variables	Regression results	
m, b	0.097	0.000
se_m, se_b	0.004	0.001
r^2, se_y	0.995	0.001
F, df	548	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep	average mg/mL	known QC Concentration
11540-6A	0.354	0.235	0.022	200	1	1	0.112	0.109	0.11
11540-6A	0.342	0.223	0.021	200	1	1	0.106		
11540-6A									
11540-6B	0.129	0.010	0.001	200	1	1	0.003	0.006	0.012
11540-6B	0.142	0.023	0.002	200	1	1	0.009		
11540-6B									
11540-53B	0.194	0.075	0.007	200	1	320	11.0	11.3	
11540-53B	0.205	0.086	0.008	200	1	320	12.7		
11540-53B	0.190	0.071	0.006	200	1	320	10.3		
11345-23H	0.346	0.227	0.022	200	1	50	5.4	5.6	
11345-23H	0.360	0.241	0.023	200	1	50	5.7		
11345-23H	0.354	0.235	0.022	200	1	50	5.6		
11345-23I	0.188	0.069	0.006	200	1	600	18.8	18.6	
11345-23I	0.185	0.066	0.006	200	1	600	17.9		
11345-23I	0.189	0.070	0.006	200	1	600	19.1		

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	2

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	11.3
Protein Concentration (dilution added to assay, mg/mL):	0.035387

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	5.00E-04
3	2.50E-04
4	1.00E-04
5	5.00E-05
6	1.00E-05
7	1.00E-06
8	1.00E-07

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5387	10774	10795	21590	0.1	177112	12.19	21399	0.0243	1	0.018	15	0.0458
			0.5	2	5408	10816											
	2	2	0.5	1	5326	10652	10695	21390	0.1	177112	12.08	21199	0.0241	1	0.018	15	0.0453
			0.5	2	5369	10738											
	3	2	0.5	1	4566	9132	9243	18486	0.1	177112	10.44	18295	0.0208	1	0.018	15	0.0391
			0.5	2	4677	9354											
	4	2	0.5	1	4679	9358	9467	18934	0.1	177112	10.69	18743	0.0213	1	0.018	15	0.0401
			0.5	2	4788	9576											
Background control	1	2	0.5	1	36	72	78	156	0.1	177112	0.09	-36	0.0000	1	0.018	15	-0.0001
			0.5	2	42	84											
	2	2	0.5	1	66	132	131	262	0.1	177112	0.15	71	0.0001	1	0.018	15	0.0002
			0.5	2	65	130											
	3	2	0.5	1	41	82	81	162	0.1	177112	0.09	-30	0.0000	1	0.018	15	-0.0001
			0.5	2	40	80											
	4	2	0.5	1	41	82	93	186	0.1	177112	0.11	-6	0.0000	1	0.018	15	0.0000
			0.5	2	52	104											
Positive control	1	2	0.5	1	2208	4416	4510	9020	0.1	177112	5.09	8829	0.0100	1	0.018	15	0.0189
			0.5	2	2302	4604											
	2	2	0.5	1	2161	4322	4283	8566	0.1	177112	4.84	8375	0.0095	1	0.018	15	0.0179
			0.5	2	2122	4244											
	3	2	0.5	1	2042	4084	4082	8164	0.1	177112	4.61	7973	0.0090	1	0.018	15	0.0170
			0.5	2	2040	4080											
	4	2	0.5	1	2017	4034	3996	7992	0.1	177112	4.51	7801	0.0089	1	0.018	15	0.0167
			0.5	2	1979	3958											
Negative Control	1	2	0.5	1	5034	10068	10094	20188	0.1	177112	11.40	19997	0.0227	1	0.018	15	0.0428
			0.5	2	5060	10120											
	2	2	0.5	1	4846	9692	9700	19400	0.1	177112	10.95	19209	0.0218	1	0.018	15	0.0411
			0.5	2	4854	9708											
	3	2	0.5	1	4639	9278	9086	18172	0.1	177112	10.26	17981	0.0204	1	0.018	15	0.0384
			0.5	2	4447	8894											
	4	2	0.5	1	4503	9006	9028	18056	0.1	177112	10.19	17865	0.0203	1	0.018	15	0.0382
			0.5	2	4525	9050											
Ref Chem 10	1-1	2	0.5	1	3733	7466	7451	14902	0.1	177112	8.41	14711	0.0167	1	0.018	15	0.0315
			0.5	2	3718	7436											
	1-2	2	0.5	1	3754	7508	7613	15226	0.1	177112	8.60	15035	0.0171	1	0.018	15	0.0321
			0.5	2	3859	7718											
	1-3	2	0.5	1	3645	7290	7355	14710	0.1	177112	8.31	14519	0.0165	1	0.018	15	0.0310
			0.5	2	3710	7420											
	2-1	2	0.5	1	3926	7852	7752	15504	0.1	177112	8.75	15313	0.0174	1	0.018	15	0.0327
			0.5	2	3826	7652											
	2-2	2	0.5	1	3893	7786	7804	15608	0.1	177112	8.81	15417	0.0175	1	0.018	15	0.0330
			0.5	2	3911	7822											
	2-3	2	0.5	1	3885	7770	7747	15494	0.1	177112	8.75	15303	0.0174	1	0.018	15	0.0327
			0.5	2	3862	7724											
	3-1	2	0.5	1	4297	8594	8596	17192	0.1	177112	9.71	17001	0.0193	1	0.018	15	0.0363
			0.5	2	4299	8598											
	3-2	2	0.5	1	4048	8096	8225	16450	0.1	177112	9.29	16259	0.0185	1	0.018	15	0.0348
			0.5	2	4177	8354											
	3-3	2	0.5	1	4190	8380	8440	16880	0.1	177112	9.53	16689	0.0189	1	0.018	15	0.0357
			0.5	2	4250	8500											
	4-1	2	0.5	1	4250	8500	8533	17066	0.1	177112	9.64	16875	0.0192	1	0.018	15	0.0361
			0.5	2	4283	8566											
	4-2	2	0.5	1	4437	8874	8717	17434	0.1	177112	9.84	17243	0.0196	1	0.018	15	0.0369
			0.5	2	4280	8560											
	4-3	2	0.5	1	4350	8700	8952	17904	0.1	177112	10.11	17713	0.0201	1	0.018	15	0.0379
			0.5	2	4602	9204											
	5-1	2	0.5	1	4618	9236	9133	18266	0.1	177112	10.31	18075	0.0205	1	0.018	15	0.0386
			0.5	2	4515	9030											
	5-2	2	0.5	1	4824	9648	9572	19144	0.1	177112	10.81	18953	0.0215	1	0.018	15	0.0405
			0.5	2	4748	9496											
	5-3	2	0.5	1	4677	9354	9251	18502	0.1	177112	10.45	18311	0.0208	1	0.018	15	0.0391
			0.5	2	4574	9148											
	6-1	2	0.5	1	4661	9322	9414	18828	0.1	177112	10.63	18637	0.0212	1	0.018	15	0.0398
			0.5	2	4753	9506											
	6-2	2	0.5	1	4788	9576	9588	19176	0.1	177112	10.83	18985	0.0215	1	0.018	15	0.0406
			0.5	2	4800	9600											
	6-3	2	0.5	1	4574	9148	9291	18582	0.1	177112	10.49	18391	0.0209	1	0.018	15	0.0393
			0.5	2	4717	9434											
	7-1	2	0.5	1	4716	9432	9574	19148	0.1	177112	10.81	18957	0.0215	1	0.018	15	0.0405
			0.5	2	4858	9716											
	7-2	2	0.5	1	4775	9550	9449	18898	0.1	177112	10.67	18707	0.0212	1	0.018	15	0.0400
			0.5	2	4674	9348											
	7-3	2	0.5	1	4710	9420	9425	18850	0.1	177112	10.64	18659	0.0212	1	0.018	15	0.0399
			0.5	2	4715	9430											
	8-1	2	0.5	1	4778	9556	9586	19172	0.1	177112	10.82	18981	0.0215	1	0.018	15	0.0406
			0.5	2	4808	9616											
	8-2	2	0.5	1	4901	9802	9743	19486	0.1	177112	11.00	19295	0.0219	1	0.018	15	0.0413
			0.5	2	4842	9684											
	8-3	2	0.5	1	4564	9128	9141	18282	0.1	177112	10.32	18091	0.0205	1	0.018	15	0.0387
			0.5	2	4577	9154											

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0455	0.0003
Full activity	End	0.0396	0.0007
Full activity	Overall	0.0426	0.0035
Background	Beginning	0.0000	0.000160253
Background	End	0.0000	3.62838E-05
Background	Overall	0.0000	0.000104239
Positive	Beginning	0.0184	0.0007
Positive	End	0.0169	0.0003
Positive	Overall	0.0176	0.0010
Negative	Beginning	0.0419	0.0012
Negative	End	0.0383	0.0002
Negative	Overall	0.0401	0.0022

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 10	1	1	1.00E-03	-3.00	0.0315
Ref Chem 10	1	2	1.00E-03	-3.00	0.0321
Ref Chem 10	1	3	1.00E-03	-3.00	0.0310
Ref Chem 10	2	1	5.00E-04	-3.30	0.0327
Ref Chem 10	2	2	5.00E-04	-3.30	0.0330
Ref Chem 10	2	3	5.00E-04	-3.30	0.0327
Ref Chem 10	3	1	2.50E-04	-3.60	0.0363
Ref Chem 10	3	2	2.50E-04	-3.60	0.0348
Ref Chem 10	3	3	2.50E-04	-3.60	0.0357
Ref Chem 10	4	1	1.00E-04	-4.00	0.0361
Ref Chem 10	4	2	1.00E-04	-4.00	0.0369
Ref Chem 10	4	3	1.00E-04	-4.00	0.0379
Ref Chem 10	5	1	5.00E-05	-4.30	0.0386
Ref Chem 10	5	2	5.00E-05	-4.30	0.0405
Ref Chem 10	5	3	5.00E-05	-4.30	0.0391
Ref Chem 10	6	1	1.00E-05	-5.00	0.0398
Ref Chem 10	6	2	1.00E-05	-5.00	0.0406
Ref Chem 10	6	3	1.00E-05	-5.00	0.0393
Ref Chem 10	7	1	1.00E-06	-6.00	0.0405
Ref Chem 10	7	2	1.00E-06	-6.00	0.0400
Ref Chem 10	7	3	1.00E-06	-6.00	0.0399
Ref Chem 10	8	1	1.00E-07	-7.00	0.0406
Ref Chem 10	8	2	1.00E-07	-7.00	0.0413
Ref Chem 10	8	3	1.00E-07	-7.00	0.0387

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	73.89	75.52	72.93
2	-3.30	76.91	77.44	76.86
3	-3.60	85.39	81.67	83.83
4	-4.00	84.76	86.61	88.97
5	-4.30	90.79	95.20	91.97
6	-5.00	93.61	95.36	92.38
7	-6.00	95.22	93.96	93.72
8	-7.00	95.34	96.92	90.87

Aromatase Assay Spreadsheet

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	35194	1768543	
2	0.0196	32199	1642806	
3	0.0197	35939	1824315	
4	0.0195	33593	1722718	
5	0.0194	34266	1766289	
			Average DPM/g soln	1744934
			SD	67525
			CV	3.87
			µCi/g soln	0.786

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.2	10		1020.00
Dilution A			100	10.20
Dilution B			10	1.02

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.5236 g
Mass of dilution B used in substrate prep	4.8759 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.583488 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00890 µg/g soln.
µg/g soln.

a. µCi/g soln 0.786
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.583488 + 0.00890
= 0.592386 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.327 µCi/µg ASDN

843621 dpm/nmol

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8		
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20

	250	125	50	25	10	5	0
Standards:	0.530	0.371	0.240	0.191	0.146	0.122	0.114
	0.555	0.379	0.246	0.180	0.146	0.127	0.115
	0.562	0.377	0.248	0.189	0.146	0.120	0.118

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25.5	100	11540-54

	11540-6A	11540-6B	11540-54B	11345-24H	11345-24I
Samples:	0.359	0.145	0.190	0.362	0.140
	0.352	0.153	0.187	0.378	0.138
			0.195	0.359	0.140

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0510	0.549	0.433	0.0486
125	100	200	0.00013	200	0.0255	0.376	0.260	0.0282
50	200	1000	0.00005	200	0.0102	0.245	0.129	0.0127
25	100	1000	0.00003	200	0.0051	0.187	0.071	0.0059
10	40	1000	0.00001	200	0.0020	0.146	0.030	0.0011
5	20	1000	0.00001	200	0.0010	0.123	0.007	-0.0016

		Variables	Regression results	
Blank	0.116	m, b	0.118	-0.002
		se_m, se_b	0.007	0.002
r^2=	0.985	r^2, se_y	0.985	0.003
m=	0.118	F, df	268	4
b=	-0.002	ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.359	0.243	0.026	200	1	1	0.131	0.129	0.11
11540-6A	0.352	0.236	0.025	200	1	1	0.127		
11540-6A									
11540-6B	0.145	0.029	0.001	200	1	1	0.005	0.007	0.012
11540-6B	0.153	0.037	0.002	200	1	1	0.010		
11540-6B									
11540-54B	0.190	0.074	0.006	200	1	320	10.0	10.2	
11540-54B	0.187	0.071	0.006	200	1	320	9.5		
11540-54B	0.195	0.079	0.007	200	1	320	11.0		
11345-24H	0.362	0.246	0.027	200	1	50	6.6	6.8	
11345-24H	0.378	0.262	0.028	200	1	50	7.1		
11345-24H	0.359	0.243	0.026	200	1	50	6.6		
11345-24I	0.140	0.024	0.000	200	1	600	1.1	0.9	
11345-24I	0.138	0.022	0.000	200	1	600	0.4		
11345-24I	0.140	0.024	0.000	200	1	600	1.1		

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8						
Technician ID	1	Replicate #	3	Microsome type	Placental	Microsome ID	11540-20				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.530	0.371	0.240	0.191	0.146	0.122	0.114	25.5	100	11540-54
	0.555	0.379	0.246	0.180	0.146	0.127	0.115			
	0.562	0.377	0.248	0.189	0.146	0.120	0.118			

Samples:	11540-6A	11540-6B	11540-54B	11345-24H	11345-24I
	0.359	0.145	0.190	0.362	0.140
	0.352	0.153	0.187	0.378	0.138
			0.195	0.359	0.140

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00026	200	0.0510	0.549	0.433	0.0417
125	100	200	0.00013	200	0.0255	0.376	0.260	0.0246
50	200	1000	0.00005	200	0.0102	0.245	0.129	0.0117
25	100	1000	0.00003	200	0.0051	0.187	0.071	0.0060
10	40	1000	0.00001	200	0.0020	0.146	0.030	0.0020
5	20	1000	0.00001	200	0.0010	0.123	0.007	-0.0003

Variables	Regression results	
m, b	0.099	-0.001
se_m, se_b	0.007	0.001
r^2, se_y	0.986	0.001
F, df	214	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

			r^2=	0.986
Blank	0.116		m=	0.099
			b=	-0.001

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/mL Prep.	average mg/mL	known QC Concentration
11540-6A	0.359	0.243	0.023	200	1	1	0.115	0.113	0.11
11540-6A	0.352	0.236	0.022	200	1	1	0.111		
11540-6A									
11540-6B	0.145	0.029	0.002	200	1	1	0.009	0.011	0.012
11540-6B	0.153	0.037	0.003	200	1	1	0.013		
11540-6B									
11540-54B	0.190	0.074	0.006	200	1	320	10.1	10.2	
11540-54B	0.187	0.071	0.006	200	1	320	9.6		
11540-54B	0.195	0.079	0.007	200	1	320	10.9		
11345-24H	0.362	0.246	0.023	200	1	50	5.8	5.9	
11345-24H	0.378	0.262	0.025	200	1	50	6.2		
11345-24H	0.359	0.243	0.023	200	1	50	5.7		
11345-24I	0.140	0.024	0.001	200	1	600	4.1	3.9	
11345-24I	0.138	0.022	0.001	200	1	600	3.5		
11345-24I	0.140	0.024	0.001	200	1	600	4.1		

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	3

Microsome Dilution Details		
Dilution A	0.25	mL microsome Stock used
	80	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	10.2
Protein Concentration (dilution added to assay, mg/mL):	0.031775

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	5.00E-04
3	2.50E-04
4	1.00E-04
5	5.00E-05
6	1.00E-05
7	1.00E-06
8	1.00E-07

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	1	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4119	8238	8240	16480	0.1	174493	9.44	16316	0.0193	1	0.016	15	0.0406
			0.5	2	4121	8242											
	2	2	0.5	1	4045	8090	8181	16362	0.1	174493	9.36	16200	0.0192	1	0.016	15	0.0403
			0.5	2	4136	8272											
	3	2	0.5	1	3904	7808	7847	15694	0.1	174493	8.99	15532	0.0184	1	0.016	15	0.0386
			0.5	2	3943	7886											
	4	2	0.5	1	3917	7834	7755	15510	0.1	174493	8.89	15348	0.0182	1	0.016	15	0.0382
			0.5	2	3838	7676											
Background control	1	2	0.5	1	51	102	94	188	0.1	174493	0.11	26	0.0000	1	0.016	15	0.0001
			0.5	2	43	86											
	2	2	0.5	1	39	78	85	170	0.1	174493	0.10	8	0.0000	1	0.016	15	0.0000
			0.5	2	46	92											
	3	2	0.5	1	38	76	78	156	0.1	174493	0.09	-6	0.0000	1	0.016	15	0.0000
			0.5	2	40	80											
	4	2	0.5	1	30	60	67	134	0.1	174493	0.08	-28	0.0000	1	0.016	15	-0.0001
			0.5	2	37	74											
Positive control	1	2	0.5	1	2093	4186	4274	8548	0.1	174493	4.90	8386	0.0099	1	0.016	15	0.0209
			0.5	2	2181	4362											
	2	2	0.5	1	1976	3952	4010	8020	0.1	174493	4.60	7858	0.0093	1	0.016	15	0.0195
			0.5	2	2034	4068											
	3	2	0.5	1	1885	3770	3800	7600	0.1	174493	4.36	7438	0.0089	1	0.016	15	0.0185
			0.5	2	1915	3830											
	4	2	0.5	1	1905	3810	3797	7594	0.1	174493	4.35	7432	0.0088	1	0.016	15	0.0185
			0.5	2	1892	3784											
Negative Control	1	2	0.5	1	4086	8172	8141	16282	0.1	174493	9.33	16120	0.0191	1	0.016	15	0.0401
			0.5	2	4055	8110											
	2	2	0.5	1	4047	8094	8046	16092	0.1	174493	9.22	15930	0.0189	1	0.016	15	0.0396
			0.5	2	3999	7998											
	3	2	0.5	1	3881	7762	7783	15566	0.1	174493	8.92	15404	0.0183	1	0.016	15	0.0383
			0.5	2	3902	7804											
	4	2	0.5	1	3724	7448	7387	14774	0.1	174493	8.47	14612	0.0173	1	0.016	15	0.0363
			0.5	2	3663	7326											
Ref Chem 10	1-1	2	0.5	1	3302	6604	6465	12930	0.1	174493	7.41	12768	0.0151	1	0.016	15	0.0316
			0.5	2	3163	6326											
	1-2	2	0.5	1	3295	6590	6493	12986	0.1	174493	7.44	12824	0.0152	1	0.016	15	0.0319
			0.5	2	3198	6396											
	1-3	2	0.5	1	3196	6392	6436	12872	0.1	174493	7.38	12710	0.0151	1	0.016	15	0.0316
			0.5	2	3240	6480											
	2-1	2	0.5	1	3242	6484	6395	12790	0.1	174493	7.33	12628	0.0150	1	0.016	15	0.0314
			0.5	2	3153	6306											
	2-2	2	0.5	1	3218	6436	6455	12910	0.1	174493	7.40	12748	0.0151	1	0.016	15	0.0317
			0.5	2	3237	6474											
	2-3	2	0.5	1	3207	6414	6412	12824	0.1	174493	7.35	12662	0.0150	1	0.016	15	0.0315
			0.5	2	3205	6410											
	3-1	2	0.5	1	3552	7104	7068	14136	0.1	174493	8.10	13974	0.0166	1	0.016	15	0.0348
			0.5	2	3516	7032											
	3-2	2	0.5	1	3590	7180	7177	14354	0.1	174493	8.23	14192	0.0168	1	0.016	15	0.0353
			0.5	2	3587	7174											
	3-3	2	0.5	1	3480	6960	6889	13778	0.1	174493	7.90	13616	0.0161	1	0.016	15	0.0339
			0.5	2	3409	6818											
	4-1	2	0.5	1	3978	7956	7885	15770	0.1	174493	9.04	15608	0.0185	1	0.016	15	0.0388
			0.5	2	3907	7814											
	4-2	2	0.5	1	3798	7596	7589	15178	0.1	174493	8.70	15016	0.0178	1	0.016	15	0.0373
			0.5	2	3791	7582											
	4-3	2	0.5	1	3680	7360	7359	14718	0.1	174493	8.43	14556	0.0173	1	0.016	15	0.0362
			0.5	2	3679	7358											
	5-1	2	0.5	1	3868	7736	7731	15462	0.1	174493	8.86	15300	0.0181	1	0.016	15	0.0381
			0.5	2	3863	7726											
	5-2	2	0.5	1	3791	7582	7656	15312	0.1	174493	8.78	15150	0.0180	1	0.016	15	0.0377
			0.5	2	3865	7730											
	5-3	2	0.5	1	3733	7466	7606	15212	0.1	174493	8.72	15050	0.0178	1	0.016	15	0.0374
			0.5	2	3873	7746											
	6-1	2	0.5	1	3888	7776	8007	16014	0.1	174493	9.18	15852	0.0188	1	0.016	15	0.0394
			0.5	2	4119	8238											
	6-2	2	0.5	1	3746	7492	7618	15236	0.1	174493	8.73	15074	0.0179	1	0.016	15	0.0375
			0.5	2	3872	7744											
	6-3	2	0.5	1	3827	7654	7727	15454	0.1	174493	8.86	15292	0.0181	1	0.016	15	0.0380
			0.5	2	3900	7800											
	7-1	2	0.5	1	4021	8042	7946	15892	0.1	174493	9.11	15730	0.0186	1	0.016	15	0.0391
			0.5	2	3925	7850											
	7-2	2	0.5	1	3854	7708	7758	15516	0.1	174493	8.89	15354	0.0182	1	0.016	15	0.0382
			0.5	2	3904	7808											
	7-3	2	0.5	1	3842	7684	7688	15376	0.1	174493	8.81	15214	0.0180	1	0.016	15	0.0378
			0.5	2	3846	7692											
	8-1	2	0.5	1	3937	7874	7983	15966	0.1	174493	9.15	15804	0.0187	1	0.016	15	0.0393
			0.5	2	4046	8092											
	8-2	2	0.5	1	3894	7788	7790	15580	0.1	174493	8.93	15418	0.0183	1	0.016	15	0.0383
			0.5	2	3896	7792											
	8-3	2	0.5	1	3787	7574	7619	15239	0.1	174493	8.73	15076	0.0179	1	0.016	15	0.0375
			0.5	2	3832	7664											

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	11540-20	Technician ID	Replicate # 1 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0404	0.0002
Full activity	End	0.0384	0.0003
Full activity	Overall	0.0394	0.0012
Background	Beginning	0.0000	3.1654E-05
Background	End	0.0000	3.86882E-05
Background	Overall	0.0000	5.67117E-05
Positive	Beginning	0.0202	0.0009
Positive	End	0.0185	0.0000
Positive	Overall	0.0193	0.0011
Negative	Beginning	0.0399	0.0003
Negative	End	0.0373	0.0014
Negative	Overall	0.0386	0.0017

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 10	1	1	1.00E-03	-3.00	0.0318
Ref Chem 10	1	2	1.00E-03	-3.00	0.0319
Ref Chem 10	1	3	1.00E-03	-3.00	0.0316
Ref Chem 10	2	1	5.00E-04	-3.30	0.0314
Ref Chem 10	2	2	5.00E-04	-3.30	0.0317
Ref Chem 10	2	3	5.00E-04	-3.30	0.0315
Ref Chem 10	3	1	2.50E-04	-3.60	0.0348
Ref Chem 10	3	2	2.50E-04	-3.60	0.0353
Ref Chem 10	3	3	2.50E-04	-3.60	0.0339
Ref Chem 10	4	1	1.00E-04	-4.00	0.0388
Ref Chem 10	4	2	1.00E-04	-4.00	0.0373
Ref Chem 10	4	3	1.00E-04	-4.00	0.0362
Ref Chem 10	5	1	5.00E-05	-4.30	0.0381
Ref Chem 10	5	2	5.00E-05	-4.30	0.0377
Ref Chem 10	5	3	5.00E-05	-4.30	0.0374
Ref Chem 10	6	1	1.00E-05	-5.00	0.0394
Ref Chem 10	6	2	1.00E-05	-5.00	0.0375
Ref Chem 10	6	3	1.00E-05	-5.00	0.0380
Ref Chem 10	7	1	1.00E-06	-6.00	0.0391
Ref Chem 10	7	2	1.00E-06	-6.00	0.0382
Ref Chem 10	7	3	1.00E-06	-6.00	0.0378
Ref Chem 10	8	1	1.00E-07	-7.00	0.0393
Ref Chem 10	8	2	1.00E-07	-7.00	0.0383
Ref Chem 10	8	3	1.00E-07	-7.00	0.0375

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	80.56	80.91	80.19
2	-3.30	79.67	80.43	79.89
3	-3.60	88.17	89.54	85.91
4	-4.00	98.48	94.74	91.84
5	-4.30	96.53	95.59	94.96
6	-5.00	100.02	95.11	96.48
7	-6.00	99.25	96.87	95.99
8	-7.00	99.71	97.28	95.12

Appendix E
Statistical Analysis Report

Report on the Statistical Analysis for WA 4-16, Task 7

1.0 Concentration Response Fits for the Reference Chemicals (as described in Section 6.2.1 of the protocol) and Graphical and Analysis of Variance Comparisons Among Concentration Response Curve Fits (as described in Section 6.2.2 of the protocol)

The results of the concentration response model fits for each replicate within each reference chemical were analyzed using an ANOVA model to assess the variability in the concentration response model parameter estimates. The average values, standard errors and 95% confidence intervals, incorporating the replicate-to-replicate components of variation, for each concentration response model parameter (Top(T), Bottom(B), $\log_{10}$IC50(μ) and Slope(β)) and reference chemical are summarized in Table 1. The individual replicate parameter values and their 95% confidence intervals, along with the overall average chemical-level parameter values and their corresponding 95% confidence intervals are shown in Exhibits 1 through 32. Two of the reference chemicals, DBA and ATZ, did not have response model parameter estimates and are not shown in Table 1 or in the graphical exhibits.

Table 1. Summary Statistics for Response Model Parameters by Chemical

Chemical	Dependent	Mean	Standard Error	Lower 95% CI	Upper 95% CI
AG	Top	99.12492	1.57751	95.78075	102.4691
AG	Bottom	-1.85972	1.13561	-4.26711	0.54767
AG	Log10(IC50)	-4.17072	0.01399	-4.20038	-4.14105
AG	Slope	-0.96607	0.02946	-1.02853	-0.90360
KCZ	Top	96.41466	2.27876	91.58391	101.2454
KCZ	Bottom	-1.03624	0.82685	-2.78907	0.71660
KCZ	Log10(IC50)	-6.39491	0.01604	-6.42893	-6.36090
KCZ	Slope	-0.91331	0.02994	-0.97677	-0.84985
PCZ	Top	101.4679	3.39725	94.26607	108.6698
PCZ	Bottom	0.05692	0.37621	-0.74061	0.85446
PCZ	Log10(IC50)	-7.69838	0.03656	-7.77589	-7.62087
PCZ	Slope	-0.96598	0.01897	-1.00619	-0.92578
NYP	Top	98.96943	0.72628	97.42978	100.5091
NYP	Bottom	0.49439	0.63617	-0.85422	1.84300
NYP	Log10(IC50)	-4.60386	0.00828	-4.62142	-4.58630
NYP	Slope	-1.94856	0.05468	-2.06447	-1.83265
FRM	Top	93.06132	5.21029	82.01600	104.1066
FRM	Bottom	0.25634	0.64106	-1.10265	1.61532
FRM	Log10(IC50)	-5.22726	0.02230	-5.27454	-5.17998
FRM	Slope	-0.93144	0.02567	-0.98586	-0.87702
ECZ	Top	98.82528	1.13709	96.41475	101.2358
ECZ	Bottom	0.09514	0.22409	-0.37992	0.57020

Chemical	Dependent	Mean	Standard Error	Lower 95% CI	Upper 95% CI
ECZ	Log10(IC50)	-8.82331	0.01687	-8.85907	-8.78756
ECZ	Slope	-1.05398	0.01719	-1.09041	-1.01755
CYN	Top	93.33085	1.76650	89.58604	97.07566
CYN	Bottom	20.63057	0.79027	18.95528	22.30586
CYN	Log10(IC50)	-5.76713	0.03412	-5.83945	-5.69480
CYN	Slope	-1.39382	0.06546	-1.53258	-1.25506
DCF	Top	99.71240	2.03492	95.39856	104.0262
DCF	Bottom	-4.86033	1.78199	-8.63798	-1.08268
DCF	Log10(IC50)	-4.37915	0.21004	-4.82441	-3.93388
DCF	Slope	-0.65793	0.10547	-0.88151	-0.43435

2.0 Graphical and Analysis of Variance Comparisons of Full Enzyme Activity, Background Activity, Positive and Negative Control Percent of Control Across Reference Chemicals and Replicates (as described in Section 6.2.3 of the protocol)

The adjusted values for aromatase activity were divided by the average of the full enzyme activity control values for the corresponding reference chemical and replicate in order to calculate the percent of control values. Plots of the percent of control values across reference chemical and replicate were produced to visually assess the data for trends. Exhibits 33 through 40 show the percent of control values for each control type across chemicals and replicates. Exhibits 33 through 36 present the reference chemicals along the horizontal axis and show the percent of control values by the replicates and portion types within the graphs. Exhibits 37 through 40 present the reference chemicals and replicates along the horizontal axis and show the percent of control values by portion type within the graphs. Exhibit 37 shows percent of control values for background control that are close to 0%, with a few exceptions, and no discernable pattern in the variation of the percent of control values above and below the 0% line or by portion. The percent of control values for replicate 3 for AG and replicate 2 for KCZ are approximately ±1.5% whereas the rest of the percent of control values are between -0.3% and 0.3%. Exhibit 38 shows percent of control values for full activity control that, for the most part, vary between 90% and 110% of control. There is a discernable pattern that most of the beginning portion values are above 100% of control and most of the end portion values are below 100% control. Exhibit 39 shows percent of control values for negative control that vary between 85% and 110% of control. The same pattern of higher beginning portion values and lower end portion values as seen in the full activity control graph is also evident for the negative control values. Exhibit 40 shows percent of control values for positive control that vary between 40% and 60% and also display the pattern of higher beginning portion values and lower end portion values. The same patterns as indicated above for each control type can also be observed in Exhibits 41 through 44 which display the difference of the average beginning percent of control value subtracted from the average end percent of control value within each reference chemical and replicate. The majority of the percent of control differences for background control appear along the

zero percent reference line however, the majority of the percent of control differences for full activity, positive and negative control appear below the zero percent reference line, indicating that the average end percent of control values are smaller than the average beginning percent of control values.

The data for each control type was analyzed by a mixed effects analysis of variance (ANOVA) model. The fixed effects in the model included the reference chemical and portion effects and their interaction term. The random effects in the model included the replicate within reference chemical and portion by replicate within reference chemical interaction term. The ANOVA results for the fixed effects are presented in Table 2 and the results for the random effects are presented in Table 3. Table 2 shows that the portion effect was a significant contributor to the variation in the percent of control variation for each control type. The reference chemical effect was significant only for the negative control type. The reference chemical by portion interaction term was significant for each control type except positive control. To avoid convergence problems with the ANOVA model, the covariance parameters for Table 3 were restricted from going below 1E-30 so the covariance parameter estimates for replicate within reference chemical for background, full activity and negative control are essentially zero.

Table 2. ANOVA Results for Fixed Effects

Control	Effect	Num DF	Den DF	F Value	Pr > F
Background control	Chemical	9	20	0.00	1.0000
Background control	Portion	1	20	3.17	0.0904
Background control	Chemical*Portion	9	20	2.47	0.0444
Full activity control	Chemical	9	20	0.00	1.0000
Full activity control	Portion	1	20	285.17	<.0001
Full activity control	Chemical*Portion	9	20	5.88	0.0005
Negative Control	Chemical	9	20	3.76	0.0065
Negative Control	Portion	1	20	309.61	<.0001
Negative Control	Chemical*Portion	9	20	2.69	0.0315
Positive control	Chemical	9	20	0.32	0.9590
Positive control	Portion	1	20	71.84	<.0001
Positive control	Chemical*Portion	9	20	0.91	0.5370

Table 3. ANOVA Results for Random Effects

Control	Covariance Parameter	Estimate	Standard Error	Z Value	Pr > |Z|
Background control	Replicate(Chemical)	1E-30	.	.	.
Background control	Portion*Replicate(Chemical)	0.1316	0.03131	4.20	<.0001
Background control	Residual	0.01648	0.003009	5.48	<.0001
Full activity control	Replicate(Chemical)	1E-30	.	.	.
Full activity control	Portion*Replicate(Chemical)	2.5680	1.2385	2.07	0.0381
Full activity control	Residual	5.1217	0.9351	5.48	<.0001
Negative Control	Replicate(Chemical)	1E-30	.	.	.
Negative Control	Portion*Replicate(Chemical)	2.0604	1.5220	1.35	0.1758
Negative Control	Residual	7.7862	1.4444	5.39	<.0001
Positive control	Replicate(Chemical)	5.7365	2.7299	2.10	0.0356
Positive control	Portion*Replicate(Chemical)	3.0700	1.6339	1.88	0.0603
Positive control	Residual	3.9403	0.7194	5.48	<.0001

Since the portion by reference chemical interactions were significant for most of the control types, the portion effects within each reference chemical by control type and their standard errors are presented in Table 4. Table 5 presents the significance tests for the portion effects within each reference chemical and control type. There are significant portion effects within chemicals AG and KCZ for the variation in the background control percent of control values. The portion effects within every reference chemical for the negative control are significant. For the full activity and positive control types, every reference chemical except DBA contains significant portion effects. The significant portion effects results within most of the reference chemicals and control types support the evidence of the pattern of higher beginning portion percent of control values in the graphical displays discussed earlier.

Table 4. Portion Effects and Standard Errors within Chemical and Control Type

Control	Effect	Chemical	Portion	Estimate	Standard Error
Background control	Chemical*Portion	AG	Begin	-0.5498	0.2159
Background control	Chemical*Portion	AG	End	0.5498	0.2159
Background control	Chemical*Portion	KCZ	Begin	-0.5224	0.2159
Background control	Chemical*Portion	KCZ	End	0.5224	0.2159
Background control	Chemical*Portion	PCZ	Begin	0.03988	0.2159
Background control	Chemical*Portion	PCZ	End	-0.03988	0.2159
Background control	Chemical*Portion	NYP	Begin	-0.02315	0.2159
Background control	Chemical*Portion	NYP	End	0.02315	0.2159

Control	Effect	Chemical	Portion	Estimate	Standard Error
Background control	Chemical*Portion	DBA	Begin	0.06594	0.2159
Background control	Chemical*Portion	DBA	End	-0.06594	0.2159
Background control	Chemical*Portion	FRM	Begin	0.03988	0.2159
Background control	Chemical*Portion	FRM	End	-0.03988	0.2159
Background control	Chemical*Portion	ECZ	Begin	-0.01649	0.2159
Background control	Chemical*Portion	ECZ	End	0.01649	0.2159
Background control	Chemical*Portion	CYN	Begin	-0.01649	0.2159
Background control	Chemical*Portion	CYN	End	0.01649	0.2159
Background control	Chemical*Portion	DCF	Begin	0.06174	0.2159
Background control	Chemical*Portion	DCF	End	-0.06174	0.2159
Background control	Chemical*Portion	ATZ	Begin	0.06174	0.2159
Background control	Chemical*Portion	ATZ	End	-0.06174	0.2159
Full activity control	Chemical*Portion	AG	Begin	105.34	1.3075
Full activity control	Chemical*Portion	AG	End	94.6615	1.3075
Full activity control	Chemical*Portion	KCZ	Begin	105.36	1.3075
Full activity control	Chemical*Portion	KCZ	End	94.6436	1.3075
Full activity control	Chemical*Portion	PCZ	Begin	106.45	1.3075
Full activity control	Chemical*Portion	PCZ	End	93.5474	1.3075
Full activity control	Chemical*Portion	NYP	Begin	103.06	1.3075
Full activity control	Chemical*Portion	NYP	End	96.9357	1.3075
Full activity control	Chemical*Portion	DBA	Begin	99.2568	1.3075
Full activity control	Chemical*Portion	DBA	End	100.74	1.3075
Full activity control	Chemical*Portion	FRM	Begin	106.45	1.3075
Full activity control	Chemical*Portion	FRM	End	93.5474	1.3075
Full activity control	Chemical*Portion	ECZ	Begin	106.38	1.3075
Full activity control	Chemical*Portion	ECZ	End	93.6166	1.3075
Full activity control	Chemical*Portion	CYN	Begin	106.38	1.3075
Full activity control	Chemical*Portion	CYN	End	93.6166	1.3075
Full activity control	Chemical*Portion	DCF	Begin	105.34	1.3075
Full activity control	Chemical*Portion	DCF	End	94.6576	1.3075
Full activity control	Chemical*Portion	ATZ	Begin	105.34	1.3075
Full activity control	Chemical*Portion	ATZ	End	94.6576	1.3075
Negative Control	Chemical*Portion	AG	Begin	103.09	1.4087
Negative Control	Chemical*Portion	AG	End	90.2630	1.4087

Control	Effect	Chemical	Portion	Estimate	Standard Error
Negative Control	Chemical*Portion	KCZ	Begin	104.10	1.5119
Negative Control	Chemical*Portion	KCZ	End	91.0794	1.4087
Negative Control	Chemical*Portion	PCZ	Begin	105.30	1.4087
Negative Control	Chemical*Portion	PCZ	End	91.6542	1.5119
Negative Control	Chemical*Portion	NYP	Begin	102.58	1.4087
Negative Control	Chemical*Portion	NYP	End	96.1236	1.4087
Negative Control	Chemical*Portion	DBA	Begin	103.73	1.4087
Negative Control	Chemical*Portion	DBA	End	99.2391	1.4087
Negative Control	Chemical*Portion	FRM	Begin	105.30	1.4087
Negative Control	Chemical*Portion	FRM	End	91.6542	1.5119
Negative Control	Chemical*Portion	ECZ	Begin	102.04	1.4087
Negative Control	Chemical*Portion	ECZ	End	88.5283	1.4087
Negative Control	Chemical*Portion	CYN	Begin	102.04	1.4087
Negative Control	Chemical*Portion	CYN	End	88.5283	1.4087
Negative Control	Chemical*Portion	DCF	Begin	101.74	1.4087
Negative Control	Chemical*Portion	DCF	End	91.2358	1.4087
Negative Control	Chemical*Portion	ATZ	Begin	101.74	1.4087
Negative Control	Chemical*Portion	ATZ	End	91.2358	1.4087
Positive control	Chemical*Portion	AG	Begin	52.1718	1.8953
Positive control	Chemical*Portion	AG	End	45.9253	1.8953
Positive control	Chemical*Portion	KCZ	Begin	51.9361	1.8953
Positive control	Chemical*Portion	KCZ	End	46.3131	1.8953
Positive control	Chemical*Portion	PCZ	Begin	50.5839	1.8953
Positive control	Chemical*Portion	PCZ	End	45.7198	1.8953
Positive control	Chemical*Portion	NYP	Begin	49.2120	1.8953
Positive control	Chemical*Portion	NYP	End	45.2748	1.8953
Positive control	Chemical*Portion	DBA	Begin	47.2049	1.8953
Positive control	Chemical*Portion	DBA	End	46.4291	1.8953
Positive control	Chemical*Portion	FRM	Begin	50.5839	1.8953
Positive control	Chemical*Portion	FRM	End	45.7198	1.8953
Positive control	Chemical*Portion	ECZ	Begin	52.5466	1.8953
Positive control	Chemical*Portion	ECZ	End	45.7689	1.8953
Positive control	Chemical*Portion	CYN	Begin	52.5466	1.8953
Positive control	Chemical*Portion	CYN	End	45.7689	1.8953

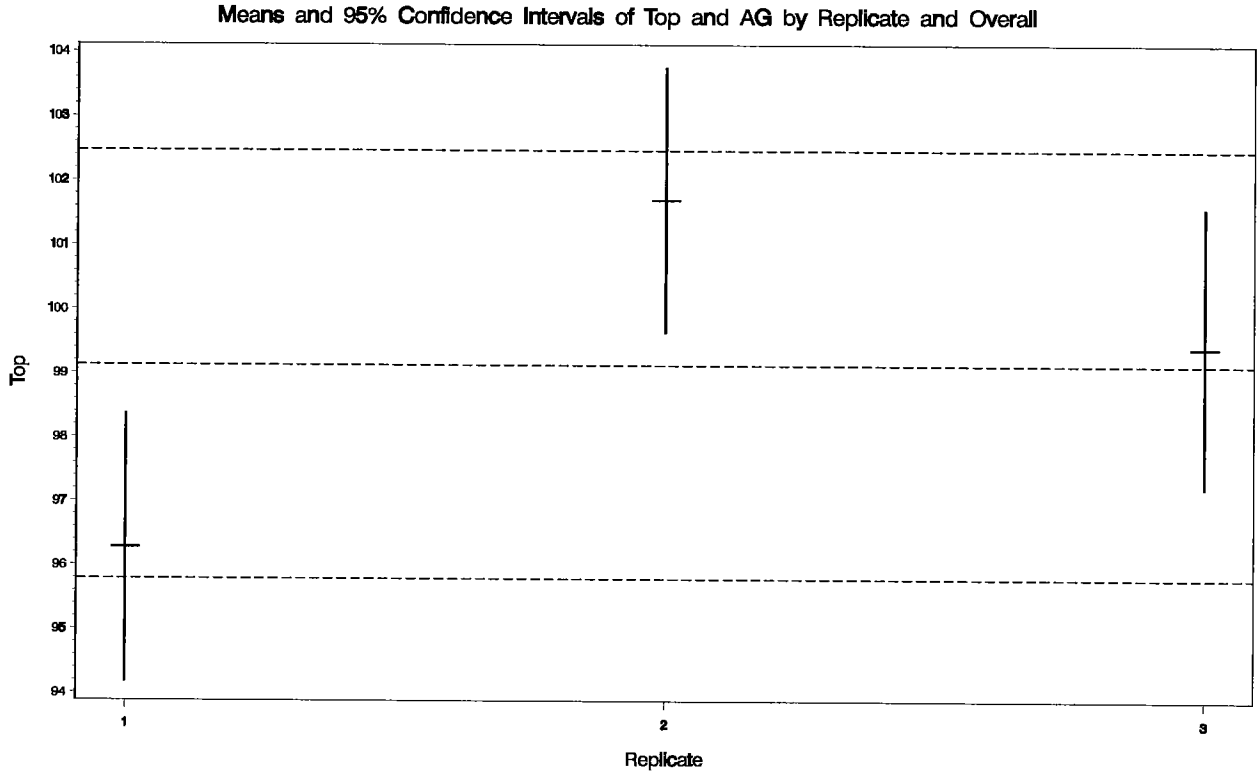
Control	Effect	Chemical	Portion	Estimate	Standard Error
Positive control	Chemical*Portion	DCF	Begin	49.5963	1.8953
Positive control	Chemical*Portion	DCF	End	44.9639	1.8953
Positive control	Chemical*Portion	ATZ	Begin	49.5963	1.8953
Positive control	Chemical*Portion	ATZ	End	44.9639	1.8953

Table 5. Significance Tests for Portion Effects within Chemical and Control Type

Control	Effect	Chemical	Num DF	Den DF	F Value	Pr > F
Background control	Chemical*Portion	AG	1	20	12.97	0.0018
Background control	Chemical*Portion	KCZ	1	20	11.71	0.0027
Background control	Chemical*Portion	PCZ	1	20	0.07	0.7966
Background control	Chemical*Portion	NYP	1	20	0.02	0.8810
Background control	Chemical*Portion	DBA	1	20	0.19	0.6704
Background control	Chemical*Portion	FRM	1	20	0.07	0.7966
Background control	Chemical*Portion	ECZ	1	20	0.01	0.9151
Background control	Chemical*Portion	CYN	1	20	0.01	0.9151
Background control	Chemical*Portion	DCF	1	20	0.16	0.6902
Background control	Chemical*Portion	ATZ	1	20	0.16	0.6902
Full activity control	Chemical*Portion	AG	1	20	33.34	<.0001
Full activity control	Chemical*Portion	KCZ	1	20	33.56	<.0001
Full activity control	Chemical*Portion	PCZ	1	20	48.71	<.0001
Full activity control	Chemical*Portion	NYP	1	20	10.98	0.0035
Full activity control	Chemical*Portion	DBA	1	20	0.65	0.4310
Full activity control	Chemical*Portion	FRM	1	20	48.71	<.0001
Full activity control	Chemical*Portion	ECZ	1	20	47.67	<.0001
Full activity control	Chemical*Portion	CYN	1	20	47.67	<.0001
Full activity control	Chemical*Portion	DCF	1	20	33.39	<.0001
Full activity control	Chemical*Portion	ATZ	1	20	33.39	<.0001
Negative Control	Chemical*Portion	AG	1	20	41.48	<.0001
Negative Control	Chemical*Portion	KCZ	1	20	39.69	<.0001
Negative Control	Chemical*Portion	PCZ	1	20	43.58	<.0001
Negative Control	Chemical*Portion	NYP	1	20	10.49	0.0041
Negative Control	Chemical*Portion	DBA	1	20	5.08	0.0356
Negative Control	Chemical*Portion	FRM	1	20	43.58	<.0001
Negative Control	Chemical*Portion	ECZ	1	20	45.98	<.0001
Negative Control	Chemical*Portion	CYN	1	20	45.98	<.0001
Negative Control	Chemical*Portion	DCF	1	20	27.82	<.0001
Negative Control	Chemical*Portion	ATZ	1	20	27.82	<.0001
Positive control	Chemical*Portion	AG	1	20	11.61	0.0028
Positive control	Chemical*Portion	KCZ	1	20	9.41	0.0061
Positive control	Chemical*Portion	PCZ	1	20	7.04	0.0152
Positive control	Chemical*Portion	NYP	1	20	4.61	0.0442

Control	Effect	Chemical	Num DF	Den DF	F Value	Pr > F
Positive control	Chemical*Portion	DBA	1	20	0.18	0.6767
Positive control	Chemical*Portion	FRM	1	20	7.04	0.0152
Positive control	Chemical*Portion	ECZ	1	20	13.67	0.0014
Positive control	Chemical*Portion	CYN	1	20	13.67	0.0014
Positive control	Chemical*Portion	DCF	1	20	6.39	0.0200
Positive control	Chemical*Portion	ATZ	1	20	6.39	0.0200

Exhibit 1.



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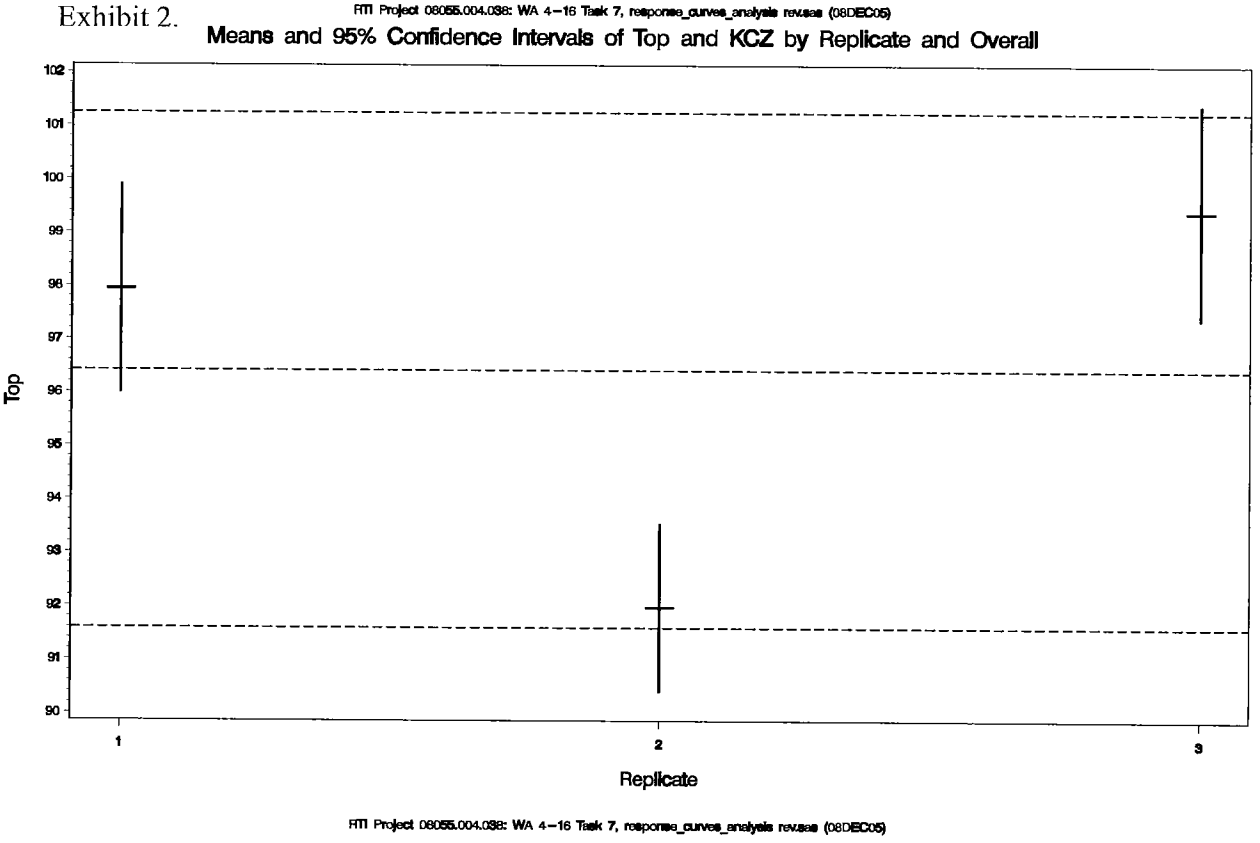

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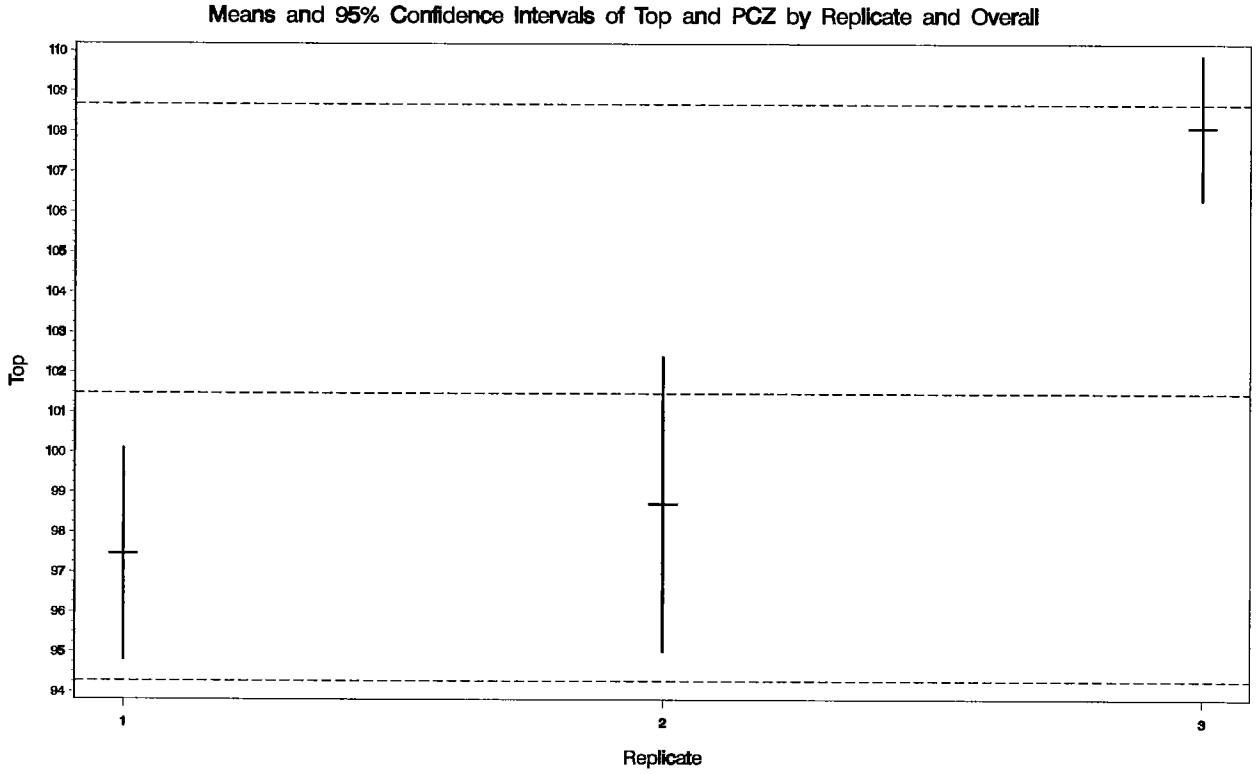

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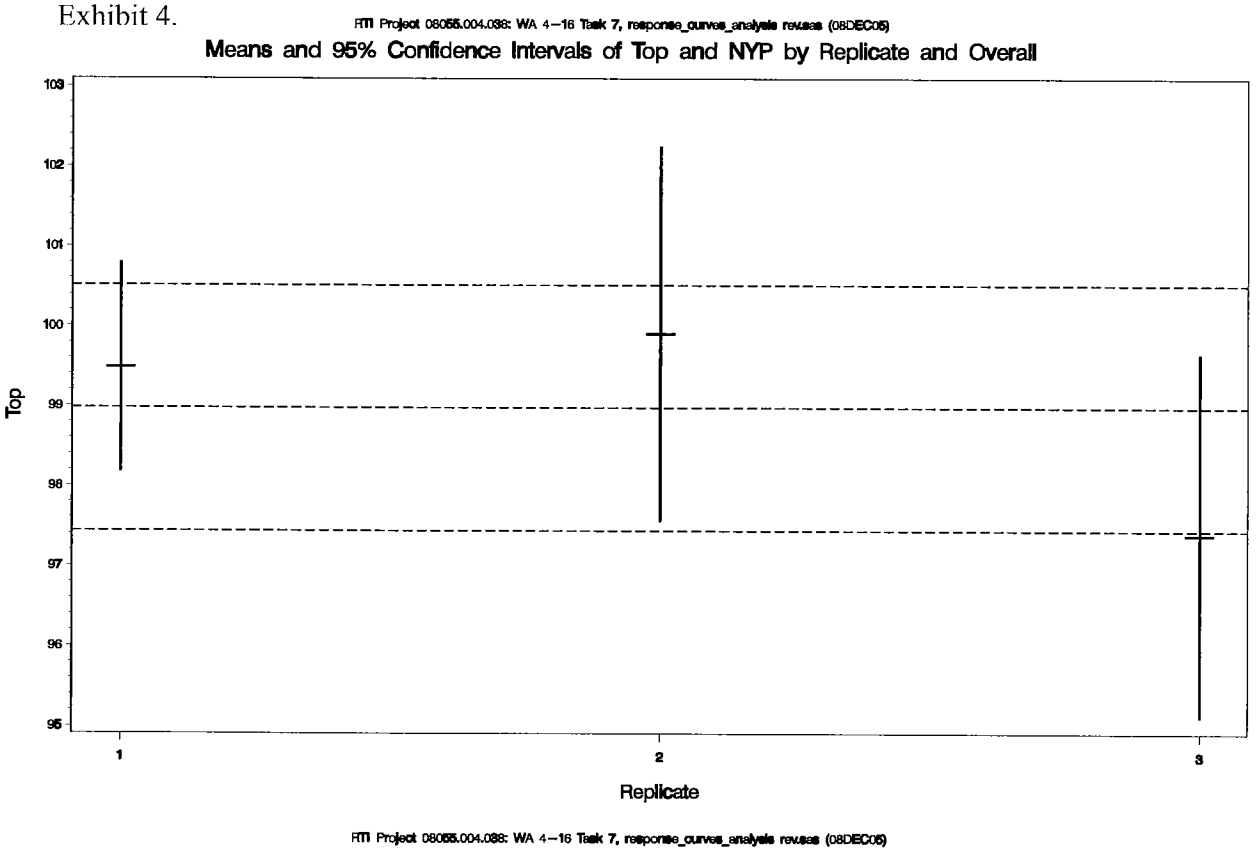

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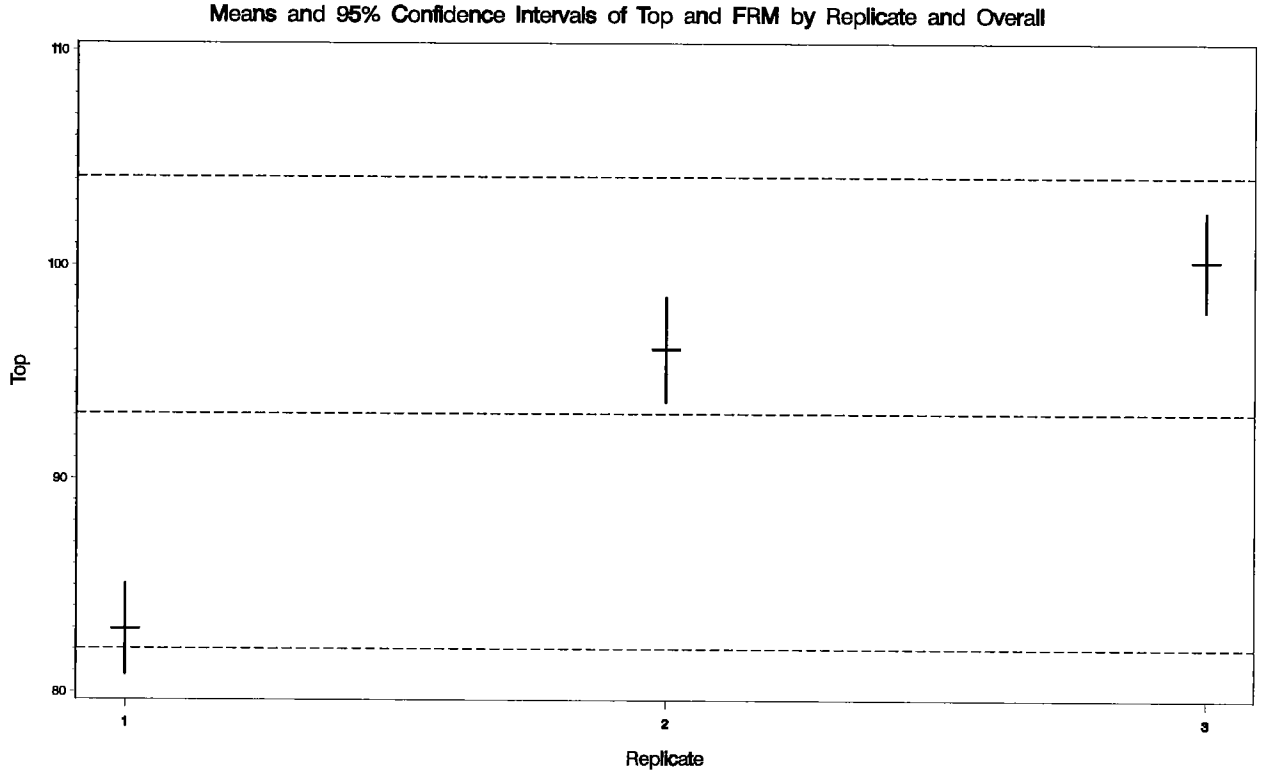

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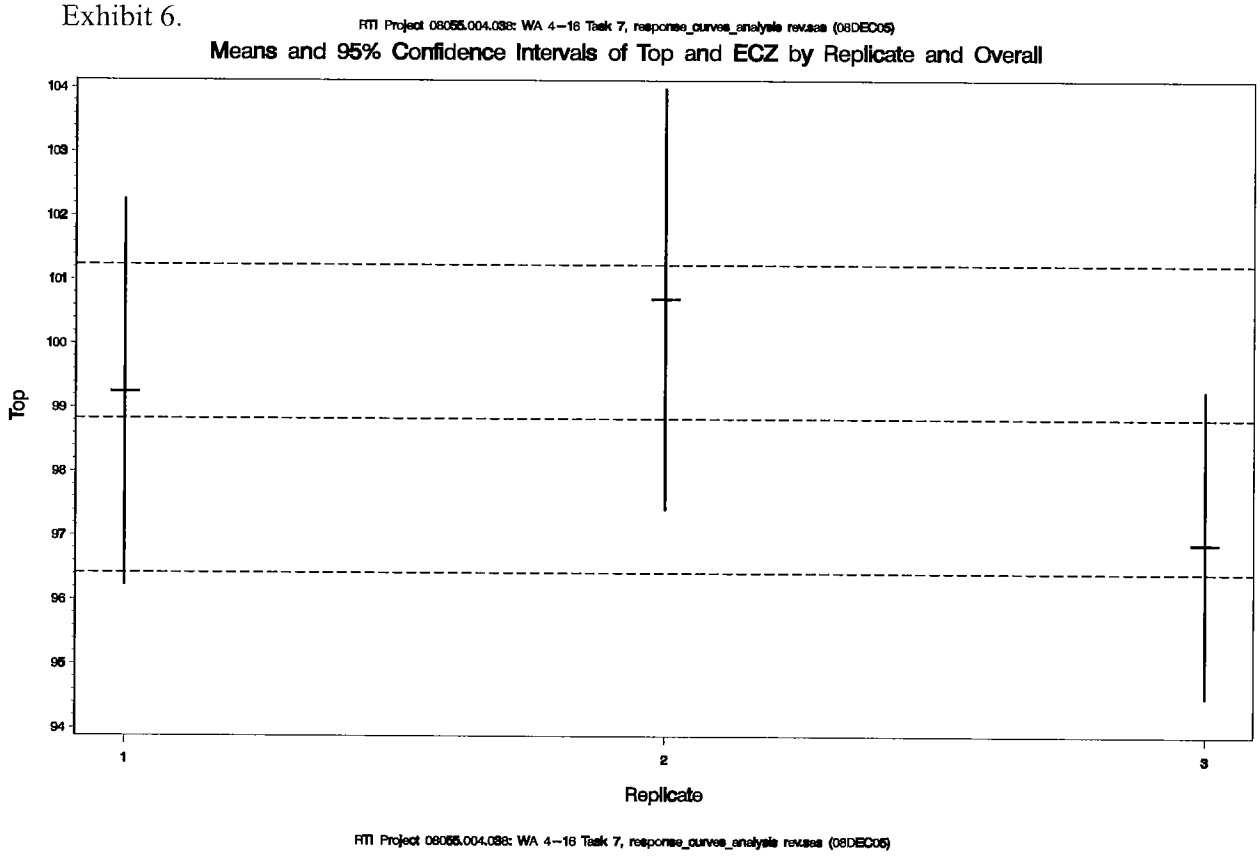

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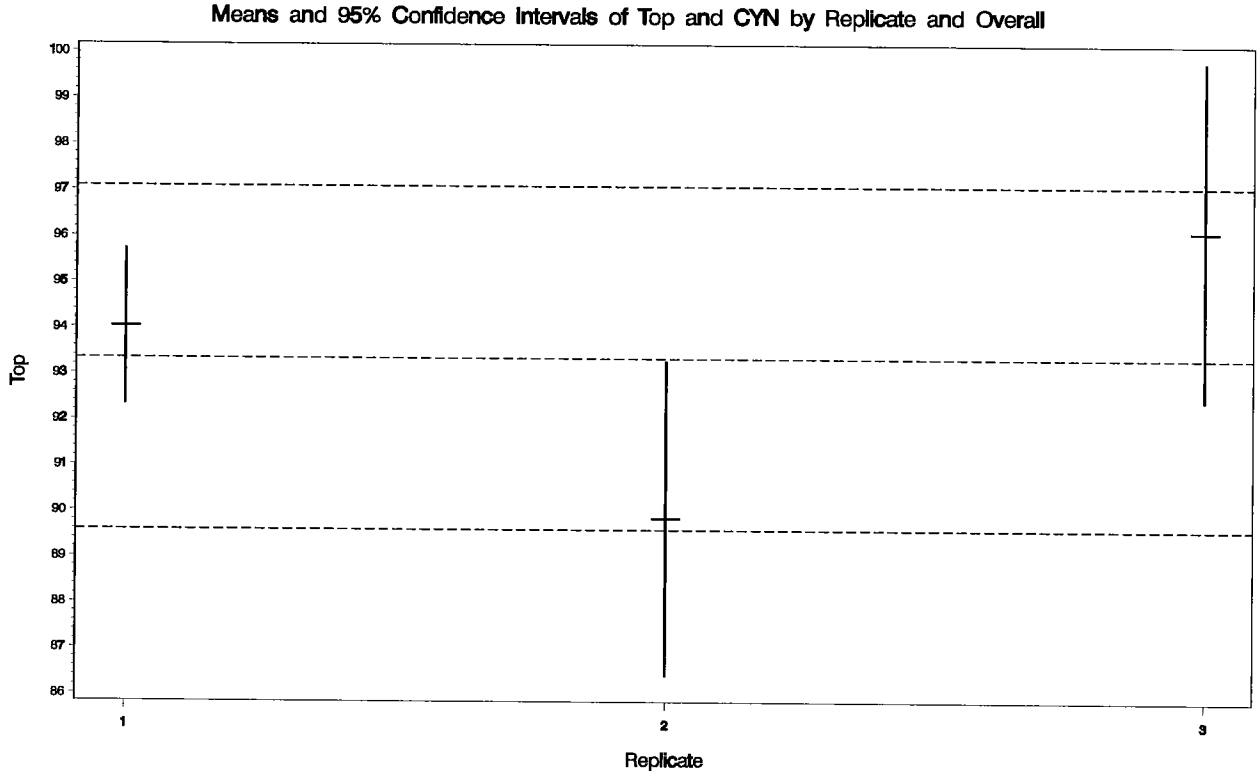

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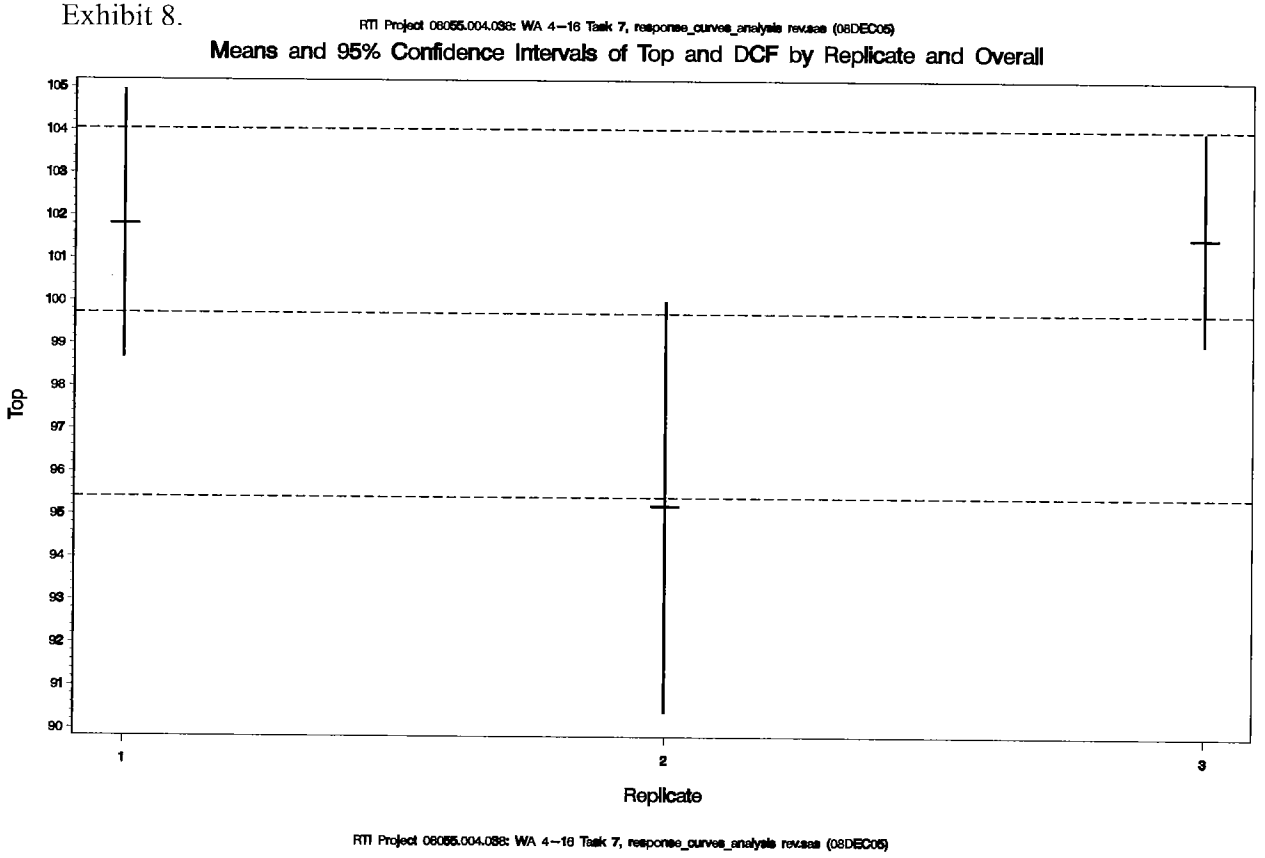

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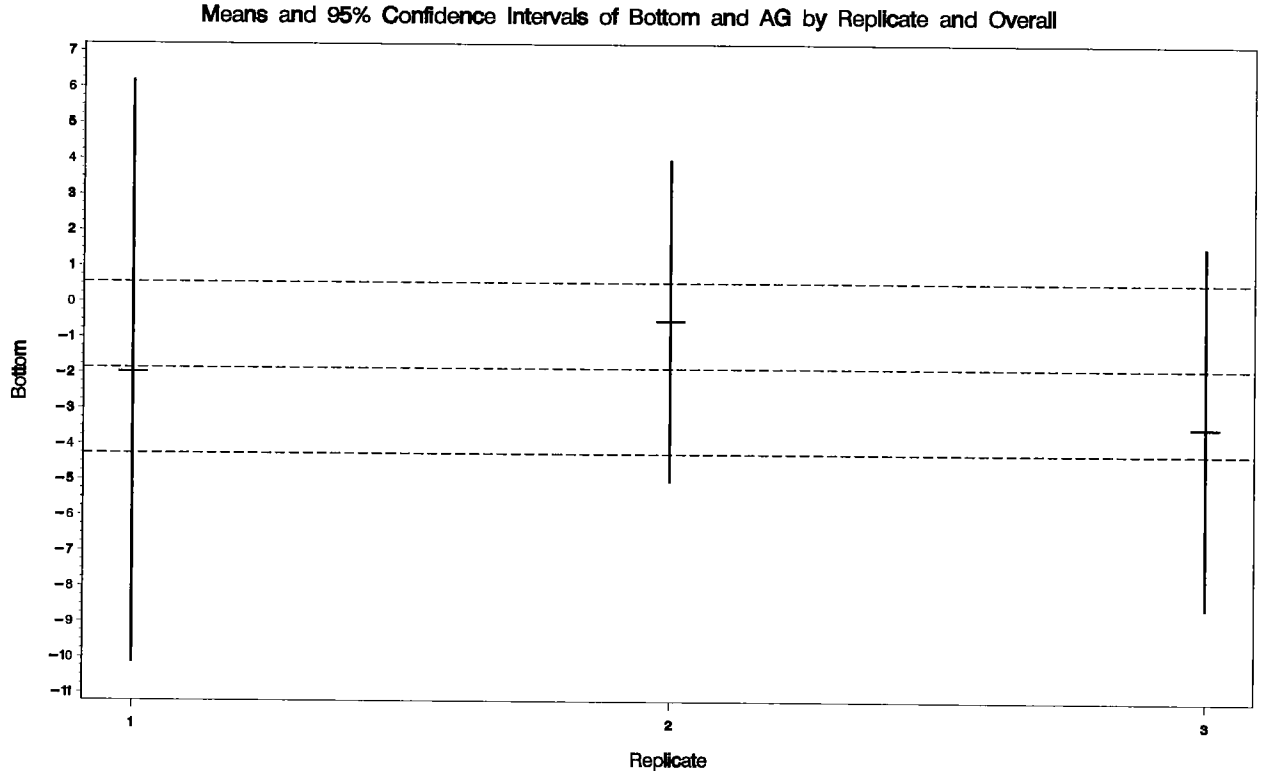
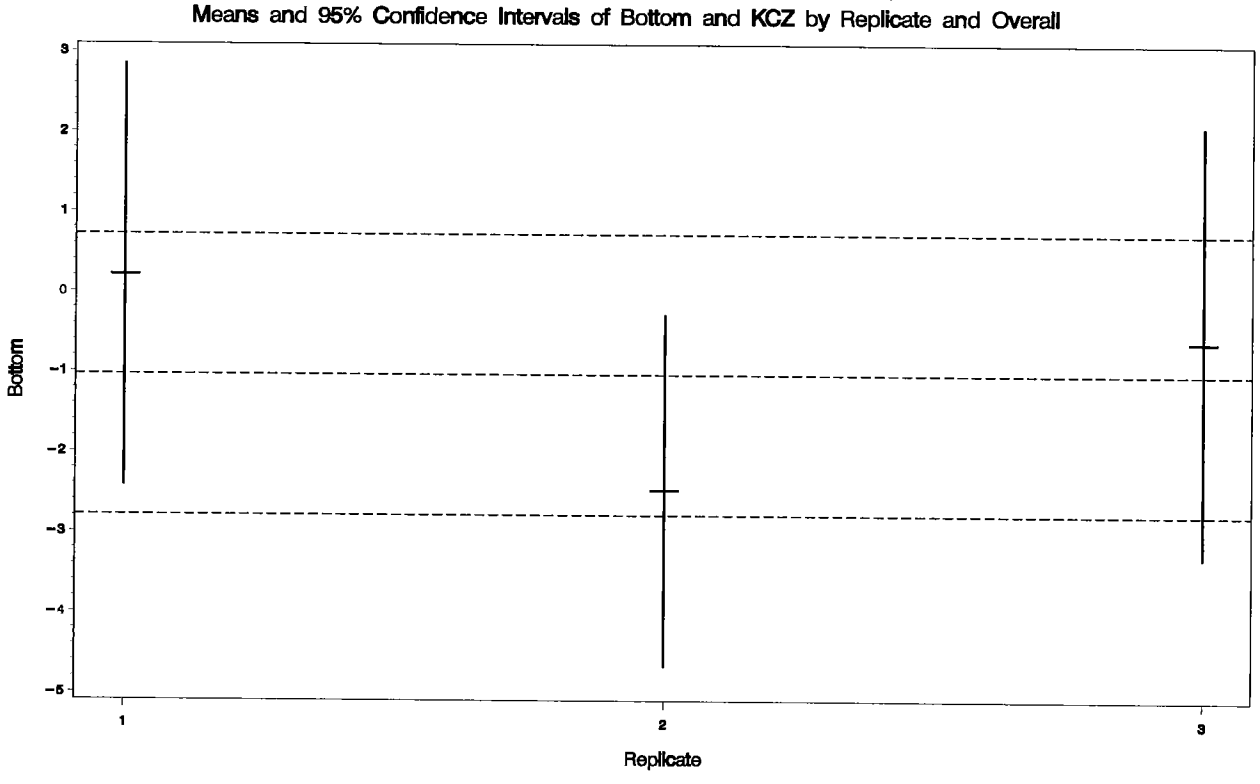


Exhibit 10.



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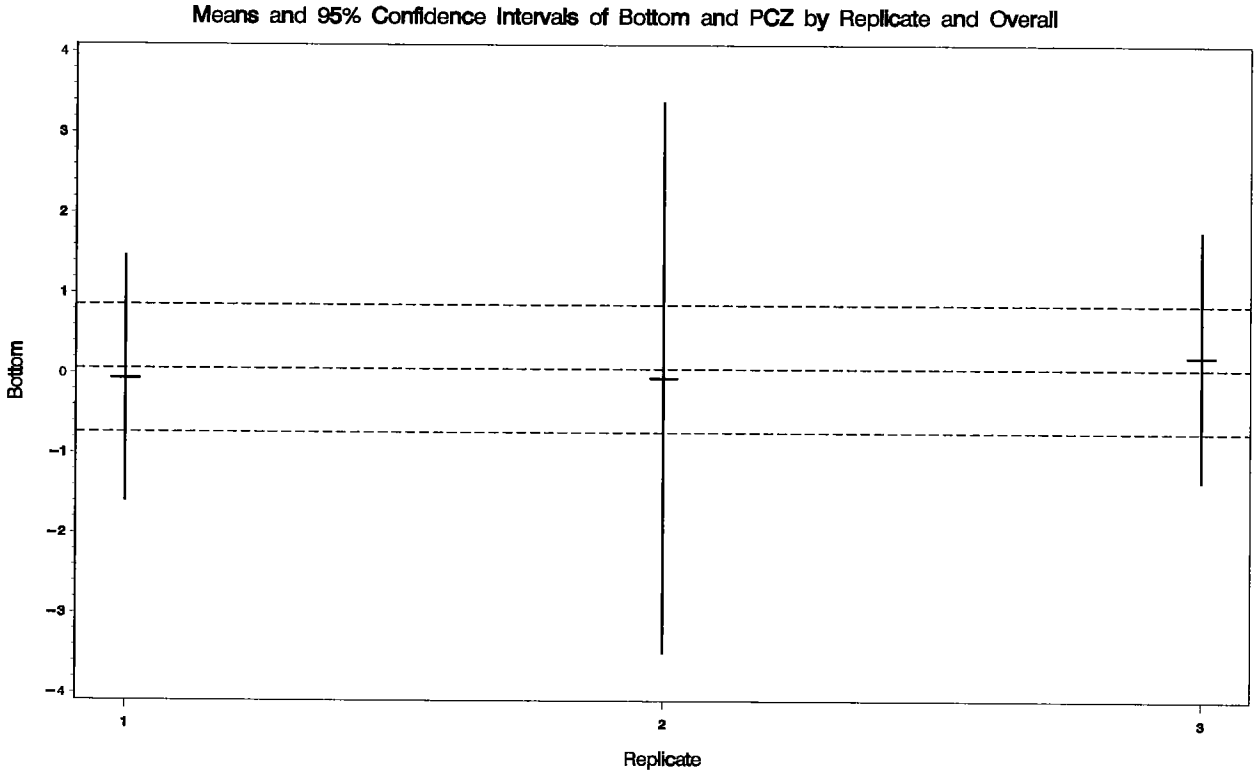

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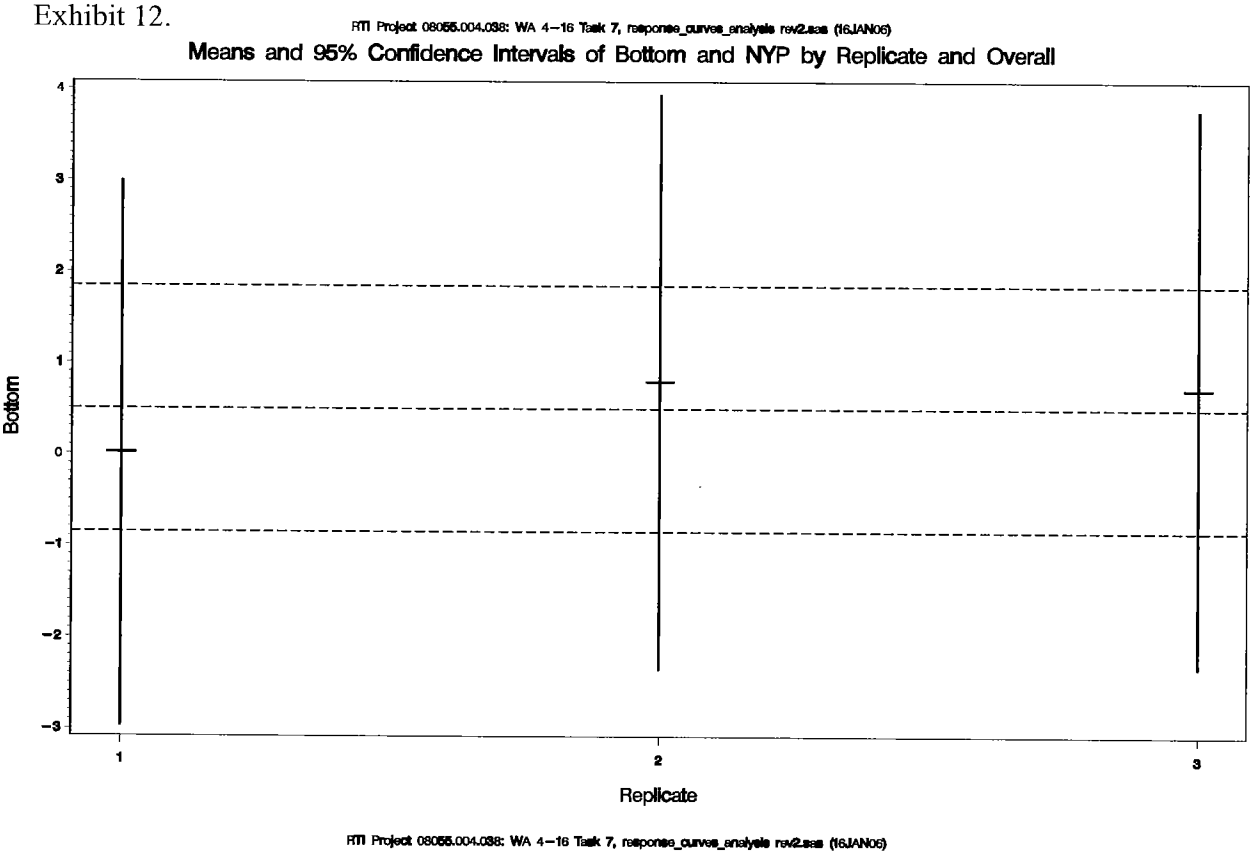

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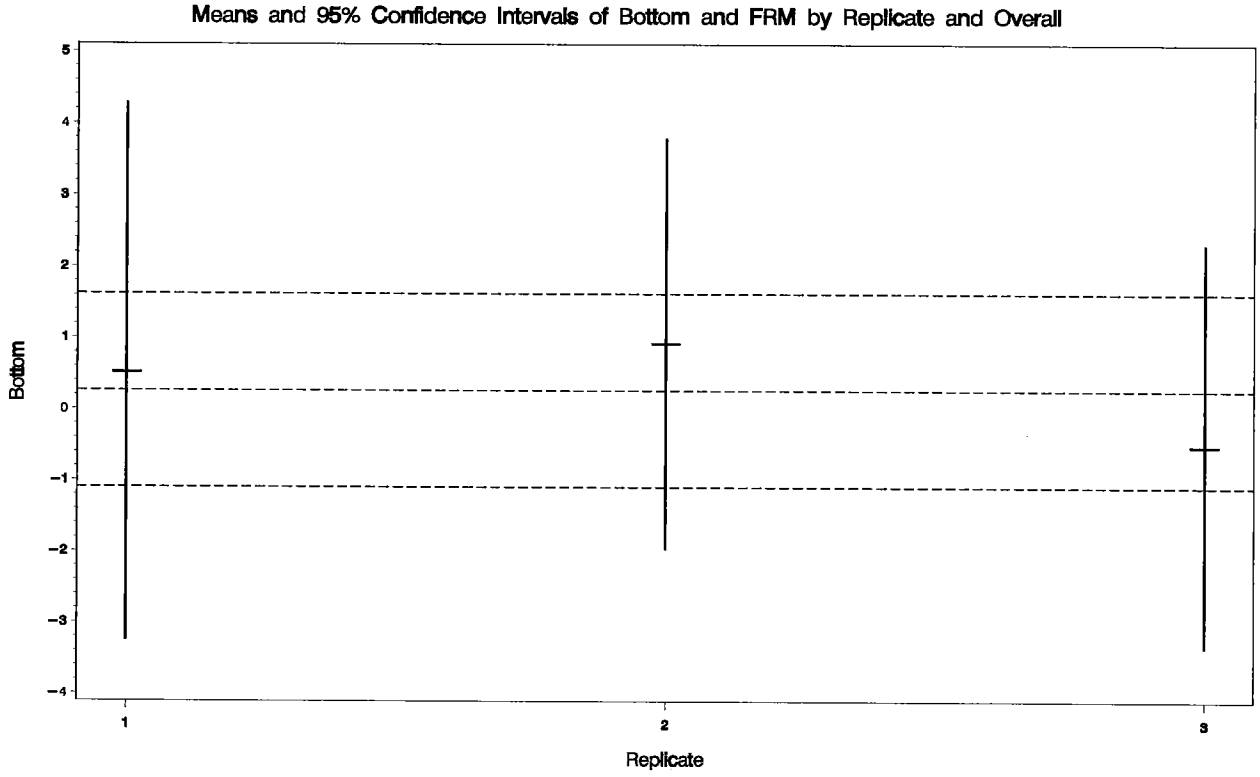

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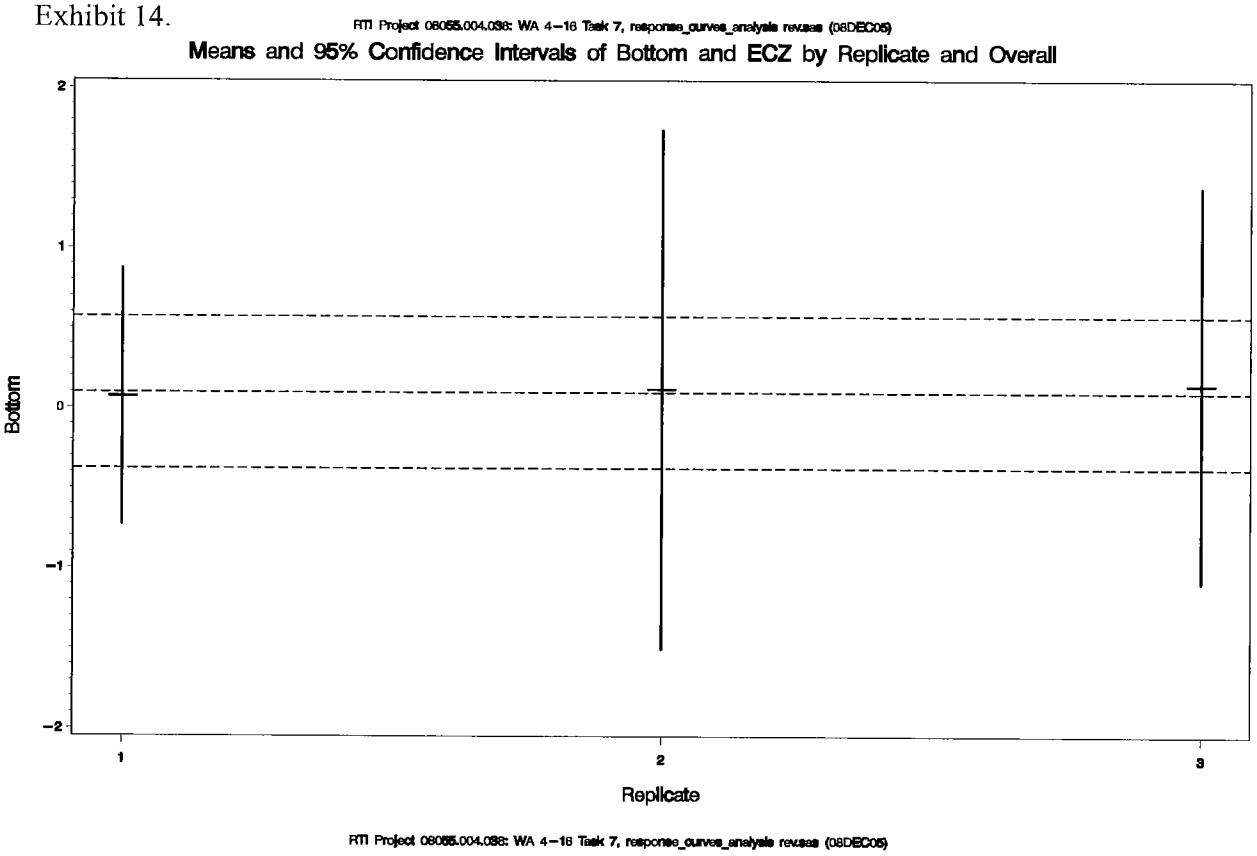

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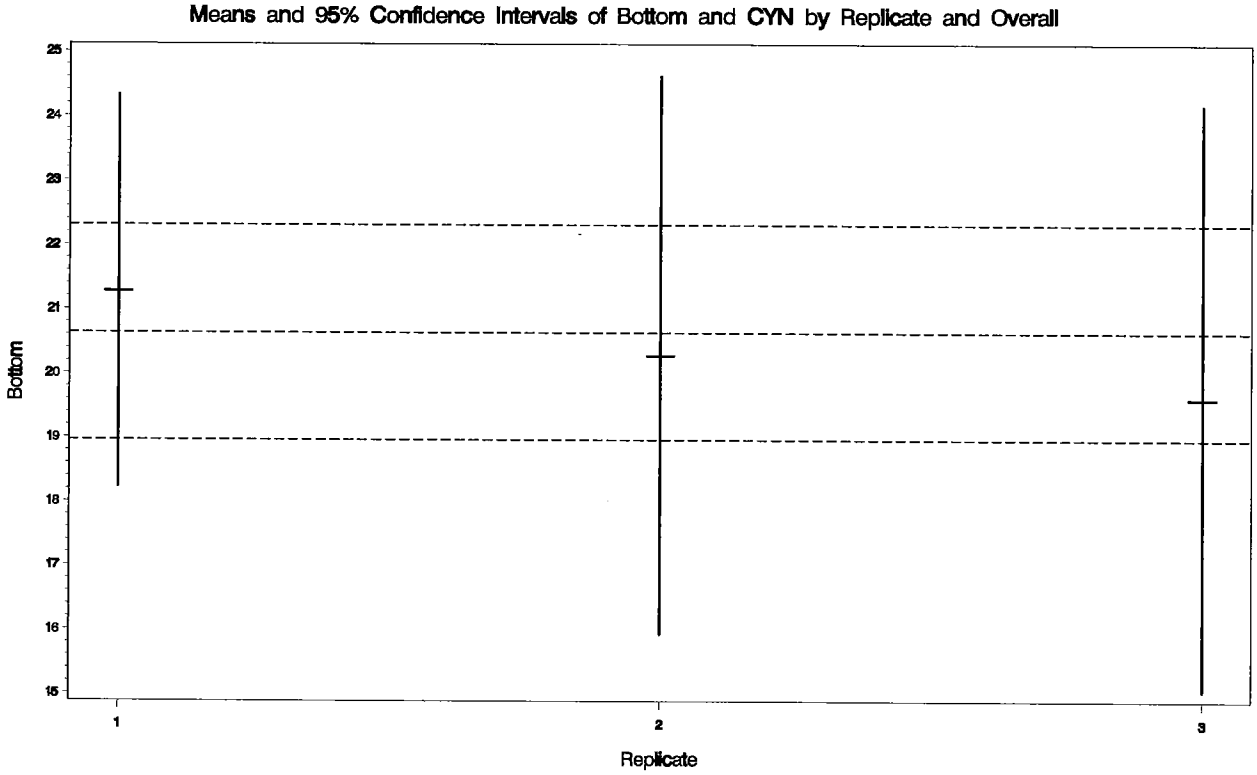

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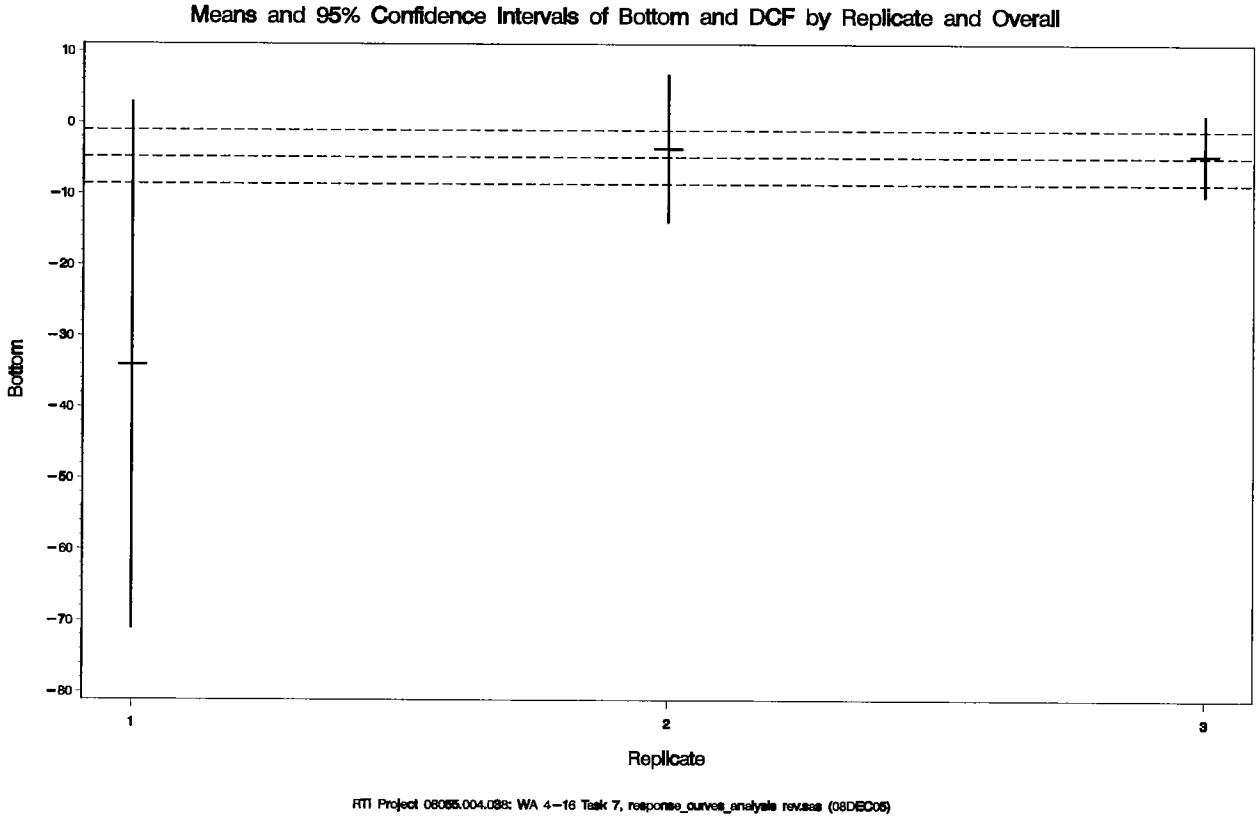

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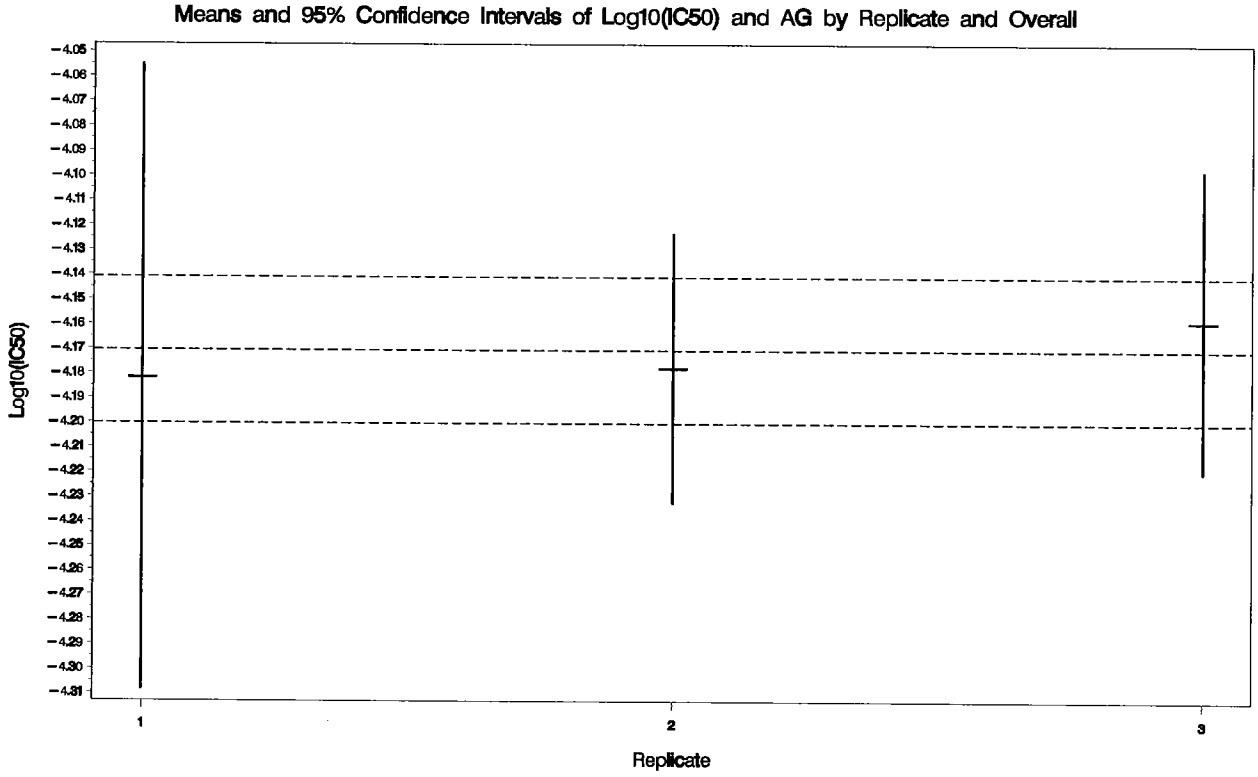

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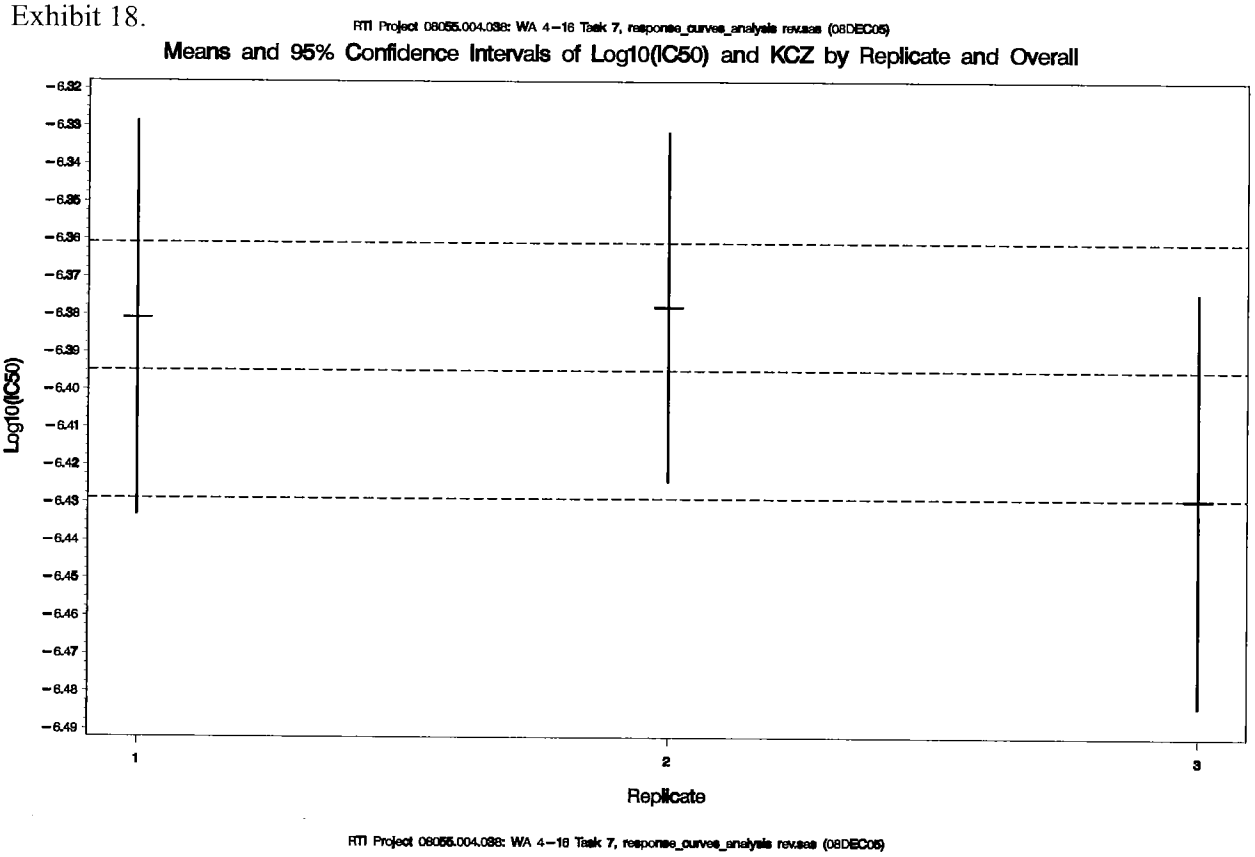

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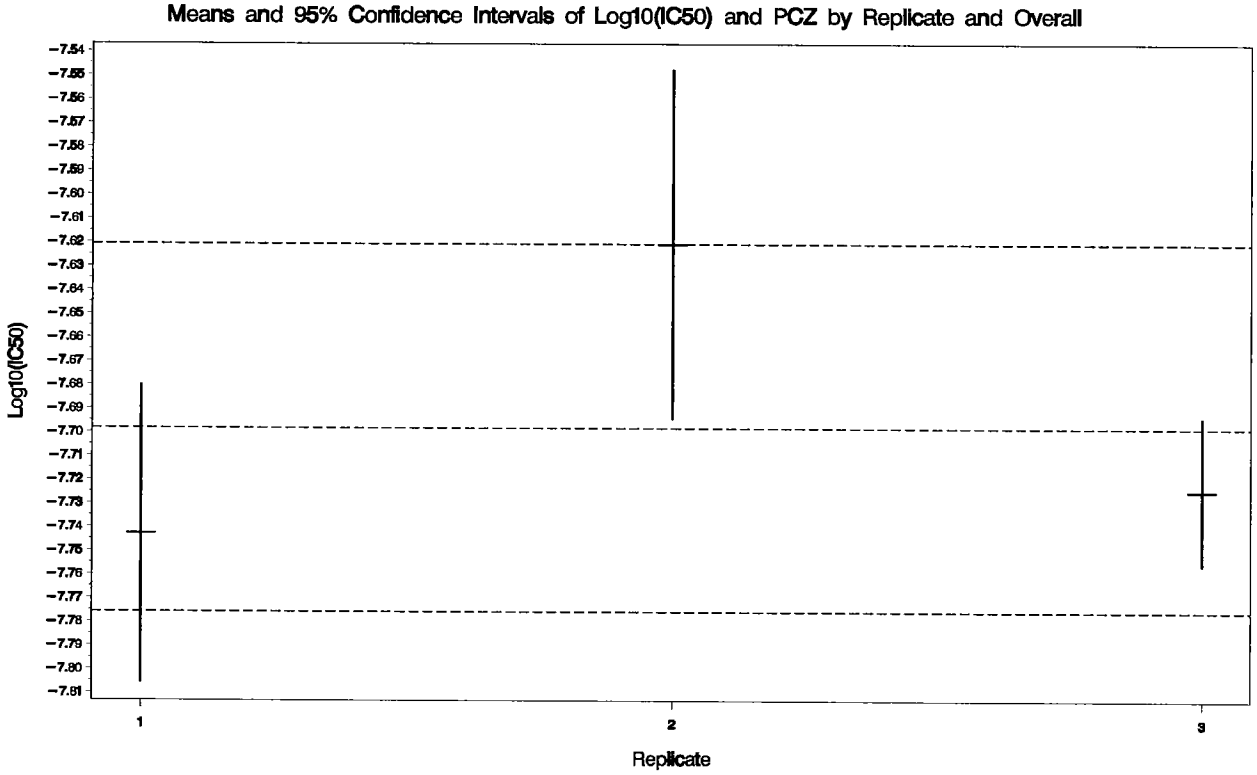

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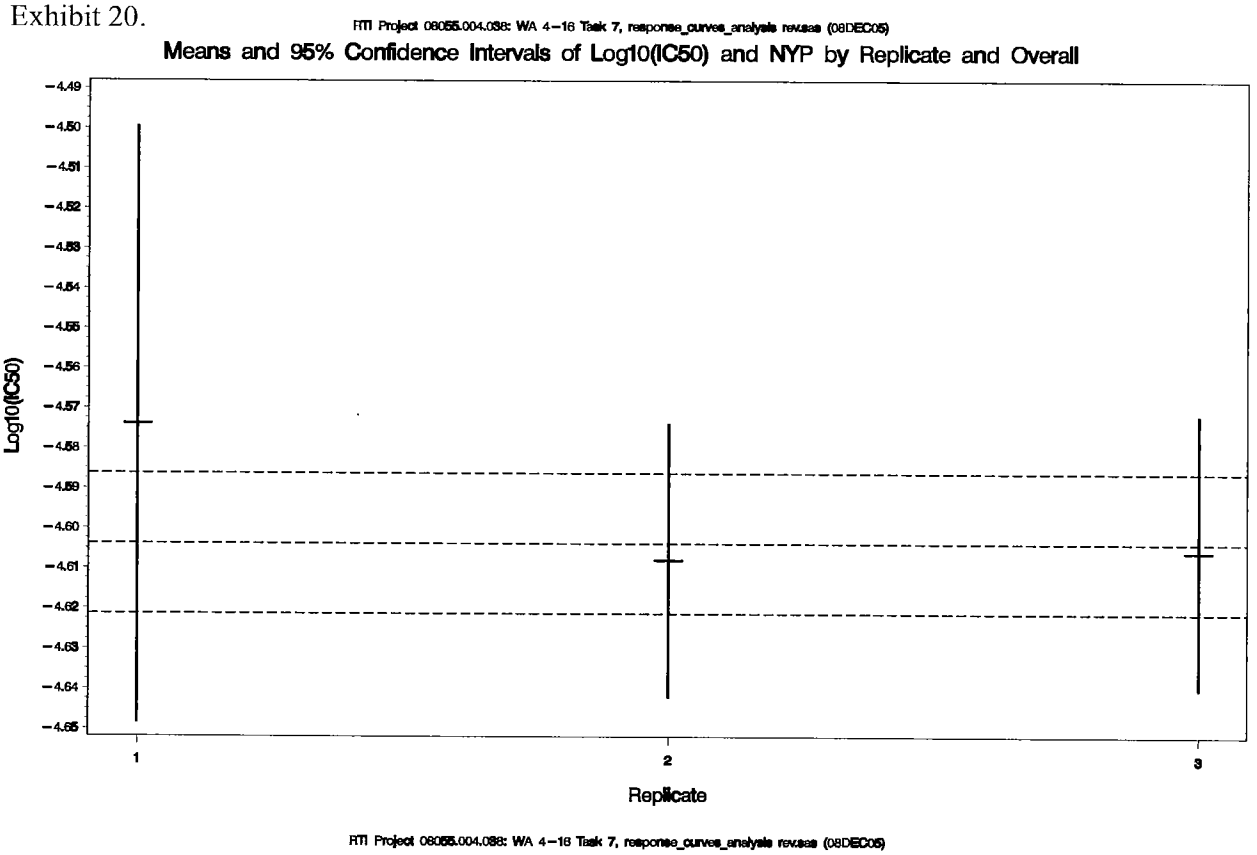

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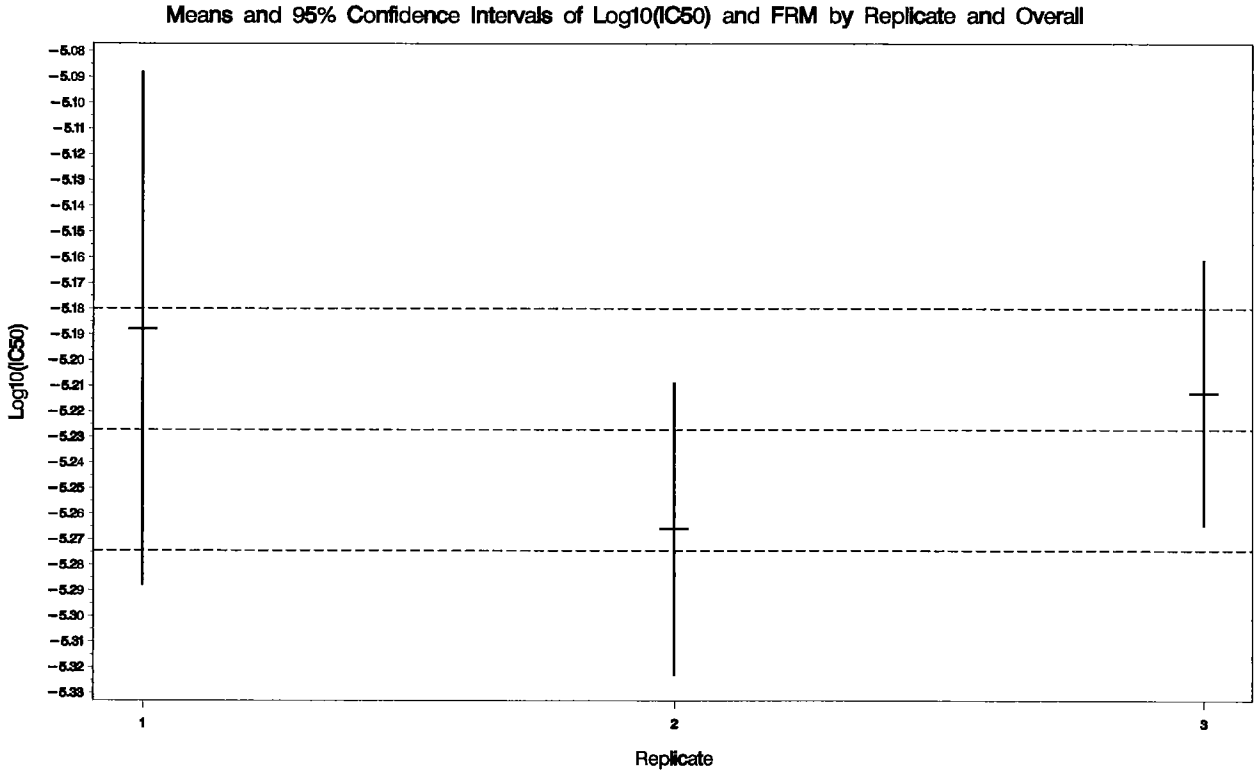

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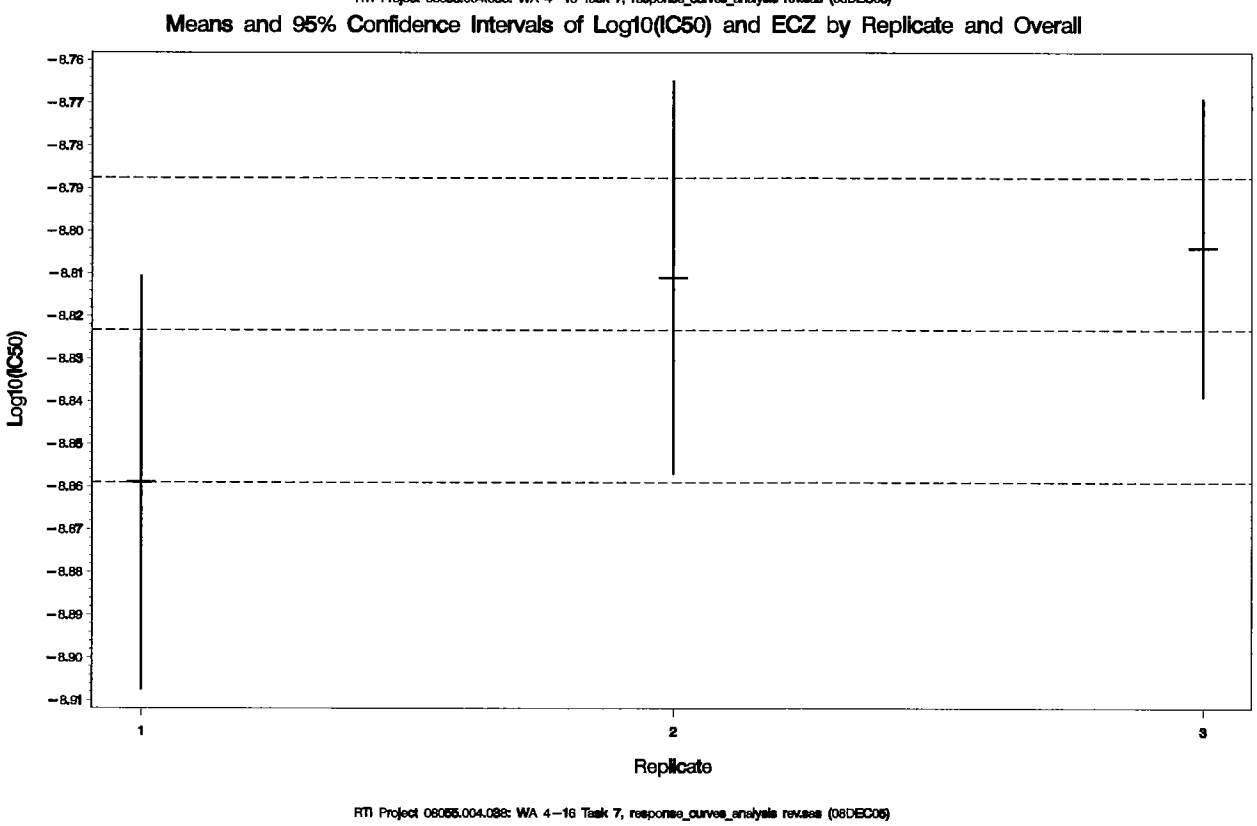


Exhibit 23.

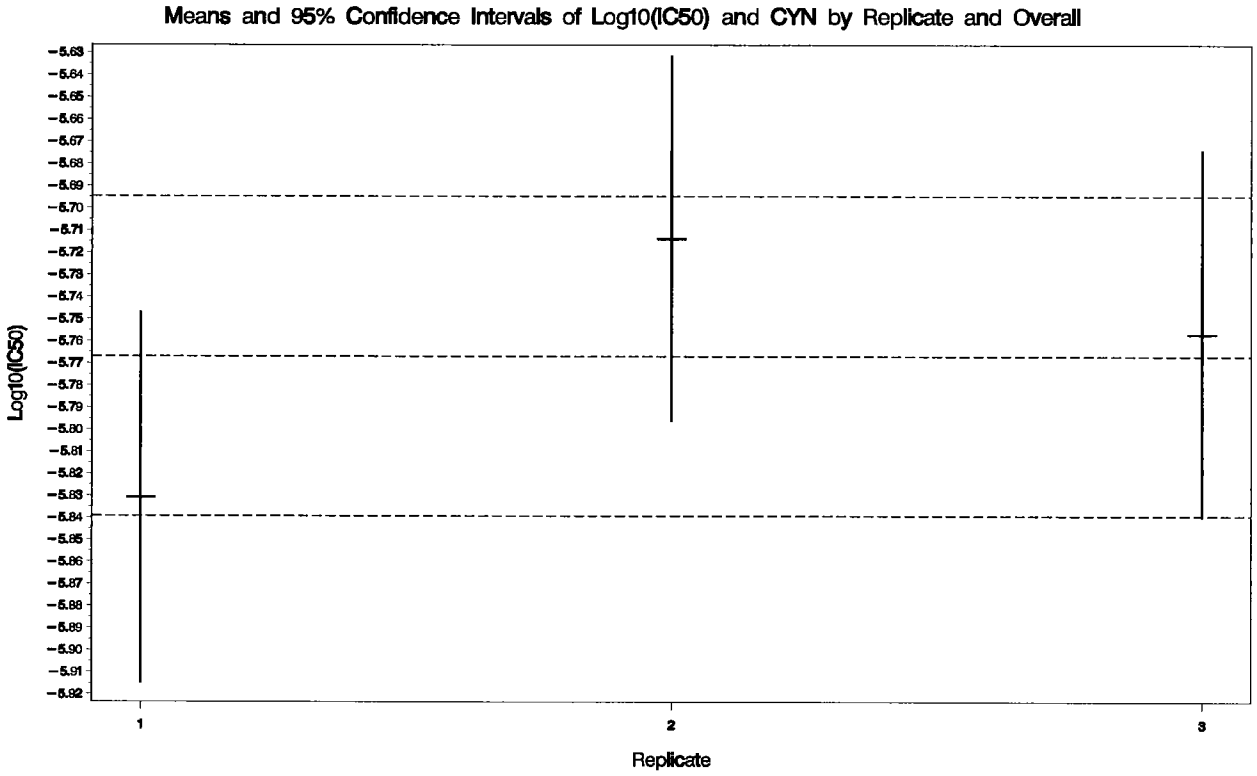


Exhibit 24.

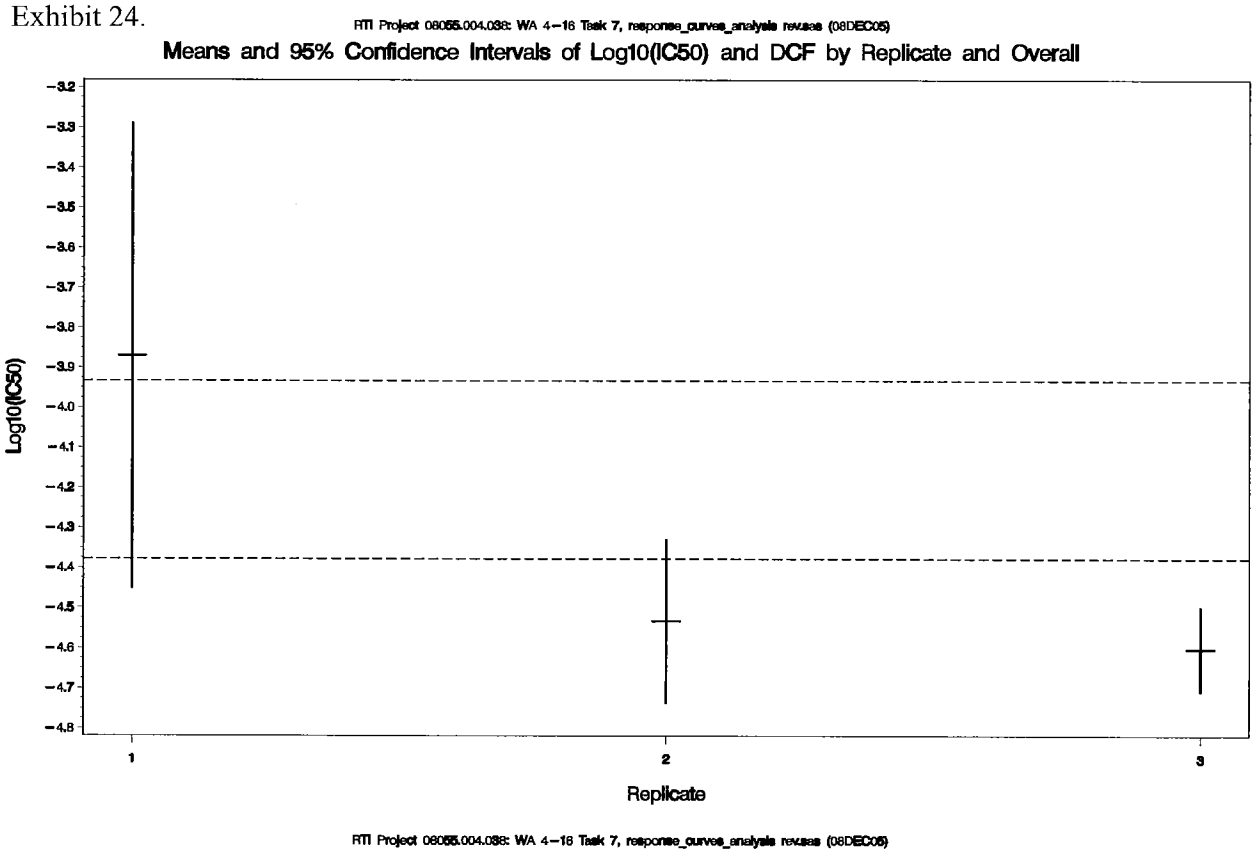


Exhibit 25.

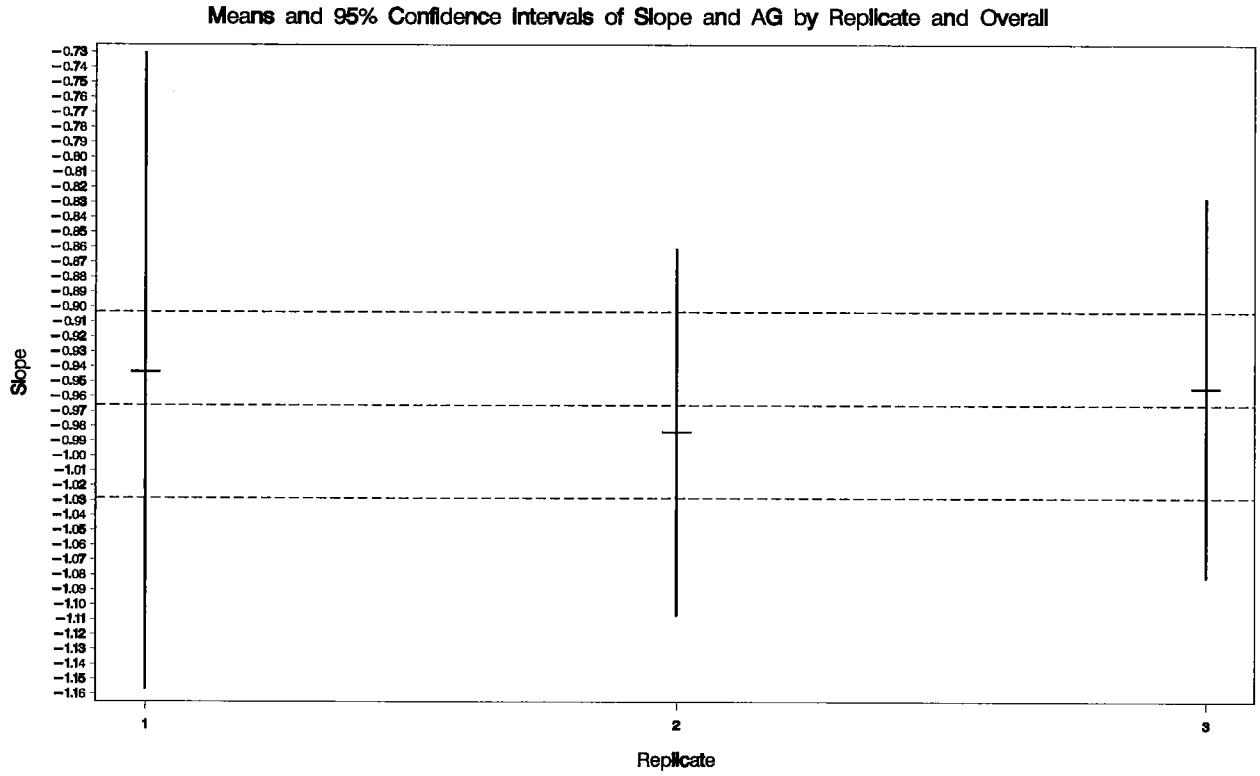


Exhibit 26.

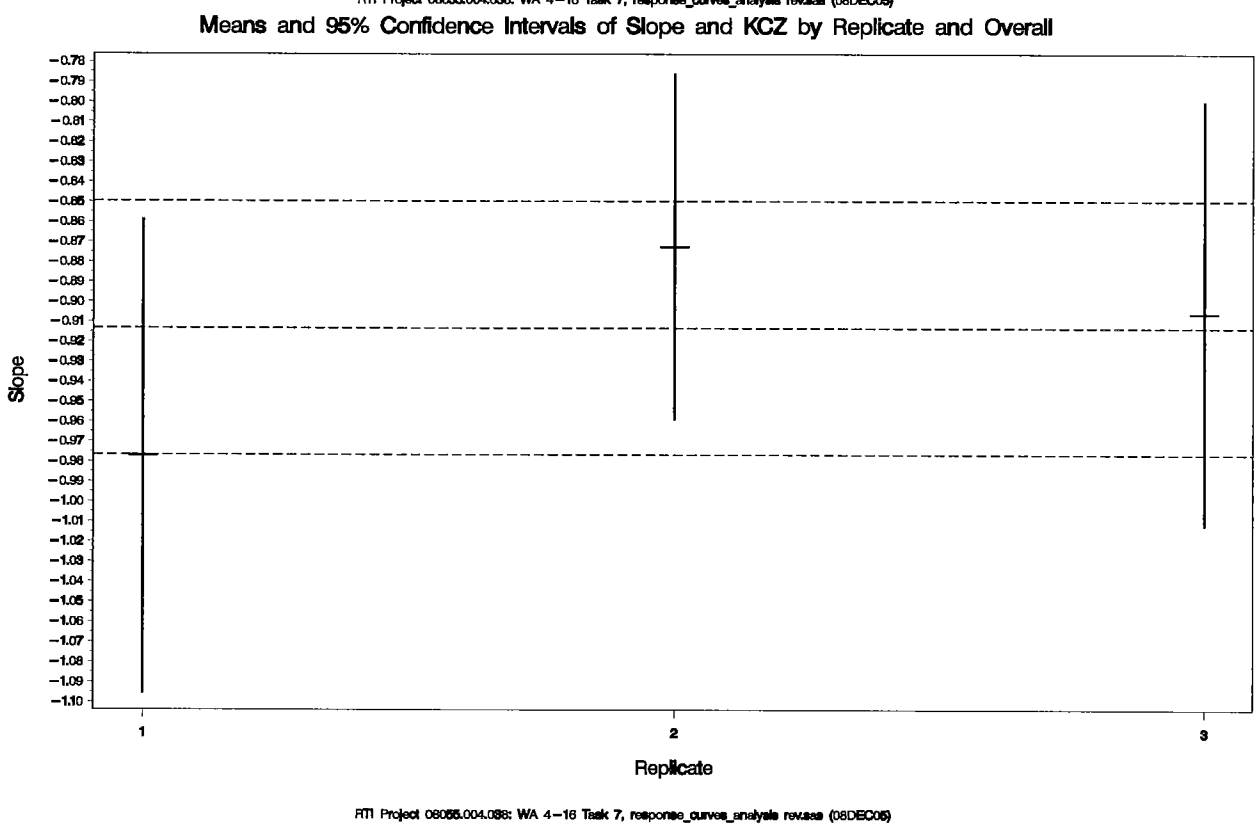


Exhibit 27.

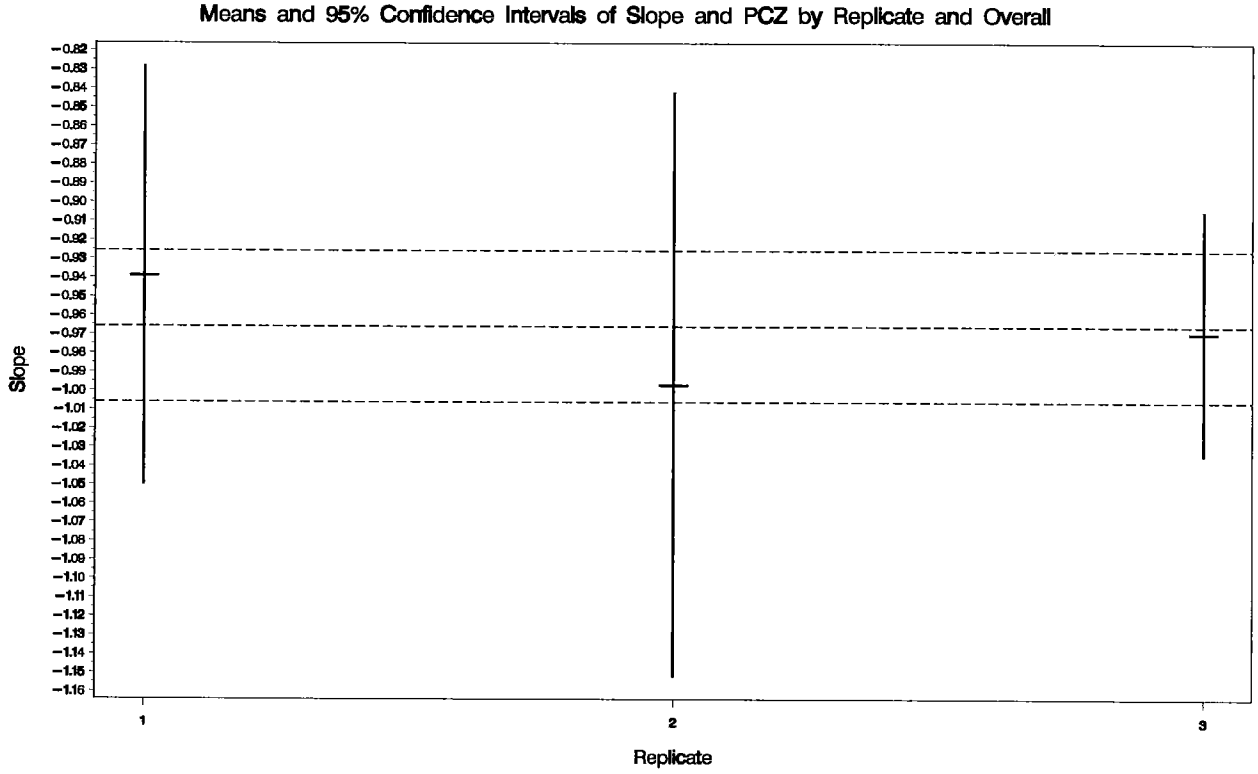
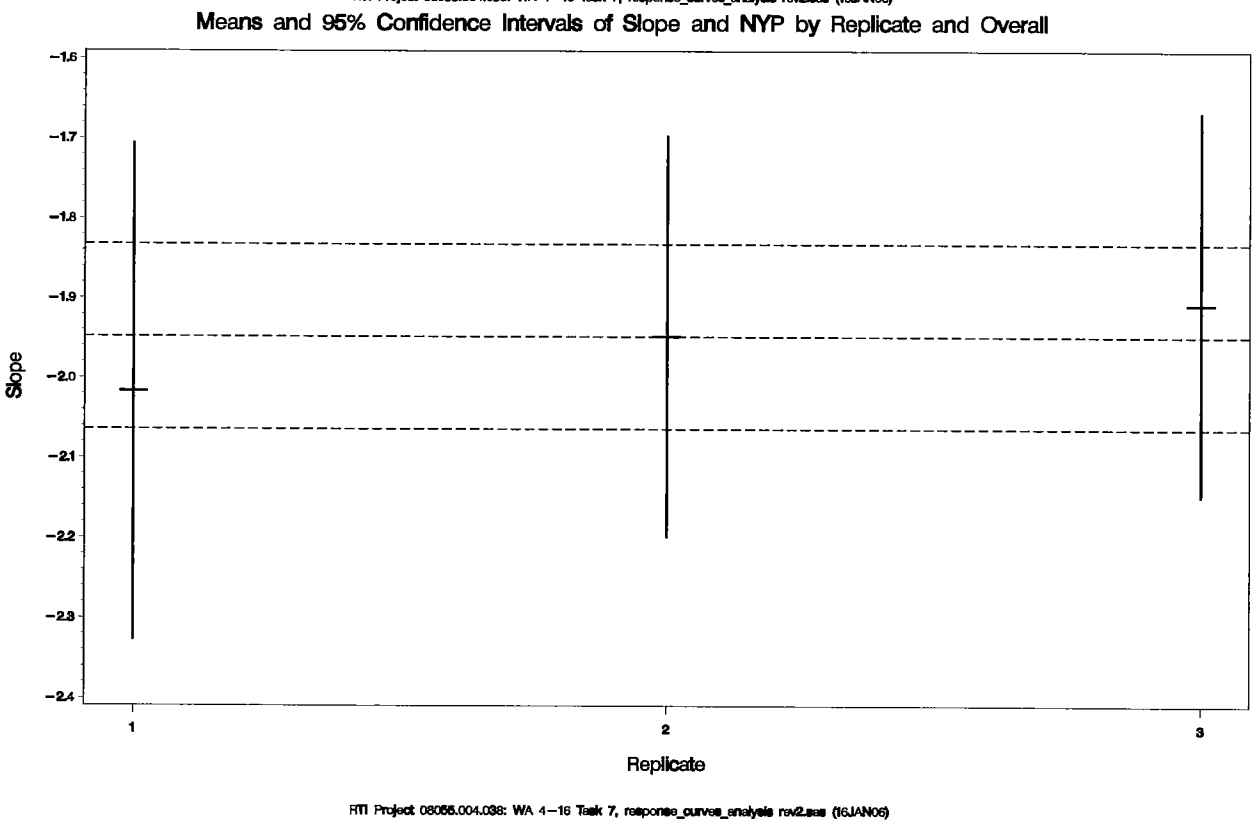


Exhibit 28.



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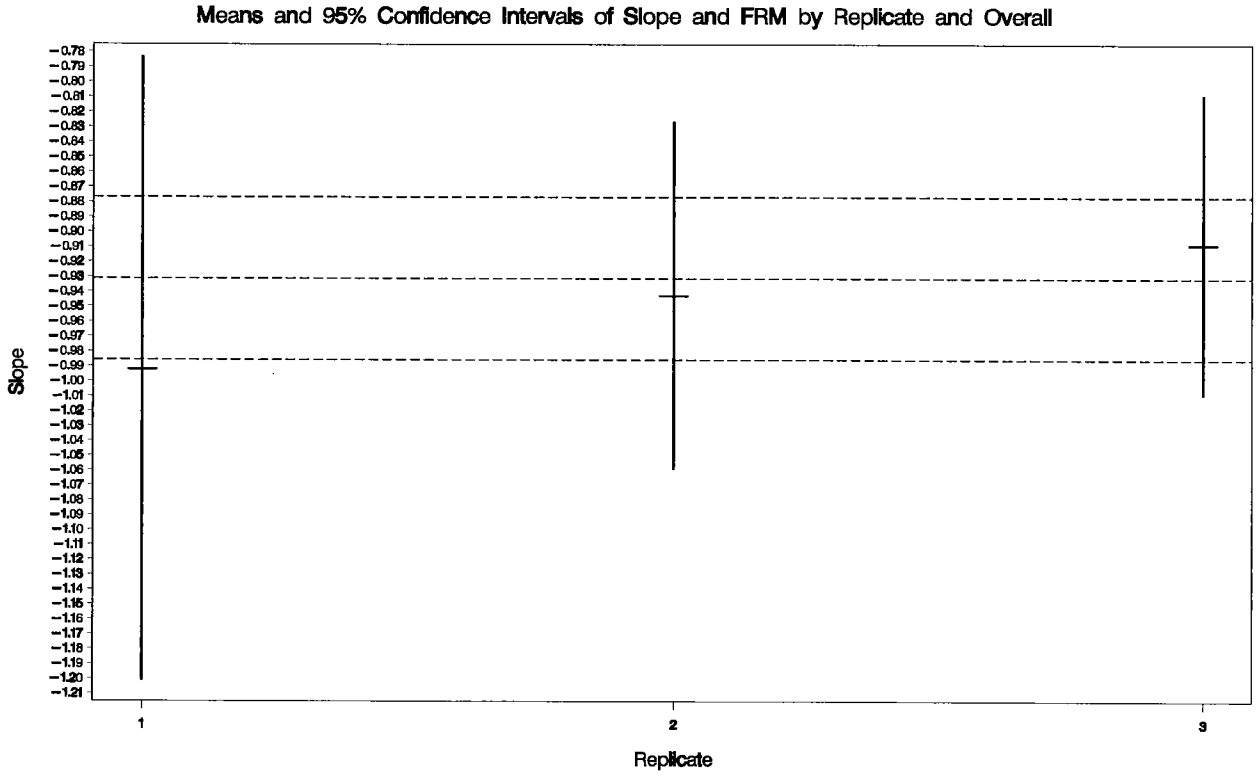


Exhibit 30.

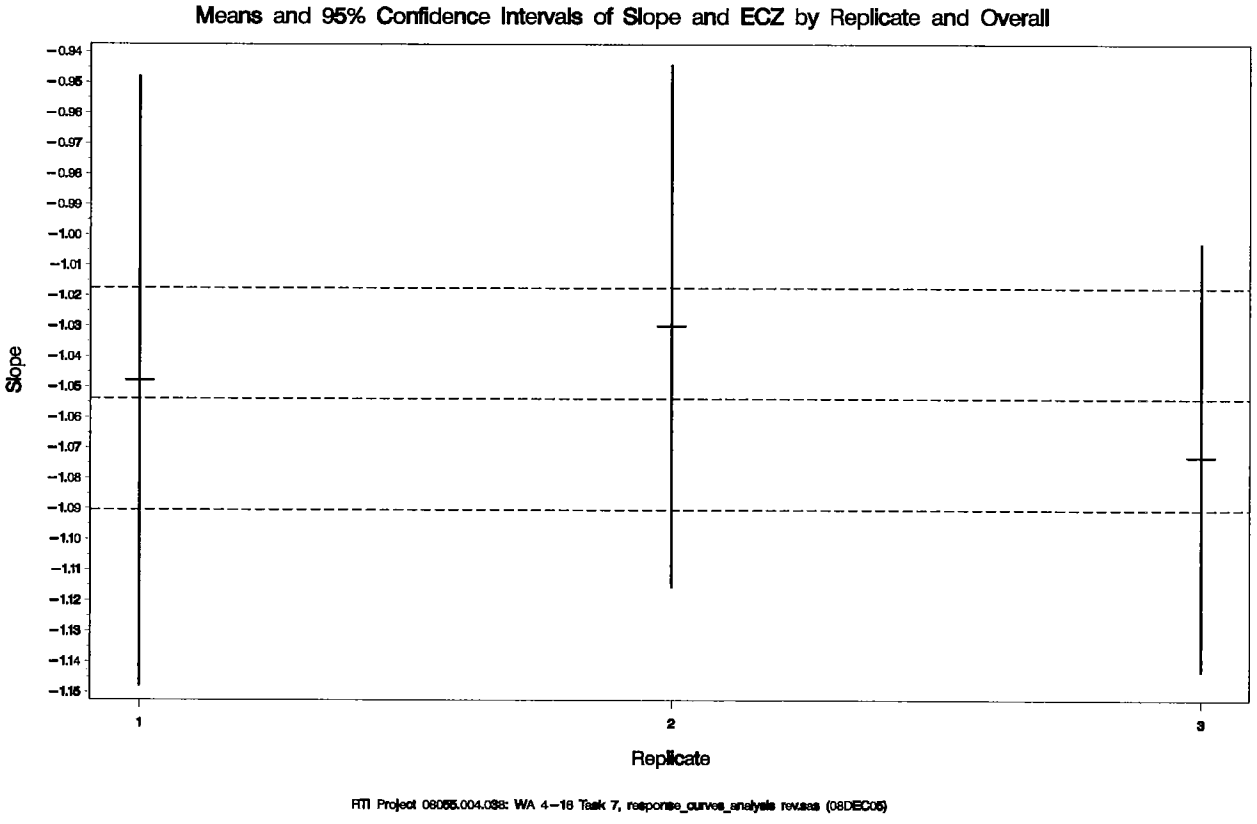
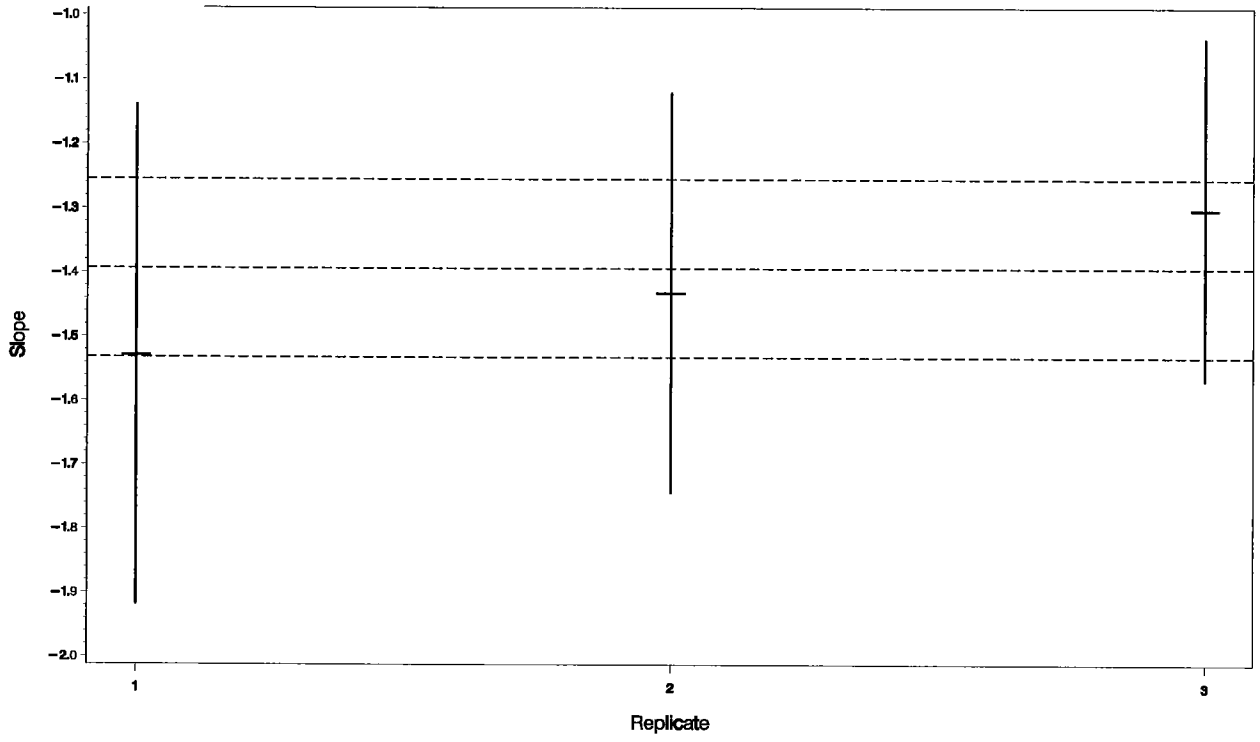
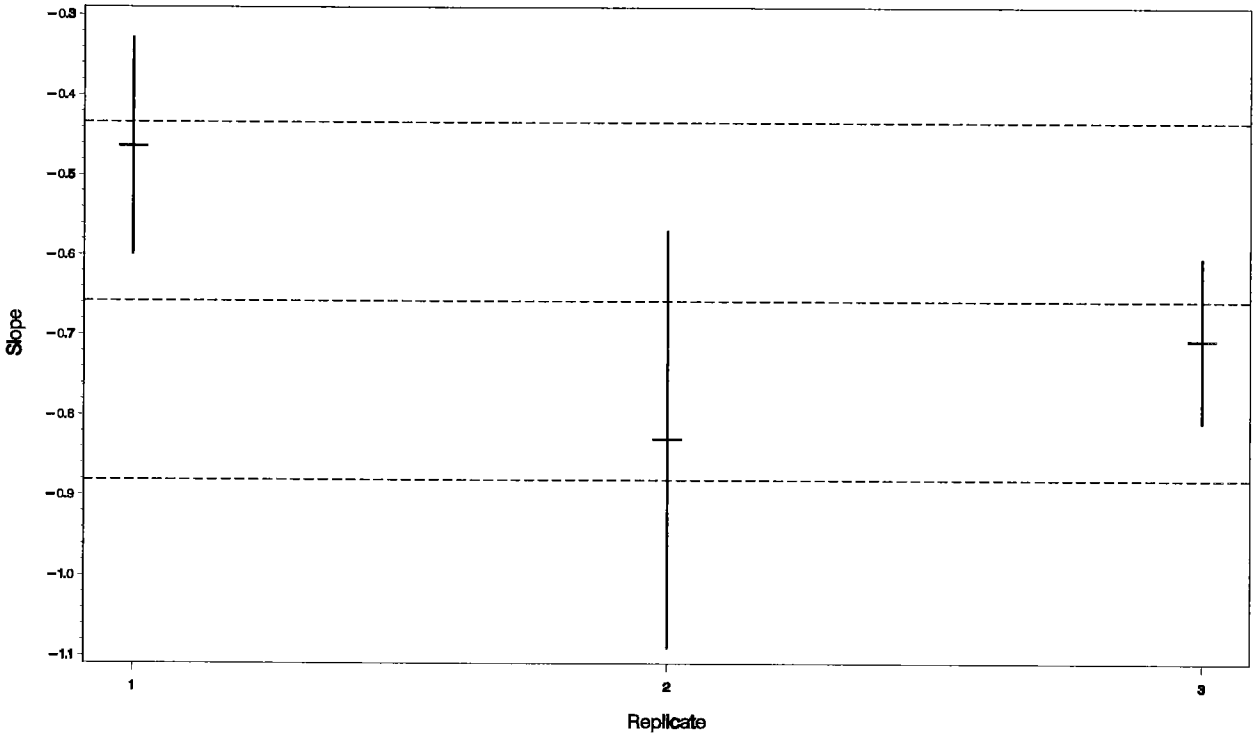


Exhibit 31. Means and 95% Confidence Intervals of Slope and CYN by Replicate and Overall



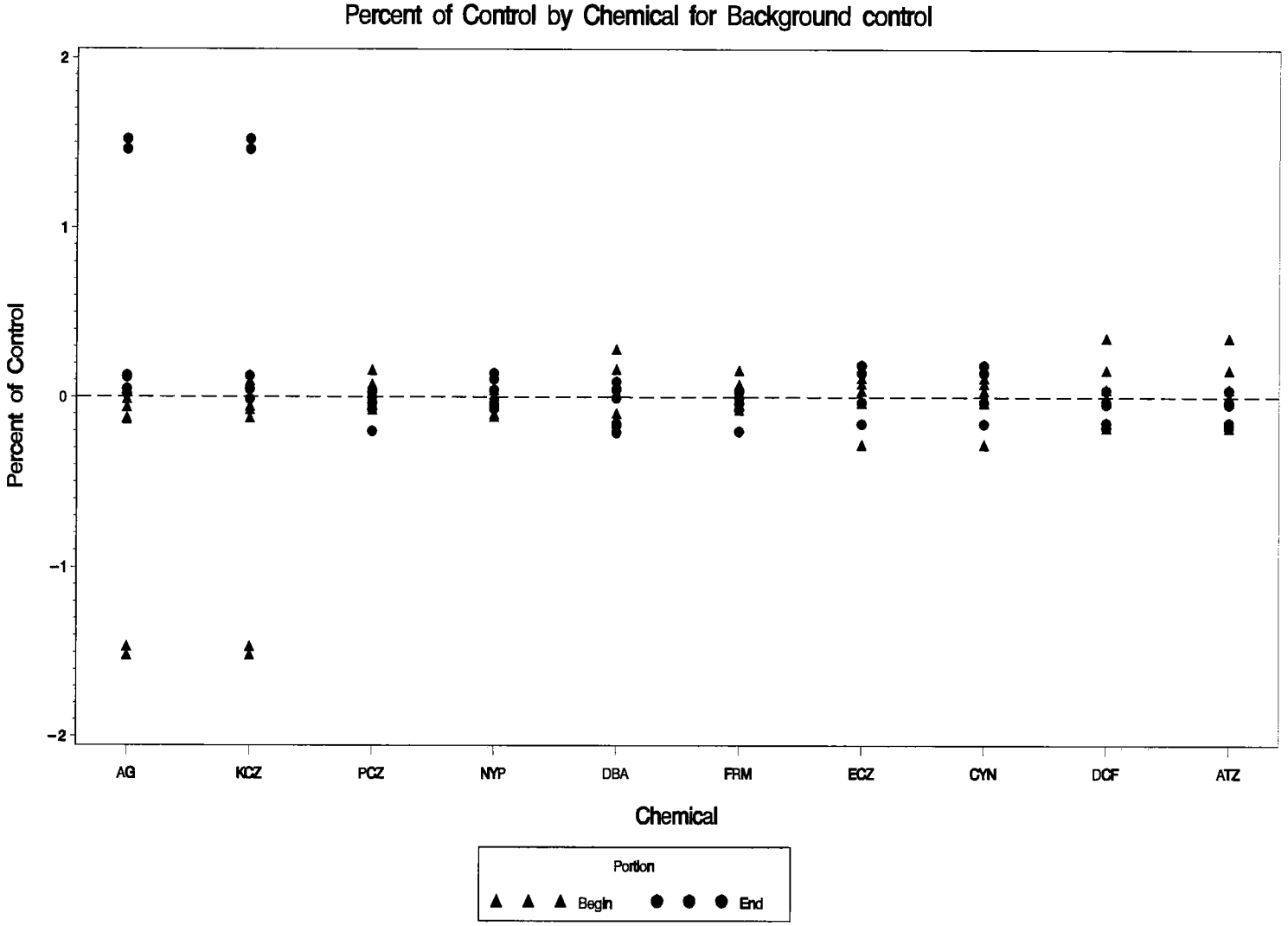
RTI Project 06055.004.038: WA 4-16 Task 7, response_curves_analysis rev.sas (08DEC05)

Exhibit 32. Means and 95% Confidence Intervals of Slope and DCF by Replicate and Overall



RTI Project 06055.004.038: WA 4-16 Task 7, response_curves_analysis rev.sas (08DEC05)

Exhibit 33.



RTI Project 08055.004.038: WA 4-16 Task 7, control_analysis.sas (07DEC05)

Exhibit 34.

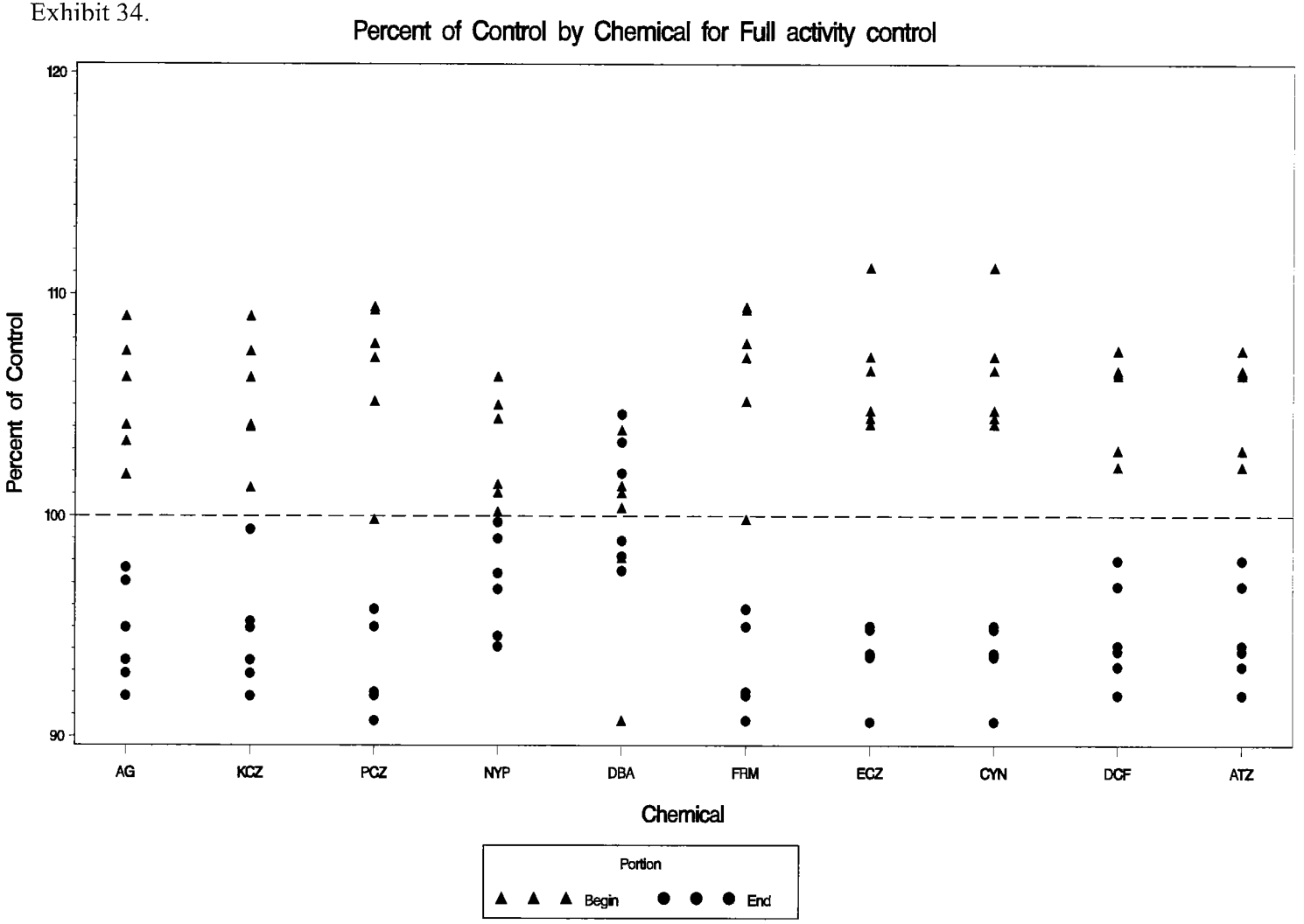

Percent of Control by Chemical for Full activity control
120
110
100
90
Percent of Control
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
Portion
Begin
End
RTI Project 08055.004.038: WA 4-16 Task 7, control_analysis.sas (07DEC05)

Exhibit 35.

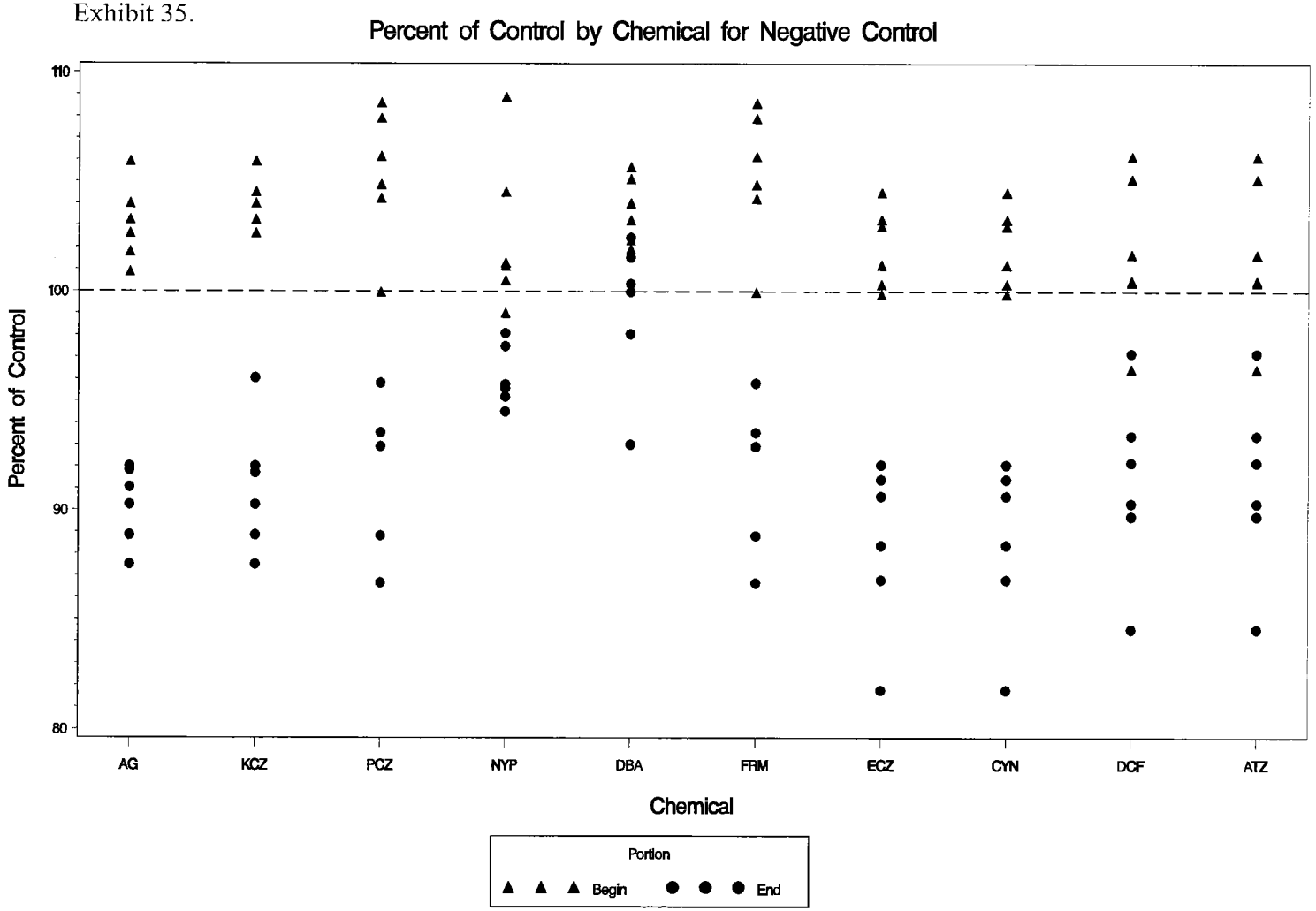

Percent of Control by Chemical for Negative Control
110
100
90
80
Percent of Control
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
Portion
Begin
End
RTI Project 08055.004.038: WA 4-16 Task 7, control_analysis.sas (07DEC05)

Exhibit 36.

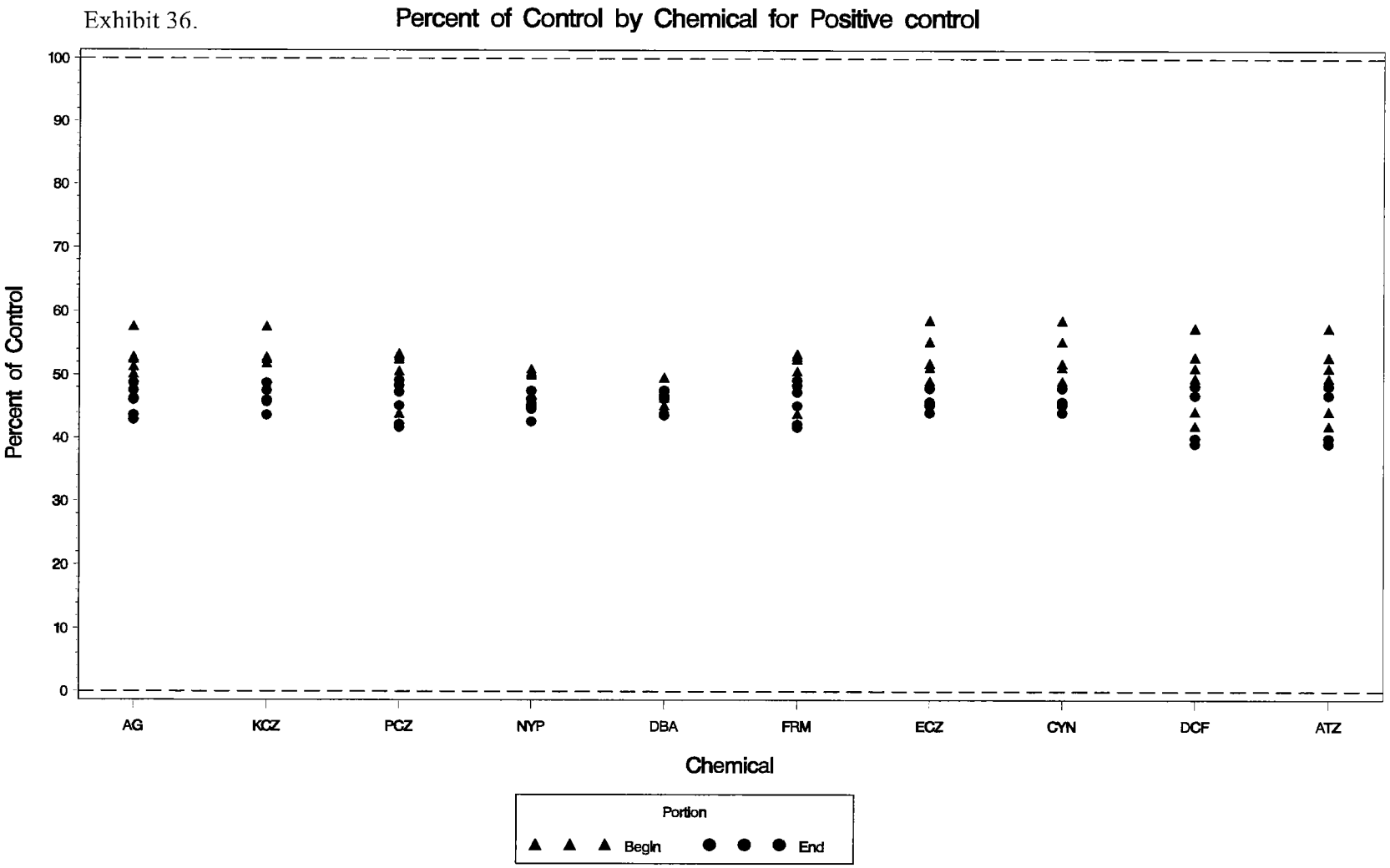
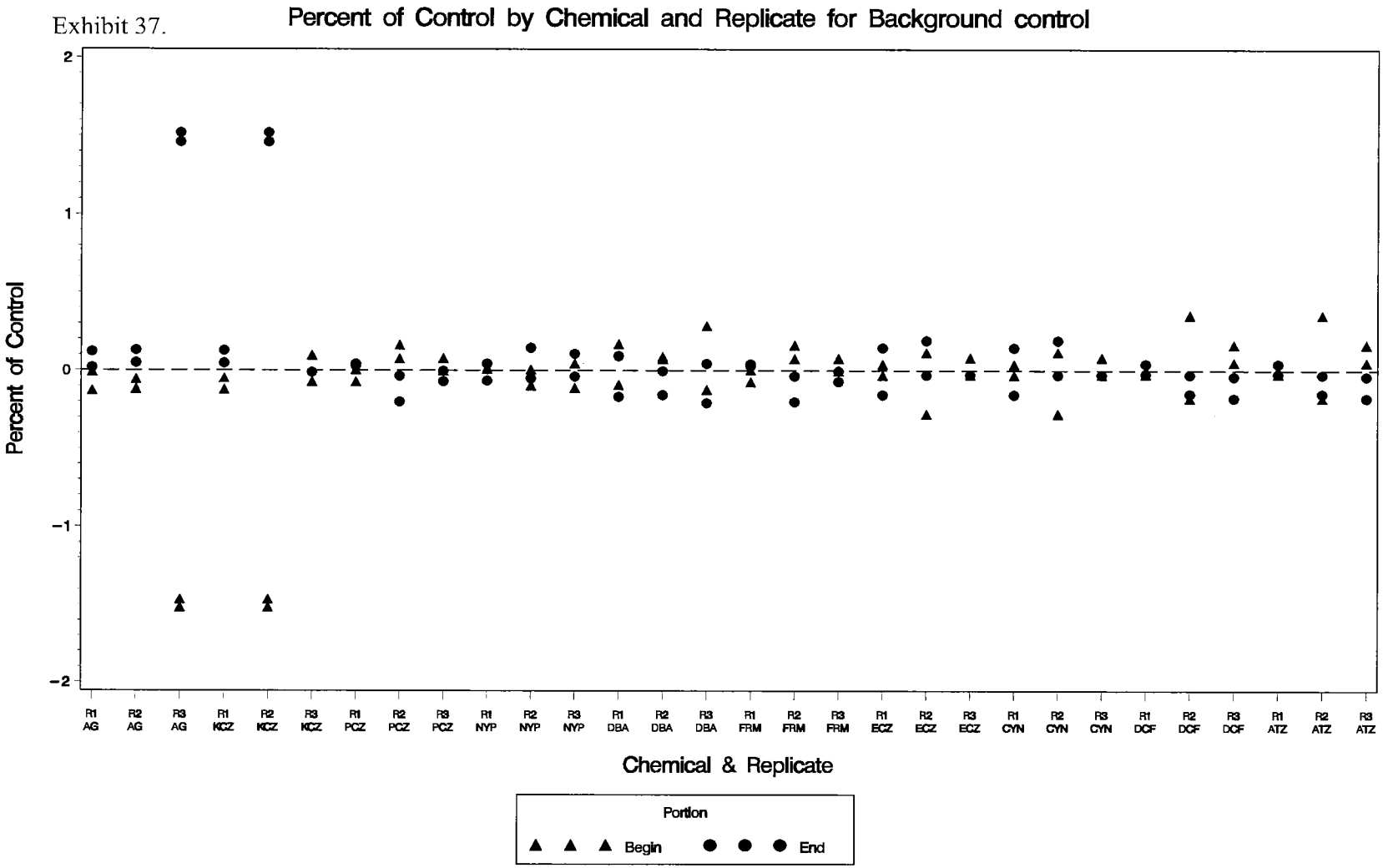
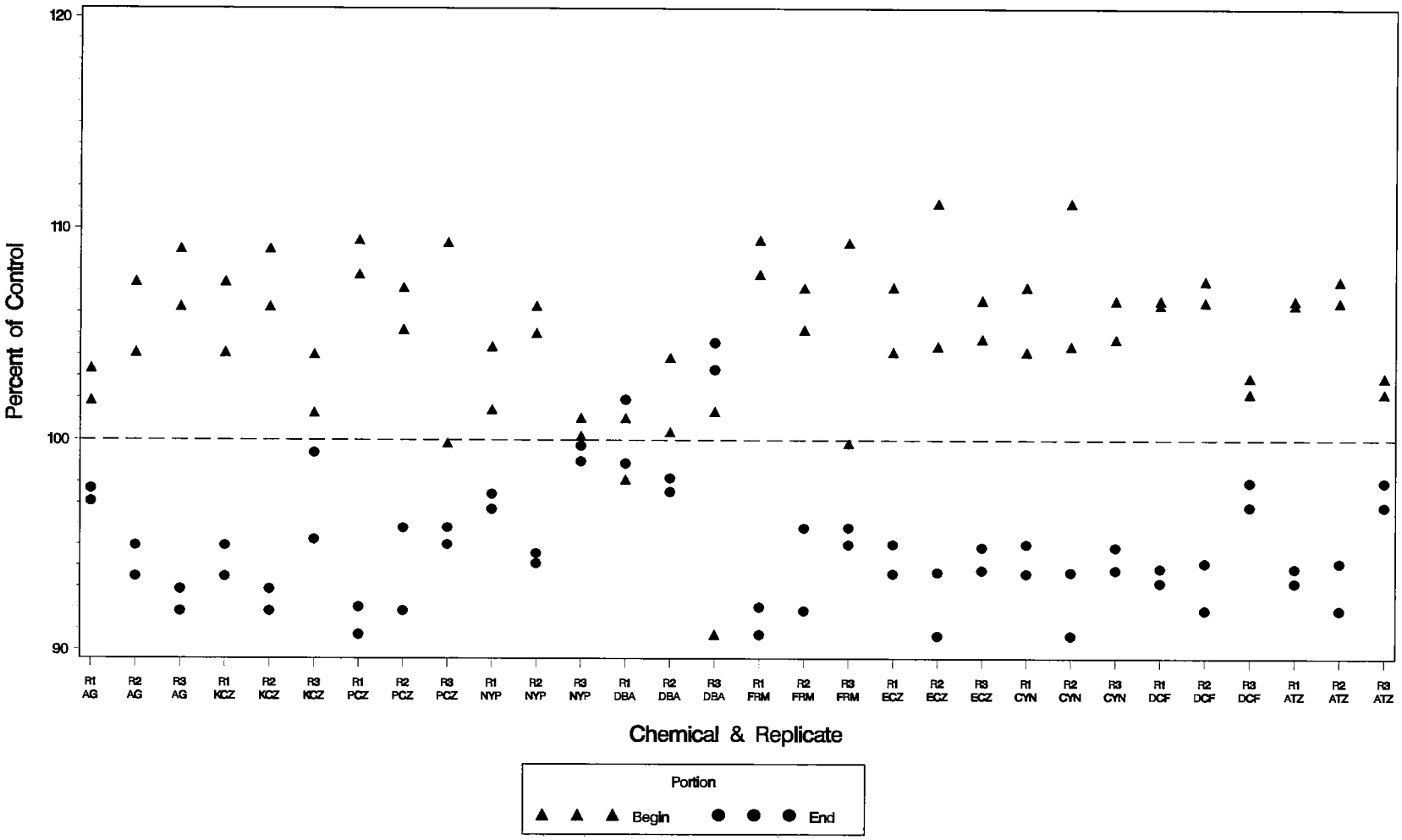

Percent of Control by Chemical for Positive control
Percent of Control
100
90
80
70
60
50
40
30
20
10
0
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
Portion
Begin
End
RTI Project 08055.004.038: WA 4-16 Task 7, control_analysis.sas (07DEC05)

Exhibit 37.



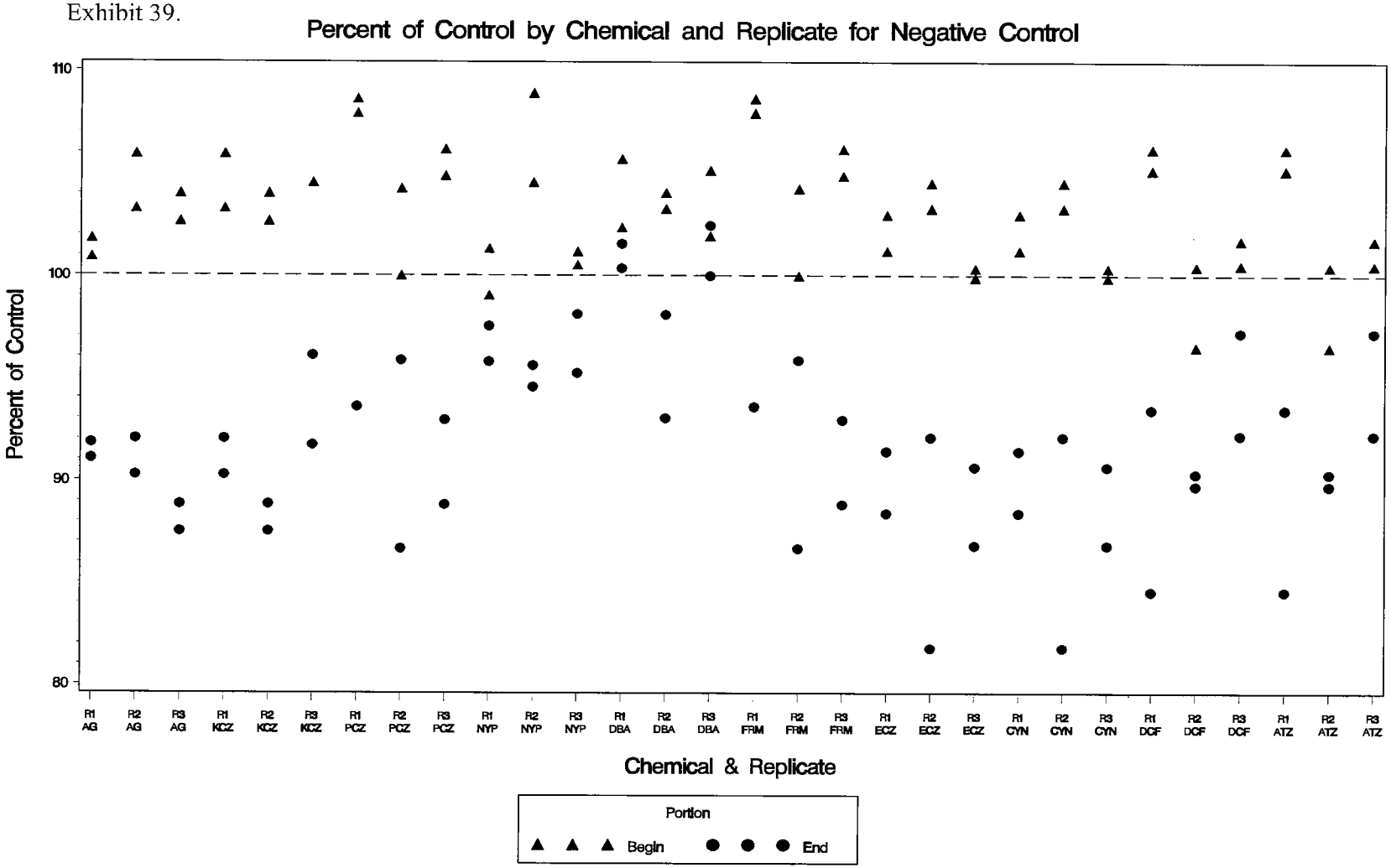
RTI Project 08055.004.038: WA 4−16 Task 7, control_analysis.sas (07DEC05)

Exhibit 38. Percent of Control by Chemical and Replicate for Full activity control



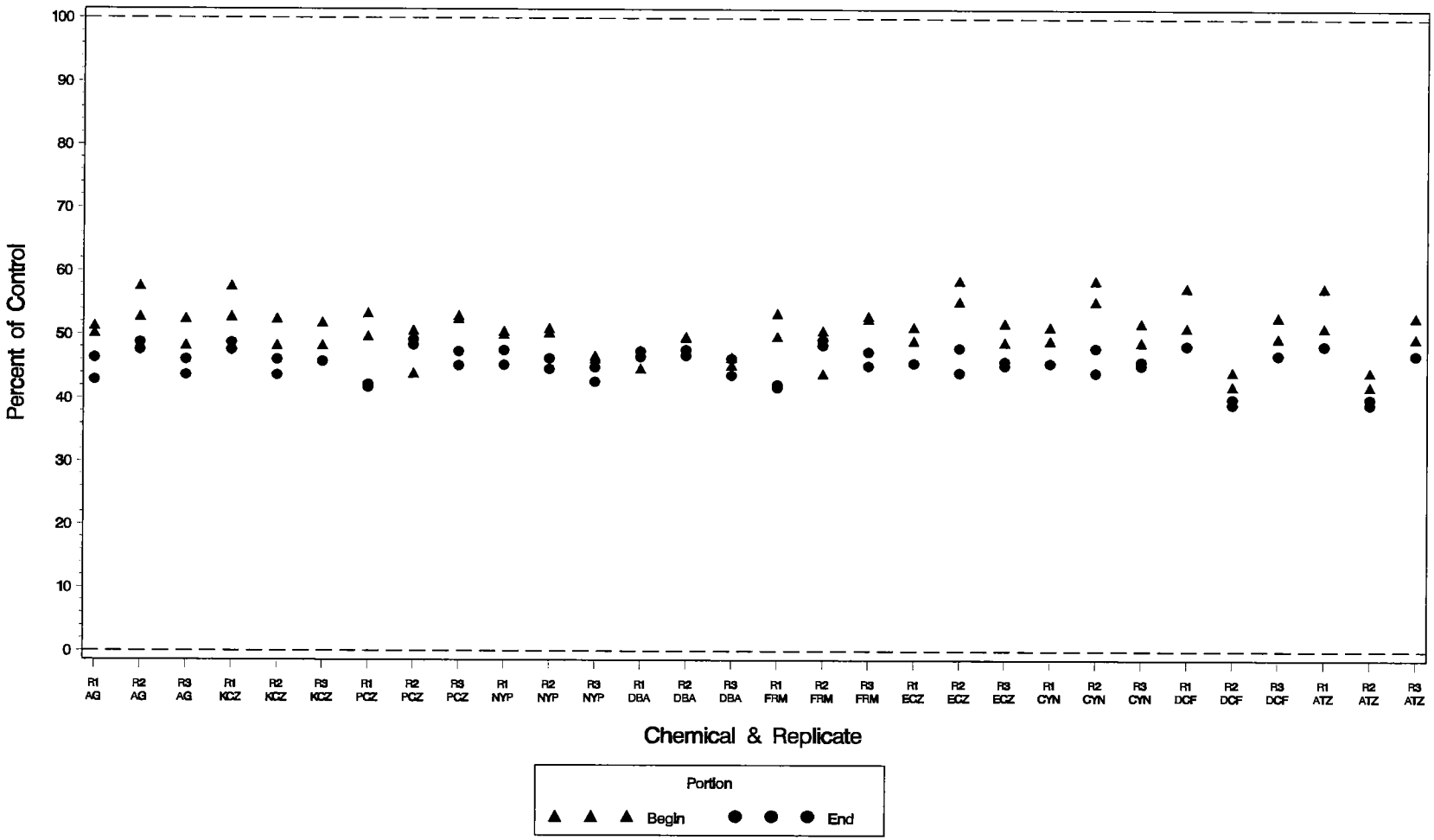
RTI Project 08055.004.038: WA 4-16 Task 7, control_analysis.sas (07DEC05)

Exhibit 39.



RTI Project 08055.004.038: WA 4-16 Task 7, control_analysis.sas (07DEC05)

Exhibit 40. Percent of Control by Chemical and Replicate for Positive control



RTI Project 08055.004.038: WA 4-16 Task 7, control_analysis.sas (07DEC05)

Exhibit 41.

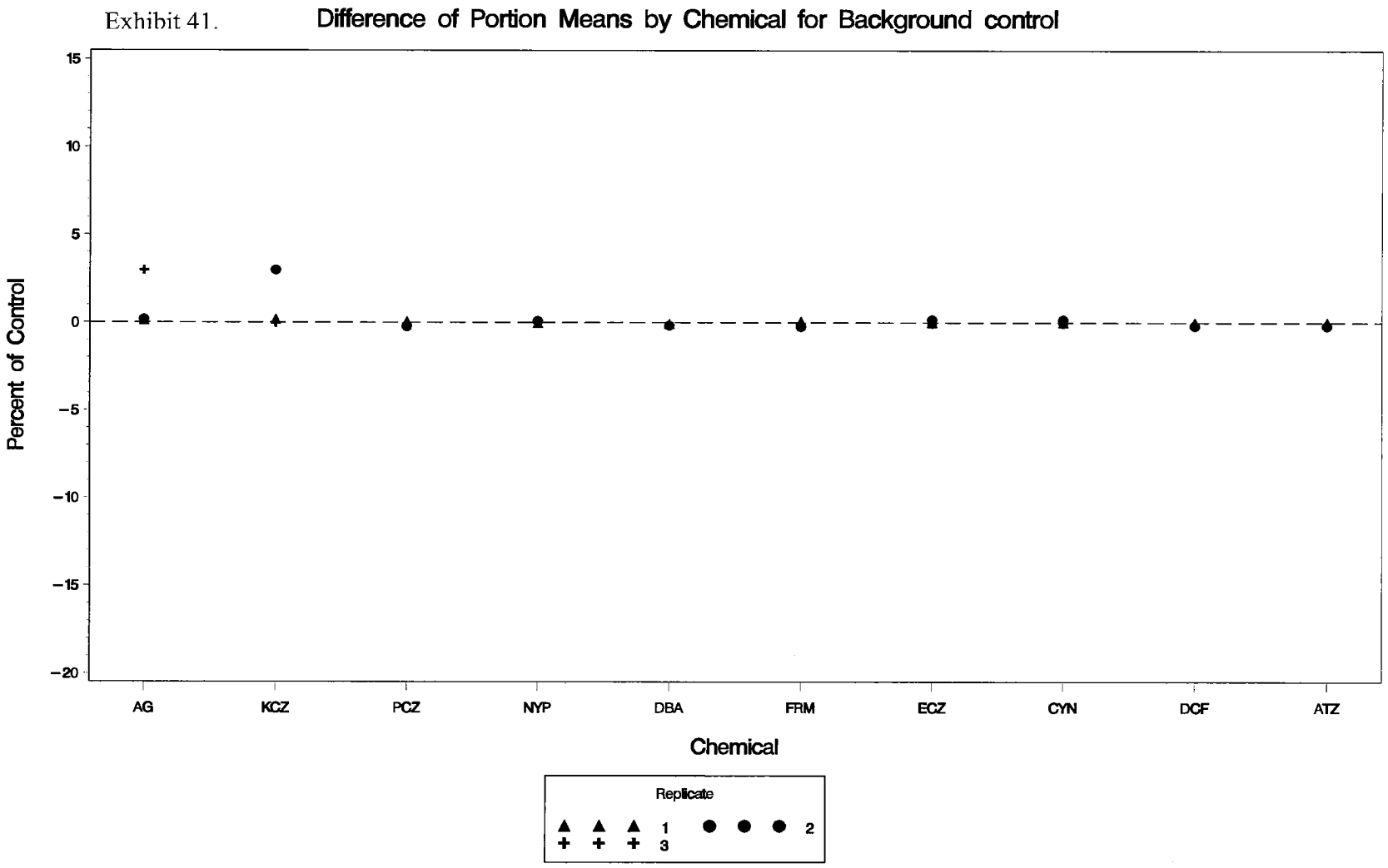

Difference of Portion Means by Chemical for Background control
Percent of Control
15
10
5
0
-5
-10
-15
-20
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
Replicate
1
2
3
RTI Project 08055.004.038: WA 4-16 Task 7, control_analysis.sas (07DEC05)

Exhibit 42.

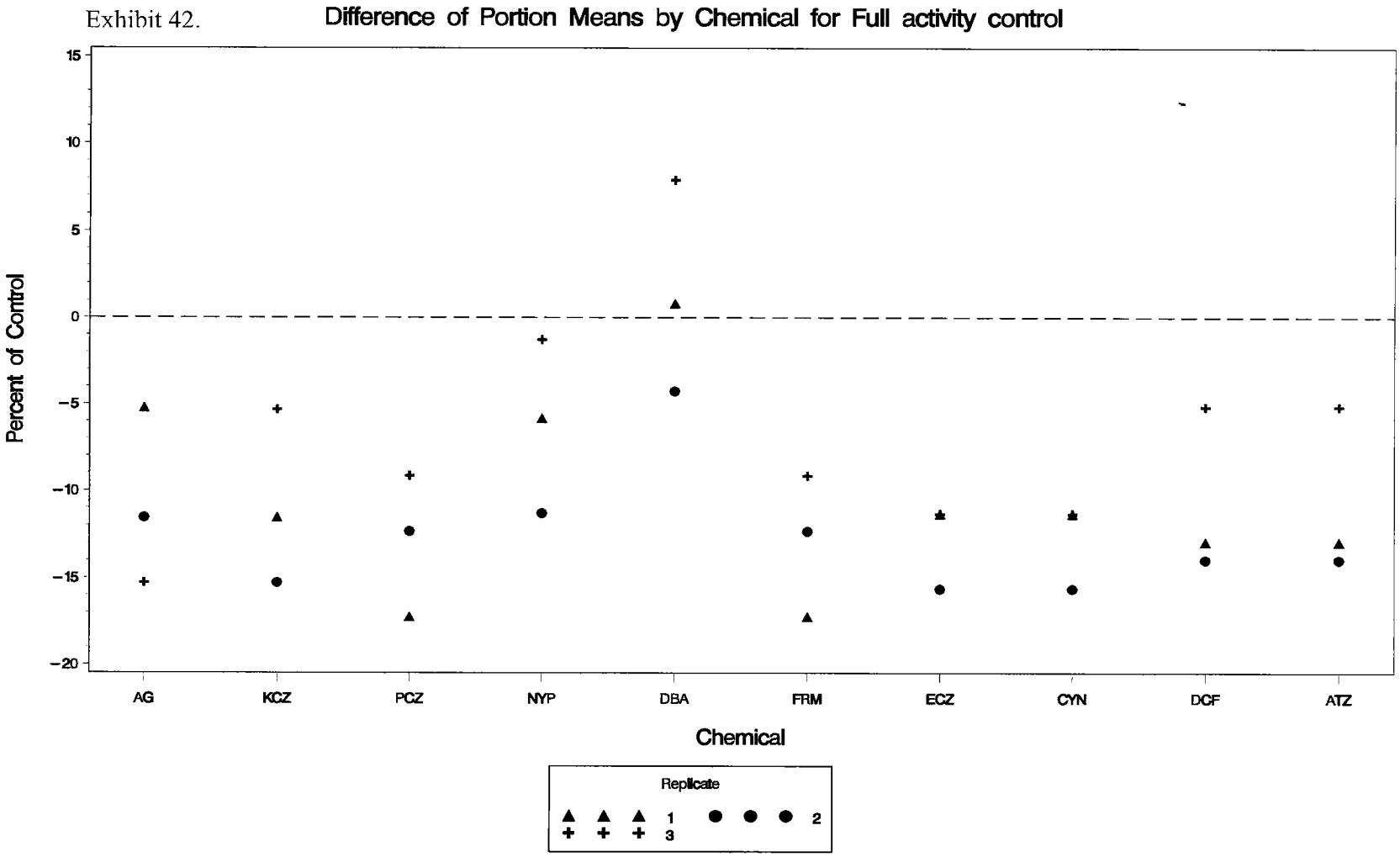

Difference of Portion Means by Chemical for Full activity control
Percent of Control
15
10
5
0
−5
−10
−15
−20
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
Replicate
1
2
3
RTI Project 08055.004.038: WA 4−16 Task 7, control_analysis.sas (07DEC05)

Exhibit 43.

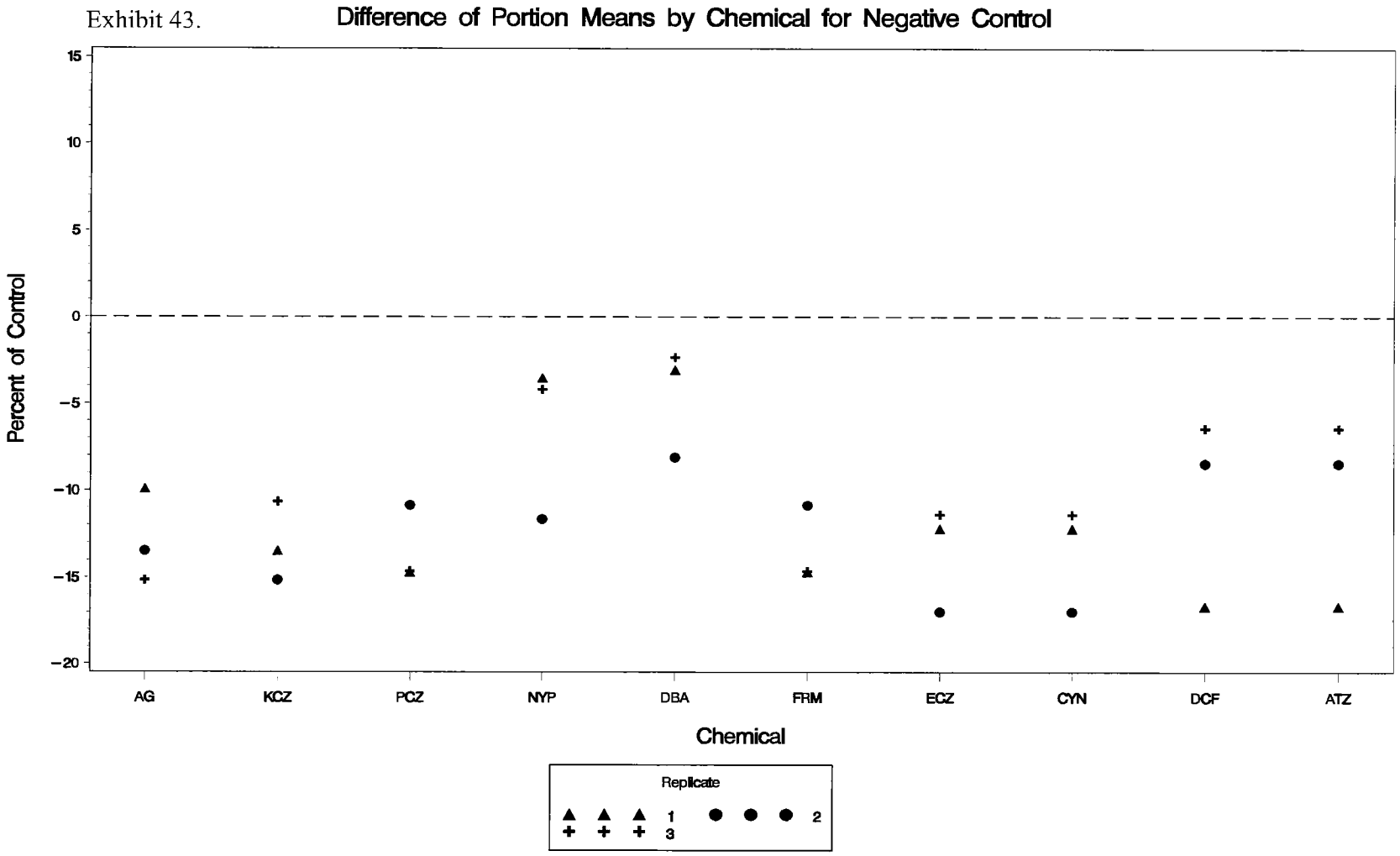
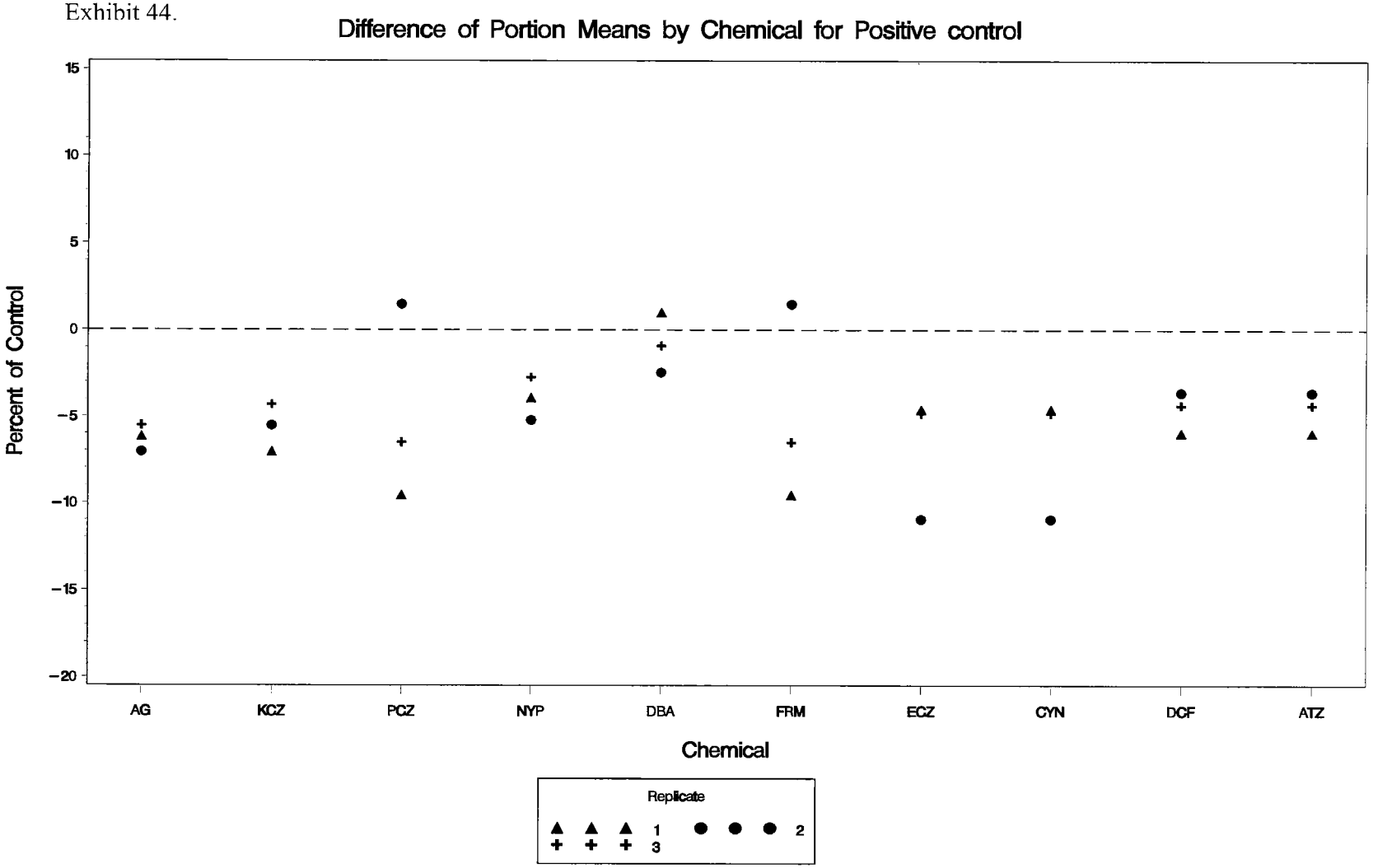
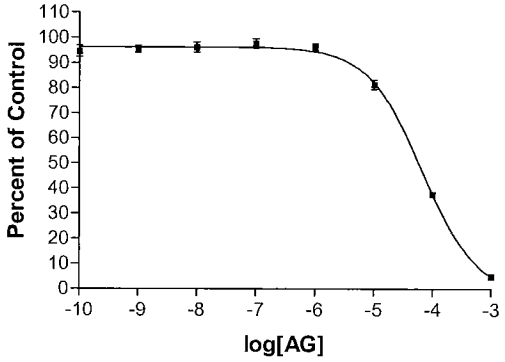

Difference of Portion Means by Chemical for Negative Control
Percent of Control
15
10
5
0
−5
−10
−15
−20
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
Replicate
1
2
3
RTI Project 08055.004.038: WA 4−16 Task 7, control_analysis.sas (07DEC05)

Exhibit 44.


Difference of Portion Means by Chemical for Positive control
Percent of Control
15
10
5
0
-5
-10
-15
-20
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
Replicate
1
2
3
RTI Project 08055.004.038: WA 4-16 Task 7, control_analysis.sas (07DEC05)

Appendix F
Concentration Response Curves

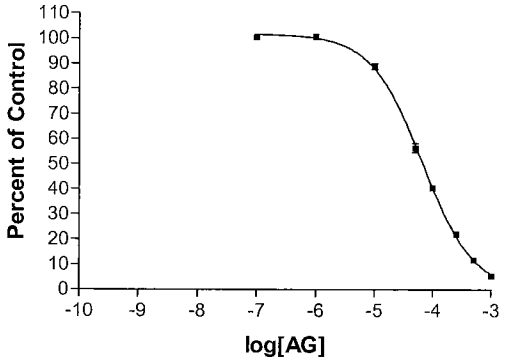
Aminoglutethimide, Replicate 1



log[AG]	Replicate 1		
	Y1	Y2	Y3
-3.0	5.1	4.8	5.0
-3.3			
-3.6			
-4.0	38.1	38.3	36.7
-4.3			
-5.0	84.4	81.8	78.1
-6.0	98.6	95.9	93.7
-7.0	101.3	96.2	95.6
-8.0	100.1	95.1	93.3
-9.0	97.8	95.3	93.0
-10.0	98.6	90.8	94.0

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	-1.984
TOP	96.27
LOGEC50	-4.182
HILLSLOPE	-0.9437
Std. Error	
BOTTOM	2.941
TOP	0.7558
LOGEC50	0.04567
HILLSLOPE	0.07679
95% Confidence Intervals	
BOTTOM	-8.119 to 4.150
TOP	94.70 to 97.85
LOGEC50	-4.278 to -4.087
HILLSLOPE	-1.104 to -0.7836
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9942
Absolute Sum of Squares	149.7
Sy.x	2.736
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

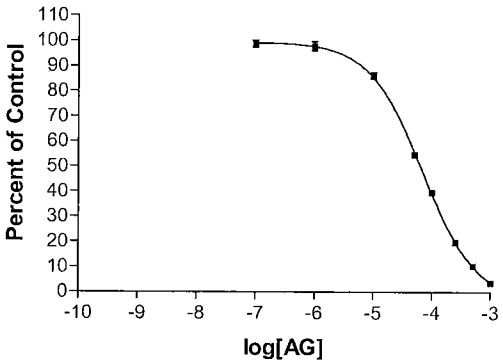
Aminoglutethimide, Replicate 2



log[AG]	Replicate 2		
	Y1	Y2	Y3
-3.0	5.1	5.5	5.9
-3.3	12.1	11.8	11.7
-3.6	22.0	22.5	21.9
-4.0	41.2	40.5	40.0
-4.3	59.6	53.4	56.6
-5.0	90.6	89.2	86.6
-6.0	102.3	100.6	99.3
-7.0	101.7	101.3	98.6
-8.0			
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	-0.5217
TOP	101.7
LOGEC50	-4.178
HILLSLOPE	-0.9842
Std. Error	
BOTTOM	1.624
TOP	0.7431
LOGEC50	0.01973
HILLSLOPE	0.04441
95% Confidence Intervals	
BOTTOM	-3.909 to 2.866
TOP	100.1 to 103.2
LOGEC50	-4.219 to -4.136
HILLSLOPE	-1.077 to -0.8915
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9984
Absolute Sum of Squares	52.71
Sy.x	1.623
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

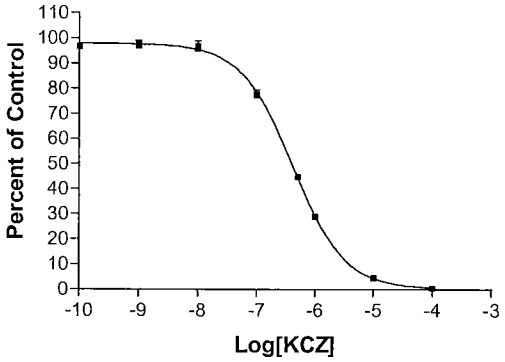
Aminoglutethimide, Replicate 3



log[AG]	Replicate 3		
	Y1	Y2	Y3
-3.0	3.8	3.5	3.5
-3.3	10.3	10.6	9.9
-3.6	20.3	18.7	20.7
-4.0	40.3	39.6	39.4
-4.3	56.8	54.2	53.2
-5.0	88.6	84.9	84.9
-6.0	97.6	101.1	94.8
-7.0	101.3	98.5	96.5
-8.0			
-9.0			
-10.0			

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	-3.500
TOP	99.40
LOGEC50	-4.159
HILLSLOPE	-0.9547
Std. Error	
BOTTOM	1.830
TOP	0.7882
LOGEC50	0.02204
HILLSLOPE	0.04587
95% Confidence Intervals	
BOTTOM	-7.318 to 0.3182
TOP	97.76 to 101.0
LOGEC50	-4.205 to -4.113
HILLSLOPE	-1.050 to -0.8590
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9982
Absolute Sum of Squares	57.79
Sy.x	1.700
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

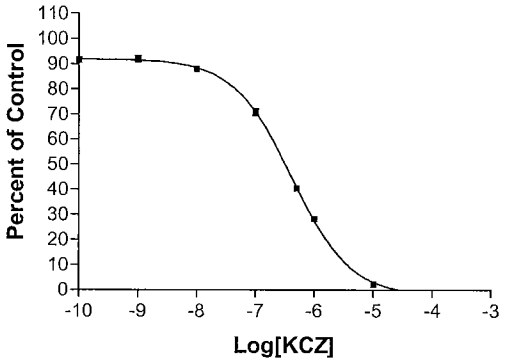
Ketoconazole, Replicate 1



Log[KCZ]	Replicate 1		
	Y1	Y2	Y3
-4.0	0.7	0.4	0.5
-5.0	4.7	4.3	4.6
-6.0	29.9	28.8	28.7
-6.3	46.0	44.8	44.1
-7.0	80.6	77.6	75.4
-8.0	100.3	97.1	93.4
-9.0	100.2	97.8	95.0
-10.0	98.2	97.3	94.9

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	0.2081
TOP	97.94
LOGEC50	-6.381
HILLSLOPE	-0.9772
Std. Error	
BOTTOM	0.9492
TOP	0.7048
LOGEC50	0.01886
HILLSLOPE	0.04268
95% Confidence Intervals	
BOTTOM	-1.772 to 2.188
TOP	96.47 to 99.41
LOGEC50	-6.420 to -6.342
HILLSLOPE	-1.066 to -0.8882
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9980
Absolute Sum of Squares	72.56
Sy.x	1.905
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

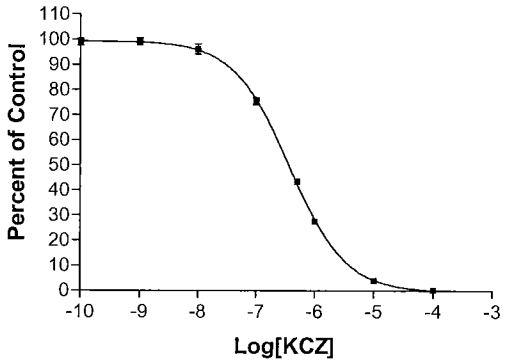
Ketoconazole, Replicate 2



Log[KCZ]	Replicate 2		
	Y1	Y2	Y3
-4.0	-1.1	-1.1	-1.1
-5.0	2.0	2.3	2.5
-6.0	27.9	28.4	28.7
-6.3	41.9	40.5	39.4
-7.0	73.5	71.1	68.4
-8.0	89.3	88.0	87.2
-9.0	94.5	91.1	90.3
-10.0	93.7	91.3	89.9

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	-2.476
TOP	91.97
LOGEC50	-6.378
HILLSLOPE	-0.8726
Std. Error	
BOTTOM	0.7907
TOP	0.5661
LOGEC50	0.01678
HILLSLOPE	0.03115
95% Confidence Intervals	
BOTTOM	-4.125 to -0.8262
TOP	90.79 to 93.15
LOGEC50	-6.413 to -6.343
HILLSLOPE	-0.9376 to -0.8076
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9987
Absolute Sum of Squares	42.90
Sy.x	1.465
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

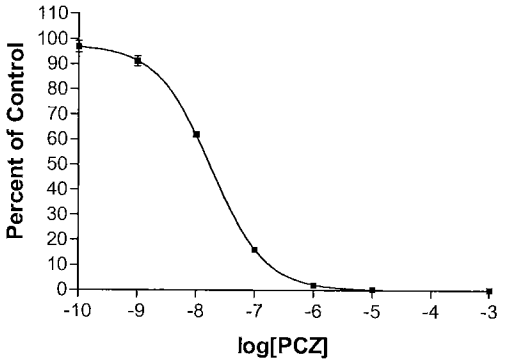
Ketoconazole, Replicate 3



Log[KCZ]	Replicate 3		
	Y1	Y2	Y3
-4.0	0.4	0.1	0.2
-5.0	4.4	3.8	4.0
-6.0	28.9	27.1	27.3
-6.3	45.2	43.3	42.4
-7.0	78.5	74.4	73.8
-8.0	99.7	95.9	92.7
-9.0	100.5	100.6	96.4
-10.0	101.1	99.1	96.7

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	-0.6192
TOP	99.39
LOGEC50	-6.429
HILLSLOPE	-0.9062
Std. Error	
BOTTOM	0.9722
TOP	0.7244
LOGEC50	0.01980
HILLSLOPE	0.03819
95% Confidence Intervals	
BOTTOM	-2.647 to 1.409
TOP	97.88 to 100.9
LOGEC50	-6.470 to -6.387
HILLSLOPE	-0.9859 to -0.8266
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9981
Absolute Sum of Squares	71.38
Sy.x	1.889
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

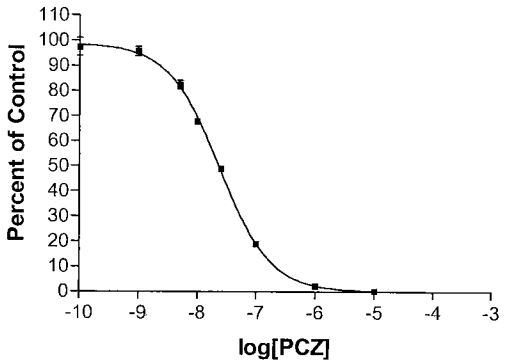
Prochloraz, Replicate 1



log[PCZ]	Replicate 1		
	Y1	Y2	Y3
-3.0	-0.1	0.1	4.0e-002
-4.0	-0.2	-0.1	0.1
-5.0	0.1	0.4	0.3
-6.0	1.9	2.3	2.0
-7.0	16.4	15.8	16.3
-7.6			
-8.0	61.6	64.1	60.3
-8.3			
-9.0	95.0	89.7	88.7
-10.0	100.2	98.0	92.5

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	-0.07196
TOP	97.44
LOGEC50	-7.743
HILLSLOPE	-0.9392
Std. Error	
BOTTOM	0.5527
TOP	0.9589
LOGEC50	0.02266
HILLSLOPE	0.03994
95% Confidence Intervals	
BOTTOM	-1.225 to 1.081
TOP	95.44 to 99.44
LOGEC50	-7.791 to -7.696
HILLSLOPE	-1.023 to -0.8559
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9984
Absolute Sum of Squares	62.58
Sy.x	1.769
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

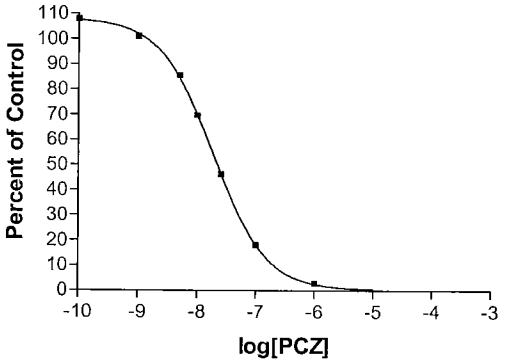
Prochloraz, Replicate 2



log[PCZ]	Replicate 2		
	Y1	Y2	Y3
-3.0			
-4.0			
-5.0	0.1	0.2	0.2
-6.0	2.0	2.4	2.3
-7.0	18.4	19.7	19.1
-7.6	49.7	49.5	47.4
-8.0	69.8	67.7	66.3
-8.3	84.1	84.2	79.1
-9.0	98.7	96.0	92.3
-10.0	90.3	102.1	99.8

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	-0.05843
TOP	98.70
LOGEC50	-7.621
HILLSLOPE	-0.9971
Std. Error	
BOTTOM	1.239
TOP	1.332
LOGEC50	0.02643
HILLSLOPE	0.05585
95% Confidence Intervals	
BOTTOM	-2.643 to 2.526
TOP	95.92 to 101.5
LOGEC50	-7.676 to -7.565
HILLSLOPE	-1.114 to -0.8806
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9958
Absolute Sum of Squares	143.1
Sy.x	2.675
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

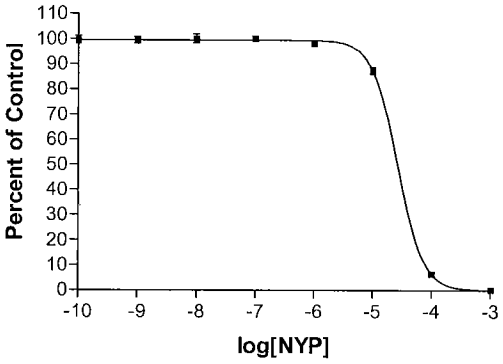
Prochloraz, Replicate 3



log[PCZ]	Replicate 3		
	Y1	Y2	Y3
-3.0			
-4.0			
-5.0	-1.0e-002	-0.2	0.2
-6.0	3.0	3.0	2.9
-7.0	18.6	18.3	17.5
-7.6	47.3	47.2	44.8
-8.0	68.2	69.9	71.5
-8.3	85.2	86.3	85.8
-9.0	102.8	100.7	99.8
-10.0	109.0	108.8	106.1

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	0.2152
TOP	108.1
LOGEC50	-7.725
HILLSLOPE	-0.9697
Std. Error	
BOTTOM	0.5643
TOP	0.6502
LOGEC50	0.01124
HILLSLOPE	0.02336
95% Confidence Intervals	
BOTTOM	-0.9618 to 1.392
TOP	106.8 to 109.5
LOGEC50	-7.749 to -7.702
HILLSLOPE	-1.018 to -0.9210
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9992
Absolute Sum of Squares	30.44
Sy.x	1.234
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

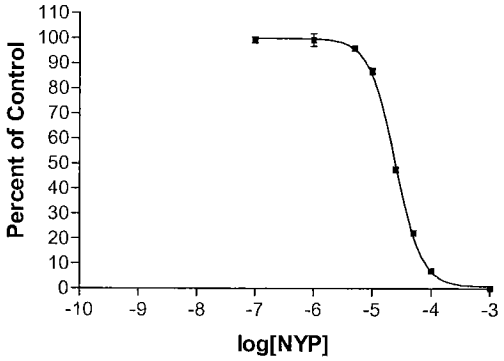
4-Nonylphenol, Replicate 1



log[NYP]	Replicate 1		
	Y1	Y2	Y3
-3.0	2.0e-002	0.2	0.1
-4.0	7.4	5.7	6.3
-4.3			
-4.6			
-5.0	89.1	88.3	84.9
-5.3			
-6.0	98.2	98.6	97.8
-7.0	102.0	98.6	99.1
-8.0	103.6	97.9	98.9
-9.0	101.3	100.3	96.8
-10.0	102.3	99.2	97.3

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	0.01138
TOP	99.48
LOGEC50	-4.574
HILLSLOPE	-2.017
Std. Error	
BOTTOM	1.075
TOP	0.4728
LOGEC50	0.02684
HILLSLOPE	0.1120
95% Confidence Intervals	
BOTTOM	-2.230 to 2.253
TOP	98.49 to 100.5
LOGEC50	-4.630 to -4.518
HILLSLOPE	-2.250 to -1.783
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9984
Absolute Sum of Squares	66.46
Sy.x	1.823
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

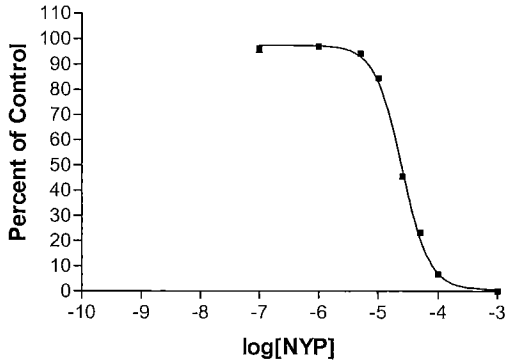
4-Nonylphenol, Replicate 2



log[NYP]	Replicate 2		
	Y1	Y2	Y3
-3.0	0.2	0.1	1.0e-002
-4.0	7.0	7.2	7.0
-4.3	22.6	22.6	22.0
-4.6	48.8	48.5	45.3
-5.0	88.5	87.9	84.2
-5.3	97.2	94.6	96.2
-6.0	104.4	97.1	96.5
-7.0	100.8	100.0	96.9
-8.0			
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	0.7939
TOP	99.90
LOGEC50	-4.608
HILLSLOPE	-1.948
Std. Error	
BOTTOM	1.134
TOP	0.8444
LOGEC50	0.01230
HILLSLOPE	0.09056
95% Confidence Intervals	
BOTTOM	-1.572 to 3.160
TOP	98.14 to 101.7
LOGEC50	-4.633 to -4.582
HILLSLOPE	-2.137 to -1.759
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9974
Absolute Sum of Squares	99.99
Sy.x	2.236
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

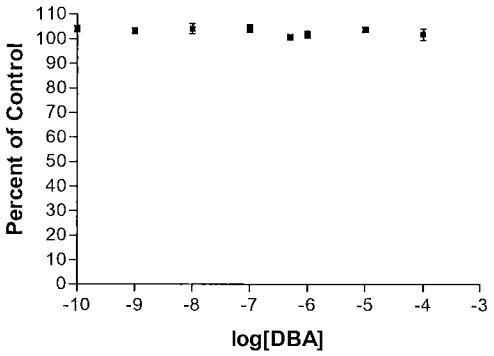
4-Nonylphenol, Replicate 3



log[NYP]	Replicate 3		
	Y1	Y2	Y3
-3.0	-3.0e-002	-0.1	0.1
-4.0	7.1	6.7	6.7
-4.3	23.9	23.6	22.5
-4.6	45.1	47.1	44.9
-5.0	84.6	86.1	82.8
-5.3	94.3	95.5	92.8
-6.0	95.4	96.9	98.8
-7.0	93.4	96.8	97.2
-8.0			
-9.0			
-10.0			

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	0.7179
TOP	97.38
LOGEC50	-4.606
HILLSLOPE	-1.908
Std. Error	
BOTTOM	1.099
TOP	0.8158
LOGEC50	0.01233
HILLSLOPE	0.08676
95% Confidence Intervals	
BOTTOM	-1.575 to 3.011
TOP	95.68 to 99.08
LOGEC50	-4.632 to -4.580
HILLSLOPE	-2.089 to -1.727
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9975
Absolute Sum of Squares	92.36
Sy.x	2.149
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

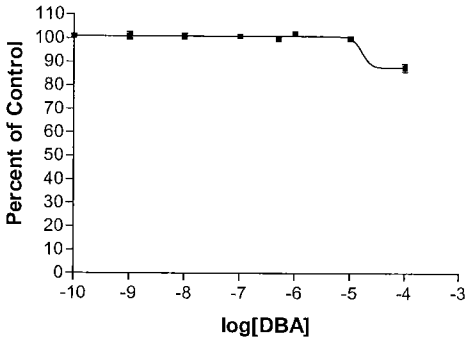
Dibenz[a,h]anthracene, Replicate 1



log[DBA]	Replicate 1		
	Y1	Y2	Y3
-4.0	104.9	103.3	97.2
-5.0	105.4	104.1	101.7
-6.0	101.3	104.3	99.5
-6.3	103.0	99.2	100.0
-7.0	106.8	104.7	101.4
-8.0	104.7	107.4	100.4
-9.0	101.3	105.3	103.1
-10.0	106.5	103.1	102.6

	Replicate 1
Equation 1	Floating point error.
Best-fit values	
BOTTOM	
TOP	
LOGEC50	
HILLSLOPE	
Std. Error	
BOTTOM	
TOP	
LOGEC50	
HILLSLOPE	
95% Confidence Intervals	
BOTTOM	
TOP	
LOGEC50	
HILLSLOPE	
Goodness of Fit	
Degrees of Freedom	
R^2	
Absolute Sum of Squares	
Sy.x	
Data	
Number of X values	
Number of Y replicates	
Total number of values	
Number of missing values	

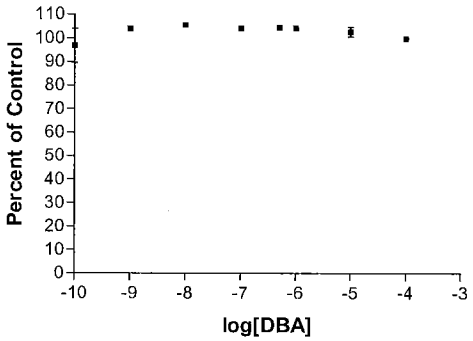
Dibenz[a,h]anthracene, Replicate 2



log[DBA]	Replicate 2		
	Y1	Y2	Y3
-4.0	89.2	89.2	84.2
-5.0	99.8	100.9	98.9
-6.0	103.0	102.7	100.3
-6.3	99.8	100.9	98.4
-7.0	101.5	101.9	98.9
-8.0	102.0	102.2	98.5
-9.0	104.2	99.0	99.6
-10.0	100.0	102.7	100.0

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	87.54
TOP	100.9
LOGEC50	-4.792
HILLSLOPE	-5.269
Std. Error	
BOTTOM	185.0
TOP	0.4865
LOGEC50	3237
HILLSLOPE	80507
95% Confidence Intervals	
BOTTOM	-298.3 to 473.4
TOP	99.85 to 101.9
LOGEC50	-6757 to 6747
HILLSLOPE	-167900 to 167932
Goodness of Fit	
Degrees of Freedom	20
R^2	0.8676
Absolute Sum of Squares	70.01
Sy.x	1.871
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

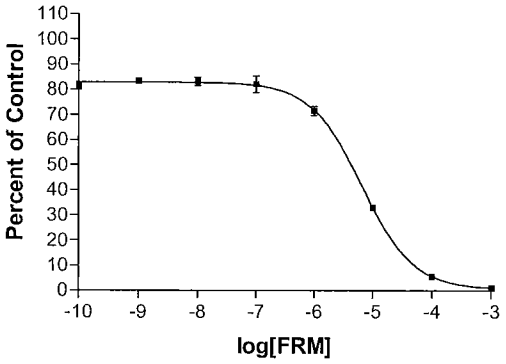
Dibenz[a,h]anthracene, Replicate 3



log[DBA]	Replicate 3		
	Y1	Y2	Y3
-4.0	100.1	101.0	98.6
-5.0	102.6	106.2	99.2
-6.0	104.0	104.8	103.8
-6.3	104.1	106.2	103.5
-7.0	102.8	104.6	105.4
-8.0	104.8	105.7	106.3
-9.0	103.3	105.8	103.1
-10.0	82.2	105.1	103.0

	Replicate 3
Equation 1	Floating point error.
Best-fit values	
BOTTOM	
TOP	
LOGEC50	
HILLSLOPE	
Std. Error	
BOTTOM	
TOP	
LOGEC50	
HILLSLOPE	
95% Confidence Intervals	
BOTTOM	
TOP	
LOGEC50	
HILLSLOPE	
Goodness of Fit	
Degrees of Freedom	
R^2	
Absolute Sum of Squares	
Sy.x	
Data	
Number of X values	
Number of Y replicates	
Total number of values	
Number of missing values	

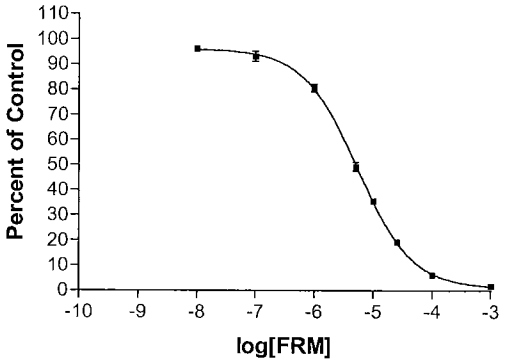
Fenarimol, Replicate 1



log[FRM]	Replicate 1		
	Y1	Y2	Y3
-3.0	1.0	1.0	1.1
-4.0	5.7	6.0	5.2
-4.6			
-5.0	33.9	32.5	32.7
-5.3			
-6.0	75.2	70.1	69.6
-7.0	88.4	80.7	77.3
-8.0	86.6	81.2	82.0
-9.0	84.5	84.1	82.0
-10.0	83.7	81.9	78.9

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	0.5092
TOP	82.93
LOGEC50	-5.188
HILLSLOPE	-0.9924
Std. Error	
BOTTOM	1.358
TOP	0.7732
LOGEC50	0.03603
HILLSLOPE	0.07539
95% Confidence Intervals	
BOTTOM	-2.323 to 3.342
TOP	81.32 to 84.55
LOGEC50	-5.263 to -5.113
HILLSLOPE	-1.150 to -0.8351
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9954
Absolute Sum of Squares	126.9
Sy.x	2.519
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

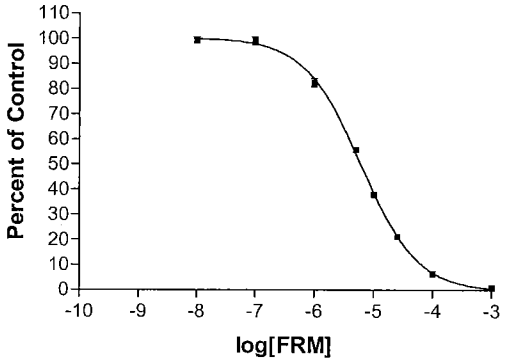
Fenarimol, Replicate 2



log[FRM]	Replicate 2		
	Y1	Y2	Y3
-3.0	1.3	1.5	2.6
-4.0	6.4	6.3	5.9
-4.6	19.6	19.5	18.7
-5.0	35.8	36.7	34.7
-5.3	51.1	51.1	45.7
-6.0	83.0	81.2	77.7
-7.0	97.0	90.8	91.7
-8.0	98.2	95.3	95.3
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	0.9139
TOP	96.07
LOGEC50	-5.266
HILLSLOPE	-0.9430
Std. Error	
BOTTOM	1.037
TOP	0.8917
LOGEC50	0.02056
HILLSLOPE	0.04181
95% Confidence Intervals	
BOTTOM	-1.249 to 3.077
TOP	94.21 to 97.93
LOGEC50	-5.309 to -5.223
HILLSLOPE	-1.030 to -0.8558
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9976
Absolute Sum of Squares	73.54
Sy.x	1.918
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

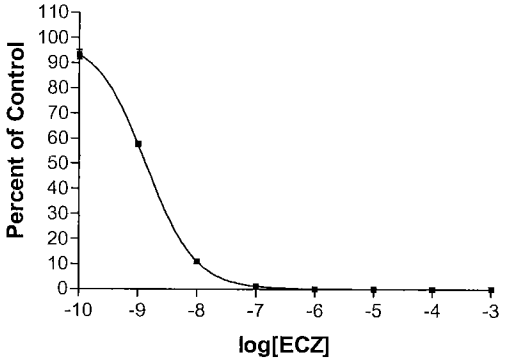
Fenarimol, Replicate 3



log[FRM]	Replicate 3		
	Y1	Y2	Y3
-3.0	1.0	0.9	0.7
-4.0	6.5	6.3	6.4
-4.6	21.1	22.1	20.5
-5.0	39.1	37.1	38.0
-5.3	56.2	56.0	55.7
-6.0	79.2	85.0	83.3
-7.0	102.0	98.1	97.1
-8.0	101.4	99.5	97.3
-9.0			
-10.0			

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	-0.5225
TOP	100.2
LOGEC50	-5.213
HILLSLOPE	-0.9089
Std. Error	
BOTTOM	1.020
TOP	0.8354
LOGEC50	0.01867
HILLSLOPE	0.03605
95% Confidence Intervals	
BOTTOM	-2.649 to 1.605
TOP	98.46 to 101.9
LOGEC50	-5.252 to -5.174
HILLSLOPE	-0.9841 to -0.8337
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9981
Absolute Sum of Squares	63.98
Sy.x	1.789
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

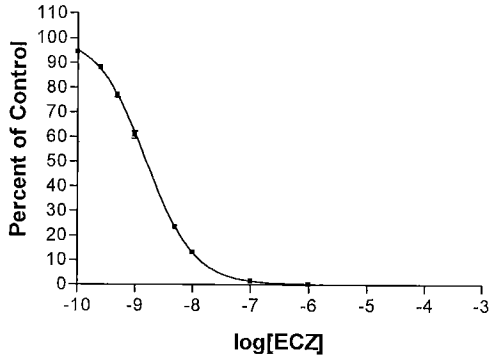
Econazole, Replicate 1



log[ECZ]	Replicate 1		
	Y1	Y2	Y3
-3.0	0.1	0.1	0.1
-4.0	0.3	-3.0e-002	-0.1
-5.0	-4.0e-002	0.1	-2.0e-002
-6.0	0.2	0.1	0.2
-7.0	1.2	1.2	1.3
-8.0	11.3	11.1	11.0
-8.3			
-9.0	59.0	~~-8.8~~	57.0
-9.3			
-9.6			
-10.0	96.9	91.5	91.5

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	0.06986
TOP	99.24
LOGEC50	-8.859
HILLSLOPE	-1.048
Std. Error	
BOTTOM	0.2891
TOP	1.088
LOGEC50	0.01745
HILLSLOPE	0.03602
95% Confidence Intervals	
BOTTOM	-0.5352 to 0.6749
TOP	96.96 to 101.5
LOGEC50	-8.896 to -8.823
HILLSLOPE	-1.124 to -0.9730
Goodness of Fit	
Degrees of Freedom	19
R^2	0.9991
Absolute Sum of Squares	21.62
Sy.x	1.067
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	23
Number of missing values	10

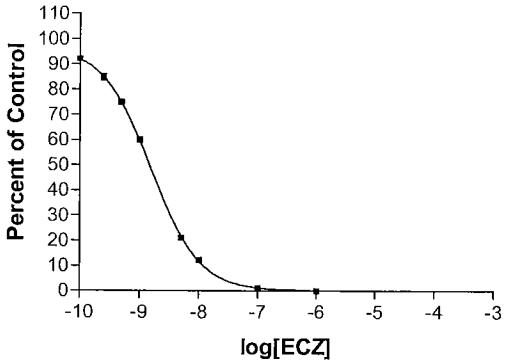
Econazole, Replicate 2



log[ECZ]	Replicate 2		
	Y1	Y2	Y3
-3.0			
-4.0			
-5.0			
-6.0	0.1	0.1	0.1
-7.0	1.4	1.4	1.4
-8.0	12.9	13.7	12.9
-8.3	23.4	23.6	23.1
-9.0	63.6	58.8	59.8
-9.3	78.2	77.9	74.9
-9.6	89.5	87.3	87.4
-10.0	96.3	93.4	93.4

	Replicate 2
Top down to Bottom, variable slope	
Best-fit values	
BOTTOM	0.1160
TOP	100.7
LOGEC50	-8.811
HILLSLOPE	-1.030
Std. Error	
BOTTOM	0.5851
TOP	1.187
LOGEC50	0.01661
HILLSLOPE	0.03087
95% Confidence Intervals	
BOTTOM	-1.104 to 1.336
TOP	98.18 to 103.1
LOGEC50	-8.846 to -8.776
HILLSLOPE	-1.094 to -0.9655
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9990
Absolute Sum of Squares	34.41
Sy.x	1.312
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

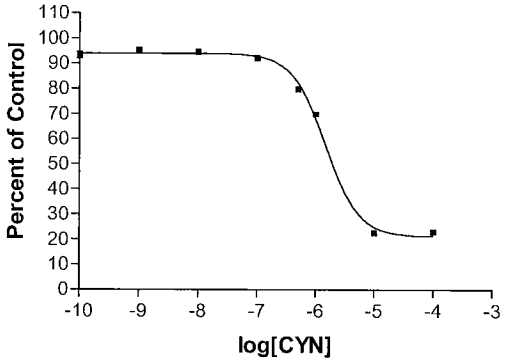
Econazole, Replicate 3



log[ECZ]	Replicate 3		
	Y1	Y2	Y3
-3.0			
-4.0			
-5.0			
-6.0	-0.2	3.0e-002	0.2
-7.0	1.3	1.3	1.5
-8.0	12.2	12.5	12.2
-8.3	21.8	20.9	21.3
-9.0	60.3	61.0	58.9
-9.3	76.2	74.2	75.0
-9.6	86.8	84.9	82.7
-10.0	94.0	91.3	91.1

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	0.1432
TOP	96.88
LOGEC50	-8.804
HILLSLOPE	-1.073
Std. Error	
BOTTOM	0.4460
TOP	0.8613
LOGEC50	0.01259
HILLSLOPE	0.02527
95% Confidence Intervals	
BOTTOM	-0.7873 to 1.074
TOP	95.08 to 98.67
LOGEC50	-8.830 to -8.778
HILLSLOPE	-1.126 to -1.020
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9993
Absolute Sum of Squares	20.65
Sy.x	1.016
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

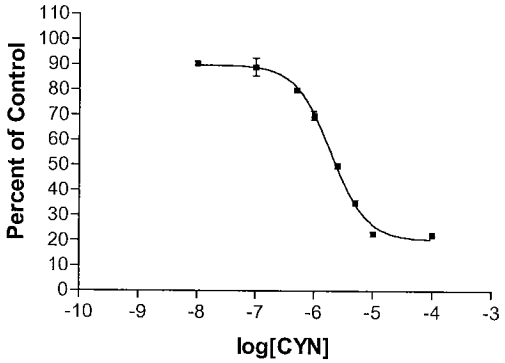
Chrysin, Replicate 1



log[CYN]	Replicate 1		
	Y1	Y2	Y3
-4.0	23.0	23.0	23.6
-5.0	22.8	22.6	22.9
-5.3			
-5.6			
-6.0	69.9	71.0	69.1
-6.3	78.8	81.0	80.3
-7.0	91.8	92.1	93.0
-8.0	93.0	94.9	96.4
-9.0	94.7	95.8	95.7
-10.0	91.1	93.4	95.3

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	21.27
TOP	94.02
LOGEC50	-5.831
HILLSLOPE	-1.528
Std. Error	
BOTTOM	1.100
TOP	0.6105
LOGEC50	0.03025
HILLSLOPE	0.1406
95% Confidence Intervals	
BOTTOM	18.97 to 23.56
TOP	92.75 to 95.30
LOGEC50	-5.894 to -5.768
HILLSLOPE	-1.822 to -1.235
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9960
Absolute Sum of Squares	81.68
Sy.x	2.021
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

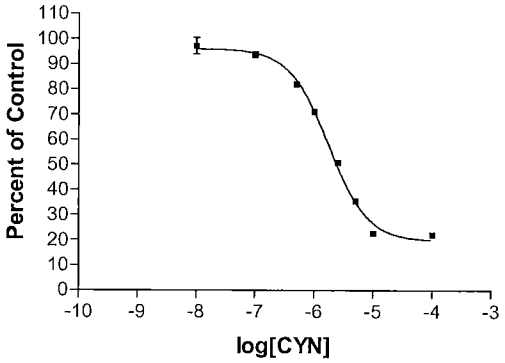
Chrysin, Replicate 2



log[CYN]	Replicate 2		
	Y1	Y2	Y3
-4.0	22.1	23.7	21.5
-5.0	23.5	22.5	22.5
-5.3	36.8	34.6	34.2
-5.6	51.5	50.4	48.1
-6.0	72.4	70.5	66.8
-6.3	80.5	79.8	79.6
-7.0	95.2	88.8	83.0
-8.0	90.7	90.8	89.7
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	20.27
TOP	89.84
LOGEC50	-5.714
HILLSLOPE	-1.432
Std. Error	
BOTTOM	1.568
TOP	1.241
LOGEC50	0.02965
HILLSLOPE	0.1124
95% Confidence Intervals	
BOTTOM	17.00 to 23.54
TOP	87.25 to 92.42
LOGEC50	-5.776 to -5.652
HILLSLOPE	-1.667 to -1.198
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9904
Absolute Sum of Squares	165.1
Sy.x	2.873
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

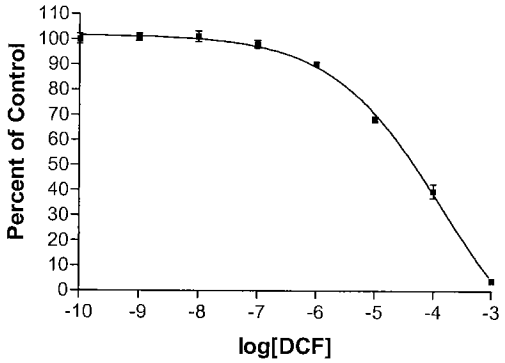
Chrysin, Replicate 3



log[CYN]	Replicate 3		
	Y1	Y2	Y3
-4.0	23.2	21.7	21.8
-5.0	22.7	23.1	22.4
-5.3	37.0	35.7	34.4
-5.6	50.8	52.4	49.7
-6.0	71.2	72.0	70.1
-6.3	81.6	83.3	81.4
-7.0	91.7	95.2	94.5
-8.0	103.8	94.2	93.8
-9.0			
-10.0			

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	19.60
TOP	96.10
LOGEC50	-5.757
HILLSLOPE	-1.302
Std. Error	
BOTTOM	1.645
TOP	1.327
LOGEC50	0.02984
HILLSLOPE	0.09583
95% Confidence Intervals	
BOTTOM	16.17 to 23.03
TOP	93.33 to 98.87
LOGEC50	-5.819 to -5.695
HILLSLOPE	-1.502 to -1.103
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9914
Absolute Sum of Squares	170.9
Sy.x	2.923
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

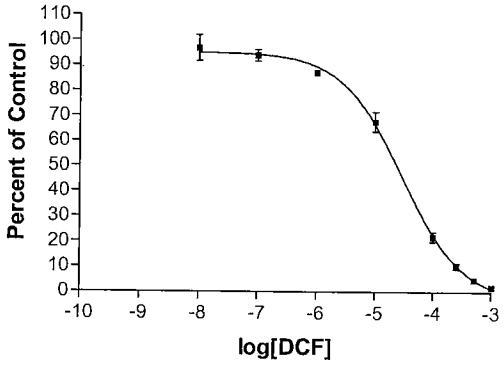
Dicofol, Replicate 1



log[DCF]	Replicate 1		
	Y1	Y2	Y3
-3.0	3.0	4.3	4.7
-3.3			
-3.6			
-4.0	35.5	45.0	38.8
-5.0	70.7	67.4	66.8
-6.0	91.2	91.3	88.6
-7.0	99.7	99.5	94.9
-8.0	105.2	99.8	98.2
-9.0	102.4	102.2	98.0
-10.0	103.3	100.7	96.4

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	-34.12
TOP	101.8
LOGEC50	-3.871
HILLSLOPE	-0.4643
Std. Error	
BOTTOM	13.34
TOP	1.130
LOGEC50	0.2096
HILLSLOPE	0.04879
95% Confidence Intervals	
BOTTOM	-61.95 to -6.291
TOP	99.42 to 104.1
LOGEC50	-4.308 to -3.434
HILLSLOPE	-0.5660 to -0.3625
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9936
Absolute Sum of Squares	176.2
Sy.x	2.968
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

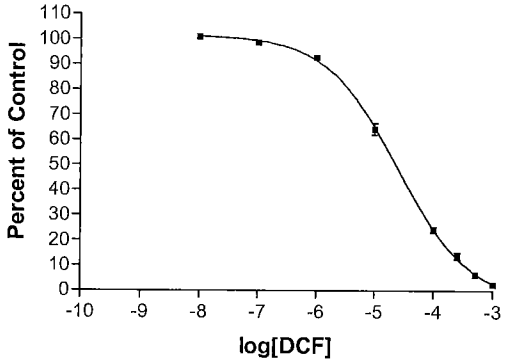
Dicofol, Replicate 2



log[DCF]	Replicate 2		
	Y1	Y2	Y3
-3.0	1.9	2.0	2.0
-3.3	3.9	4.8	5.6
-3.6	7.8	11.7	11.3
-4.0	19.1	25.9	20.9
-5.0	74.2	68.4	60.6
-6.0	86.8	87.5	87.4
-7.0	93.4	98.3	90.2
-8.0	102.9	101.1	86.8
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	-3.624
TOP	95.20
LOGEC50	-4.534
HILLSLOPE	-0.8303
Std. Error	
BOTTOM	3.744
TOP	1.733
LOGEC50	0.07350
HILLSLOPE	0.09404
95% Confidence Intervals	
BOTTOM	-11.43 to 4.185
TOP	91.59 to 98.82
LOGEC50	-4.687 to -4.381
HILLSLOPE	-1.026 to -0.6341
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9907
Absolute Sum of Squares	353.1
Sy.x	4.202
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

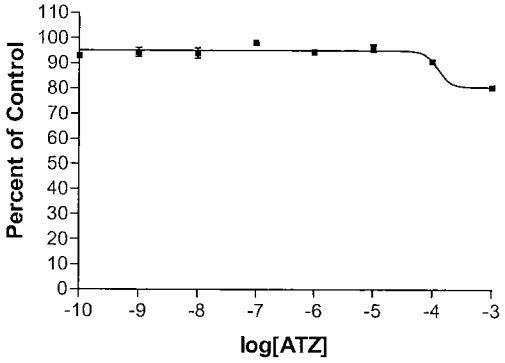
Dicofol, Replicate 3



log[DCF]	Replicate 3		
	Y1	Y2	Y3
-3.0	2.5	2.6	2.2
-3.3	6.5	5.6	7.0
-3.6	14.6	11.1	15.8
-4.0	25.6	25.1	22.0
-5.0	68.4	64.5	60.2
-6.0	92.8	93.2	92.3
-7.0	98.4	99.1	98.7
-8.0	101.4	102.6	99.1
-9.0			
-10.0			

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	-4.540
TOP	101.5
LOGEC50	-4.604
HILLSLOPE	-0.7067
Std. Error	
BOTTOM	2.050
TOP	0.8974
LOGEC50	0.03790
HILLSLOPE	0.03725
95% Confidence Intervals	
BOTTOM	-8.815 to -0.2641
TOP	99.64 to 103.4
LOGEC50	-4.683 to -4.525
HILLSLOPE	-0.7844 to -0.6290
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9982
Absolute Sum of Squares	72.21
Sy.x	1.900
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

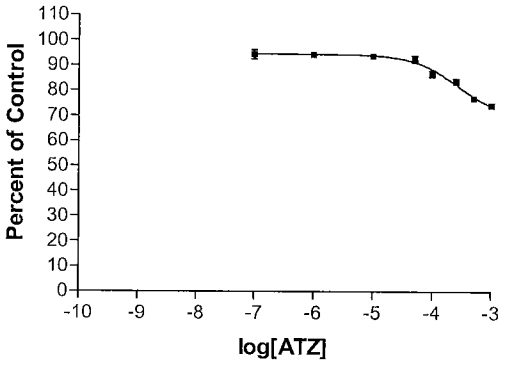
Atrazine, Replicate 1



log[ATZ]	Replicate 1		
	Y1	Y2	Y3
-3.0	81.2	80.6	79.8
-3.3			
-3.6			
-4.0	90.0	90.8	91.7
-4.3			
-5.0	98.0	97.3	93.2
-6.0	93.3	96.1	94.6
-7.0	99.5	98.2	96.8
-8.0	97.2	90.2	94.9
-9.0	95.6	97.0	90.8
-10.0	93.3	94.4	91.7

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	80.57
TOP	95.12
LOGEC50	-3.904
HILLSLOPE	-3.931
Std. Error	
BOTTOM	8.953
TOP	0.6369
LOGEC50	20.82
HILLSLOPE	825.3
95% Confidence Intervals	
BOTTOM	61.90 to 99.25
TOP	93.79 to 96.45
LOGEC50	-47.34 to 39.53
HILLSLOPE	-1726 to 1718
Goodness of Fit	
Degrees of Freedom	20
R^2	0.8207
Absolute Sum of Squares	121.7
Sy.x	2.467
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

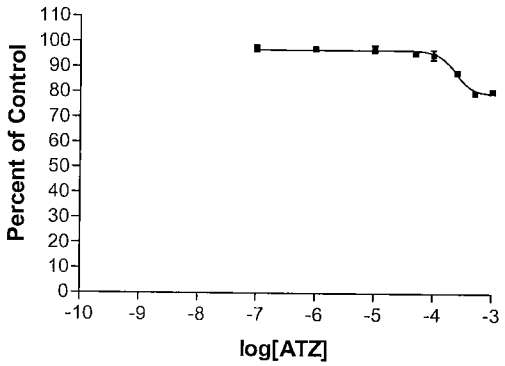
Atrazine, Replicate 2



log[ATZ]	Replicate 2		
	Y1	Y2	Y3
-3.0	73.9	75.5	72.9
-3.3	76.9	77.4	76.9
-3.6	85.4	81.7	83.8
-4.0	84.8	86.6	89.0
-4.3	90.8	95.2	92.0
-5.0	93.6	95.4	92.4
-6.0	95.2	94.0	93.7
-7.0	95.3	96.9	90.9
-8.0			
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	69.51
TOP	94.43
LOGEC50	-3.569
HILLSLOPE	-1.163
Std. Error	
BOTTOM	4.407
TOP	0.7146
LOGEC50	0.1641
HILLSLOPE	0.3000
95% Confidence Intervals	
BOTTOM	60.32 to 78.71
TOP	92.94 to 95.93
LOGEC50	-3.911 to -3.226
HILLSLOPE	-1.789 to -0.5372
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9486
Absolute Sum of Squares	74.10
Sy.x	1.925
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

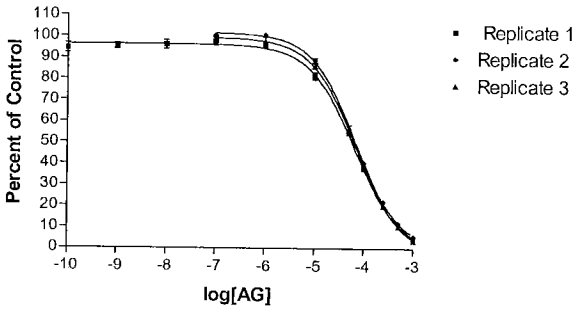
Atrazine, Replicate 3



log[ATZ]	Replicate 3		
	Y1	Y2	Y3
-3.0	80.6	80.9	80.2
-3.3	79.7	80.4	79.9
-3.6	88.2	89.5	85.9
-4.0	98.5	94.7	91.8
-4.3	96.5	95.6	95.0
-5.0	100.0	95.1	96.5
-6.0	99.3	96.9	96.0
-7.0	99.7	97.3	95.1
-8.0			
-9.0			
-10.0			

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	79.55
TOP	96.84
LOGEC50	-3.629
HILLSLOPE	-3.042
Std. Error	
BOTTOM	1.198
TOP	0.5652
LOGEC50	0.04352
HILLSLOPE	1.029
95% Confidence Intervals	
BOTTOM	77.05 to 82.05
TOP	95.66 to 98.02
LOGEC50	-3.719 to -3.538
HILLSLOPE	-5.188 to -0.8949
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9419
Absolute Sum of Squares	72.56
Sy.x	1.905
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

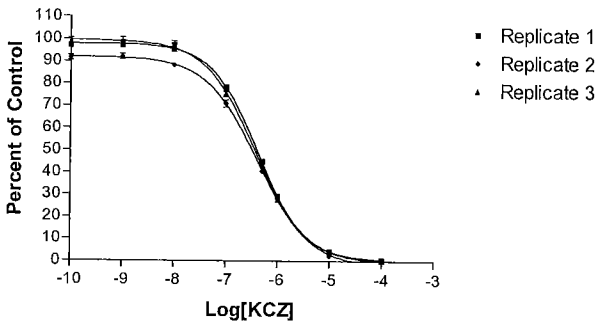
Aminoglutethimide



log[AG]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	5.1	4.8	5.0	5.1	5.5	5.9	3.8	3.5	3.5
-3.3				12.1	11.8	11.7	10.3	10.6	9.9
-3.6				22.0	22.5	21.9	20.3	18.7	20.7
-4.0	38.1	38.3	36.7	41.2	40.5	40.0	40.3	39.6	39.4
-4.3				59.6	53.4	56.6	56.8	54.2	53.2
-5.0	84.4	81.8	78.1	90.6	89.2	86.6	88.6	84.9	84.9
-6.0	98.6	95.9	93.7	102.3	100.6	99.3	97.6	101.1	94.8
-7.0	101.3	96.2	95.6	101.7	101.3	98.6	101.3	98.5	96.5
-8.0	100.1	95.1	93.3						
-9.0	97.8	95.3	93.0						
-10.0	98.6	90.8	94.0						

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	-1.984	-0.5217	-3.500
TOP	96.27	101.7	99.40
LOGEC50	-4.182	-4.178	-4.159
HILLSLOPE	-0.9437	-0.9842	-0.9547
Std. Error			
BOTTOM	2.941	1.624	1.830
TOP	0.7558	0.7431	0.7882
LOGEC50	0.04567	0.01973	0.02204
HILLSLOPE	0.07679	0.04441	0.04587
95% Confidence Intervals			
BOTTOM	-8.119 to 4.150	-3.909 to 2.866	-7.318 to 0.3182
TOP	94.70 to 97.85	100.1 to 103.2	97.76 to 101.0
LOGEC50	-4.278 to -4.087	-4.219 to -4.136	-4.205 to -4.113
HILLSLOPE	-1.104 to -0.7836	-1.077 to -0.8915	-1.050 to -0.8590
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9942	0.9984	0.9982
Absolute Sum of Squares	149.7	52.71	57.79
Sy.x	2.736	1.623	1.700
Data			
Number of X values	11	8	8
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	9	0	0

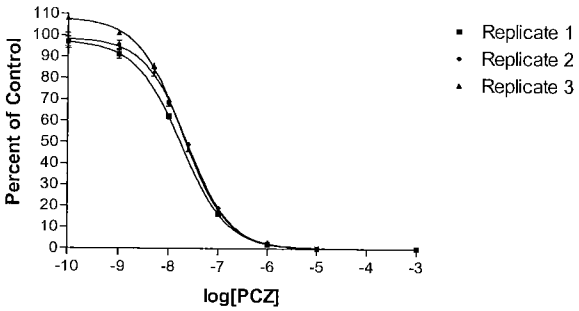
Ketoconazole



Log[KCZ]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.0	0.7	0.4	0.5	-1.1	-1.1	-1.1	0.4	0.1	0.2
-5.0	4.7	4.3	4.6	2.0	2.3	2.5	4.4	3.8	4.0
-6.0	29.9	28.8	28.7	27.9	28.4	28.7	28.9	27.1	27.3
-6.3	46.0	44.8	44.1	41.9	40.5	39.4	45.2	43.3	42.4
-7.0	80.6	77.6	75.4	73.5	71.1	68.4	78.5	74.4	73.8
-8.0	100.3	97.1	93.4	89.3	88.0	87.2	99.7	95.9	92.7
-9.0	100.2	97.8	95.0	94.5	91.1	90.3	100.5	100.6	96.4
-10.0	98.2	97.3	94.9	93.7	91.3	89.9	101.1	99.1	96.7

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	0.2081	-2.476	-0.6192
TOP	97.94	91.97	99.39
LOGEC50	-6.381	-6.378	-6.429
HILLSLOPE	-0.9772	-0.8726	-0.9062
Std. Error			
BOTTOM	0.9492	0.7907	0.9722
TOP	0.7048	0.5661	0.7244
LOGEC50	0.01886	0.01678	0.01980
HILLSLOPE	0.04268	0.03115	0.03819
95% Confidence Intervals			
BOTTOM	-1.772 to 2.188	-4.125 to -0.8262	-2.647 to 1.409
TOP	96.47 to 99.41	90.79 to 93.15	97.88 to 100.9
LOGEC50	-6.420 to -6.342	-6.413 to -6.343	-6.470 to -6.387
HILLSLOPE	-1.066 to -0.8882	-0.9376 to -0.8076	-0.9859 to -0.8266
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9980	0.9987	0.9981
Absolute Sum of Squares	72.56	42.90	71.38
Sy.x	1.905	1.465	1.889
Data			
Number of X values	8	8	8
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	0	0	0

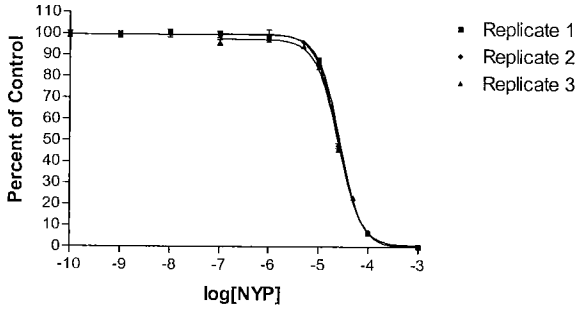
Prochloraz



log[PCZ]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	-0.1	0.1	4.0e-002						
-4.0	-0.2	-0.1	0.1						
-5.0	0.1	0.4	0.3	0.1	0.2	0.2	-1.0e-002	-0.2	0.2
-6.0	1.9	2.3	2.0	2.0	2.4	2.3	3.0	3.0	2.9
-7.0	16.4	15.8	16.3	18.4	19.7	19.1	18.6	18.3	17.5
-7.6				49.7	49.5	47.4	47.3	47.2	44.8
-8.0	61.6	64.1	60.3	69.8	67.7	66.3	68.2	69.9	71.5
-8.3				84.1	84.2	79.1	85.2	86.3	85.8
-9.0	95.0	89.7	88.7	98.7	96.0	92.3	102.8	100.7	99.8
-10.0	100.2	98.0	92.5	90.3	102.1	99.8	109.0	108.8	106.1

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	-0.07196	-0.05843	0.2152
TOP	97.44	98.70	108.1
LOGEC50	-7.743	-7.621	-7.725
HILLSLOPE	-0.9392	-0.9971	-0.9697
Std. Error			
BOTTOM	0.5527	1.239	0.5643
TOP	0.9589	1.332	0.6502
LOGEC50	0.02266	0.02643	0.01124
HILLSLOPE	0.03994	0.05585	0.02336
95% Confidence Intervals			
BOTTOM	-1.225 to 1.081	-2.643 to 2.526	-0.9618 to 1.392
TOP	95.44 to 99.44	95.92 to 101.5	106.8 to 109.5
LOGEC50	-7.791 to -7.696	-7.676 to -7.565	-7.749 to -7.702
HILLSLOPE	-1.023 to -0.8559	-1.114 to -0.8806	-1.018 to -0.9210
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9984	0.9958	0.9992
Absolute Sum of Squares	62.58	143.1	30.44
Sy.x	1.769	2.675	1.234
Data			
Number of X values	10	10	10
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	6	6	6

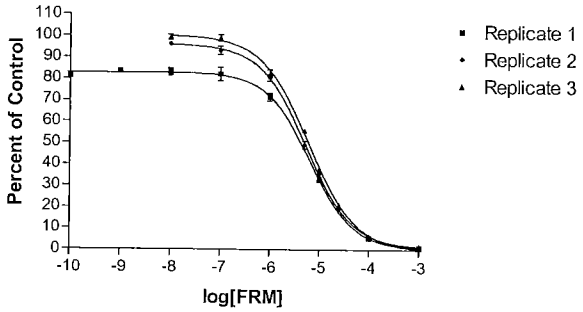
4-Nonylphenol



log[NYP]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	2.0e-002	0.2	0.1	0.2	0.1	1.0e-002	-3.0e-002	-0.1	0.1
-4.0	7.4	5.7	6.3	7.0	7.2	7.0	7.1	6.7	6.7
-4.3				22.6	22.6	22.0	23.9	23.6	22.5
-4.6				48.8	48.5	45.3	45.1	47.1	44.9
-5.0	89.1	88.3	84.9	88.5	87.9	84.2	84.6	86.1	82.8
-5.3				97.2	94.6	96.2	94.3	95.5	92.8
-6.0	98.2	98.6	97.8	104.4	97.1	96.5	95.4	96.9	98.8
-7.0	102.0	98.6	99.1	100.8	100.0	96.9	93.4	96.8	97.2
-8.0	103.6	97.9	98.9						
-9.0	101.3	100.3	96.8						
-10.0	102.3	99.2	97.3						

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	0.01138	0.7939	0.7179
TOP	99.48	99.90	97.38
LOGEC50	-4.574	-4.608	-4.606
HILLSLOPE	-2.017	-1.948	-1.908
Std. Error			
BOTTOM	1.075	1.134	1.099
TOP	0.4728	0.8444	0.8158
LOGEC50	0.02684	0.01230	0.01233
HILLSLOPE	0.1120	0.09056	0.08676
95% Confidence Intervals			
BOTTOM	-2.230 to 2.253	-1.572 to 3.160	-1.575 to 3.011
TOP	98.49 to 100.5	98.14 to 101.7	95.68 to 99.08
LOGEC50	-4.630 to -4.518	-4.633 to -4.582	-4.632 to -4.580
HILLSLOPE	-2.250 to -1.783	-2.137 to -1.759	-2.089 to -1.727
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9984	0.9974	0.9975
Absolute Sum of Squares	66.46	99.99	92.36
Sy.x	1.823	2.236	2.149
Data			
Number of X values	11	8	8
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	9	0	0

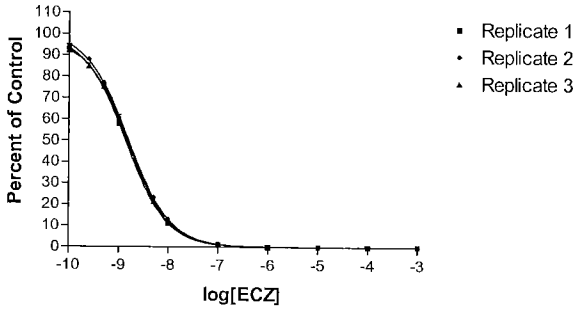
Fenarimol



log[FRM]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	1.0	1.0	1.1	1.3	1.5	2.6	1.0	0.9	0.7
-4.0	5.7	6.0	5.2	6.4	6.3	5.9	6.5	6.3	6.4
-4.6				19.6	19.5	18.7	21.1	22.1	20.5
-5.0	33.9	32.5	32.7	35.8	36.7	34.7	39.1	37.1	38.0
-5.3				51.1	51.1	45.7	56.2	56.0	55.7
-6.0	75.2	70.1	69.6	83.0	81.2	77.7	79.2	85.0	83.3
-7.0	88.4	80.7	77.3	97.0	90.8	91.7	102.0	98.1	97.1
-8.0	86.6	81.2	82.0	98.2	95.3	95.3	101.4	99.5	97.3
-9.0	84.5	84.1	82.0						
-10.0	83.7	81.9	78.9						

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	0.5092	0.9139	-0.5225
TOP	82.93	96.07	100.2
LOGEC50	-5.188	-5.266	-5.213
HILLSLOPE	-0.9924	-0.9430	-0.9089
Std. Error			
BOTTOM	1.358	1.037	1.020
TOP	0.7732	0.8917	0.8354
LOGEC50	0.03603	0.02056	0.01867
HILLSLOPE	0.07539	0.04181	0.03605
95% Confidence Intervals			
BOTTOM	-2.323 to 3.342	-1.249 to 3.077	-2.649 to 1.605
TOP	81.32 to 84.55	94.21 to 97.93	98.46 to 101.9
LOGEC50	-5.263 to -5.113	-5.309 to -5.223	-5.252 to -5.174
HILLSLOPE	-1.150 to -0.8351	-1.030 to -0.8558	-0.9841 to -0.8337
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9954	0.9976	0.9981
Absolute Sum of Squares	126.9	73.54	63.98
Sy.x	2.519	1.918	1.789
Data			
Number of X values	10	8	8
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	6	0	0

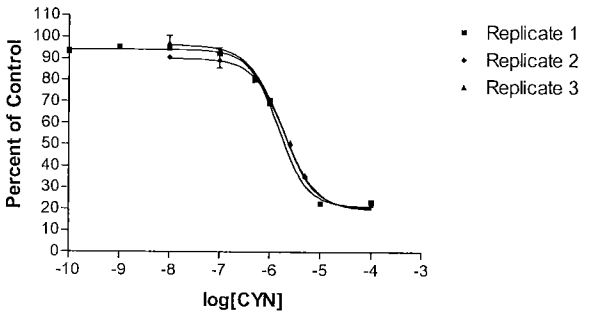
Econazole



log[ECZ]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	0.1	0.1	0.1						
-4.0	0.3	-3.0e-002	-0.1						
-5.0	-4.0e-002	0.1	-2.0e-002						
-6.0	0.2	0.1	0.2	0.1	0.1	0.1	-0.2	3.0e-002	0.2
-7.0	1.2	1.2	1.3	1.4	1.4	1.4	1.3	1.3	1.5
-8.0	11.3	11.1	11.0	12.9	13.7	12.9	12.2	12.5	12.2
-8.3				23.4	23.6	23.1	21.8	20.9	21.3
-9.0	59.0	~~8.8~~	57.0	63.6	58.8	59.8	60.3	61.0	58.9
-9.3				78.2	77.9	74.9	76.2	74.2	75.0
-9.6				89.5	87.3	87.4	86.8	84.9	82.7
-10.0	96.9	91.5	91.5	96.3	93.4	93.4	94.0	91.3	91.1

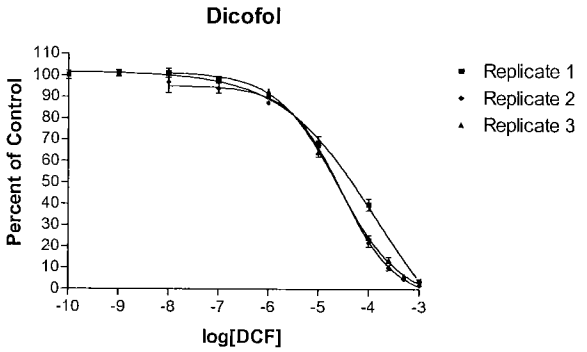
	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	0.06986	0.1160	0.1432
TOP	99.24	100.7	96.88
LOGEC50	-8.859	-8.811	-8.804
HILLSLOPE	-1.048	-1.030	-1.073
Std. Error			
BOTTOM	0.2891	0.5851	0.4460
TOP	1.088	1.187	0.8613
LOGEC50	0.01745	0.01661	0.01259
HILLSLOPE	0.03602	0.03087	0.02527
95% Confidence Intervals			
BOTTOM	-0.5352 to 0.6749	-1.104 to 1.336	-0.7873 to 1.074
TOP	96.96 to 101.5	98.18 to 103.1	95.08 to 98.67
LOGEC50	-8.896 to -8.823	-8.846 to -8.776	-8.830 to -8.778
HILLSLOPE	-1.124 to -0.9730	-1.094 to -0.9655	-1.126 to -1.020
Goodness of Fit			
Degrees of Freedom	19	20	20
R^2	0.9991	0.9990	0.9993
Absolute Sum of Squares	21.62	34.41	20.65
Sy.x	1.067	1.312	1.016
Data			
Number of X values	11	11	11
Number of Y replicates	3	3	3
Total number of values	23	24	24
Number of missing values	10	9	9

Chrysin



log[CYN]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.0	23.0	23.0	23.6	22.1	23.7	21.5	23.2	21.7	21.8
-5.0	22.8	22.6	22.9	23.5	22.5	22.5	22.7	23.1	22.4
-5.3				36.8	34.6	34.2	37.0	35.7	34.4
-5.6				51.5	50.4	48.1	50.8	52.4	49.7
-6.0	69.9	71.0	69.1	72.4	70.5	66.8	71.2	72.0	70.1
-6.3	78.8	81.0	80.3	80.5	79.8	79.6	81.6	83.3	81.4
-7.0	91.8	92.1	93.0	95.2	88.8	83.0	91.7	95.2	94.5
-8.0	93.0	94.9	96.4	90.7	90.8	89.7	103.8	94.2	93.8
-9.0	94.7	95.8	95.7						
-10.0	91.1	93.4	95.3						

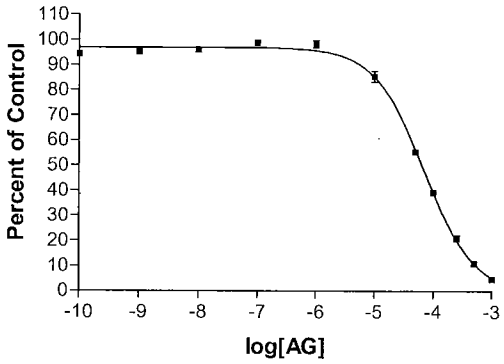
	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	21.27	20.27	19.60
TOP	94.02	89.84	96.10
LOGEC50	-5.831	-5.714	-5.757
HILLSLOPE	-1.528	-1.432	-1.302
Std. Error			
BOTTOM	1.100	1.568	1.645
TOP	0.6105	1.241	1.327
LOGEC50	0.03024	0.02965	0.02984
HILLSLOPE	0.1407	0.1124	0.09583
95% Confidence Intervals			
BOTTOM	18.97 to 23.56	17.00 to 23.54	16.17 to 23.03
TOP	92.75 to 95.30	87.25 to 92.42	93.33 to 98.87
LOGEC50	-5.894 to -5.768	-5.776 to -5.652	-5.819 to -5.695
HILLSLOPE	-1.822 to -1.235	-1.667 to -1.198	-1.502 to -1.103
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9960	0.9904	0.9914
Absolute Sum of Squares	81.68	165.1	170.9
Sy.x	2.021	2.873	2.923
Data			
Number of X values	10	8	8
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	6	0	0



log[DCF]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	3.0	4.3	4.7	1.9	2.0	2.0	2.5	2.6	2.2
-3.3				3.9	4.8	5.6	6.5	5.6	7.0
-3.6				7.8	11.7	11.3	14.6	11.1	15.8
-4.0	35.5	45.0	38.8	19.1	25.9	20.9	25.6	25.1	22.0
-5.0	70.7	67.4	66.8	74.2	68.4	60.6	68.4	64.5	60.2
-6.0	91.2	91.3	88.6	86.8	87.5	87.4	92.8	93.2	92.3
-7.0	99.7	99.5	94.9	93.4	98.3	90.2	98.4	99.1	98.7
-8.0	105.2	99.8	98.2	102.9	101.1	86.8	101.4	102.6	99.1
-9.0	102.4	102.2	98.0						
-10.0	103.3	100.7	96.4						

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	-34.12	-3.624	-4.540
TOP	101.8	95.20	101.5
LOGEC50	-3.871	-4.534	-4.604
HILLSLOPE	-0.4643	-0.8303	-0.7067
Std. Error			
BOTTOM	13.34	3.744	2.050
TOP	1.130	1.733	0.8974
LOGEC50	0.2096	0.07350	0.03790
HILLSLOPE	0.04879	0.09404	0.03725
95% Confidence Intervals			
BOTTOM	-61.95 to -6.291	-11.43 to 4.185	-8.815 to -0.2641
TOP	99.42 to 104.1	91.59 to 98.82	99.64 to 103.4
LOGEC50	-4.308 to -3.434	-4.687 to -4.381	-4.683 to -4.525
HILLSLOPE	-0.5660 to -0.3625	-1.026 to -0.6341	-0.7844 to -0.6290
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9936	0.9907	0.9982
Absolute Sum of Squares	176.2	353.1	72.21
Sy.x	2.968	4.202	1.900
Data			
Number of X values	10	8	8
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	6	0	0

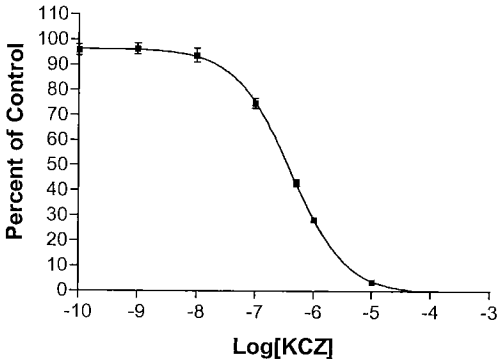
Aminoglutethimide



log[AG]	Data Set-A		
	Y	SEM	N
-3.0	4.7	0.6	3
-3.3	11.1	0.8	2
-3.6	21.0	1.1	2
-4.0	39.3	0.8	3
-4.3	55.6	0.9	2
-5.0	85.5	2.2	3
-6.0	98.2	1.4	3
-7.0	99.0	0.8	3
-8.0	96.2	0.0	1
-9.0	95.4	0.0	1
-10.0	94.5	0.0	1

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	-0.6067
TOP	96.89
LOGEC50	-4.155
HILLSLOPE	-1.025
Std. Error	
BOTTOM	2.884
TOP	0.8194
LOGEC50	0.03689
HILLSLOPE	0.08293
95% Confidence Intervals	
BOTTOM	-7.427 to 6.213
TOP	94.95 to 98.83
LOGEC50	-4.242 to -4.067
HILLSLOPE	-1.221 to -0.8292
Goodness of Fit	
Degrees of Freedom	7
R^2	0.9985
Absolute Sum of Squares	21.96
Sy.x	1.771
Data	
Number of X values	11
# of Y replicates (mean analyzed)	1
Total number of values	11
Number of missing values	0

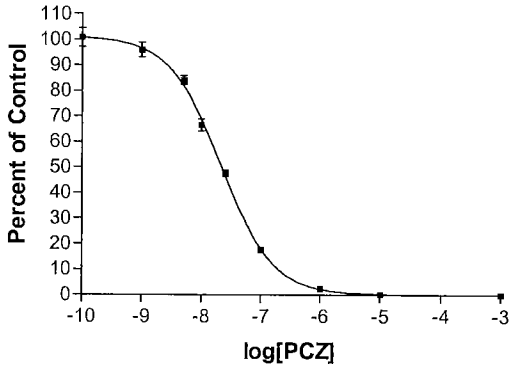
Ketoconazole



Log[KCZ]	Data Set-A		
	Y	SEM	N
-4.0	-0.1	0.5	3
-5.0	3.6	0.7	3
-6.0	28.4	0.4	3
-6.3	43.1	1.3	3
-7.0	74.8	2.0	3
-8.0	93.7	2.8	3
-9.0	96.3	2.2	3
-10.0	95.8	2.2	3

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	-0.9384
TOP	96.41
LOGEC50	-6.396
HILLSLOPE	-0.9187
Std. Error	
BOTTOM	0.4359
TOP	0.3205
LOGEC50	0.008931
HILLSLOPE	0.01797
95% Confidence Intervals	
BOTTOM	-2.149 to 0.2718
TOP	95.52 to 97.30
LOGEC50	-6.421 to -6.371
HILLSLOPE	-0.9686 to -0.8688
Goodness of Fit	
Degrees of Freedom	4
R^2	0.9999
Absolute Sum of Squares	0.9517
Sy.x	0.4878
Data	
Number of X values	8
# of Y replicates (mean analyzed)	1
Total number of values	8
Number of missing values	0

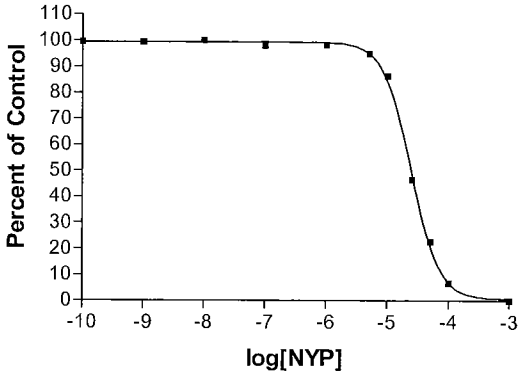
Prochloraz



log[PCZ]	Data Set-A		
	Y	SEM	N
-3.0	1.0e-002	0.0	1
-4.0	-0.1	0.0	1
-5.0	0.1	0.1	3
-6.0	2.4	0.3	3
-7.0	17.8	0.9	3
-7.6	47.6	1.2	2
-8.0	66.6	2.4	3
-8.3	84.1	1.7	2
-9.0	96.0	2.9	3
-10.0	100.8	3.6	3

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	0.007779
TOP	101.2
LOGEC50	-7.668
HILLSLOPE	-0.9956
Std. Error	
BOTTOM	0.7428
TOP	1.223
LOGEC50	0.02179
HILLSLOPE	0.04626
95% Confidence Intervals	
BOTTOM	-1.810 to 1.825
TOP	98.18 to 104.2
LOGEC50	-7.721 to -7.614
HILLSLOPE	-1.109 to -0.8824
Goodness of Fit	
Degrees of Freedom	6
R^2	0.9993
Absolute Sum of Squares	11.84
Sy.x	1.405
Data	
Number of X values	10
# of Y replicates (mean analyzed)	1
Total number of values	10
Number of missing values	0

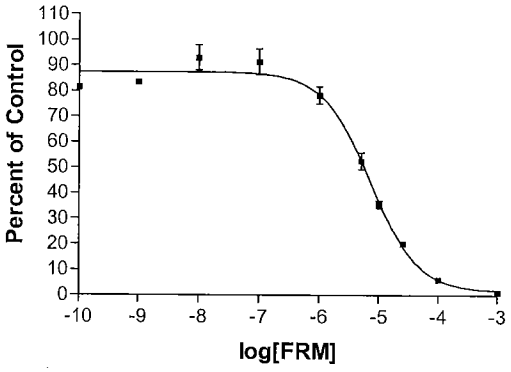
4-Nonylphenol



log[NYP]	Data Set-A		
	Y	SEM	N
-3.0	0.1	3.2e-002	3
-4.0	6.8	0.2	3
-4.3	22.9	0.5	2
-4.6	46.6	0.9	2
-5.0	86.3	0.9	3
-5.3	95.1	0.9	2
-6.0	98.2	0.7	3
-7.0	98.3	1.3	3
-8.0	100.1	0.0	1
-9.0	99.5	0.0	1
-10.0	99.6	0.0	1

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	0.6543
TOP	99.38
LOGEC50	-4.610
HILLSLOPE	-1.916
Std. Error	
BOTTOM	1.356
TOP	0.6711
LOGEC50	0.01434
HILLSLOPE	0.09936
95% Confidence Intervals	
BOTTOM	-2.553 to 3.862
TOP	97.80 to 101.0
LOGEC50	-4.644 to -4.576
HILLSLOPE	-2.151 to -1.681
Goodness of Fit	
Degrees of Freedom	7
R^2	0.9990
Absolute Sum of Squares	16.74
Sy.x	1.547
Data	
Number of X values	11
# of Y replicates (mean analyzed)	1
Total number of values	11
Number of missing values	0

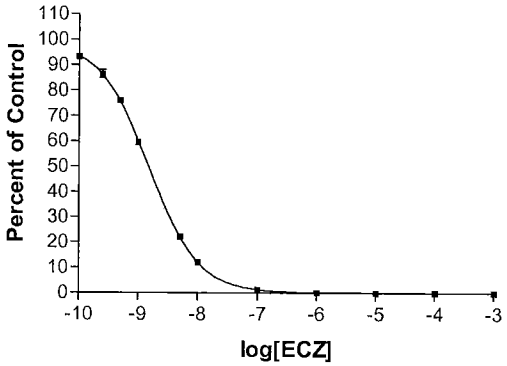
Fenarimol



log[FRM]	Data Set-A		
	Y	SEM	N
-3.0	1.2	0.3	3
-4.0	6.1	0.2	3
-4.6	20.3	1.0	2
-5.0	35.6	1.5	3
-5.3	52.6	3.3	2
-6.0	78.3	3.4	3
-7.0	91.5	5.0	3
-8.0	93.0	4.9	3
-9.0	83.5	0.0	1
-10.0	81.5	0.0	1

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	1.398
TOP	87.50
LOGEC50	-5.146
HILLSLOPE	-1.087
Std. Error	
BOTTOM	3.812
TOP	2.162
LOGEC50	0.07037
HILLSLOPE	0.1939
95% Confidence Intervals	
BOTTOM	-7.929 to 10.73
TOP	82.21 to 92.79
LOGEC50	-5.318 to -4.974
HILLSLOPE	-1.562 to -0.6126
Goodness of Fit	
Degrees of Freedom	6
R^2	0.9907
Absolute Sum of Squares	108.7
Sy.x	4.257
Data	
Number of X values	10
# of Y replicates (mean analyzed)	1
Total number of values	10
Number of missing values	0

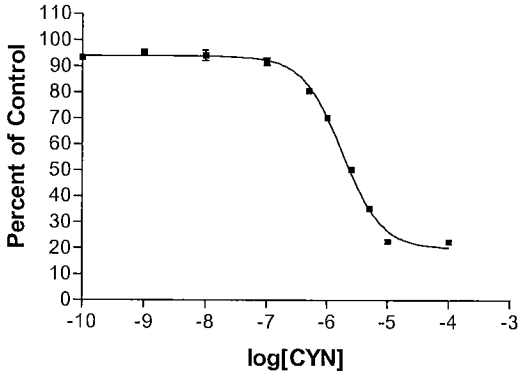
Econazole



log[ECZ]	Data Set-A		
	Y	SEM	N
-3.0	0.1	0.0	1
-4.0	0.1	0.0	1
-5.0	1.0e-002	0.0	1
-6.0	0.1	4.6e-002	3
-7.0	1.3	0.1	3
-8.0	12.2	0.6	3
-8.3	22.3	1.0	2
-9.0	59.6	0.8	3
-9.3	76.1	0.9	2
-9.6	86.4	1.6	2
-10.0	93.3	0.6	3

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	0.07620
TOP	99.04
LOGEC50	-8.818
HILLSLOPE	-1.049
Std. Error	
BOTTOM	0.1839
TOP	0.5995
LOGEC50	0.008488
HILLSLOPE	0.01519
95% Confidence Intervals	
BOTTOM	-0.3587 to 0.5111
TOP	97.62 to 100.5
LOGEC50	-8.838 to -8.797
HILLSLOPE	-1.085 to -1.013
Goodness of Fit	
Degrees of Freedom	7
R^2	0.9999
Absolute Sum of Squares	1.112
Sy.x	0.3986
Data	
Number of X values	11
# of Y replicates (mean analyzed)	1
Total number of values	11
Number of missing values	0

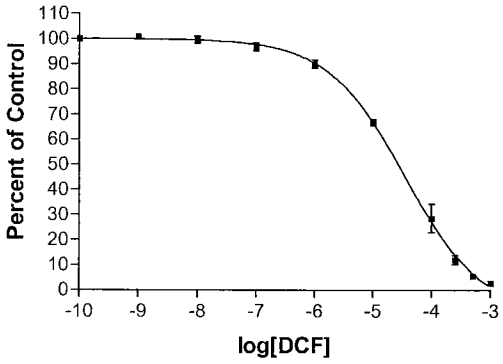
Chrysin



log[CYN]	Data Set-A		
	Y	SEM	N
-4.0	22.6	0.3	3
-5.0	22.8	2.5e-002	3
-5.3	35.5	0.2	2
-5.6	50.5	0.5	2
-6.0	70.3	0.4	3
-6.3	80.7	0.7	3
-7.0	91.7	1.4	3
-8.0	94.1	2.0	3
-9.0	95.4	0.0	1
-10.0	93.3	0.0	1

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	19.99
TOP	93.86
LOGEC50	-5.750
HILLSLOPE	-1.325
Std. Error	
BOTTOM	2.169
TOP	1.182
LOGEC50	0.03857
HILLSLOPE	0.1255
95% Confidence Intervals	
BOTTOM	14.68 to 25.29
TOP	90.97 to 96.75
LOGEC50	-5.845 to -5.656
HILLSLOPE	-1.632 to -1.018
Goodness of Fit	
Degrees of Freedom	6
R^2	0.9963
Absolute Sum of Squares	30.56
Sy.x	2.257
Data	
Number of X values	10
# of Y replicates (mean analyzed)	1
Total number of values	10
Number of missing values	0

Dicofol



log[DCF]	Data Set-A		
	Y	SEM	N
-3.0	2.8	0.6	3
-3.3	5.6	0.8	2
-3.6	12.1	1.8	2
-4.0	28.7	5.6	3
-5.0	66.8	1.2	3
-6.0	90.1	1.6	3
-7.0	96.9	1.5	3
-8.0	99.7	1.4	3
-9.0	100.9	0.0	1
-10.0	100.1	0.0	1

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	-9.782
TOP	100.1
LOGEC50	-4.452
HILLSLOPE	-0.6562
Std. Error	
BOTTOM	3.178
TOP	0.7530
LOGEC50	0.05625
HILLSLOPE	0.03993
95% Confidence Intervals	
BOTTOM	-17.56 to -2.005
TOP	98.26 to 101.9
LOGEC50	-4.589 to -4.314
HILLSLOPE	-0.7539 to -0.5585
Goodness of Fit	
Degrees of Freedom	6
R^2	0.9993
Absolute Sum of Squares	11.37
Sy.x	1.377
Data	
Number of X values	10
# of Y replicates (mean analyzed)	1
Total number of values	10
Number of missing values	0

APPENDIX B

BATTELLE REPORT

ERRATA

To the Draft Final Report
For WA 4-16, Task 7

The aminoglutethimide and ketoconazole IC_{50} values reported in the individual laboratory reports are incorrect by a factor of 10, i.e., the IC_{50} for aminoglutethimide is 10-fold high and for ketoconazole is 10-fold low. This error occurred due to a miscommunication by the Chemical Repository as to the identity of these two reference chemicals and their respective stock concentrations. In this overall report, the errors were corrected for each of the laboratories in order for an evaluation of the intralaboratory and interlaboratory results to be made without having to be concerned about this discrepancy. The individual laboratory reports will be corrected by their respective study directors when the draft final reports are finalized.

DRAFT REPORT

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES

Prepared By:

Battelle Memorial Institute
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Prepared For:

EPA Contract: 68-W-01-023
Work Assignment: WA 4-16, Task 7

Battelle Study No. G608316

DRAFT REPORT

Title:	AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES
Author:	Bozena D. Lusiak, Ph.D.
Performing Laboratory:	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693
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Study Initiation Date:	July 11, 2005
Experimental Dates:	July 20, 2005 through October 4, 2005

Author:	Approved:
_____	_____
Signature Date	Signature Date

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit (QAU) inspected this study and reports were submitted to the Study Director and management as follows:

Phase Inspected	Date Inspected	Date of Report to Study Director/Management
Protocol review	7/6/2005	7/6/2005
Sample preparation	8/8/2005	8/9/2005
Test system preparation	8/8/2005	8/9/2005
Test substance preparation	8/8/2005	8/9/2005
Dilution	8/8/2005	8/9/2005
Centrifugation	8/8/2005	8/9/2005
Extraction	8/8/2005	8/9/2005
Dispensing	8/8/2005	8/9/2005
Protein analysis	8/8/2005	8/9/2005
Solution preparation	8/9/2005	8/9/2005
Protocol amendment review	11/18/2005	11/18/2005
Protocol amendment review	12/16/2005	12/16/2005
Audit study file	3/15/2006	3/15/2006
Audit draft final report	3/15/2006	3/15/2006
Protocol amendment review	3/20/2006	3/20/2006

Quality Assurance Unit — 5/24/06 Date

COMPLIANCE STATEMENT

This study, Battelle Study No. G608316, was conducted in compliance with the United States (U.S.) Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) Standards (40 CFR, Part 160), October 16, 1989; the standard operating procedures (SOPs) of Battelle Memorial Institute; and the protocol as approved by the Sponsor.

Bozena D. Lusiak, Ph.D.
Study Director

Date

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1.0 EXECUTIVE SUMMARY

The primary objective of this task was to evaluate the responsiveness of the aromatase assay to various chemicals using microsomes prepared by Battelle and In Vitro Technologies laboratories.

Ten reference chemicals, including aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, dibenz[a,h]anthracene, fenarimol, econazole, chrysin, dicofol, and atrazine, were tested for their ability to affect CYP19 aromatase activity in human placental microsomes using the classic ^3H_2O method. Briefly, each inhibitor was incubated with microsomes in the presence of ^{3}H-androstenedione (substrate for aromatase) at pH 7.4 and 37 ± 1°C, for 15 minutes. Three independent replicates were conducted for each reference chemical. Eight concentrations of each reference chemical were tested in the aromatase assay to construct a dose-response curve from which IC_{50} was calculated. Four types of control samples were included for each replicate: full and background enzyme activity control, positive (with 4-hydroxyandrostenedione as a known aromatase inhibitor) and negative (with lindane a known non-inhibitor) controls. The average IC_{50} for aminoglutethimide was 81.02 μM, for ketoconazole 0.4984 μM, for prochloraz 0.02688 μM, for 4-nonylphenol 37.98 μM, for fenarimol 6.045 μM, for econazole 2.433 nM, for chrysin 2.207 μM, and for dicofol 24.14 μM. The IC_{50} values were generated by fitting the mean percent of control aromatase activity values for each replicate (three repetitions for each replicate, across three replicates for each of the reference chemicals). From all tested potential aromatase inhibitors, econazole was the most potent. Two tested reference chemicals, dibenz[a,h]- anthracene and atrazine, exhibited no aromatase activity inhibition properties.

2.0 INTRODUCTION

2.1 Background

The Food Quality Protection Act of 1996 was enacted by Congress to authorize the Environmental Protection Agency (EPA) to implement a screening program on pesticides and other chemicals found in food or water sources for endocrine effects in humans. Thus, the U.S. EPA is implementing an Endocrine Disruptor Screening Program (EDSP). In this program, comprehensive toxicological and ecotoxicological screens and tests are being developed for identifying and characterizing the endocrine effects of various environmental contaminants, industrial chemicals, and pesticides. The aim of the program is to develop a two-tiered approach, e.g., a combination of *in vitro* and *in vivo* mammalian and ecotoxicological screens (Tier 1) and a set of *in vivo* tests (Tier 2) for identifying and characterizing endocrine effects of pesticides, industrial chemicals, and environmental contaminants. Validation of the individual screens and tests is required, and the Endocrine Disruptor Methods Validation Advisory Committee (EDMVAC) will provide advice and counsel on the validation assays.

Estrogens are sex steroid hormones that are necessary for female reproduction and affect the development of secondary sex characteristics of females. Estrogens are biosynthesized from cholesterol by a series of enzymatic steps, with the last step involving the conversion of androgens into estrogens by the enzyme aromatase. Estrogen biosynthesis occurs primarily in the ovary in mature, premenopausal women. During pregnancy, the placenta is the main source of estrogen biosynthesis and pathways for production change. Small amounts of these hormones are also synthesized by the testes in the male and by the adrenal cortex, the hypothalamus, and the anterior pituitary in both sexes. The major source of estrogens in both postmenopausal women and men occurs in extraglandular sites,

particularly in adipose tissue. One potential endocrine target for environmental chemicals is the enzyme aromatase, which catalyzes the biosynthesis of estrogens. An aromatase assay is proposed as one of the Tier 1 Screening Battery Alternate Methods. A detailed literature review on aromatase was performed and encompassed: (1) searching the literature databases, (2) contacting individuals to obtain information on unpublished research, and (3) evaluating the literature and personal communications.

Aromatase is a cytochrome P450 enzyme complex responsible for estrogen biosynthesis and converts androgens, such as testosterone and androstenedione, into the estrogens estradiol and estrone. Aromatase is present in the ovary, placenta, uterus, testis, brain, and extraglandular adipose tissues. Two proteins, cytochrome $P450_{arom}$ and NADPH-cytochrome P450 reductase, are necessary for enzymatic activity, and the enzyme complex is localized in the smooth endoplasmic reticulum. The aromatase gene, designated CYP19, encodes the cytochrome $P450_{arom}$ and consists of ten exons, with the exact size of the gene exceeding 70 kilobases. Aromatase is found in breast tissue, and the importance of intratumoral aromatase and local estrogen production is being unraveled. Effective aromatase inhibitors have been developed as therapeutic agents for estrogen-dependent breast cancer to reduce the growth stimulatory effects of estrogens in breast cancer. Investigations on the development of aromatase inhibitors began in the 1970's and have expanded greatly in the past three decades.

An *in vitro* aromatase assay could easily be utilized as an alternative screening method in the Tier 1 Screening Battery to assess the potential effects of various environmental toxicants on aromatase activity. Both *in vitro* subcellular (microsomal) assays and cell-based assays are available for measuring aromatase activity. The *in vitro* subcellular assay using human placental microsomes is commonly used to evaluate the ability of pharmaceuticals and environmental chemicals to inhibit aromatase activity. In addition, human JEG-3 and JAR choriocarcinoma cell culture lines, originally isolated from cytotrophoblasts of malignant placental tissues, have been used as *in vitro* systems for measuring the effects of compounds on aromatase activity. These cell lines are also utilized for investigations on the effects of agents in placental toxicology.

Numerous flavonoids and related phytoestrogen derivatives have been extensively evaluated for their ability to inhibit aromatase activity for two primary reasons: (1) these natural plant products can serve as possible leads for the development of new nonsteroidal aromatase inhibitors, and (2) humans and other animals are exposed to these agents through the diet. In general, the flavonoids and related analogs demonstrate aromatase inhibition with IC_{50} values in the micromolar range; however, these compounds lack both the potency and specificity of aromatase inhibitors developed for breast cancer therapy. Several pesticides have also demonstrated inhibition of aromatase activity in the human placental microsomal assay system.

The human placental microsomal aromatase assay was recommended as the *in vitro* aromatase screening assay to be included in the Tier 1 Screening Battery. This assay will detect environmental toxicants that possess the ability to inhibit aromatase activity. Prevalidation studies on recombinant aromatase (WA 2-24) were conducted to optimize the microsomal aromatase assay protocol for human placenta, demonstrate the utility of the microsomal assay to detect known aromatase inhibitors, and compare the performance of a recombinant assay system and the placental microsomal assays.

2.2 Task Description and Objectives

Three independent replicates of aromatase assay were conducted (on separate days) for each of the ten reference chemicals. Each reference chemical was tested at eight concentrations and there were three repetitions for each concentration of a given replicate. Two different human placental microsomal preparations (prepared by Battelle and In Vitro Technologies) were used.

IC_{50} for each replicate were calculated using Prism software as specified in the protocol (Version 4.0).

The objective of the presented study was to demonstrate the responsiveness of the aromatase assay to reference chemicals. An additional aim of the study was to use the optimized assay to obtain intralaboratory assay variability estimates. The study protocol can be found in Appendix A.

3.0 MATERIALS AND METHODS

3.1 Substrate

The substrate for the aromatase assay was androstenedione (ASDN). Non-radiolabeled and radiolabeled ASDN were used. The non-radiolabeled ASDN (Lot No. 024K0809) was obtained from Sigma-Aldrich, St. Louis, MO by the Sponsor's chemical repository (CR) and was then distributed to the participating laboratories. It had a reported purity of 100%. The radiolabeled androstenedione ([1β-^{3}H]-androstenedione, [^{3}H]ASDN, Lot No. 3538496), was obtained from PerkinElmer Life Sciences, Inc., Boston, MA and had a reported specific activity of 25.3 Ci/mmol. Radiochemical purity was reported by the supplier to be > 97%. Radiochemical purity was assessed by high performance liquid chromatography (HPLC) by the lead laboratory (see Results section).

Preparing the substrate solution involved the mixing of non-radiolabeled and radiolabeled [^{3}H]ASDN in order to achieve 100 nM final concentration of ASDN in the assay. The amount of tritium added to each incubation tube was about 0.1 µCi. This substrate solution should have had a concentration of 2 µM with a radiochemical content of about 1 µCi/mL.

The following illustrates the preparation of a substrate solution using a stock of [^{3}H]ASDN with a specific activity of 25.3 Ci/mmol and a concentration of 1 mCi/mL. A 1:100 dilution of the radiolabeled stock solution in buffer and a 1 mg/mL solution of ASDN in ethanol were prepared. Subsequently, the 1 mg/mL ASDN in ethanol solution was diluted in buffer to a final concentration of 1 µg/mL. Five and four-tenth (5.4) mL of the 1 µg/mL solution of ASDN, 960 µL of the [^{3}H]ASDN buffer dilution and 3.24 mL buffer to make 9.6 mL were combined. The weight of each component added to the substrate solution was recorded. After mixing the solution, five aliquots of ca. 20 µL were weighed out and combined with scintillation cocktail for radiochemical content analysis.

3.2 Reference Chemicals

The Sponsor's CR was responsible for chemistry activities required to perform this study. Their responsibilities included chemical procurement, solubility, formulation stability assessment, formulation preparation, formulation analysis and shipment of stock formulation to the participating laboratories (see Results section).

The reference chemical formulations and vehicles were received from the Sponsor's CR. To each solution of the reference chemical, a specific code was assigned to ensure that replicates were conducted blind for reference chemical identity. Table 1 summarizes all information about the used reference chemicals.

Table 1 - Reference Chemicals

Reference Chemical Name	Mfr. Purity (%)	CAS No.	Molecular Formula	Stock Solution Code	Stock Solution Concentration (M)	Vehicle
Aminoglutethimide	>99	125-84-8	$C_{13}H_{16}N_2O_2$	RC# 1	0.1	DMSO
Chrysin	98.2-101.4	480-40-0	$C_{15}H_{10}O_4$	RC# 8	0.01	DMSO
Dicofol	96.5	115-32-2	$C_{14}H_9Cl_5O$	RC# 9	0.1	DMSO
Econazole (nitrate)	98	24169-02-6	$C_{18}H_{15}Cl_3N_2O$-HNO_3	RC# 7	0.1	DMSO
Ketoconazole	> 99	65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	RC# 2	0.01	DMSO
Atrazine	98	1912-24-9	$C_8H_{14}ClN_5$	RC# 10	0.1	DMSO
Fenarimol	99	60168-88-9	$C_{17}H_{12}Cl_2N_2O$	RC# 6	0.1	DMSO
4-Nonylphenol	>98.5	84852-15-3	$C_{15}H_{24}O$	RC# 4	0.1	DMSO
Prochloraz	99.5	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	RC# 3	0.1	DMSO
Dibenz[a,h]anthracene	97	53-70-3	$C_{22}H_{14}$	RC# 5	0.01	DMSO

Dilutions of each reference chemical solution were prepared fresh each day for use in the assay. In each case, the same lot number of vehicle used to prepare the stock solution was used in the preparations of the dilutions. Suppliers and lot numbers for reference chemical vehicles are presented in Table 18 (Section 3.5).

The results of first replicate for each reference chemical were reviewed and adjustments were made (at the direction of the Study Director) to the tested concentrations in subsequent replicates of the assay in order to obtain data that better described the dose-dependent curve. The dilution schemes described in Tables 2 through 14 were used in the preparations of appropriate dilutions of all ten tested reference chemicals.

Table 2 - Dilution Scheme for Replicate 1 of Aminoglutethimide and Prochloraz (RC# 1 and RC# 3)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	900	100	Stock Solution	1.00E+01	1.00E-04
2	900	100	Diln. 1	1.00E+00	1.00E-05
3	900	100	Diln. 2	1.00E-01	1.00E-06
4	900	100	Diln. 3	1.00E-02	1.00E-07
5	900	100	Diln. 4	1.00E-03	1.00E-08
6	900	100	Diln. 5	1.00E-04	1.00E-09
7	900	100	Diln. 6	1.00E-05	1.00E-10
8	900	100	Diln. 7	1.00E-06	1.00E-11

Table 3 - Dilution Scheme for Replicate 1 of Ketoconazole (RC# 2), Replicate 1 of Chrysin (RC# 8)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	0	1000	Stock Solution	1.00E+01	1.00E-04
2	900	100	Diln. 1	1.00E+00	1.00E-05
3	900	100	Diln. 2	1.00E-01	1.00E-06
4	750	250	Diln. 3	2.50E-02	2.50E-07
5	900	100	Diln. 3	1.00E-02	1.00E-07
6	900	100	Diln. 5	1.00E-03	1.00E-08
7	900	100	Diln. 6	1.00E-04	1.00E-09
8	900	100	Diln. 7	1.00E-05	1.00E-10

Table 4 - Dilution Scheme for the Replicate 1 of 4-Nonylphenol (RC# 4), Fenarimol (RC# 6), Econazole (RC# 7), Dicofol (RC# 9), and Atrazine (RC# 10)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	0	1000	Stock Solution	1.00E+02	1.00E-03
2	900	100	Diln. 1	1.00E+01	1.00E-04
3	900	100	Diln. 2	1.00E+00	1.00E-05
4	900	100	Diln. 3	1.00E-01	1.00E-06
5	900	100	Diln. 4	1.00E-02	1.00E-07
6	900	100	Diln. 5	1.00E-03	1.00E-08
7	900	100	Diln. 6	1.00E-04	1.00E-09
8	900	100	Diln. 7	1.00E-05	1.00E-10

Table 5 - Dilution Scheme for Replicates 2 and 3 of Fenarimol (RC# 6)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	0	1000	Stock Solution	1.00E+02	1.00E-03
2	900	100	Stock Solution	1.00E+01	1.00E-04
3	900	100	Diln. 2	1.00E+00	1.00E-05
4	750	250	Diln. 3	2.50E-01	2.50E-06
5	900	100	Diln. 3	1.00E-01	1.00E-06
6	900	100	Diln. 5	1.00E-02	1.00E-07
7	900	100	Diln. 6	1.00E-03	1.00E-08
8	900	100	Diln. 7	1.00E-04	1.00E-09

Table 6 - Dilution Scheme for Replicates 1, 2, and 3 of Dibenz[a,h]anthracene (RC# 5)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	0	1000	Stock Solution	1.00E+01	1.00E-04
2	900	100	Diln. 1	1.00E+00	1.00E-05
3	900	100	Diln. 2	1.00E-01	1.00E-06
4	750	250	Diln. 3	2.50E-02	2.50E-07
5	900	100	Diln. 3	1.00E-02	1.00E-07
6	900	100	Diln. 5	1.00E-03	1.00E-08
7	900	100	Diln. 6	1.00E-04	1.00E-09
8	900	100	Diln. 7	1.00E-05	1.00E-10

Table 7 - Dilution Scheme for Replicates 2 and 3 of Aminoglutethimide (RC# 1)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	0	1000	Stock Solution	1.00E+02	1.00E-03
2	750	250	Stock Solution	2.50E+01	2.50E-04
3	900	100	Stock Solution	1.00E+01	1.00E-04
4	500	500	Diln. 3	5.00E+00	5.00E-05
5	500	500	Diln. 4	2.50E+00	2.50E-05
6	900	100	Diln. 3	1.00E+00	1.00E-05
7	750	250	Diln. 6	2.50E-01	2.50E-06
8	900	100	Diln. 6	1.00E-01	1.00E-06

Table 8 - Dilution Scheme for Replicates 2 and 3 of Ketoconazole (RC# 2)[a]

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	0	1000	Stock Solution	1.00E+01	1.00E-04
2	900	100	Diln. 1	1.00E+00	1.00E-05
3	900	100	Diln. 2	1.00E-01	1.00E-06
4	500	500	Diln. 3	5.00E-02	5.00E-07
5	750	250	Diln. 3	2.50E-02	2.50E-07
6	900	100	Diln. 3	1.00E-02	1.00E-07
7	900	100	Diln. 6	1.00E-03	1.00E-08
8	900	100	Diln. 7	1.00E-04	1.00E-09

a. For ID #6, the solution concentration may have been 2.50E-03 mM for replicate #2. However, the results suggest that the solution concentration was 1.00E-02 mM as reported in the table.

Table 9 - Dilution Scheme for Replicates 2 and 3 of Prochloraz (RC# 3)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
A	900	100	Stock Solution	1.00E+01	--
1	900	100	Diln. A	1.00E+00	1.00E-05
2	900	100	Diln. 1	1.00E-01	1.00E-06
3	900	100	Diln. 2	1.00E-02	1.00E-07
4	750	250	Diln. 3	2.50E-03	2.50E-08
5	900	100	Diln. 3	1.00E-03	1.00E-08
6	750	250	Diln. 5	2.50E-04	2.50E-09
7	900	100	Diln. 5	1.00E-04	1.00E-09
8	900	100	Diln. 7	1.00E-05	1.00E-10

Table 10 - Dilution Scheme for Replicates 2 and 3 of 4-Nonylphenol (RC# 4)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	0	1000	Stock Solution	1.00E+02	1.00E-03
2	900	100	Diln. 1	1.00E+01	1.00E-04
3	500	500	Diln. 2	5.00E+00	5.00E-05
4	750	250	Diln. 2	2.50E+00	2.50E-05
5	900	100	Diln. 2	1.00E+00	1.00E-05
6	900	100	Diln. 5	1.00E-01	1.00E-06
7	900	100	Diln. 6	1.00E-02	1.00E-07
8	900	100	Diln. 7	1.00E-03	1.00E-08

Table 11 - Dilution Scheme for Replicates 2 and 3 of Econazole (RC# 7)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	900	100	Stock Solution	1.00E+01	1.00E-04
2	900	100	Diln. 1	1.00E+00	1.00E-05
3	900	100	Diln. 2	1.00E-01	1.00E-06
4	900	100	Diln. 3	1.00E-02	1.00E-07
5	900	100	Diln. 4	1.00E-03	1.00E-08
6	750	250	Diln. 5	2.50E-04	2.50E-09
7	900	100	Diln. 5	1.00E-04	1.00E-09
8	900	100	Diln. 7	1.00E-05	1.00E-10

Table 12 - Dilution Scheme for Replicates 2 and 3 of Chrysin (RC# 8)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	0	1000	Stock Solution	1.00E+01	1.00E-04
2	900	100	Diln. 1	1.00E+00	1.00E-05
3	500	500	Diln. 2	5.00E-01	5.00E-06
4	750	250	Diln. 2	2.50E-01	2.50E-06
5	900	100	Diln. 2	1.00E-01	1.00E-06
6	900	100	Diln. 5	1.00E-02	1.00E-07
7	900	100	Diln. 6	1.00E-03	1.00E-08
8	900	100	Diln. 7	1.00E-04	1.00E-09

Table 13 - Dilution Scheme for Replicates 2 and 3 of Dicofol (RC# 9)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	0	1000	Stock Solution	1.00E+02	1.00E-03
2	900	100	Diln. 1	1.00E+01	1.00E-04
3	500	500	Diln. 2	5.00E+00	5.00E-05
4	900	100	Diln. 2	1.00E+00	1.00E-05
5	900	100	Diln. 4	1.00E-01	1.00E-06
6	900	100	Diln. 5	1.00E-02	1.00E-07
7	900	100	Diln. 6	1.00E-03	1.00E-08
8	900	100	Diln. 7	1.00E-04	1.00E-09

Table 14 - Dilution Scheme for Replicates 2 and 3 of Atrazine (RC# 10)

Dilution ID	Vehicle (µL)	Sample (µL)	Sample Used	Soln Concentration (mM)	Final Concentration in Assay (M)
1	0	1000	Stock Solution	1.00E+02	1.00E-03
2	100	900	Stock Solution	9.00E+01	9.00E-04
3	200	800	Stock Solution	8.00E+01	8.00E-04
4	250	750	Stock Solution	7.50E+01	7.50E-04
5	500	500	Stock Solution	5.00E+01	5.00E-04
6	900	100	Stock Solution	1.00E+01	1.00E-04
7	900	100	Diln. 6	1.00E+00	1.00E-05
8	900	100	Diln. 7	1.00E-01	1.00E-06

3.3 Control Substances

The known aromatase inhibitor, 4-hydroxyandrostenedione (4-OH ASDN), was used as a positive control and the known aromatase non-inhibitor, lindane, was used as a negative control. Stock solutions of control substances (Table 15) were supplied by Sponsor's CR and were stored refrigerated. Dilutions were made fresh each day of use in the same vehicle and lot number that was used to prepare the stock solutions (see Table 18, Section 3.5). Tables 16 and 17 present the dilution scheme for 4-OH ASDN and lindane, respectively.

Table 15 - Control Substances

Control Substance	Chemical Code	Mfr. Purity	CAS No.	Molecular Formula	Molecular Weight (g/mol)	Stock Solution ID	Vehicle	Storage Conditions (°C)
4- hydroxyandrostenedione	4-OH ASDN	99%	566-48-3	$C_{19}H_{26}O_3$	302.4	4-ASDN-1	95% ethanol	2-8
Lindane	Lindane	99.6%	58-89-9	$C_6H_6Cl_6$	290.8	3-LIN-1	DMSO	2-8

Table 16 - Dilution Scheme for 4-OH ASDN (Positive Control)

Dilution ID	Volume of EtOH (µL)	Volume of Solution (µL)	Solution Used	Solution Concentration (M)	Final Concentration In Assay (M)
1	9900	100	4-ASDN-1 (0.01 M)	1.00E-04	NA
2	1900	100	Diln.1	5.00E-06	5.00E-08

Table 17 - Dilution Scheme for Lindane (Negative Control)

Dilution ID	Volume of DMSO (µL)	Volume of Solution (µL)	Solution Used	Solution Concentration (M)	Final Concentration In Assay (M)
1	9900	100	3-LIN-1 (0.1 M)	1.00E-03	NA
2	900	100	Diln.1	1.00E-04	1.00E-06

3.4 Microsomes

Human placental microsomes provided by Battelle (Lot No. 6-041305) and In Vitro Technologies (Lot No. BAA) were stored at approximately -70°C until the time of the assay. On the day of use, microsomes were thawed rapidly in a 37 ± 1°C water bath, rehomogenized by brief vortexing and then kept on ice until used. The time between thawing of the microsomes and their use in the assay was limited to less than 1 hour and, in most cases was about 30 to 50 minutes.

The protein concentrations in stock microsomes were approximately 21.0 mg/mL for Battelle-prepared microsomes, and approximately 8 mg/mL for In Vitro Technologies-prepared microsomes. Battelle microsomes were diluted in assay buffer in two serial dilutions. The first dilution (1:50) was achieved by gently mixing 0.1 mL of microsomes stock suspension with 4.9 mL of buffer (total volume 5 mL). The second dilution (1:19) was obtained by gently mixing 2.5 mL of first microsome dilution with 45 mL of buffer. The first dilution was kept on ice until the protein concentration was measured. In the second dilution, a target protein concentration was ca. 0.025 mg/mL to achieve a final protein concentration in the incubation mixture ca. 0.0125 mg/mL. The second dilution was also kept on ice until it was placed in the water bath just prior to its addition to the incubation mixture to start the reaction. In Vitro Technologies microsomes were diluted only once (1:350); 120 µL of microsome stock solution was diluted with 41.88 mL of buffer and gently mixed. These dilutions resulted in a final approximate protein concentration of 0.0125 mg/mL in each assay tube.

According to Section 5 in the study protocol regarding microsomes distribution and matrix testing, Battelle-prepared microsomes were used with following reference chemicals: aminoglutethimide (RC #1), ketoconazole (RC #2), dibenz[a,h]anthracene (RC #5), dicofol (RC #9), and atrazine (RC #10). In Vitro Technologies-prepared microsomes were used with following reference chemicals: prochloraz (RC #3), 4-nonylphenol (RC #4), fenarimol (RC #6), econazole (RC #7), and chrysin (RC #8).

3.5 Other Assay Components

In addition to substrate, reference chemicals or control substances, vehicle, and microsomes, the aromatase assay contained nicotinamide adenine dinucleotide phosphate reduced form (NADPH), propylene glycol and phosphate buffer. Suppliers and lot numbers for other aromatase assay components are presented in Table 18.

Table 18 - Suppliers and Lot Numbers for Aromatase Assay Components

Chemical	Supplier	Lot Number
NADPH	Sigma	074K7057
Propylene glycol	Spectrum Chemical	SQ0397
Sodium phosphate dibasic	Sigma	083K0120
Sodium phosphate monobasic	Sigma	054K0144
Ethanol, 95%	Spectrum Chemical	SW0045
Vehicle (DMSO) for Reference Chemicals 1-7, 9, and 10 and Lindane	Mallinckrodt	2969A46428
Vehicle (DMSO) for Chemical 8	EMD	44155

3.5.1 Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form (NADPH)

NADPH is the required co-factor for aromatase. The final concentration in the assay was 0.3 mM. Typically, a 6 mM stock solution was prepared fresh each day by dissolving ca. 20 mg of NADPH in 4 mL of assay buffer and kept on ice until use.

3.5.2 Assay Buffer

The assay buffer was 0.1 M sodium phosphate buffer, pH 7.4. One liter of 0.1 M solution of sodium phosphate monobasic (NaH_2PO_4) in Milli-Q water and one liter of 0.1 M solution of sodium phosphate dibasic (Na_2HPO_4) in Milli-Q water were prepared. The solutions were combined in the approximate ratio 80:20 (dibasic: monobasic sodium phosphate) to achieve a pH of 7.4. The assay buffer was stored for up to one month in the refrigerator (2 to 8°C).

3.6 Protein Determination

The protein concentration in the microsomes was determined each day of microsomes use by using a DC Protein Assay kit from BioRad (Hercules, CA). The six-point standard curve was prepared using bovine serum albumin (BSA) reconstituted in Milli-Q water.

The standard curve range was from 5 to 250 μg protein/mL. Each standard and unknown sample was run in triplicate; quality control (QC) standards (target protein concentrations of 10 and 100 μg/mL) were run in duplicate with each protein assay. To a 200 μL unknown, standard and QC aliquot 100 μL of BioRad DC Protein Kit Reagent A was added and mixed. Next, 0.8 mL of BioRad DC Protein Kit Reagent B was added. The samples were (standard and unknown) gently mixed. The samples were incubated in room temperature for at least 15 minutes. Each sample was transferred to disposable polystyrene cuvettes and the absorbance at 750 nm was measured using a spectrophotometer. The protein concentration of each microsomal sample and QC standard was determined by interpolation, reading the protein concentration on the standard curve that corresponded to its absorbance.

3.7 Cytochrome P450 Aromatase (CYP19) Activity

Three independent replicates were conducted (on separate days) for each reference chemical with all replicates for a given reference chemical conducted by the same technician. Each reference chemical was tested at eight concentrations and there were three (triplicate) repetitions for each concentration of a given replicate. A single replicate experiment of an example reference chemical is described in Table 19.

Four types of control samples were included with each replicate. These included:

- Full enzyme (aromatase) activity controls (substrate, NADPH, propylene glycol, buffer, vehicle [used for preparation of reference chemical solutions] and microsomes.
- Background activity controls (all components that were in the full aromatase activity control, except NADPH).
- Positive controls (all components that were in the full aromatase activity controls, except vehicle, and with the addition of 4-OH ASDN at the concentration of 5×10^{-8} M.
- Negative controls (all components that were in full aromatase activity controls, except vehicle, and with the addition of lindane at the concentration of 1×10^{-6} M.

Four test tubes of each type of control were included with each replicate and were treated the same as the other samples. The control sets were split that two tubes (of each control type) were run at the beginning and two at the end of each replicate set.

The assays were performed in 13 x 100 mm test tubes maintained at 37 ± 1°C in a shaking water bath. Propylene glycol, [^{3}H]ASDN, NADPH, and assay buffer were combined in the test tubes with or without inhibitor (as described below) to the total volume of 1.0 mL. The volume of the reference chemical or control substance solutions or vehicle used was 20 μL (1% of total assay volume). The final concentrations for the assay major components are presented in Table 20. The tubes and the microsomal suspension were placed at 37 ± 1°C in the water bath for approximately 5 minutes prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume was 2.0 mL, and the tubes were incubated for 15 minutes. The incubations were stopped by the addition of methylene chloride (2.0 mL); the tubes were vortex-mixed for approximately 5 seconds and placed on ice. The tubes were then vortex-mixed an additional 20 to 25 seconds, then centrifuged using a Beckman GS-6 centrifuge with GH-3.8 rotor for 10 minutes at a setting of 1000 rpm. After

centrifugation, the methylene chloride layer was removed and discarded; the aqueous layers were extracted again with methylene chloride (2.0 mL). This extraction procedure was performed one additional time, each time discarding the methylene chloride layer. The aqueous layers were transferred to vials and duplicate aliquots (0.5 mL) were transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Packard, 10 mL) was added to each counting vial and shaken to mix the solution.

Analysis of the samples was performed using liquid scintillation spectrometry (LSS). Radioactivity found in the aqueous fractions represented amount of formed ^3H_2O. The aromatization of one mole of [^{3}H]ASDN resulted in the production of one mole of estrone (non-radiolabeled) and one mole of ^3H_2O.

Table 19 - Reference Chemical Study Design

Sample Type	Repetitions (Test Tubes)	Description	Reference Chemical Concentration (M)
Full Enzyme Activity Control	4	Complete assay[a] with reference chemical vehicle control	N/A
Background Activity Control	4	Complete assay with reference chemical vehicle control omitting NADPH	N/A
Positive Control	4	Complete assay with positive control chemical (4-OH ASDN) added	5×10^{-8}
Negative Control	4	Complete assay with negative control chemical (Lindane) added	1×10^{-6}
Reference Chemical Concentration 1	3	Complete assay with Reference Chemical added	1×10^{-3}
Reference Chemical Concentration 2	3	Complete assay with Reference Chemical added	1×10^{-4}
Reference Chemical Concentration 3	3	Complete assay with Reference Chemical added	1×10^{-5}
Reference Chemical Concentration 4	3	Complete assay with Reference Chemical added	1×10^{-6}
Reference Chemical Concentration 5	3	Complete assay with Reference Chemical added	1×10^{-7}
Reference Chemical Concentration 6	3	Complete assay with Reference Chemical added	1×10^{-8}
Reference Chemical Concentration 7	3	Complete assay with Reference Chemical added	1×10^{-9}
Reference Chemical Concentration 8	3	Complete assay with Reference Chemical added	1×10^{-10}

[a] The complete assay contained buffer, propylene glycol, microsomal protein, [^{3}H]ASDN and NADPH.

Table 20 - Aromatase Assay Conditions using Human Placental Microsomes

Assay Component	Component Volume Added to the Assay	Final Concentration in the Assay
Microsomal protein	1.0 mL	0.0125 mg/mL
NADPH	100 µL	0.3 mM
[^{3}H]ASDN	100 µL	100 nM
Propylene glycol	100 µL	5 % (v/v)
Reference Chemical, Control Substance or Vehicle	20 µL	Varies[a]

[a] See Table 19 for details.

3.8 Data Analysis

3.8.1 Aromatase Activity and Percent of Control Calculations

Data were entered into an Excel spreadsheet for calculation of aromatase activity and percent of control. The master spreadsheet used was titled Aromatase_Master_Version 1.2.xls.

For each repeat tube (full enzyme activity control, background activity control, positive and negative controls and each reference chemical concentration), the Excel spreadsheet included total observed

(uncorrected) disintegration per minute (dpm) per tube and total aromatase activity per tube. The dpm and aromatase activity values were corrected for the background dpms, as measured by the average of the background activity control tubes. The aromatase activity was calculated as the corrected dpm, normalized by the specific activity of the [^{3}H]ASDN, the mg of protein, and the incubation time.

For each tube, percent of control was determined by dividing the background corrected aromatase activity for that tube by the average background corrected aromatase activity for the four full enzyme activity control tubes and multiplying by 100. Nominally, one may expect for an inhibitor the percent of control activity values to vary between approximately 0% near the high inhibition concentrations and approximately 100% near the low inhibition concentrations. However due to experimental variation, individual observed percent of control values sometimes extended below 0% or above 100%.

The spreadsheet calculated dpm/mL for each aliquot of extracted aqueous incubation mixture and average dpm/mL and total dpm for each aqueous portion (after extraction). Multiplication of the volume (mL) of substrate solution added to the incubation by the substrate solution radiochemical content (dpm/mL) yielded the total dpm present in the assay tube at initiation. The total dpm remaining in the aqueous portion after extraction divided by the total dpm present in the assay tube at initiation times 100 yielded the percent of the substrate that was converted to product. The total dpm remaining in the aqueous portion after extraction was corrected for background by subtracting the average dpm present in the aqueous portion of the background activity control tubes (for that day/assay). This corrected dpm was then converted to nmol product formed by dividing by the substrate specific activity (dpm/nmol). The activity of the enzyme was expressed in nmol (mg protein) $^{-1}$min^{-1} and was calculated by dividing the amount of estrogen formed (nmol) by the amount of microsomal protein used (in mg) times the incubation time (in min). Average activity in the full activity control samples was calculated. Percent of activity remaining in the presence of various inhibitor concentrations was calculated by dividing the aromatase activity at a given inhibitor concentration by the average positive full activity control and multiplying by 100.

3.8.2 Statistical Analyses

3.8.2.1 Concentration Response Fits for the Reference Chemicals

For the reference chemicals, an independent concentration response curve fit was carried out for each replicate. Concentration response trend curves were fitted to the percent of control activity values within each of the repeat tubes at each reference chemical concentration.

Concentration was expressed on the log scale. In agreement with past convention, logarithms were common logarithms (i.e., base 10). Let X denote the logarithm of the concentration of reference chemical (e.g. if concentration = 10^{-5} then X = -5). Let:

Y = percent of control activity in the inhibitor tube
X = logarithm (base 10) of the concentration
T = top of plateau
B = bottom of plateau
H = Hill slope
$\mu = \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

The following concentration response curve was fitted to relate percent of control activity to logarithm of concentration within each replicate:

$Y = B + (T-B)/[1 + 10^{(\mu - X)*H}]$.

The response curve was fitted by non-weighted least squares nonlinear regression analysis. Model fits were carried out using Prism software (Version 4.0).

Concentration response models were fitted for each replicate test within each reference chemical. Based on the results of the fit within each replicate the extent of aromatase inhibition was summarized as top (T), bottom (B), $\log_{10}IC_{50}$ (μ) and slope (β). The estimated T, B, $\log_{10}IC_{50}$ and β for a reference chemical was a (weighted) mean across the replicates. The "Status" of each response curve was indicated:

- Complete curve –"inhibitor"- data were available up to at least 80% inhibition-IC_{50} was calculated.
- Incomplete curve- "presumed inhibitor" – data were available to at least 50% inhibition but not beyond 80% inhibition – IC_{50} was calculated.
- Incomplete curve –"equivocal" –data were available to between 20% and 50 % inhibition – IC_{50} was not calculated.
- "No inhibition" – no data were available above 20% inhibition – IC_{50} was not calculated.

3.8.2.2 Graphical and Analysis of Variance Comparisons Among Concentration Response Curve Fits

For each replicate, the individual percent of control values were plotted versus logarithm of the reference chemical concentration. The fitted concentration response curve was superimposed on the plot. Individual plots were prepared for each replicate.

Additional plots were prepared to compare the percent of control activity values across replicates. For each replicate, the average percent of control values were plotted versus logarithm of reference chemical concentration on the same plot. Plotting symbols distinguished among replicates. The fitted concentration response curves for each replicate were superimposed on the plots. On a separate plot the average percent of control values for each replicate was plotted versus logarithm of reference chemical concentration. The average concentration response curve across replicates was superimposed on the same plot.

Graphs were prepared for the parameter estimates from the response curve model fits to visually assess the estimates and their variations.

3.8.2.3 Graphical and Analysis of Variance Comparisons of Full Enzyme Activity, Background Activity, Positive and Negative Control Percent of Control Across Reference Chemicals and Replicates

The means of the full enzyme activity control values within each replicate and chemical were calculated as the reference value for 100% of control for the replicate and chemical. The percent of control for each repetition within each replicate and chemical was calculated as the ratio of the repetition value divided by the corresponding 100% of control reference value. Graphs of the percent of control values indicating the

repetition portion by replicate and chemical for background activity, full activity, negative and positive controls were prepared. Graphs displaying the differences of the means of the beginning repetitions and the means of the end repetitions, within each replicate and chemical were prepared. A mixed effects model was fit to the percent of control values for each control. The fixed effects were assigned as the chemical type, portion, and portion by chemical interaction. The random effects were assigned as replicated within reference chemical and portion by replicate interaction within chemical.

3.8.2.4 Statistical Software

Concentration response curves were fitted to the data using the non-linear regression analysis features in the Prism statistical analysis package, Version 4.0. Supplemental statistical analyses and displays such as summary tables, graphical displays, analysis of variance, and multiple comparisons were carried out using the SAS statistical analysis system, Version 9.0.

3.9 Retention of Records

All study records, including the final report, are retained in the archives as specified in the study protocol.

4.0 RESULTS

4.1 Radiochemical Purity

The radiochemical purity for the substrate androstenedione was 97% as reported by RTI International.

4.2 Stock Formulation Analysis

The formulation stability and formulation analysis results for all ten reference chemicals and the positive and negative control substances from Battelle CR are included in the study file. Some of the formulation analysis data are summarized in Table 21. The RC#5 formulation was used one day beyond its expiration date.

Table 21 - Formulation Analysis Data

Chemical Code	Reference Chemical ID	Stock Solution ID	Manufacturer's Purity (%)	Stock Solution Preparation Date	Known Stability (days)	Date of Last Use on WA4-16, Task 7
1	Aminoglutethimide	RC #1	>99	6/30/2005	59	7/22/2005
2	Ketoconazole	RC #2	> 99	6/29/2005	60	8/15/2005
3	Prochloraz	RC #3	99.5	7/25/2005	63	8/4/2005
4	4-Nonylphenol	RC #4	>98.5	9/13/2005	20	9/29/2005
5	Dibenz[a,h]anthracene	RC #5	97	9/13/2005	20	10/4/2005
6	Fenarimol	RC #6	99	7/25/2005	30	8/9/2005
7	Econazole (nitrate)	RC #7	98	8/8/2005	56	8/19/2005
8	Chrysin	RC #8	98.2-101.4	8/9/2005	100	8/25/2005
9	Dicofol	RC #9	96.5	8/22/2005	29	8/31/2005
10	Atrazine	RC #10	98	8/23/2005	28	9/8/2005
N/A	Lindane	3-LIN-1	99.6	7/1/2005	168	10/4/2005
N/A	4-OH ASDN	4-ASDN-1	99	6/27/2005	173	10/4/2005

4.3 Protein Analysis

Protein concentration measurements were done according to the procedure provided in Section 3.6 of this report. To measure the protein concentration, 1:50 microsomes dilution in assay buffer (for Battelle prepared microsomes) and 1:350 microsomes dilution in assay buffer (for In Vitro Technologies-prepared microsomes) were processed. The results for measuring protein concentration in human placental microsomes for each day of use are presented in Table 22. The average measured protein content of the human placental microsomes stock solution was 25.02 ± 1.64 mg/mL (6.55% CV) and 9.222 ± 0.526 mg/mL (5.71% CV). The poor standard curve linearity and poor QC sample performance was observed when all six standards from 5 to 250 µg/mL were used (this phenomenon was also observed during the previous study, Task 6). After omitting the highest standard (250 µg/mL) the constructed 5-point standard curve had much better linearity and improved QC sample performance. The 5-point (5 to 125 µg/mL) standard curve was used during the presented study.

In order to better characterize the protein assay, QC samples were included in all runs (see Section 3.6). The QC results are presented in Table 23. The overall mean value for the low QC standard (across 30 replicates) was determined as 0.011 ± 0.001 mg/mL (10.9% CV, RTI reported value was 0.012 mg/mL) and overall mean value for the high QC standard (2.43% CV, RTI reported value was 0.110 mg/mL) was determined as 0.107 ± 0.003 mg/mL (2.43% CV).

Table 22 - Protein Concentration Data

Microsomes	Run by Reference Chemical	Protein Concentration (mg/mL) Rep. 1	Rep. 2	Rep. 3	Protein Concentration (mg/mL) Mean	SD	SEM	%CV
Battelle	Aminoglutethimide (RC #1)	20.91	26.04	27.26	25.02	1.64	0.42	6.55
	Ketoconazole (RC #2)	24.23	26.17	24.87				
	Dibenz[a,h]anthracene (RC #5)	26.67	25.46	26.18				
	Dicofol (RC #9)	24.61	26.22	23.87				
	Atrazine (RC #10)	25.39	22.74	24.64				
In Vitro	Prochloraz (RC #3)	8.767	9.159	8.934	9.222	0.526	0.14	5.71
	4-Nonylphenol (RC #4)	10.11	9.618	9.897				
	Fenarimol (RC #6)	9.843	9.424	8.156				
	Econazole (RC #7)	9.176	8.636	9.026				
	Chrysin (RC #8)	8.803	9.405	9.380				

Table 23 - Protein Concentration in QC Samples

Nominal Concentration (mg/mL)*	Assay Date	Measured Concentration (mg/mL)	Mean	SD	SEM	% CV	% Difference From Known
0.012 (Low)	7/20/2005	0.012	0.011	0.001	0.0002	10.9	-11.39
	7/21/2005	0.014					
	7/22/2005	0.012					
	8/11/2005	0.011					
	8/12/2005	0.012					
	8/15/2005	0.010					
	8/2/2005	0.011					
	8/3/2005	0.011					
	8/4/2005	0.011					
	9/27/2005	0.011					
	9/28/2005	0.009					
	9/29/2005	0.011					
	9/30/2005	0.011					
	10/3/2005	0.010					
	10/4/2005	0.010					
	8/5/2005	0.011					
	8/8/2005	0.011					
	8/9/2005	0.012					
	8/17/2005	0.011					
	8/18/2005	0.011					
	8/19/2005	0.011					
	8/23/2005	0.009					
	8/24/2005	0.010					
	8/25/2005	0.010					
	8/29/2005	0.010					
	8/30/2005	0.010					
	8/31/2005	0.010					
	9/6/2005	0.010					
	9/7/2005	0.009					
	9/8/2005	0.008					
0.110 (High)	7/20/2005	0.107	0.107	0.003	0.0005	2.43	-2.88
	7/21/2005	0.109					
	7/22/2005	0.114					
	8/11/2005	0.108					
	8/12/2005	0.108					
	8/15/2005	0.108					
	8/2/2005	0.105					
	8/3/2005	0.106					

* Concentration reported by RTI.

Table 23 - Protein Concentration in QC Samples (Continued)

Nominal Concentration (mg/mL)*	Assay Date	Measured Concentration (mg/mL)	Mean	SD	SEM	% CV	% Difference From Known
0.110 (High) (Continued)	8/4/2005	0.104	0.107	0.003	0.0005	2.43	-2.88
	9/27/2005	0.107					
	9/28/2005	0.106					
	9/29/2005	0.107					
	9/30/2005	0.114					
	10/3/2005	0.109					
	10/4/2005	0.106					
	8/5/2005	0.109					
	8/8/2005	0.108					
	8/9/2005	0.104					
	8/17/2005	0.106					
	8/18/2005	0.107					
	8/19/2005	0.104					
	8/23/2005	0.103					
	8/24/2005	0.106					
	8/25/2005	0.107					
	8/29/2005	0.105					
	8/30/2005	0.108					
	8/31/2005	0.106					
	9/6/2005	0.107					
	9/7/2005	0.104					
	9/8/2005	0.103					

* Concentration reported by RTI.

4.4 Aromatase Activity

Aromatase activity was measured in the presence of eight concentrations of each of ten reference chemicals. Each assay set also included four types of controls (see Section 3.7). Three replicates were run for each reference chemical. Information regarding assay dates, technicians, protein concentration, substrate specific activity and tested reference chemical concentration ranges is presented in Table 24. Radioactivity remaining in each assay tube after extraction of the unreacted substrate represented formed estrone. The aromatase activity was calculated by normalizing the radioactivity present in each tube by the amount of microsomal protein and the reaction time. The units are nmol/mg protein/min.

Table 24 - Aromatase Assay Summary

Reference Chemical Code	Reference Chemical ID	Replicate	Assay Date	Technician	Protein Stock Conc. (measured) (mg/mL)	Substrate Specific Activity (µCi/µg ASDN)	Reference Chemical Concentration Range (M)
RC# 1	Aminoglutethimide	1	7/20/2005	TD	20.91	1.122	1.00E-04 to 1.00E-11
RC# 1	Aminoglutethimide	2	7/21/2005	TD	26.04	1.146	1.00E-03 to 1.00E-06
RC# 1	Aminoglutethimide	3	7/22/2005	TD	27.26	1.234	1.00E-03 to 1.00E-06
RC# 2	Ketoconazole	1	8/11/2005	TD	24.23	1.212	1.00E-04 to 1.00E-10
RC# 2	Ketoconazole	2	8/12/2005	TD	26.17	1.203	1.00E-04 to 1.00E-09
RC# 2	Ketoconazole	3	8/15/2005	TD	24.87	1.176	1.00E-04 to 1.00E-09
RC# 3	Prochloraz	1	8/2/2005	TD	8.767	1.182	1.00E-04 to 1.00E-11
RC# 3	Prochloraz	2	8/3/2005	TD	9.159	1.129	1.00E-05 to 1.00E-10
RC# 3	Prochloraz	3	8/4/2005	TD	8.934	1.147	1.00E-05 to 1.00E-10
RC# 4	4-Nonylphenol	1	9/27/2005	TD	10.11	0.927	1.00E-03 to 1.00E-10
RC# 4	4-Nonylphenol	2	9/28/2005	TD	9.618	0.895	1.00E-03 to 1.00E-08
RC# 4	4-Nonylphenol	3	9/29/2005	TD	9.897	0.948	1.00E-03 to 1.00E-08
RC# 5	Dibenz[a,h]anthracene	1	9/30/2005	TD	26.67	0.954	1.00E-04 to 1.00E-10
RC# 5	Dibenz[a,h]anthracene	2	10/3/2005	TD	25.46	0.869	1.00E-04 to 1.00E-10
RC# 5	Dibenz[a,h]anthracene	3	10/4/2005	TD	26.18	0.871	1.00E-04 to 1.00E-10
RC# 6	Fenarimol	1	8/5/2005	TD	9.843	1.157	1.00E-03 to 1.00E-10
RC# 6	Fenarimol	2	8/8/2005	TD	9.424	1.094	1.00E-03 to 1.00E-09
RC# 6	Fenarimol	3	8/9/2005	TD	8.156	1.177	1.00E-03 to 1.00E-09
RC# 7	Econazole	1	8/17/2005	TD	9.176	1.221	1.00E-03 to 1.00E-10
RC# 7	Econazole	2	8/18/2005	TD	8.636	1.039	1.00E-04 to 1.00E-10
RC# 7	Econazole	3	8/19/2005	TD	9.026	1.151	1.00E-04 to 1.00E-10
RC# 8	Chrysin	1	8/23/2005	TD	8.803	1.196	1.00E-04 to 1.00E-10
RC# 8	Chrysin	2	8/24/2005	TD	9.405	1.151	1.00E-04 to 1.00E-09
RC# 8	Chrysin	3	8/25/2005	TD	9.380	1.140	1.00E-04 to 1.00E-09
RC# 9	Dicofol	1	8/29/2005	TD	24.61	1.085	1.00E-03 to 1.00E-10
RC# 9	Dicofol	2	8/30/2005	TD	26.22	1.092	1.00E-03 to 1.00E-09
RC# 9	Dicofol	3	8/31/2005	TD	23.87	1.222	1.00E-03 to 1.00E-09
RC# 10	Atrazine	1	9/6/2005	TD	25.39	1.151	1.00E-03 to 1.00E-10
RC# 10	Atrazine	2	9/7/2005	TD	22.74	0.919	1.00E-03 to 1.00E-06
RC# 10	Atrazine	3	9/8/2005	TD	24.64	0.942	1.00E-03 to 1.00E-06

4.4.1 Control Results

Each replicate set for each reference chemical included four types of controls, each run in quadruplicate. The control types were full aromatase activity, background activity, positive and negative controls. The positive control tubes contained the known aromatase inhibitor 4-OH ASDN at a concentration of 5×10^{-8} M and negative control tubes contained the known aromatase non-inhibitor, lindane, at a concentration of 1×10^{-6} M. The control tubes were divided so that two of each type were run at the beginning of the set and two were run at the end of the set. In general, there was approximately a 7 minute interval between the beginning and the end of each replicate set. The aromatase activity in the full aromatase activity controls represented 100% activity and since all aromatase activities were corrected for background, the background activity controls were necessarily set to 0%. The mean activities for each type of control (except background) for the beginning and end groups and the overall means, the standard deviation (SD), the standard error of the mean (SEM) and the coefficient of variation (%CV) across replicates and across chemicals are presented in Table 25. The overall control values for the full enzyme activity control and the negative control were very close, 0.0555 ± 0.0118 nmol/mg/min (21.34% CV) and 0.0538 ± 0.0113 nmol/mg/min

(20.99% CV), respectively for Battelle-prepared microsomes (the overall negative control value was approximately 97% of the full activity control value). The percent of control values of the positive control were consistent, as the overall value of 0.0270 ± 0.0037 nmol/mg/min (13.55% CV) was of approximately 49% of the full enzyme activity control (for Battelle-prepared microsomes). The overall control values for the full enzyme activity control and the negative control for In Vitro Technologies-prepared microsomes were also very close, 0.0459 ± 0.0031 nmol/mg/min (6.83% CV) and 0.0448 ± 0.0036 nmol/mg/min (7.99% CV), respectively (the overall negative control value was approximately 98% of the full activity control value). The percent of control values of the positive control for In Vitro Technologies-prepared microsomes were consistent, with the overall value of 0.0220 ± 0.0012 nmol/mg/min (5.52% CV) (approximately 48% of the full enzyme activity control).

Table 25 - Aromatase Activity in Controls

Reference Chemical	Microsome Source	Replicate	Mean Beginning Control[a]	Mean Ending Control[a]	Mean Overall[a]	SD	SEM	%CV	Overall By Chemical Mean[a]	± SD	SEM	%CV
Full Activity Controls												
Aminoglutethimide	Battelle	1	0.0925	0.0888	0.0907	0.0026	0.0013	2.92	0.0734	0.0149	0.0086	20.37
	Battelle	2	0.0655	0.0644	0.0650	0.0013	0.0007	2.06				
	Battelle	3	0.0658	0.0634	0.0646	0.0023	0.0012	3.58				
Ketoconazole	Battelle	1	0.0691	0.0664	0.0678	0.0019	0.0009	2.79	0.0587	0.0091	0.0052	15.41
	Battelle	2	0.0616	0.0559	0.0588	0.0037	0.0018	6.28				
	Battelle	3	0.0522	0.0471	0.0497	0.0032	0.0016	6.52				
Dibenz[a,h]anthracene	Battelle	1	0.0552	0.0514	0.0533	0.0023	0.0011	4.26	0.0551	0.0027	0.0016	4.94
	Battelle	2	0.0563	0.0512	0.0537	0.0055	0.0027	10.18				
	Battelle	3	0.0596	0.0569	0.0582	0.0018	0.0009	3.06				
Dicofol	Battelle	1	0.0538	0.0479	0.0509	0.0041	0.0020	7.96	0.0478	0.0027	0.0015	5.54
	Battelle	2	0.0478	0.0444	0.0461	0.0020	0.0010	4.44				
	Battelle	3	0.0474	0.0456	0.0465	0.0013	0.0007	2.85				
Atrazine	Battelle	1	0.0467	0.0456	0.0462	0.0021	0.0010	4.50	0.0424	0.0033	0.0019	7.70
	Battelle	2	0.0438	0.0365	0.0401	0.0043	0.0021	10.64				
	Battelle	3	0.0418	0.0402	0.0410	0.0015	0.0008	3.66				
Overall									0.0555	0.0118	0.0053	21.34
Positive Controls												
Aminoglutethimide	Battelle	1	0.0410	0.0392	0.0401	0.0012	0.0006	2.90	0.0325	0.0066	0.0038	20.17
	Battelle	2	0.0295	0.0288	0.0292	0.0005	0.0002	1.55				
	Battelle	3	0.0287	0.0280	0.0283	0.0007	0.0003	2.37				
Ketoconazole	Battelle	1	0.0302	0.0288	0.0295	0.0008	0.0004	2.75	0.0271	0.0022	0.0013	8.25
	Battelle	2	0.0265	0.0266	0.0266	0.0005	0.0003	2.02				
	Battelle	3	0.0250	0.0252	0.0251	0.0013	0.0006	4.98				
Dibenz[a,h]anthracene	Battelle	1	0.0258	0.0255	0.0257	0.0014	0.0007	5.32	0.0280	0.0020	0.0012	7.27
	Battelle	2	0.0290	0.0285	0.0287	0.0006	0.0003	2.19				
	Battelle	3	0.0299	0.0290	0.0295	0.0009	0.0005	3.15				
Dicofol	Battelle	1	0.0260	0.0263	0.0261	0.0011	0.0005	4.21	0.0246	0.0015	0.0009	6.06
	Battelle	2	0.0249	0.0238	0.0244	0.0008	0.0004	3.26				
	Battelle	3	0.0235	0.0228	0.0232	0.0005	0.0003	2.34				
Atrazine	Battelle	1	0.0233	0.0228	0.0231	0.0004	0.0002	1.76	0.0230	0.0022	0.0013	9.76
	Battelle	2	0.0220	0.0195	0.0207	0.0016	0.0008	7.57				
	Battelle	3	0.0261	0.0244	0.0252	0.0013	0.0007	5.16				
Overall									0.0270	0.0037	0.0016	13.55

a. Units are nmol/mg protein/min.

Table 25 - Aromatase Activity in Controls (Continued)

Reference Chemical	Microsome Source	Replicate	Mean Beginning Control[a]	Mean Ending Control[a]	Mean Overall[a]	SD	SEM	%CV	Overall By Chemical Mean[a]	± SD	SEM	%CV
Negative Controls												
Aminoglutethimide	Battelle	1	0.0916	0.0843	0.0879	0.0060	0.0030	6.81	0.0689	0.0165	0.0095	23.96
	Battelle	2	0.0609	0.0603	0.0606	0.0011	0.0006	1.89				
	Battelle	3	0.0593	0.0571	0.0582	0.0016	0.0008	2.69				
Ketoconazole	Battelle	1	0.0709	0.0675	0.0692	0.0020	0.0010	2.90	0.0592	0.0101	0.0058	17.07
	Battelle	2	0.0593	0.0593	0.0593	0.0007	0.0004	1.24				
	Battelle	3	0.0496	0.0484	0.0490	0.0007	0.0004	1.43				
Dibenz[a,h]anthracene	Battelle	1	0.0540	0.0513	0.0527	0.0016	0.0008	3.09	0.0543	0.0034	0.0020	6.30
	Battelle	2	0.0522	0.0517	0.0520	0.0026	0.0013	4.97				
	Battelle	3	0.0597	0.0567	0.0582	0.0021	0.0010	3.61				
Dicofol	Battelle	1	0.0513	0.0489	0.0501	0.0022	0.0011	4.32	0.0474	0.0023	0.0013	4.89
	Battelle	2	0.0472	0.0452	0.0462	0.0015	0.0008	3.32				
	Battelle	3	0.0481	0.0438	0.0459	0.0026	0.0013	5.74				
Atrazine	Battelle	1	0.0442	0.0435	0.0439	0.0011	0.0006	2.54	0.0393	0.0056	0.0032	14.27
	Battelle	2	0.0380	0.0281	0.0330	0.0092	0.0046	27.96				
	Battelle	3	0.0436	0.0382	0.0409	0.0038	0.0019	9.29				
Overall									0.0538	0.0113	0.0051	20.99
Full Activity Controls												
Prochloraz	In Vitro Technologies	1	0.0604	0.0578	0.0591	0.0016	0.0008	2.71	0.0478	0.0101	0.0058	21.08
	In Vitro Technologies	2	0.0451	0.0436	0.0444	0.0020	0.0010	4.50				
	In Vitro Technologies	3	0.0429	0.0367	0.0398	0.0041	0.0020	10.21				
4-Nonylphenol	In Vitro Technologies	1	0.0366	0.0357	0.0361	0.0009	0.0004	2.49	0.0422	0.0063	0.0036	14.92
	In Vitro Technologies	2	0.0509	0.0466	0.0487	0.0028	0.0014	5.75				
	In Vitro Technologies	3	0.0428	0.0407	0.0418	0.0021	0.0010	4.97				
Fenarimol	In Vitro Technologies	1	0.0488	0.0465	0.0476	0.0014	0.0007	2.87	0.0498	0.0027	0.0016	5.50
	In Vitro Technologies	2	0.0568	0.0489	0.0528	0.0046	0.0023	8.69				
	In Vitro Technologies	3	0.0525	0.0453	0.0489	0.0043	0.0022	8.86				
Econazole	In Vitro Technologies	1	0.0517	0.0215	0.0366	0.0174	0.0087	47.67	0.0433	0.0064	0.0037	14.86
	In Vitro Technologies	2	0.0515	0.0473	0.0494	0.0029	0.0015	5.89				
	In Vitro Technologies	3	0.0471	0.0405	0.0438	0.0058	0.0029	13.16				
Chrysin	In Vitro Technologies	1	0.0497	0.0456	0.0476	0.0024	0.0012	5.01	0.0463	0.0044	0.0025	9.42
	In Vitro Technologies	2	0.0435	0.0394	0.0414	0.0037	0.0019	8.94				
	In Vitro Technologies	3	0.0531	0.0466	0.0499	0.0042	0.0021	8.34				
Overall									0.0459	0.0031	0.0014	6.83

a. Units are nmol/mg protein/min.

Table 25 - Aromatase Activity in Controls (Continued)

Reference Chemical	Microsome Source	Replicate	Mean Beginning Control[a]	Mean Ending Control[a]	Mean Overall[a]	SD	SEM	%CV	Overall By Chemical Mean[a]	± SD	SEM	%CV
Positive Controls												
Prochloraz	In Vitro Technologies	1	0.0270	0.0261	0.0265	0.0006	0.0003	2.42	0.0240	0.0023	0.0013	9.65
	In Vitro Technologies	2	0.0229	0.0239	0.0234	0.0007	0.0003	2.86				
	In Vitro Technologies	3	0.0210	0.0230	0.0220	0.0013	0.0006	5.82				
4-Nonylphenol	In Vitro Technologies	1	0.0206	0.0191	0.0198	0.0010	0.0005	4.94	0.0210	0.0018	0.0010	8.59
	In Vitro Technologies	2	0.0236	0.0225	0.0231	0.0008	0.0004	3.60				
	In Vitro Technologies	3	0.0209	0.0194	0.0201	0.0012	0.0006	6.21				
Fenarimol	In Vitro Technologies	1	0.0214	0.0203	0.0208	0.0007	0.0004	3.50	0.0223	0.0016	0.0009	6.95
	In Vitro Technologies	2	0.0236	0.0242	0.0239	0.0006	0.0003	2.66				
	In Vitro Technologies	3	0.0224	0.0221	0.0223	0.0005	0.0002	2.08				
Econazole	In Vitro Technologies	1	0.0240	0.0132	0.0186	0.0063	0.0031	33.64	0.0216	0.0027	0.0016	12.55
	In Vitro Technologies	2	0.0247	0.0230	0.0239	0.0012	0.0006	4.93				
	In Vitro Technologies	3	0.0221	0.0225	0.0223	0.0005	0.0002	2.07				
Chrysin	In Vitro Technologies	1	0.0216	0.0204	0.0210	0.0012	0.0006	5.60	0.0212	0.0015	0.0009	7.16
	In Vitro Technologies	2	0.0199	0.0196	0.0197	0.0005	0.0002	2.43				
	In Vitro Technologies	3	0.0232	0.0222	0.0227	0.0006	0.0003	2.66				
Overall									0.0220	0.0012	0.0005	5.52
Negative Controls												
Prochloraz	In Vitro Technologies	1	0.0597	0.0564	0.0580	0.0021	0.0011	3.66	0.0472	0.0096	0.0055	20.32
	In Vitro Technologies	2	0.0436	0.0436	0.0436	0.0010	0.0005	2.28				
	In Vitro Technologies	3	0.0408	0.0389	0.0399	0.0013	0.0006	3.18				
4-Nonylphenol	In Vitro Technologies	1	0.0338	0.0333	0.0336	0.0006	0.0003	1.66	0.0401	0.0061	0.0035	15.29
	In Vitro Technologies	2	0.0467	0.0448	0.0457	0.0014	0.0007	3.04				
	In Vitro Technologies	3	0.0427	0.0394	0.0411	0.0021	0.0010	5.01				
Fenarimol	In Vitro Technologies	1	0.0493	0.0434	0.0464	0.0043	0.0021	9.22	0.0493	0.0034	0.0020	6.86
	In Vitro Technologies	2	0.0539	0.0520	0.0530	0.0013	0.0006	2.41				
	In Vitro Technologies	3	0.0501	0.0471	0.0486	0.0018	0.0009	3.75				
Econazole	In Vitro Technologies	1	0.0503	0.0216	0.0360	0.0167	0.0084	46.55	0.0430	0.0067	0.0039	15.53
	In Vitro Technologies	2	0.0502	0.0482	0.0492	0.0015	0.0008	3.11				
	In Vitro Technologies	3	0.0441	0.0437	0.0439	0.0020	0.0010	4.52				
Chrysin	In Vitro Technologies	1	0.0492	0.0438	0.0465	0.0038	0.0019	8.20	0.0442	0.0027	0.0016	6.12
	In Vitro Technologies	2	0.0427	0.0398	0.0412	0.0027	0.0013	6.52				
	In Vitro Technologies	3	0.0481	0.0419	0.0450	0.0055	0.0028	12.31				
Overall									0.0448	0.0036	0.0016	7.99

a. Units are nmol/mg protein/min.

4.4.2 Percent of Control

The aromatase activity found in each assay tube was normalized to percent of control by dividing by the average full enzyme activity for the replicate. The percent of control values for each reference chemical replicate and tube along with the mean, SD, SEM and % CV of the percent of control across tubes within a replicate are presented in Table 26.

Table 26 - Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control						
			Tube 1	Tube 2	Tube 3	Mean	SD	SEM	%CV
Aminoglutethimide	1	-4.00	36.01	36.81	36.46	36.43	0.40	0.23	1.10
		-5.00	85.78	85.88	84.07	85.24	1.01	0.59	1.19
		-6.00	97.89	98.33	96.29	97.50	1.07	0.62	1.10
		-7.00	97.78	97.67	97.05	97.50	0.39	0.23	0.40
		-8.00	98.38	100.01	97.30	98.57	1.37	0.79	1.39
		-9.00	98.68	98.37	99.65	98.90	0.67	0.39	0.67
		-10.00	99.02	101.95	101.75	100.9	1.64	0.94	1.62
		-11.00	98.20	101.10	96.83	98.71	2.18	1.26	2.21
	2	-3.00	6.05	6.25	6.32	6.21	0.14	0.08	2.22
		-3.60	20.22	20.74	19.91	20.29	0.42	0.24	2.07
		-4.00	38.48	39.25	39.20	38.98	0.43	0.25	1.11
		-4.30	55.19	56.76	55.97	55.97	0.78	0.45	1.39
		-4.60	70.96	71.04	71.00	71.00	0.04	0.02	0.06
		-5.00	82.66	84.80	85.26	84.24	1.39	0.80	1.65
		-5.60	90.57	91.08	87.94	89.86	1.68	0.97	1.87
		-6.00	95.57	90.29	95.77	93.88	3.11	1.79	3.31
	3	-3.00	5.20	4.89	4.52	4.87	0.34	0.20	6.96
		-3.60	19.48	19.18	19.20	19.29	0.17	0.10	0.88
		-4.00	38.50	38.15	37.97	38.21	0.27	0.16	0.71
		-4.30	54.71	53.35	54.02	54.03	0.68	0.39	1.26
		-4.60	70.16	69.23	68.75	69.38	0.72	0.42	1.04
		-5.00	85.86	83.42	82.88	84.05	1.59	0.92	1.89
		-5.60	93.87	91.84	91.63	92.44	1.24	0.71	1.34
		-6.00	94.25	97.63	96.82	96.23	1.77	1.02	1.84

Table 26 - Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control						
			Tube 1	Tube 2	Tube 3	Mean	SD	SEM	%CV
Ketoconazole	1	-4.00	0.99	0.51	0.41	0.64	0.31	0.18	48.70
		-5.00	4.18	4.04	4.02	4.08	0.09	0.05	2.09
		-6.00	29.99	30.99	29.90	30.30	0.61	0.35	2.00
		-6.60	65.23	62.69	63.33	63.75	1.32	0.76	2.07
		-7.00	79.09	79.07	75.89	78.02	1.84	1.06	2.36
		-8.00	100.54	98.52	94.56	97.87	3.04	1.75	3.10
		-9.00	101.23	100.10	100.34	100.56	0.60	0.34	0.59
		-10.00	100.73	99.89	98.37	99.66	1.20	0.69	1.20
	2	-4.00	0.64	-0.16	-0.15	0.11	0.46	0.26	412.5
		-5.00	4.26	3.74	4.28	4.09	0.31	0.18	7.52
		-6.00	32.27	31.66	31.46	31.80	0.42	0.24	1.33
		-6.30	47.76	46.32	47.07	47.05	0.72	0.42	1.54
		-6.60	64.02	64.88	63.11	64.00	0.88	0.51	1.38
		-7.00	81.67	82.06	78.97	80.90	1.68	0.97	2.08
		-8.00	95.02	95.29	94.80	95.04	0.24	0.14	0.26
		-9.00	99.66	98.69	98.40	98.92	0.66	0.38	0.67
	3	-4.00	0.29	0.36	0.36	0.34	0.04	0.02	11.31
		-5.00	5.15	5.42	5.21	5.26	0.14	0.08	2.66
		-6.00	34.86	36.07	35.59	35.51	0.61	0.35	1.71
		-6.30	50.71	51.54	51.34	51.20	0.43	0.25	0.84
		-6.60	68.86	63.11	68.67	66.88	3.27	1.89	4.88
		-7.00	82.27	83.24	82.02	82.51	0.64	0.37	0.78
		-8.00	95.59	95.54	82.61	91.25	7.48	4.32	8.20
		-9.00	98.20	97.18	94.28	96.55	2.04	1.18	2.11
Prochloraz	1	-4.00	0.29	0.00	-0.08	0.07	0.20	0.11	276.8
		-5.00	0.49	0.18	0.15	0.27	0.19	0.11	69.07
		-6.00	2.43	2.18	2.27	2.29	0.13	0.07	5.50
		-7.00	18.41	18.91	17.34	18.22	0.81	0.47	4.43
		-8.00	65.10	66.92	65.66	65.89	0.93	0.54	1.41
		-9.00	92.77	93.70	96.38	94.29	1.88	1.08	1.99
		-10.00	95.86	95.04	97.70	96.20	1.36	0.79	1.41
		-11.00	96.59	96.48	97.06	96.71	0.31	0.18	0.32
	2	-5.00	0.15	-0.06	0.12	0.07	0.11	0.07	162.3
		-6.00	2.60	2.42	2.58	2.53	0.10	0.06	3.89
		-7.00	22.18	22.46	21.58	22.07	0.45	0.26	2.04
		-7.60	47.61	50.00	51.41	49.67	1.92	1.11	3.87
		-8.00	71.75	71.24	71.97	71.65	0.37	0.22	0.52
		-8.60	89.05	89.33	87.07	88.48	1.23	0.71	1.39
		-9.00	95.87	95.30	94.35	95.17	0.77	0.44	0.81
		-10.00	99.21	99.06	98.22	98.83	0.53	0.31	0.54
	3	-5.00	-0.05	0.08	0.22	0.09	0.13	0.08	156.9
		-6.00	3.24	3.15	3.23	3.21	0.05	0.03	1.52
		-7.00	24.60	23.97	24.19	24.25	0.32	0.18	1.31
		-7.60	56.57	54.05	54.20	54.94	1.42	0.82	2.58
		-8.00	75.66	71.97	73.67	73.77	1.85	1.07	2.50
		-8.60	87.05	89.33	91.55	89.31	2.25	1.30	2.52
		-9.00	93.51	96.35	94.03	94.63	1.51	0.87	1.60
		-10.00	100.16	94.50	96.66	97.11	2.86	1.65	2.94

Table 26 - Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control						
			Tube 1	Tube 2	Tube 3	Mean	SD	SEM	%CV
4-Nonylphenol	1	-3.00	3.81	-1.58	-0.50	0.58	2.85	1.65	492.7
		-4.00	17.68	15.47	17.09	16.75	1.15	0.66	6.85
		-5.00	96.33	96.37	96.64	96.45	0.17	0.10	0.17
		-6.00	101.51	100.03	98.72	100.08	1.40	0.81	1.40
		-7.00	107.22	102.08	106.41	105.24	2.77	1.60	2.63
		-8.00	93.18	93.10	96.17	94.15	1.75	1.01	1.86
		-9.00	80.56	96.55	93.56	90.22	8.50	4.91	9.42
		-10.00	94.81	92.81	96.56	94.73	1.88	1.08	1.98
	2	-3.00	1.51	0.01	-0.77	0.25	1.16	0.67	458.6
		-4.00	11.99	11.12	13.10	12.07	0.99	0.57	8.24
		-4.30	26.66	27.96	26.71	27.11	0.74	0.43	2.72
		-4.60	45.81	46.13	46.38	46.11	0.29	0.17	0.63
		-5.00	83.98	85.93	82.09	84.00	1.92	1.11	2.28
		-6.00	94.08	95.59	95.94	95.20	0.99	0.57	1.04
		-7.00	88.00	93.52	91.24	90.92	2.77	1.60	3.05
		-8.00	90.81	91.22	95.46	92.50	2.58	1.49	2.79
	3	-3.00	0.29	0.31	0.41	0.34	0.06	0.04	18.16
		-4.00	12.94	13.90	15.36	14.07	1.22	0.70	8.66
		-4.30	32.58	32.19	33.63	32.80	0.75	0.43	2.27
		-4.60	46.98	48.25	50.00	48.41	1.52	0.88	3.14
		-5.00	87.59	85.67	88.61	87.29	1.49	0.86	1.71
		-6.00	99.41	99.13	96.45	98.33	1.64	0.95	1.66
		-7.00	95.54	96.66	102.95	98.38	3.99	2.31	4.06
		-8.00	98.49	98.38	96.40	97.76	1.17	0.68	1.20
Dibenz[a,h,] anthracene	1	-4.00	99.72	98.94	102.33	100.33	1.77	1.02	1.77
		-5.00	105.92	104.93	105.15	105.33	0.52	0.30	0.49
		-6.00	107.50	107.77	110.72	108.66	1.79	1.03	1.65
		-6.60	107.45	104.55	104.15	105.39	1.80	1.04	1.71
		-7.00	106.33	98.22	104.73	103.09	4.30	2.48	4.17
		-8.00	102.85	100.76	104.85	102.82	2.04	1.18	1.99
		-9.00	105.47	101.82	100.07	102.46	2.76	1.59	2.69
		-10.00	97.59	105.47	101.18	101.41	3.94	2.28	3.89
	2	-4.00	107.58	102.70	108.55	106.28	3.14	1.81	2.95
		-5.00	100.69	106.43	103.51	103.54	2.87	1.66	2.77
		-6.00	102.22	107.97	106.00	105.40	2.92	1.69	2.78
		-6.60	106.80	108.95	106.64	107.47	1.29	0.74	1.20
		-7.00	103.33	98.57	109.09	103.66	5.27	3.04	5.08
		-8.00	100.44	94.40	103.21	99.35	4.50	2.60	4.53
		-9.00	107.45	103.19	100.77	103.80	3.39	1.95	3.26
		-10.00	93.48	101.72	102.08	99.09	4.87	2.81	4.91
	3	-4.00	100.89	97.59	100.59	99.69	1.83	1.06	1.83
		-5.00	95.47	96.49	98.99	96.98	1.81	1.05	1.87
		-6.00	101.72	102.46	96.64	100.27	3.17	1.83	3.16
		-6.60	96.79	100.40	101.81	99.66	2.59	1.50	2.60
		-7.00	97.86	99.46	99.95	99.09	1.09	0.63	1.10
		-8.00	99.34	99.68	101.84	100.29	1.36	0.78	1.36
		-9.00	97.75	99.67	96.20	97.87	1.73	1.00	1.77
		-10.00	97.75	96.30	74.54	89.53	13.00	7.51	14.52

Table 26 - Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control						
			Tube 1	Tube 2	Tube 3	Mean	SD	SEM	%CV
Fenarimol	1	-3.00	1.26	1.06	1.32	1.21	0.13	0.08	10.97
		-4.00	6.42	6.05	5.88	6.11	0.28	0.16	4.50
		-5.00	33.80	39.37	39.61	37.60	3.29	1.90	8.74
		-6.00	83.57	84.50	85.60	84.55	1.02	0.59	1.20
		-7.00	97.86	102.56	99.85	100.09	2.36	1.36	2.36
		-8.00	97.69	95.75	98.98	97.47	1.62	0.94	1.66
		-9.00	97.46	99.51	100.18	99.05	1.42	0.82	1.43
		-10.00	96.98	100.08	99.68	98.91	1.68	0.97	1.70
	2	-3.00	1.45	1.71	1.62	1.60	0.13	0.08	8.31
		-4.00	6.40	6.13	5.85	6.13	0.28	0.16	4.53
		-5.00	36.62	38.25	37.42	37.43	0.82	0.47	2.18
		-5.60	68.35	67.04	71.03	68.81	2.04	1.18	2.96
		-6.00	79.10	82.58	80.91	80.86	1.74	1.01	2.16
		-7.00	97.38	93.82	93.36	94.85	2.20	1.27	2.32
		-8.00	95.44	100.80	96.30	97.51	2.88	1.66	2.95
		-9.00	96.69	98.33	97.47	97.50	0.82	0.47	0.84
	3	-3.00	1.30	1.27	1.17	1.25	0.07	0.04	5.48
		-4.00	5.77	5.66	5.90	5.78	0.12	0.07	2.10
		-5.00	38.04	38.22	37.86	38.04	0.18	0.10	0.47
		-5.60	70.27	73.37	66.50	70.05	3.44	1.99	4.92
		-6.00	86.32	87.94	88.02	87.43	0.96	0.55	1.10
		-7.00	96.73	98.05	97.21	97.33	0.67	0.39	0.69
		-8.00	101.56	95.65	98.25	98.49	2.96	1.71	3.01
		-9.00	98.23	101.29	99.36	99.63	1.54	0.89	1.55
Econazole	1	-3.00	-0.37	-0.40	-0.26	-0.34	0.07	0.04	-21.45
		-4.00	-0.38	-0.66	-0.59	-0.54	0.15	0.08	-26.94
		-5.00	0.10	-0.75	-0.37	-0.34	0.43	0.25	-126.0
		-6.00	-0.40	0.07	-0.31	-0.21	0.25	0.14	-116.7
		-7.00	1.29	1.25	1.25	1.26	0.03	0.01	2.02
		-8.00	13.18	11.64	11.99	12.27	0.81	0.47	6.57
		-9.00	40.68	43.03	44.08	42.60	1.74	1.00	4.08
		-10.00	57.01	57.86	50.24	55.03	4.18	2.41	7.59
	2	-4.00	-0.39	0.09	-0.39	-0.23	0.28	0.16	-119.7
		-5.00	-0.37	0.34	-0.22	-0.08	0.37	0.21	-452.8
		-6.00	-0.33	-0.26	-0.48	-0.36	0.11	0.06	-31.27
		-7.00	1.48	1.91	1.47	1.62	0.25	0.14	15.30
		-8.00	16.07	16.44	16.28	16.26	0.19	0.11	1.15
		-8.60	45.70	44.67	43.61	44.66	1.04	0.60	2.34
		-9.00	63.51	65.02	67.62	65.38	2.08	1.20	3.18
		-10.00	83.75	90.14	93.56	89.15	4.98	2.87	5.58
	3	-4.00	-0.11	-0.44	-0.38	-0.31	0.18	0.10	-57.53
		-5.00	-0.36	-0.56	0.16	-0.25	0.37	0.21	-146.5
		-6.00	-0.08	-0.32	-0.14	-0.18	0.13	0.07	-70.51
		-7.00	2.28	2.15	1.79	2.07	0.26	0.15	12.40
		-8.00	16.94	15.84	16.01	16.26	0.60	0.34	3.67
		-8.60	45.08	41.79	45.58	44.15	2.06	1.19	4.67
		-9.00	69.42	68.39	68.29	68.70	0.63	0.36	0.91
		-10.00	94.06	100.48	96.44	96.99	3.24	1.87	3.34

Table 26 - Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control						
			Tube 1	Tube 2	Tube 3	Mean	SD	SEM	%CV
Chrysin	1	-4.00	20.54	20.28	25.14	21.98	2.73	1.58	12.44
		-5.00	25.00	25.28	23.80	24.70	0.79	0.45	3.18
		-6.00	71.56	76.56	76.28	74.80	2.81	1.62	3.76
		-6.60	86.28	86.93	87.77	86.99	0.74	0.43	0.86
		-7.00	92.20	90.66	88.60	90.49	1.81	1.04	2.00
		-8.00	85.40	92.99	88.69	89.03	3.80	2.20	4.27
		-9.00	88.71	93.99	94.51	92.40	3.21	1.85	3.48
		-10.00	93.26	90.36	87.09	90.23	3.09	1.78	3.42
	2	-4.00	19.50	17.12	20.78	19.13	1.85	1.07	9.70
		-5.00	27.72	26.71	27.42	27.28	0.52	0.30	1.91
		-5.30	37.61	39.81	40.15	39.19	1.38	0.79	3.51
		-5.60	51.06	52.96	53.79	52.60	1.40	0.81	2.66
		-6.00	74.76	74.52	78.20	75.83	2.06	1.19	2.72
		-7.00	98.23	88.77	88.73	91.91	5.47	3.16	5.96
		-8.00	94.82	96.98	93.28	95.03	1.86	1.07	1.96
		-9.00	76.98	102.13	104.57	94.56	15.27	8.82	16.15
	3	-4.00	19.83	20.79	20.76	20.46	0.55	0.31	2.66
		-5.00	27.89	26.81	25.56	26.75	1.16	0.67	4.35
		-5.30	39.98	39.89	39.68	39.85	0.15	0.09	0.38
		-5.60	53.85	54.80	55.92	54.86	1.04	0.60	1.89
		-6.00	72.26	68.66	70.28	70.40	1.80	1.04	2.56
		-7.00	90.20	56.70	90.53	79.14	19.44	11.22	24.56
		-8.00	94.70	88.10	95.79	92.87	4.16	2.40	4.48
		-9.00	93.84	94.74	91.43	93.34	1.71	0.99	1.83
Dicofol	1	-3.00	2.56	2.63	3.68	2.96	0.63	0.36	21.30
		-4.00	20.00	20.16	21.39	20.52	0.76	0.44	3.70
		-5.00	61.78	59.69	54.70	58.72	3.64	2.10	6.19
		-6.00	89.09	94.22	89.73	91.01	2.80	1.61	3.07
		-7.00	95.14	98.50	102.65	98.76	3.76	2.17	3.81
		-8.00	97.40	95.93	98.11	97.15	1.11	0.64	1.15
		-9.00	94.89	96.20	96.28	95.79	0.78	0.45	0.82
		-10.00	100.76	100.48	92.89	98.05	4.46	2.58	4.55
	2	-3.00	3.05	2.58	2.75	2.79	0.24	0.14	8.67
		-4.00	26.03	21.58	20.71	22.77	2.85	1.64	12.51
		-4.30	34.86	44.55	30.66	36.69	7.12	4.11	19.41
		-5.00	62.19	60.34	63.59	62.04	1.63	0.94	2.63
		-6.00	92.56	92.16	94.31	93.01	1.14	0.66	1.23
		-7.00	96.36	97.80	97.37	97.18	0.74	0.43	0.76
		-8.00	98.88	97.23	98.81	98.31	0.93	0.54	0.95
		-9.00	94.73	96.80	96.84	96.13	1.21	0.70	1.25
	3	-3.00	2.27	1.58	2.75	2.20	0.59	0.34	26.66
		-4.00	23.25	22.85	22.42	22.84	0.42	0.24	1.82
		-4.30	28.43	29.71	29.95	29.36	0.82	0.47	2.78
		-5.00	82.79	82.93	81.24	82.32	0.94	0.54	1.14
		-6.00	91.55	88.89	90.18	90.21	1.33	0.77	1.48
		-7.00	93.37	95.67	100.84	96.63	3.83	2.21	3.96
		-8.00	92.62	102.07	98.56	97.75	4.78	2.76	4.89
		-9.00	92.52	100.04	97.90	96.82	3.88	2.24	4.01

Table 26 - Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control						
			Tube 1	Tube 2	Tube 3	Mean	SD	SEM	%CV
Atrazine	1	-3.00	81.18	82.20	80.87	81.42	0.70	0.40	0.86
		-4.00	93.40	95.39	95.88	94.89	1.31	0.76	1.38
		-5.00	102.09	101.78	102.58	102.15	0.40	0.23	0.40
		-6.00	100.55	99.61	101.27	100.48	0.83	0.48	0.83
		-7.00	96.57	97.97	98.25	97.59	0.90	0.52	0.92
		-8.00	95.67	99.69	98.29	97.89	2.04	1.18	2.09
		-9.00	96.02	95.78	95.02	95.60	0.52	0.30	0.55
		-10.00	91.11	95.00	95.01	93.71	2.25	1.30	2.40
	2	-3.00	79.19	83.47	84.22	82.29	2.71	1.57	3.30
		-3.05	85.38	81.39	79.58	82.12	2.97	1.71	3.61
		-3.10	76.45	75.60	81.69	77.91	3.30	1.90	4.23
		-3.12	78.20	86.60	85.15	83.32	4.49	2.59	5.39
		-3.30	82.94	82.32	85.30	83.52	1.57	0.91	1.89
		-4.00	96.53	91.58	91.05	93.05	3.03	1.75	3.25
		-5.00	90.65	95.89	96.41	94.32	3.19	1.84	3.38
		-6.00	93.48	96.02	95.87	95.13	1.43	0.82	1.50
	3	-3.00	85.30	84.77	86.21	85.43	0.73	0.42	0.85
		-3.05	83.19	85.17	87.05	85.14	1.93	1.11	2.27
		-3.10	86.58	85.31	86.34	86.08	0.67	0.39	0.78
		-3.12	84.40	84.06	86.85	85.10	1.52	0.88	1.79
		-3.30	85.99	85.84	84.06	85.30	1.07	0.62	1.26
		-4.00	93.85	93.29	92.74	93.29	0.56	0.32	0.59
		-5.00	93.96	89.72	98.91	94.20	4.60	2.66	4.88
		-6.00	94.46	97.89	96.97	96.44	1.78	1.03	1.84

The % CV at the majority of the tested concentrations (except for the highest) of the tested reference chemicals were below 10% (mostly < 5% with few exceptions). For the third replicate of dibenz[a,h]anthracene (con. 1 x 10^{-10} M), the second and third replicate of chrysin (con. 1 x 10^{-9} and 1 x 10^{-7} M, respectively) and the second replicate for dicofol (con. 1 x 10^{-4} and 5 x 10^{-5} M) % CV was between 12.51 and 24.56. For all three replicates of econazole, the highest concentration levels were observed to have very high % CV. The same high % CV values were also noticed for the highest concentration of 4-nonylphenol in Replicates 1 and 2, prochloraz in Replicates 1, 2 and 3; and the second replicate for ketoconazole. Those high % CV values are the result of the nature of the calculated numbers (very low and /or negative values for percent of control) than high assay variability.

The mean percent of control activity for each replicate and the overall mean, SD, SEM and % CV across replicates are presented in Table 27.

Table 27 - Mean Percent of Control per Replicate

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	SD	SEM	%CV
Aminoglutethimide	-3.00	NA	6.21	4.87	5.54	NC	NC	NC
	-3.60	NA	20.29	19.29	19.79	NC	NC	NC
	-4.00	36.43	38.98	38.21	37.87	1.31	0.76	3.46
	-4.30	NA	55.97	54.03	55.00	NC	NC	NC
	-4.60	NA	71.00	69.38	70.19	NC	NC	NC
	-5.00	85.24	84.24	84.05	84.51	0.64	0.37	0.76
	-5.60	NA	89.86	92.44	91.15	NC	NC	NC
	-6.00	97.50	93.88	96.23	95.87	1.84	1.06	1.92
	-7.00	97.50	NA	NA	NC	NC	NC	NC
	-8.00	98.57	NA	NA	NC	NC	NC	NC
	-9.00	98.90	NA	NA	NC	NC	NC	NC
	-10.00	100.9	NA	NA	NC	NC	NC	NC
	-11.00	98.71	NA	NA	NC	NC	NC	NC
Ketoconazole	-4.00	0.64	0.11	0.34	0.36	0.26	0.15	72.68
	-5.00	4.08	4.09	5.26	4.48	0.68	0.39	15.11
	-6.00	30.30	31.80	35.51	32.53	2.68	1.55	8.24
	-6.30	NA	47.05	51.20	49.12	NC	NC	NC
	-6.60	63.75	64.00	66.88	64.88	1.74	1.00	2.68
	-7.00	78.02	80.90	82.51	80.48	2.28	1.31	2.83
	-8.00	97.87	95.04	91.25	94.72	3.32	1.92	3.51
	-9.00	100.56	98.92	96.55	98.68	2.01	1.16	2.04
	-10.00	99.66	NA	NA	NC	NC	NC	NC
Prochloraz	-4.00	0.07	NA	NA	NC	NC	NC	NC
	-5.00	0.27	0.07	0.09	0.14	0.11	0.07	79.54
	-6.00	2.29	2.53	3.21	2.68	0.47	0.27	17.70
	-7.00	18.22	22.07	24.25	21.52	3.05	1.76	14.20
	-7.60	NA	49.67	54.94	52.31	NC	NC	NC
	-8.00	65.89	71.65	73.77	70.44	4.08	2.35	5.79
	-8.60	NA	88.48	89.31	88.90	NC	NC	NC
	-9.00	94.29	95.17	94.63	94.70	0.45	0.26	0.47
	-10.00	96.20	98.83	97.11	97.38	1.34	0.77	1.37
	-11.00	96.71	NA	NA	NC	NC	NC	NC
4-Nonylphenol	-3.00	0.58	0.25	0.34	0.39	0.17	0.10	43.48
	-4.00	16.75	12.07	14.07	14.30	2.35	1.35	16.41
	-4.30	NA	27.11	32.80	29.96	NC	NC	NC
	-4.60	NA	46.11	48.41	47.26	NC	NC	NC
	-5.00	96.45	84.00	87.29	89.25	6.45	3.72	7.23
	-6.00	100.08	95.20	98.33	97.87	2.47	1.43	2.53
	-7.00	105.24	90.92	98.38	98.18	7.16	4.13	7.29
	-8.00	94.15	92.50	97.76	94.80	2.69	1.55	2.84
	-9.00	90.22	NA	NA	NC	NC	NC	NC
	-10.00	94.73	NA	NA	NC	NC	NC	NC
Dibenz[a,h]anthracene	-4.00	100.33	106.28	99.69	102.10	3.63	2.10	3.56
	-5.00	105.33	103.54	96.98	101.95	4.40	2.54	4.31
	-6.00	108.66	105.40	100.27	104.78	4.23	2.44	4.04
	-6.60	105.39	107.47	99.66	104.17	4.04	2.33	3.88
	-7.00	103.09	103.66	99.09	101.95	2.49	1.44	2.44
	-8.00	102.82	99.35	100.29	100.82	1.80	1.04	1.78
	-9.00	102.46	103.80	97.87	101.38	3.11	1.80	3.07
	-10.00	101.41	99.09	89.53	96.68	6.30	3.64	6.51

NA = Not applicable – this test concentration was not assayed in this replicate.
NC = Not calculated – only one data point (no mean, SD, SEM of % CV calculated) or two data points (only mean was calculated).

Table 27 - Mean Percent of Control per Replicate (Continued)

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control					Overall	
		Rep 1	Rep 2	Rep 3	Mean	SD	SEM	%CV
Fenarimol	-3.00	1.21	1.60	1.25	1.35	0.21	0.12	15.73
	-4.00	6.11	6.13	5.78	6.01	0.20	0.11	3.30
	-5.00	37.60	37.43	38.04	37.69	0.31	0.18	0.83
	-5.60	NA	68.81	70.05	69.43	NC	NC	NC
	-6.00	84.55	80.86	87.43	84.28	3.29	1.90	3.91
	-7.00	100.09	94.85	97.33	97.43	2.62	1.51	2.69
	-8.00	97.47	97.51	98.49	97.82	0.57	0.33	0.59
	-9.00	99.05	97.50	99.63	98.72	1.10	0.64	1.12
	-10.00	98.91	NA	NA	NC	NC	NC	NC
Econazole	-3.00	-0.34	NA	NA	NC	NC	NC	NC
	-4.00	-0.54	-0.23	-0.31	-0.36	0.16	0.09	-45.47
	-5.00	-0.34	-0.08	-0.25	-0.23	0.13	0.08	-58.41
	-6.00	-0.21	-0.36	-0.18	-0.25	0.09	0.05	-37.41
	-7.00	1.26	1.62	2.07	1.65	0.41	0.23	24.52
	-8.00	12.27	16.26	16.26	14.93	2.30	1.33	15.42
	-8.60	NA	44.66	44.15	44.40	NC	NC	NC
	-9.00	42.60	65.38	68.70	58.89	14.21	8.20	24.13
	-10.00	55.03	89.15	96.99	80.39	22.31	12.88	27.75
Chrysin	-4.00	21.98	19.13	20.46	20.52	1.43	0.82	6.95
	-5.00	24.70	27.28	26.75	26.24	1.37	0.79	5.21
	-5.30	NA	39.19	39.85	39.52	0.47	0.27	1.18
	-5.60	NA	52.60	54.86	53.73	1.59	0.92	2.96
	-6.00	74.80	75.83	70.40	73.68	2.88	1.66	3.91
	-6.60	86.99	NA	NA	86.99	NC	NC	NC
	-7.00	90.49	91.91	79.14	87.18	7.00	4.04	8.03
	-8.00	89.03	95.03	92.87	92.31	3.04	1.75	3.29
	-9.00	92.40	94.56	93.34	93.43	1.08	0.63	1.16
	-10.00	90.23	NC	NC	NC	NC	NC	NC
Dicofol	-3.00	2.96	2.79	2.20	2.65	0.40	0.23	15.01
	-4.00	20.52	22.77	22.84	22.04	1.32	0.76	6.00
	-4.30	NA	36.69	29.36	33.03	NC	NC	NC
	-5.00	58.72	62.04	82.32	67.69	12.77	7.38	18.87
	-6.00	91.01	93.01	90.21	91.41	1.45	0.83	1.58
	-7.00	98.76	97.18	96.63	97.52	1.11	0.64	1.14
	-8.00	97.15	98.31	97.75	97.74	0.58	0.33	0.59
	-9.00	95.79	96.13	96.82	96.25	0.52	0.30	0.54
	-10.00	98.05	NA	NA	NC	NC	NC	NC
Atrazine	-3.00	81.42	82.29	85.43	83.05	2.11	1.22	2.54
	-3.05	NA	82.12	85.14	83.63	NC	NC	NC
	-3.10	NA	77.91	86.08	81.99	NC	NC	NC
	-3.12	NA	83.32	85.10	84.21	NC	NC	NC
	-3.30	NA	83.52	85.30	84.41	NC	NC	NC
	-4.00	94.89	93.05	93.29	93.74	1.00	0.58	1.07
	-5.00	102.15	94.32	94.20	96.89	4.56	2.63	4.70
	-6.00	100.48	95.13	96.44	97.35	2.79	1.61	2.86
	-7.00	97.59	NA	NA	NC	NC	NC	NC
	-8.00	97.89	NA	NA	NC	NC	NC	NC
	-9.00	95.60	NA	NA	NC	NC	NC	NC
	-10.00	93.71	NA	NA	NC	NC	NC	NC

NA = Not applicable – this test concentration was not assayed in this replicate.
NC = Not calculated – only one data point (no mean, SD, SEM of % CV calculated) or two data points (only mean was calculated).

4.4.3 IC_{50} Values

For each reference chemical and replicate the percent of control aromatase activity values were fitted to the equation presented in Section 3.8.2.1 using Prism 4.0 and the IC_{50} and slope were calculated. The concentration response curves for all ten reference chemicals are presented in Figures 1 through 10.

For aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, fenarimol, econazole, and dicofol the percent of control activity values for the tested concentrations essentially span the range 0 to 95%. In the case of chrysin, the percent of control data for the tested concentrations range from approximately 19 to 95%, for dibenz[a,h]anthracene and atrazine the percent of control data ranges from approximately 80 to 100%. All mentioned above compounds, except dibenz[a,h]anthracene and atrazine, showed inhibitor properties and IC_{50} values for those compounds were calculated. Dibenz[a,h]anthracene and atrazine were non-inhibitors and IC_{50} values were not calculated for them.

Figure 11 shows the concentration response curve fits of each replicate per reference chemical superimposed on one plot. It is evident that the replicate-to-replicate variation in curve fit is low. In the case of 4-nonylphenol the concentration curve fits for second and third replicates were almost identical. The first replicate for this reference chemical was a little different because it was run in different concentration ranges than the second and third replicates (after the first run, the chemical concentrations were adjusted by adding mid-log concentrations near the estimated IC_{50} based on first replicate results).

There is a higher degree of replicate-to-replicate variance for econazole, most of which is associated with the first replicate. It is very probable that during the preparation of econazole dilutions at 1×10^{-9} M and 1×10^{-10} M a technical error occurred.

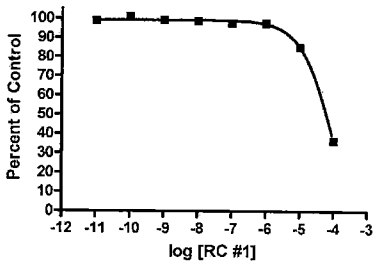
Figure 12 presents the results of curve fitting across the replicates for each reference chemical. The mean percent of control data for the repetitions within a replicate (as presented in Table 27) were fit to the model.

The IC_{50} and slope data are presented in Table 28 by reference chemical and replicate. The status of each reference chemical is also indicated in the table.

The calculated arithmetic mean for IC_{50} values for eight reference chemicals (showing inhibitor properties) are presented in Table 29.

Placental Microsomes Assay 7-20-05

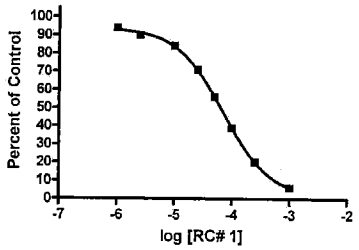
Replicate 1 (Aminoglutethimide)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-29.54
TOP	99.04
LOGEC50	-3.975
HILLSLOPE	-0.8987
EC50	0.0001060
Std. Error	
BOTTOM	86.82
TOP	0.3968
LOGEC50	0.6421
HILLSLOPE	0.2421
95% Confidence Intervals	
BOTTOM	-210.6 to 151.6
TOP	98.21 to 99.87
LOGEC50	-5.314 to -2.635
HILLSLOPE	-1.404 to -0.3937
EC50	4.851e-006 to 0.002315
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9959
Absolute Sum of Squares	41.39
Sy.x	1.439
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental Microsomes Assay 7-21-05

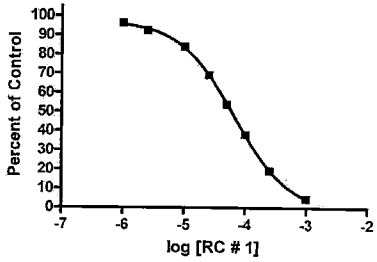
Replicate 2 (Aminoglutethimide)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	1.114
TOP	93.81
LOGEC50	-4.148
HILLSLOPE	-1.073
EC50	7.114e-005
Std. Error	
BOTTOM	1.386
TOP	0.7374
LOGEC50	0.01712
HILLSLOPE	0.04396
95% Confidence Intervals	
BOTTOM	-1.778 to 4.006
TOP	92.27 to 95.35
LOGEC50	-4.184 to -4.112
HILLSLOPE	-1.164 to -0.9810
EC50	6.553e-005 to 7.724e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9982
Absolute Sum of Squares	41.11
Sy.x	1.434
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental Microsomes Assay 7-22-05

Replicate 3 (Aminoglutethimide)

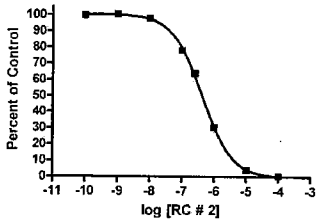


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-1.797
TOP	97.33
LOGEC50	-4.181
HILLSLOPE	-0.9702
EC50	6.592e-005
Std. Error	
BOTTOM	1.116
TOP	0.6089
LOGEC50	0.01319
HILLSLOPE	0.02912
95% Confidence Intervals	
BOTTOM	-4.124 to 0.5309
TOP	96.06 to 98.60
LOGEC50	-4.208 to -4.153
HILLSLOPE	-1.031 to -0.9095
EC50	6.188e-005 to 7.024e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9991
Absolute Sum of Squares	21.32
Sy.x	1.032
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Figure 1 - Fitted Concentration Response for Aminoglutethimide

Placental Microsomes (08-11-2005)

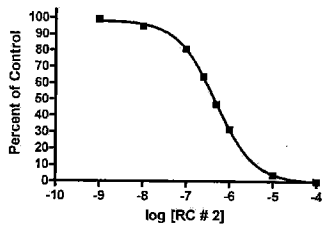
Replicate 1 (Ketoconazole)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.4299
TOP	100.3
LOGEC50	-6.371
HILLSLOPE	-0.9384
EC50	4.258e-007
Std. Error	
BOTTOM	0.8579
TOP	0.6107
LOGEC50	0.01735
HILLSLOPE	0.03367
95% Confidence Intervals	
BOTTOM	-2.219 to 1.360
TOP	99.01 to 101.6
LOGEC50	-6.407 to -6.335
HILLSLOPE	-1.009 to -0.8682
EC50	3.917e-007 to 4.628e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9986
Absolute Sum of Squares	54.34
Sy.x	1.648
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-12-2005)

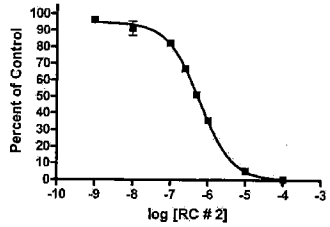
Replicate 2 (Ketoconazole)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.5072
TOP	98.31
LOGEC50	-6.325
HILLSLOPE	-0.9801
EC50	4.733e-007
Std. Error	
BOTTOM	0.4716
TOP	0.4283
LOGEC50	0.008478
HILLSLOPE	0.02055
95% Confidence Intervals	
BOTTOM	-1.491 to 0.4767
TOP	97.42 to 99.21
LOGEC50	-6.343 to -6.307
HILLSLOPE	-1.023 to -0.9373
EC50	4.544e-007 to 4.930e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9995
Absolute Sum of Squares	17.07
Sy.x	0.9239
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-15-2005)

Replicate 3 (Ketoconazole)

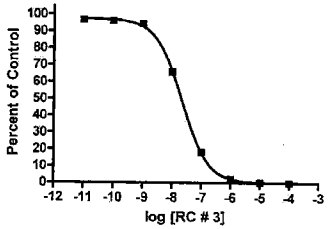


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.09188
TOP	95.03
LOGEC50	-6.225
HILLSLOPE	-1.009
EC50	5.961e-007
Std. Error	
BOTTOM	1.526
TOP	1.307
LOGEC50	0.02796
HILLSLOPE	0.07104
95% Confidence Intervals	
BOTTOM	-3.276 to 3.092
TOP	92.31 to 97.76
LOGEC50	-6.283 to -6.166
HILLSLOPE	-1.158 to -0.8612
EC50	5.212e-007 to 6.818e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9942
Absolute Sum of Squares	170.9
Sy.x	2.923
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Figure 2 - Fitted Concentration Response for Ketoconazole

Placental microsomes 08-02-05

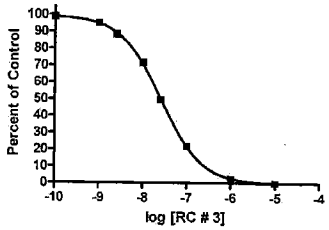
Replicate 1 (Prochloraz)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.1382
TOP	97.27
LOGEC50	-7.662
HILLSLOPE	-0.9853
EC50	2.176e-008
Std. Error	
BOTTOM	0.4018
TOP	0.4193
LOGEC50	0.01321
HILLSLOPE	0.02416
95% Confidence Intervals	
BOTTOM	-0.6999 to 0.9763
TOP	96.40 to 98.14
LOGEC50	-7.690 to -7.635
HILLSLOPE	-1.036 to -0.9349
EC50	2.042e-008 to 2.319e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9995
Absolute Sum of Squares	23.89
Sy.x	1.093
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes 08-03-05

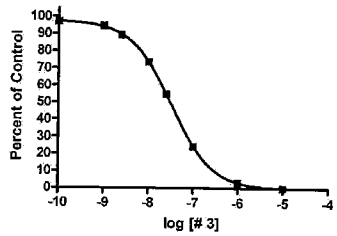
Replicate 2 (Prochloraz)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.4169
TOP	99.28
LOGEC50	-7.580
HILLSLOPE	-0.9386
EC50	2.629e-008
Std. Error	
BOTTOM	0.5029
TOP	0.5226
LOGEC50	0.01087
HILLSLOPE	0.02175
95% Confidence Intervals	
BOTTOM	-1.466 to 0.6322
TOP	98.19 to 100.4
LOGEC50	-7.603 to -7.558
HILLSLOPE	-0.9840 to -0.8932
EC50	2.495e-008 to 2.770e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9994
Absolute Sum of Squares	21.45
Sy.x	1.036
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

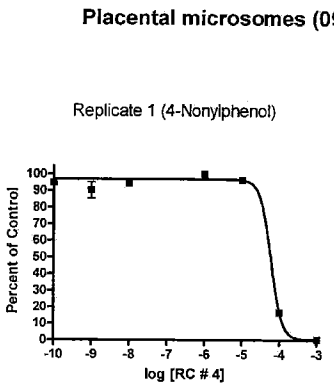
Placental microsomes 08-04-05

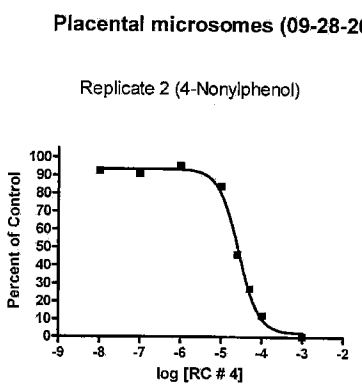
Replicate 3 (Prochloraz)

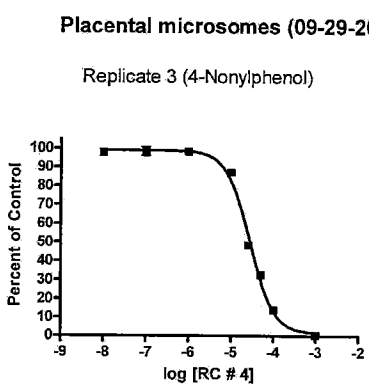


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.3238
TOP	97.48
LOGEC50	-7.487
HILLSLOPE	-0.9641
EC50	3.260e-008
Std. Error	
BOTTOM	0.7289
TOP	0.6999
LOGEC50	0.01558
HILLSLOPE	0.03239
95% Confidence Intervals	
BOTTOM	-1.844 to 1.197
TOP	96.02 to 98.94
LOGEC50	-7.519 to -7.454
HILLSLOPE	-1.032 to -0.8966
EC50	3.025e-008 to 3.513e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9987
Absolute Sum of Squares	43.88
Sy.x	1.481
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Figure 3 - Fitted Concentration Response for Prochloraz







Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.5591
TOP	96.88
LOGEC50	-4.228
HILLSLOPE	-3.042
EC50	5.911e-005
Std. Error	
BOTTOM	3.187
TOP	1.421
LOGEC50	0.2662
HILLSLOPE	3.520
95% Confidence Intervals	
BOTTOM	-6.088 to 7.206
TOP	93.92 to 99.85
LOGEC50	-4.784 to -3.673
HILLSLOPE	-10.39 to 4.302
EC50	1.646e-005 to 0.0002123
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9832
Absolute Sum of Squares	605.4
Sy.x	5.502
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

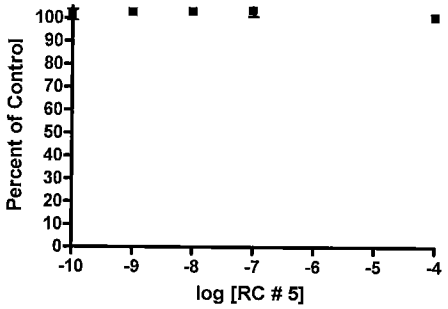
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	2.043
TOP	93.58
LOGEC50	-4.571
HILLSLOPE	-1.766
EC50	2.684e-005
Std. Error	
BOTTOM	1.802
TOP	1.114
LOGEC50	0.02105
HILLSLOPE	0.1297
95% Confidence Intervals	
BOTTOM	-1.715 to 5.801
TOP	91.26 to 95.90
LOGEC50	-4.615 to -4.527
HILLSLOPE	-2.037 to -1.496
EC50	2.425e-005 to 2.969e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9932
Absolute Sum of Squares	223.0
Sy.x	3.339
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	1.341
TOP	98.95
LOGEC50	-4.553
HILLSLOPE	-1.558
EC50	2.798e-005
Std. Error	
BOTTOM	2.095
TOP	1.239
LOGEC50	0.02412
HILLSLOPE	0.1167
95% Confidence Intervals	
BOTTOM	-3.028 to 5.710
TOP	96.37 to 101.5
LOGEC50	-4.603 to -4.503
HILLSLOPE	-1.802 to -1.315
EC50	2.492e-005 to 3.141e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9923
Absolute Sum of Squares	269.5
Sy.x	3.671
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Figure 4 - Fitted Concentration Response for 4-Nonylphenol

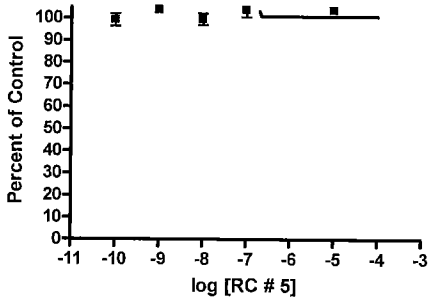
Placental microsomes (09-30-2005)

Replicate 1 (Dibenz[a,h]anthracene)



Placental microsomes (10-03-2005)

Replicate 2 (Dibenz[a,h]anthracene)



Placental microsomes (10-04-2005)

Replicate 3 (Dibenz[a,h]anthracene)

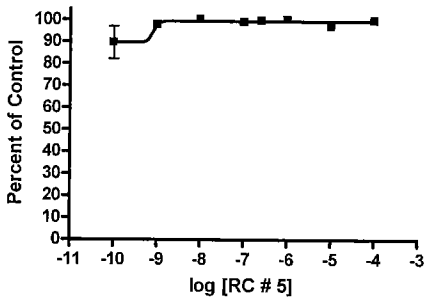
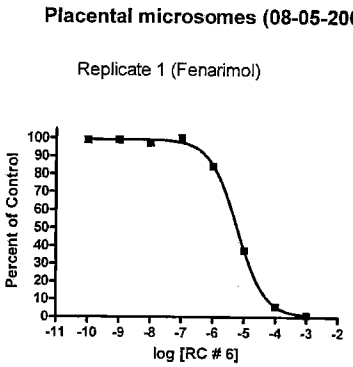
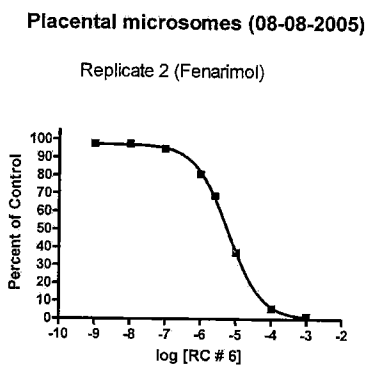
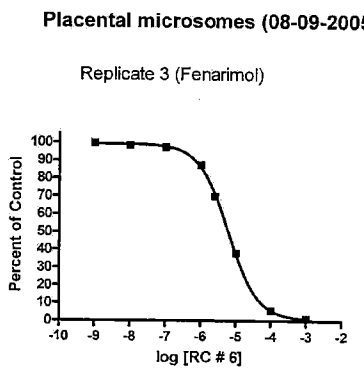


Figure 5 - Fitted Concentration Response for Dibenz[a,h]anthracene





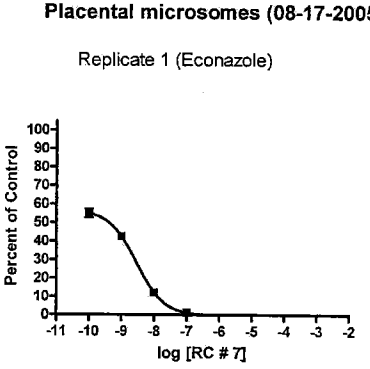


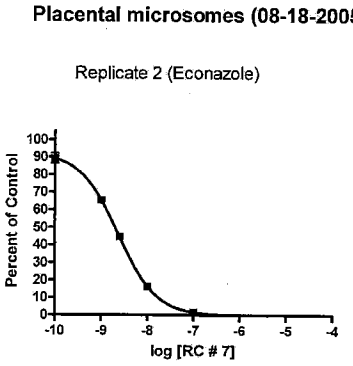
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.6679
TOP	99.20
LOGEC50	-5.224
HILLSLOPE	-1.005
EC50	5.966e-006
Std. Error	
BOTTOM	1.031
TOP	0.6040
LOGEC50	0.02338
HILLSLOPE	0.04852
95% Confidence Intervals	
BOTTOM	-1.484 to 2.819
TOP	97.94 to 100.5
LOGEC50	-5.273 to -5.176
HILLSLOPE	-1.106 to -0.9034
EC50	5.332e-006 to 6.675e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9981
Absolute Sum of Squares	77.32
Sy.x	1.966
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

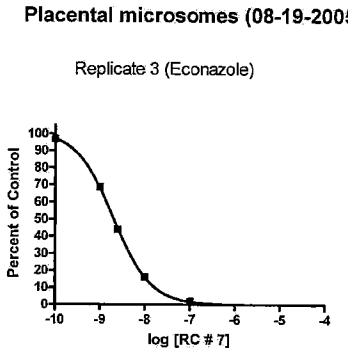
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.2286
TOP	97.27
LOGEC50	-5.218
HILLSLOPE	-0.9361
EC50	6.056e-006
Std. Error	
BOTTOM	0.9234
TOP	0.6043
LOGEC50	0.01935
HILLSLOPE	0.03584
95% Confidence Intervals	
BOTTOM	-1.698 to 2.155
TOP	96.01 to 98.53
LOGEC50	-5.258 to -5.177
HILLSLOPE	-1.011 to -0.8614
EC50	5.518e-006 to 6.646e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9984
Absolute Sum of Squares	55.49
Sy.x	1.666
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.7629
TOP	99.13
LOGEC50	-5.214
HILLSLOPE	-1.039
EC50	6.113e-006
Std. Error	
BOTTOM	0.8948
TOP	0.6117
LOGEC50	0.01790
HILLSLOPE	0.04006
95% Confidence Intervals	
BOTTOM	-1.104 to 2.629
TOP	97.85 to 100.4
LOGEC50	-5.251 to -5.176
HILLSLOPE	-1.123 to -0.9559
EC50	5.610e-006 to 6.662e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9983
Absolute Sum of Squares	60.53
Sy.x	1.740
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Figure 6 - Fitted Concentration Response for Fenarimol





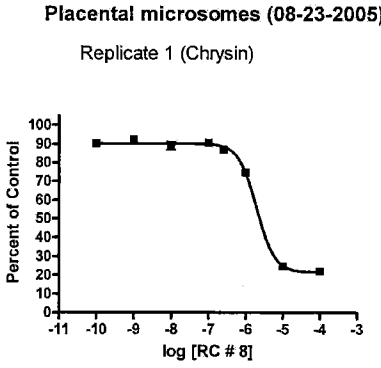


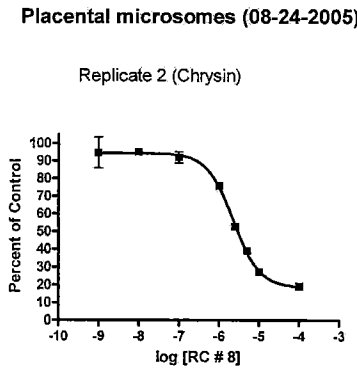
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.3735
TOP	56.78
LOGEC50	-8.531
HILLSLOPE	-1.025
EC50	2.942e-009
Std. Error	
BOTTOM	0.4024
TOP	1.155
LOGEC50	0.03646
HILLSLOPE	0.06242
95% Confidence Intervals	
BOTTOM	-1.213 to 0.4659
TOP	54.37 to 59.19
LOGEC50	-8.607 to -8.455
HILLSLOPE	-1.155 to -0.8950
EC50	2.470e-009 to 3.506e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9959
Absolute Sum of Squares	42.94
Sy.x	1.465
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

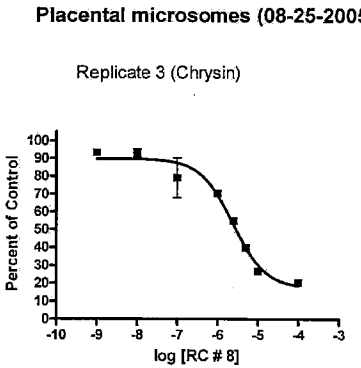
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.2682
TOP	92.38
LOGEC50	-8.628
HILLSLOPE	-1.048
EC50	2.352e-009
Std. Error	
BOTTOM	0.5394
TOP	1.388
LOGEC50	0.02032
HILLSLOPE	0.04851
95% Confidence Intervals	
BOTTOM	-1.393 to 0.8569
TOP	89.48 to 95.27
LOGEC50	-8.671 to -8.586
HILLSLOPE	-1.149 to -0.9468
EC50	2.134e-009 to 2.594e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9976
Absolute Sum of Squares	61.63
Sy.x	1.755
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.1287
TOP	101.4
LOGEC50	-8.698
HILLSLOPE	-1.038
EC50	2.005e-009
Std. Error	
BOTTOM	0.4020
TOP	1.104
LOGEC50	0.01447
HILLSLOPE	0.03437
95% Confidence Intervals	
BOTTOM	-0.9673 to 0.7099
TOP	99.13 to 103.7
LOGEC50	-8.728 to -8.668
HILLSLOPE	-1.110 to -0.9667
EC50	1.870e-009 to 2.149e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9988
Absolute Sum of Squares	34.47
Sy.x	1.313
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Figure 7 - Fitted Concentration Response for Econazole





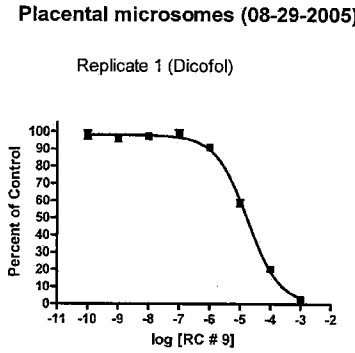


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	21.55
TOP	90.29
LOGEC50	-5.706
HILLSLOPE	-1.785
EC50	1.967e-006
Std. Error	
BOTTOM	1.520
TOP	0.7205
LOGEC50	0.05148
HILLSLOPE	0.2565
95% Confidence Intervals	
BOTTOM	18.38 to 24.72
TOP	88.79 to 91.79
LOGEC50	-5.813 to -5.599
HILLSLOPE	-2.320 to -1.250
EC50	1.536e-006 to 2.519e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9929
Absolute Sum of Squares	136.2
Sy.x	2.609
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

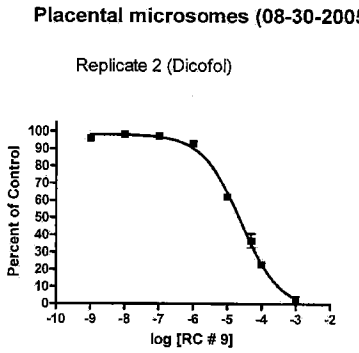
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	18.57
TOP	94.35
LOGEC50	-5.647
HILLSLOPE	-1.325
EC50	2.253e-006
Std. Error	
BOTTOM	3.176
TOP	1.876
LOGEC50	0.05260
HILLSLOPE	0.1951
95% Confidence Intervals	
BOTTOM	11.95 to 25.20
TOP	90.43 to 98.26
LOGEC50	-5.757 to -5.538
HILLSLOPE	-1.732 to -0.9180
EC50	1.750e-006 to 2.900e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9730
Absolute Sum of Squares	573.4
Sy.x	5.354
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	17.37
TOP	89.76
LOGEC50	-5.620
HILLSLOPE	-1.075
EC50	2.400e-006
Std. Error	
BOTTOM	5.169
TOP	2.807
LOGEC50	0.09618
HILLSLOPE	0.2503
95% Confidence Intervals	
BOTTOM	6.583 to 28.15
TOP	83.90 to 95.61
LOGEC50	-5.820 to -5.419
HILLSLOPE	-1.597 to -0.5525
EC50	1.512e-006 to 3.809e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9365
Absolute Sum of Squares	1150
Sy.x	7.582
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

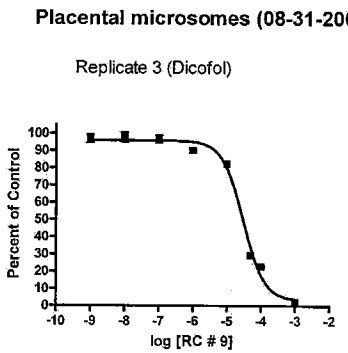
Figure 8 - Fitted Concentration Response for Chrysin



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.3831
TOP	98.01
LOGEC50	-4.759
HILLSLOPE	-0.8235
EC50	1.741e-005
Std. Error	
BOTTOM	2.418
TOP	0.8680
LOGEC50	0.04907
HILLSLOPE	0.06481
95% Confidence Intervals	
BOTTOM	-4.661 to 5.427
TOP	96.20 to 99.82
LOGEC50	-4.862 to -4.657
HILLSLOPE	-0.9587 to -0.6883
EC50	1.375e-005 to 2.204e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9948
Absolute Sum of Squares	165.8
Sy.x	2.879
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-2.536
TOP	98.27
LOGEC50	-4.606
HILLSLOPE	-0.7637
EC50	2.475e-005
Std. Error	
BOTTOM	3.025
TOP	1.129
LOGEC50	0.05031
HILLSLOPE	0.06113
95% Confidence Intervals	
BOTTOM	-8.845 to 3.773
TOP	95.91 to 100.6
LOGEC50	-4.711 to -4.501
HILLSLOPE	-0.8912 to -0.6362
EC50	1.944e-005 to 3.152e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9935
Absolute Sum of Squares	202.5
Sy.x	3.182
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

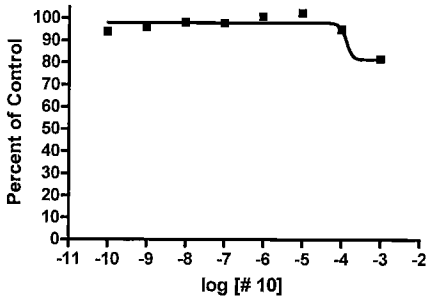


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	3.244
TOP	95.91
LOGEC50	-4.519
HILLSLOPE	-1.408
EC50	3.027e-005
Std. Error	
BOTTOM	2.584
TOP	1.263
LOGEC50	0.03954
HILLSLOPE	0.1327
95% Confidence Intervals	
BOTTOM	-2.147 to 8.635
TOP	93.27 to 98.54
LOGEC50	-4.601 to -4.436
HILLSLOPE	-1.685 to -1.131
EC50	2.504e-005 to 3.661e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9890
Absolute Sum of Squares	363.9
Sy.x	4.265
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Figure 9 - Fitted Concentration Response for Dicofol

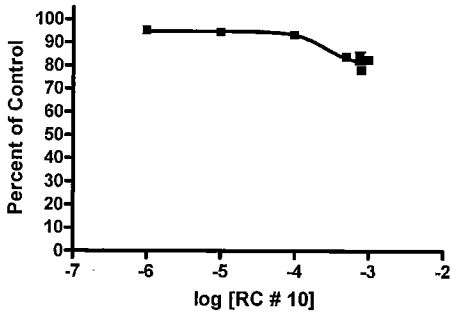
Placental microsomes (09-06-2005)

Replicate 1 (Atrazine)



Placental microsomes (09-07-2005)

Replicate 2 (Atrazine)



Placental microsomes (09-08-2005)

Replicate 3 (Atrazine)

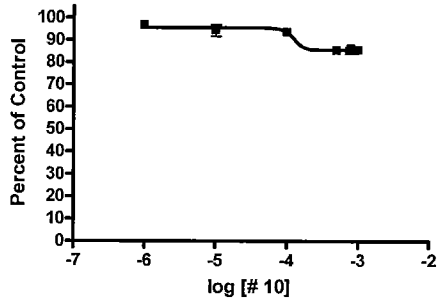


Figure 10 - Fitted Concentration Response for Atrazine

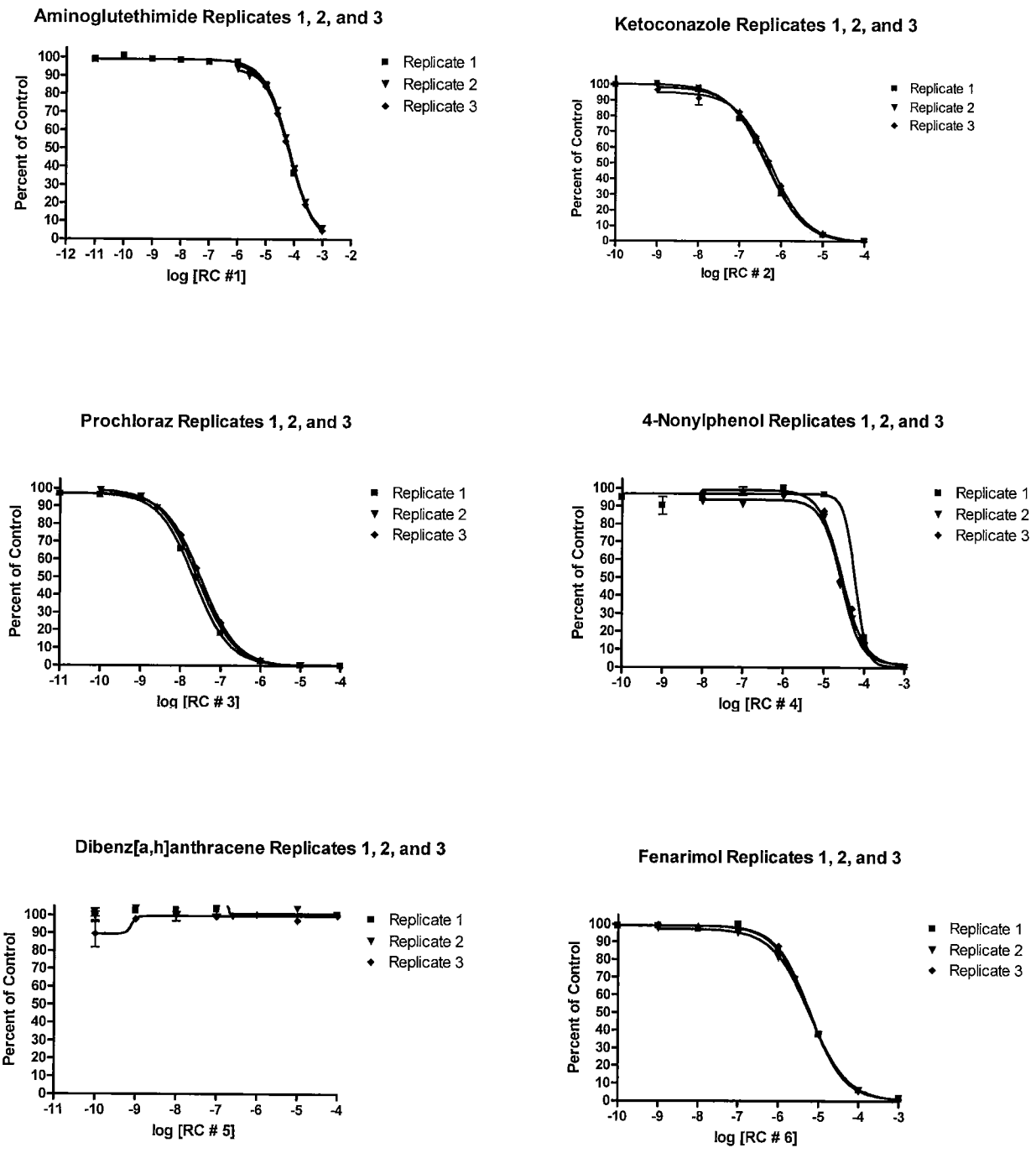


Figure 11 - Concentration Response Curve Fits – Overlay of Individual Replicate Results

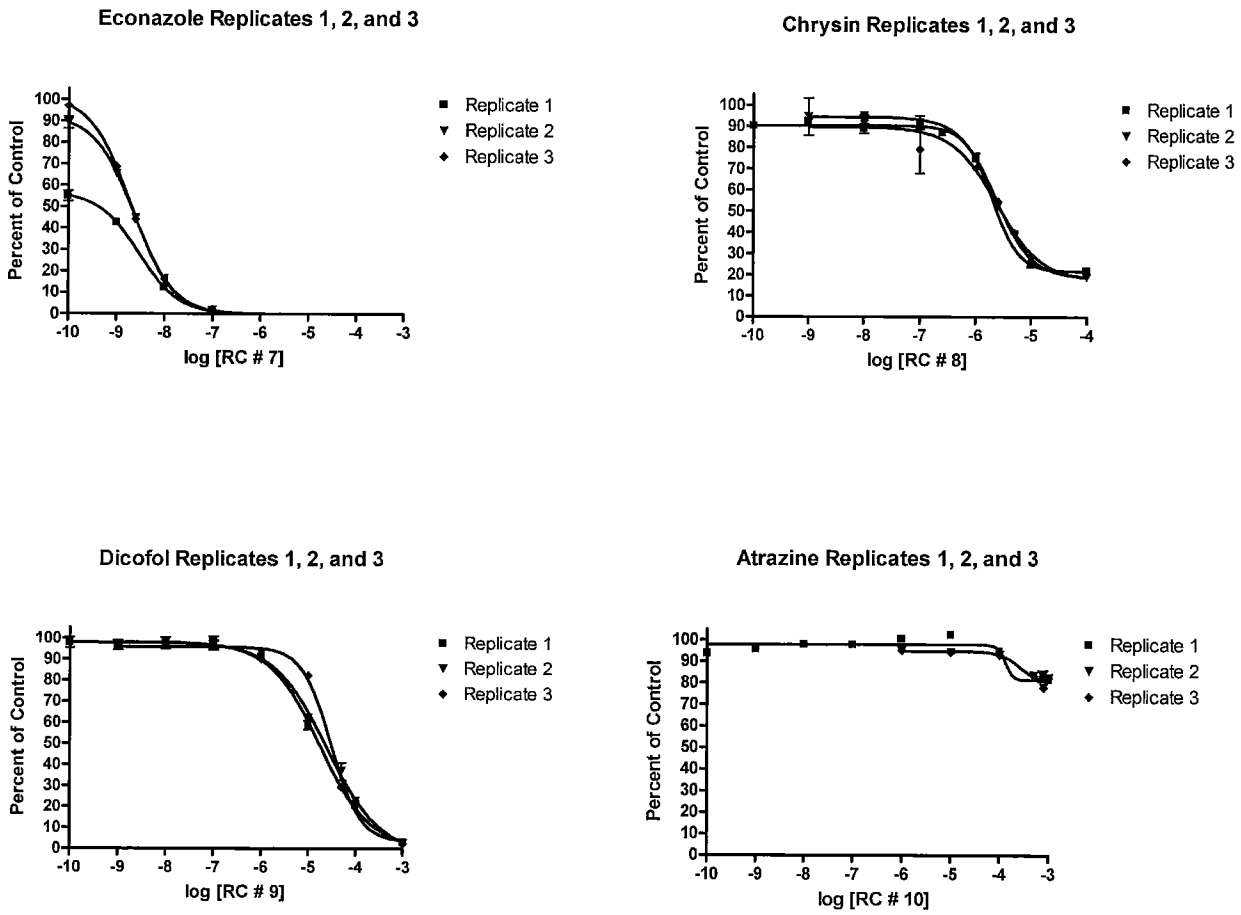
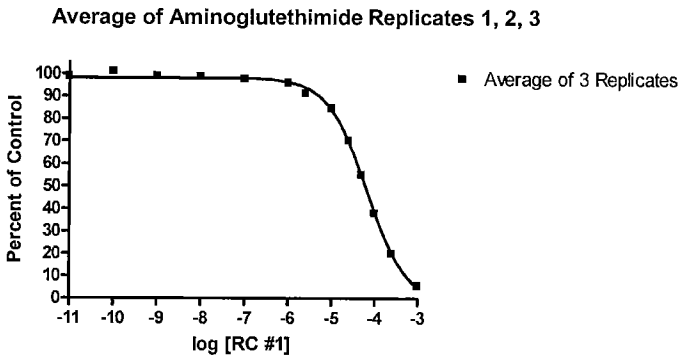
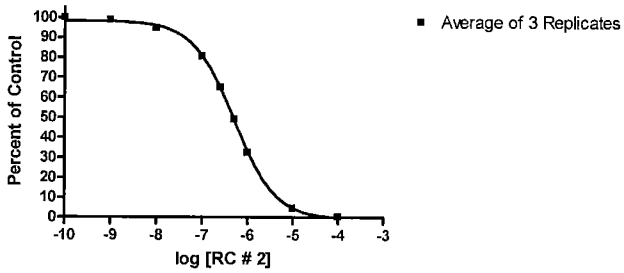
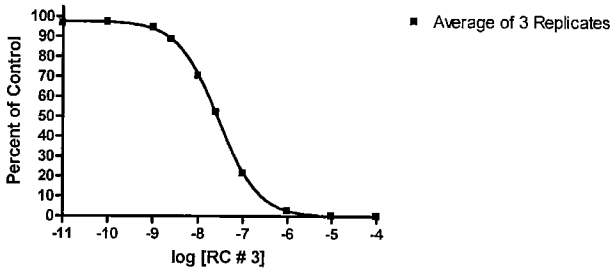


Figure 11 - Concentration Response Curve Fits – Overlay of Individual Replicate Results (Continued)







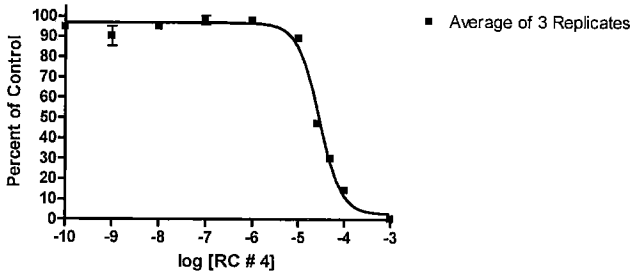
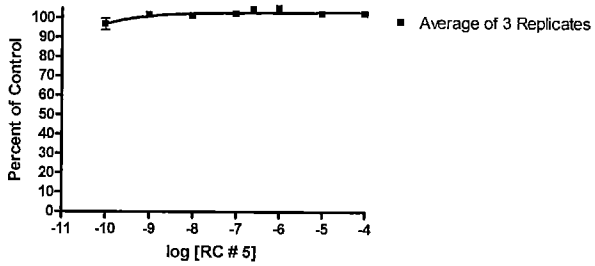
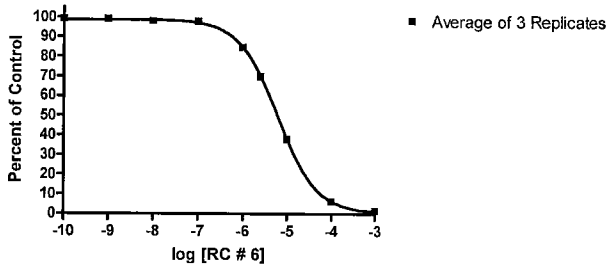


Figure 12 - Average Concentration Response Curve Fits

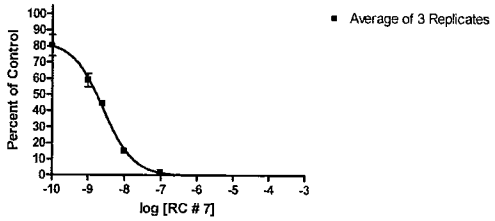
Average of Dibenz[a,h]anthracene Replicates 1, 2, and 3



Average of Fenarimol Replicates 1, 2, and 3



Average of Econazole Replicates 1, 2, and 3



Average of Chrysin Replicates 1, 2, and 3

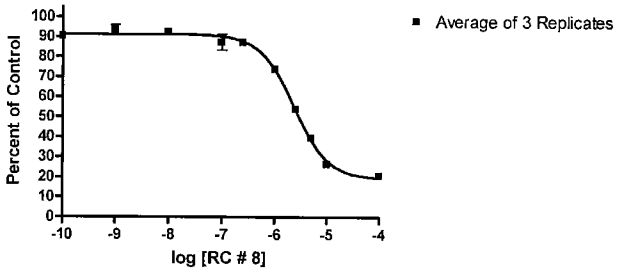


Figure 12 - Average Concentration Response Curve Fits (Continued)

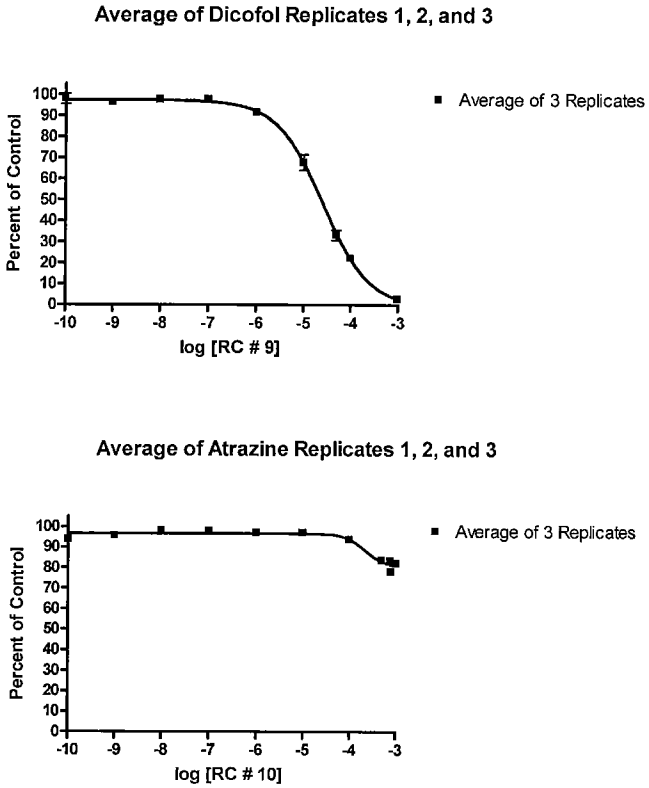


Figure 12 - Average Concentration Response Curve Fits (Continued)

Table 28 - Summary of $LogIC_{50}$ and Slope and their Associated Standard Errors (SE)

Reference Chemical	Chemical Code	Replicate	Log IC_{50}	SE $LogIC_{50}$	Slope	SE Slope	Status	IC_{50} (μM)
Aminoglutethimide	1	1	-3.975	0.6421	-0.8987	0.2421	Inhibitor	106.0
		2	-4.148	0.01712	-1.073	0.04396	Inhibitor	71.14
		3	-4.181	0.01319	-0.9702	0.02912	Inhibitor	65.92
Ketoconazole	2	1	-6.371	0.01735	-0.9384	0.03367	Inhibitor	0.4258
		2	-6.325	0.008478	-0.9801	0.02055	Inhibitor	0.4733
		3	-6.225	0.02796	-1.009	0.07104	Inhibitor	0.5961
Prochloraz	3	1	-7.662	0.01321	-0.9853	0.02416	Inhibitor	0.02176
		2	-7.580	0.01087	-0.9386	0.02175	Inhibitor	0.02629
		3	-7.487	0.01558	-0.9641	0.03239	Inhibitor	0.03260
4-Nonylphenol	4	1	-4.228	0.2662	-3.042	3.520	Inhibitor	59.11
		2	-4.571	0.02105	-1.766	0.1297	Inhibitor	26.84
		3	-4.553	0.02412	-1.558	0.1167	Inhibitor	27.98
Dibenz[a,h]anthracene	5	1-3	DNC	DNC	DNC	DNC	No inhibition	DNC
Fenarimol	6	1	-5.224	0.02338	-1.005	0.04852	Inhibitor	5.966
		2	-5.218	0.01935	-0.9361	0.03584	Inhibitor	6.056
		3	-5.214	0.01790	-1.039	0.04006	Inhibitor	6.113
Econazole	7	1	-8.531	0.03646	-1.025	0.06242	Inhibitor	0.002942
		2	-8.628	0.02032	-1.048	0.04851	Inhibitor	0.002352
		3	-8.698	0.01447	-1.038	0.03437	Inhibitor	0.002005
Chrysin	8	1	-5.706	0.05148	-1.785	0.2565	Inhibitor	1.967
		2	-5.647	0.05260	-1.325	0.1951	Inhibitor	2.253
		3	-5.620	0.09618	-1.075	0.2503	Inhibitor	2.400
Dicofol	9	1	-4.759	0.04907	-0.8235	0.06481	Inhibitor	17.41
		2	-4.606	0.05031	-0.7637	0.06113	Inhibitor	24.75
		3	-4.519	0.03954	-1.408	0.1327	Inhibitor	30.27
Atrazine	10	1-3	DNC	DNC	DNC	DNC	No inhibition	DNC

DNC- does not converge.

Table 29 - Summary Results

Reference Chemical	Chemical Code	Replicate	IC_{50}	Mean* IC_{50}	SD	SEM	%CV
Aminoglutethimide	1	1	106.0 µM	81.02 µM	21.79	12.58	26.89
		2	71.14 µM				
		3	65.92 µM				
Ketoconazole	2	1	425.8 nM	498.4 nM	87.88	50.74	17.63
		2	473.3 nM				
		3	596.1 nM				
Prochloraz	3	1	21.76 nM	26.88 nM	5.44	3.14	20.3
		2	26.29 nM				
		3	32.60 nM				
4-Nonylphenol	4	1	59.11 µM	37.98 µM	18.31	10.57	48.22
		2	26.84 µM				
		3	27.98 µM				
Fenarimol	6	1	5.966 µM	6.045 µM	0.074	0.043	1.23
		2	6.056 µM				
		3	6.113 µM				
Econazole	7	1	2.942 nM	2.433 nM	0.474	0.273	19.5
		2	2.352 nM				
		3	2.005 nM				
Chrysin	8	1	1.967 µM	2.207 µM	0.220	0.127	9.98
		2	2.253 µM				
		3	2.400 µM				
Dicofol	9	1	17.41 µM	24.14 µM	6.45	3.72	26.7
		2	24.75 µM				
		3	30.27 µM				

* Calculated (arithmetic) average value from three measurements.

5.0 DISCUSSION

The presented study (Task 7) involved testing the response of two independently prepared human placental microsomes to the presence of ten reference chemicals. Eight different concentrations of each reference chemicals generally ranging from 1 x 10^{-10} to 1 x 10^{-3} M were applied to create the dose response curves. Data from the first replicate of each reference chemical were reviewed and the target concentrations were adjusted to better define the concentration response curve. Three independent replicates for aromatase assays were performed with each reference chemical. The overall IC_{50} value for eight reference chemicals are presented in Table 29.

The microsomes used in presented study were prepared at Battelle and In Vitro Technologies laboratories.

For the presented study, the protein content of the microsomal preparation was measured each time the aromatase assay was run (total 15 runs for each microsomal preparation). The average determined protein concentration was 25.02 ± 1.64 mg/mL (6.55% CV) for the Battelle microsomal preparation and 9.222 ± 0.526 mg/mL (5.71% CV) for the In Vitro Technologies preparation. In order to better characterize the protein assay, QC samples were included in all runs (total of 30 independent measurements for each QC sample were collected). The overall mean value for the low QC protein sample was 0.011 ± 0.001 mg/mL (10.9% CV) and overall mean difference from known (0.012 mg/mL as provided by RTI) was < 11.4%. The overall mean value for the high QC protein sample was 0.107 ± 0.003 mg/mL (2.43% CV) and overall mean difference from known (0.110 mg/mL as provided by RTI) was < 2.9%.

Four types of controls were used for the aromatase assay: a full activity control which served as the 100% activity control, a background activity control which was used to correct for non-enzymatic product formation and other coincidental radiochemical content in the assay mixture, a positive control which used a known aromatase inhibitor and a negative control which used a known aromatase non-inhibitor.

All calculated aromatase activities were corrected for the radioactivity in the background activity control, so the average background control activity for a replicate was necessarily zero (0). The average full aromatase activity across all 15 runs of the aromatase assay was 0.0555 ± 0.0118 nmol/mg/min (21.3% CV) for Battelle-prepared microsomes and 0.0459 ± 0.0031 nmol/mg/min (6.83% CV) for In Vitro Technologies-prepared microsomes. The positive control activity across 15 runs averaged 0.0270 ± 0.0037 nmol/mg/min (13.6% CV) for Battelle-prepared microsomes, approximately 49% of full aromatase activity and the negative control activity averaged 0.0538 ± 0.0113 (21.0% CV), approximately 97% of full aromatase activity. For In Vitro Technologies-prepared microsomes the positive control activity across 15 runs averaged 0.0220 ± 0.0012 nmol/mg/min (5.52% CV), approximately 48% of full aromatase activity and the negative control activity averaged 0.0448 ± 0.0036 nmol/mg/min (7.99% CV), approximately 98% of full aromatase activity.

For almost all controls, a decrease in aromatase activity was noted between the controls run at the beginning of each replicate and those run at the end of the replicate. It may suggest an issue with decreasing aromatase activity over time.

Aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, dibenz[a,h]anthracene, fenarimol, econazole, chrysin, dicofol and atrazine were used as the reference chemicals during the presented study. Each of the ten reference chemicals was added to the aromatase assay at eight predefined concentrations in triplicate repetitions

(tubes) per replicate of the assay. Data from the first replicate of each reference chemical were reviewed and the target concentrations were adjusted to better define the concentration response curve. The concentrations were selected in the range to define the upper and lower plateaus with intermediate concentrations chosen to distribute as evenly as possible along the slope of the curve. The percent of control values generally ranged from < 10 to almost 100% for aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, fenarimol, econazole and dicofol. For chrysin, the percent of control values ranged from approximately 19 to 95%. For dibenz[a,h]anthracene and atrazine, the percent of control values ranged from about 80 to 100%. All reference chemicals, except dibenz[a,h]anthracene and atrazine, were found to be good aromatase activity inhibitors.

Concentration response curves were fitted to the percent of control values with Prism software using the following equation:

$$Y= B + (T-B)/[1+10^{(\mu-X)*H}]$$

Where: Y= percent of control activity in the inhibitor tube
X= logarithm (base 10) of the concentration
T= top of plateau
B= bottom of plateau
H= Hill slope
$\mu= \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

The response curve was fitted by non-weighted least square nonlinear regression analysis. The obtained curves fit the data well ($r^2 > 0.99$).

Replicate-to- replicate variation in IC_{50} was low (1.226% CV and 9.978% CV, respectively) only for fenarimol and chrysin. For the rest of the reference chemicals replicate-to replicate variation was much higher, from 17.63% CV for ketoconazole up to 48.22% CV for 4-nonylphenol, and < 26.89% CV for the remaining four inhibitors. Usually, the IC_{50} value obtained for the first replicate for each tested reference chemical was different from the other two replicates. The first replicate was assayed in a different range of inhibitor concentrations. The relatively high replicate-to-replicate variability (high % CV) in IC_{50} is the reflection of applying different inhibitor concentration ranges between the first and remaining two replicates. The IC_{50} values measured for the eight (out of ten) reference chemicals in this study compared well with those found in previous work assignments.

Eight out of ten studied reference chemicals appear to be good inhibitors of the aromatase activity while two chemicals, dibenz[a,h]anthracene and atrazine, had no inhibitor properties in the studied concentration ranges.

The full statistical analysis report is presented in Appendix D. There are some small differences in data obtained from Prism output and data presented in the Statistical Report obtained by applying the SAS statistical analysis system.

6.0 CONCLUSION

- Both microsomal preparations, prepared independently by Battelle and In Vitro Technologies from fresh human placenta, performed well in the aromatase assays.
- The responsiveness (in a concentration dependent manner) of the human placental microsome aromatase assay to aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, fenarimol, econazole, chrysin and dicofol was confirmed.
- It was determined that econazole was the most potent aromatase activity inhibitor (IC_{50} = 2.43 nM) from the group of eight studied inhibitor reference chemicals.
- Dibenz[a,h]anthracene and atrazine did not inhibit the aromatase activity in the studied range of concentrations.

APPENDIX A

Protocol (with Amendments and Deviations) A-1

BATTELLE STUDY PROTOCOL

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES

Testing Facility:

Battelle Memorial Institute
505 King Avenue
Columbus, Ohio 43201-2693

Sponsor:

Endocrine Disruptor Screening Program Office
Battelle Memorial Institute
505 King Avenue
Columbus, Ohio 43201-2693

EPA Contract: 68-W-01-023
EPA Work Assignment: WA 4-16, Task 7

Battelle
The Business *of* Innovation

Experiment Start Date: July 11, 2005
Experiment End Date: September 30, 2005

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AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES

APPROVED, BATTELLE:

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07-11-05
Date

Steven W. Graves
CTC Manager, Battelle Memorial Institute

7/11/05
Date

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Quality Assurance Auditor, Battelle Memorial Institute

7-11-05
Date

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Work Assignment Leader
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Battelle Memorial Institute

7-11-05
Date

REVIEWED BY, SPONSOR:

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EDSP Quality Assurance Manager
Battelle Memorial Institute

7-11-05
Date

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AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES

1.0 OBJECTIVES

The objectives of this task are to test 10 reference chemicals using microsomes prepared by two participating laboratories (Battelle and In Vitro Technologies) in Task 6 and determine the intra- and interlaboartory variability.

The microsomes prepared in Task 6 by Battelle and In Vitro Technologies will be distributed to each laboratory and tested as outlined in Section 2.2.

1.1 Justification for Test System

The test system for this study is human placental microsomes. This test system was selected because it provides a biological source of the aromatase enzyme and since the assay is being evaluated for its potential to serve as a screening assay, the use of human tissue enhances its predictive potential.

1.2 Test Method

This *in vitro* test method involves combining microsomes, substrate, appropriate co-factors and reference chemicals in a common reaction vessel. The effect of the reference chemicals on microsomal enzyme activity will be evaluated by measuring the amount of the product formation.

There is no applicable route of administration in the sense of a dose administration route for this *in vitro* test.

2.0 MATERIALS RECEIPT AND/OR PREPARATION

A sufficient supply of chemical reagents, radiolabeled and non-radiolabeled androstenedione and human placental microsomes will be obtained prior to initiation of the first set of experiments to ensure that sufficient quantities are available to conduct the studies.

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2.1 Substrate

2.1.1 Substrate Name/Supplier

The substrate for the aromatase assay is androstenedione (ASDN). Non-radiolabeled and radiolabeled ASDN will be used. The non-radiolabeled ASDN and the radiolabeled androstenedione ([1β-^{3}H]-androstenedione, [^{3}H]ASDN) will be provided to the laboratories by Battelle's Chemical Repository (CR). The CR will forward all applicable information regarding supplier, lot numbers and reported/measured purity for the substrate to the laboratories and this information will be included in study reports. The radiochemical purity of the [^{3}H]ASDN (of each lot that is used) was assessed by the lead laboratory (RTI) in a previous Task and was found to be 97%.

2.1.2 Preparation of Substrate Solution for use in Aromatase Assay

A solution containing a mixture of non-radiolabeled and radiolabeled [^{3}H] ASDN will be prepared to achieve 100 nM final concentration of ASDN in the assay and the amount of tritium added to each incubation about 0.1 μCi. This substrate solution should have a concentration of 2 μM with a radiochemical content of about 1 μCi/mL.

The following illustrates the preparation of a substrate solution using a stock of [^{3}H]ASDN with a specific activity of 25.3 Ci/mmol and a concentration of 1 mCi/mL. Prepare a 1:100 dilution of the radiolabeled stock in buffer. Prepare a 1 mg/mL solution of ASDN in ethanol and then prepare dilutions in buffer to a final concentration of 1 μg/mL. Combine 4.5 mL of the 1 μg/mL solution of ASDN, 800 μL of the [^{3}H]ASDN dilution and 2.7 mL buffer to make 8 mL of substrate solution (enough for 80 tubes). Record the weight of each component added to the substrate solution. After mixing the solution well, weigh aliquots (ca. 20 μL) and combine with scintillation cocktail for radiochemical content analysis. The addition of 100 μL of the substrate solution to each 2 mL assay volume yields a final [^{3}H]ASDN concentration of 100 nM with 0.1 μCi/tube.

2.2 Reference Chemicals

The reference chemicals for Task 7, their properties and rationale for selection are listed in Table 1. Each chemical will initially be tested over the concentration range 10^{-3} to 10^{-10} M (final concentration) but range may be adjusted as described in Section 5.

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Table 1. Reference Chemicals for Aromatase Assay Validation

Reference Chemical	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Basis for Selection
Aminoglutethimide	125-84-8	$C_{13}H_{16}N_2O_2$	232.2	Non-steroidal aromatase inhibitor
Chrysin	480-40-0	$C_{15}H_{10}O_4$	254.2	Potent flavonoid
Dicofol	115-32-2	$C_{14}H_9Cl_5O$	370.47	Organochlorine (insecticide)
Econazole (nitrate)	24169-02-6	$C_{18}H_{15}Cl_3N_2O \cdot HNO_3$	444.7	Potent imidazole fungicide
Ketoconazole	65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	531.43	Weak imidazole fungicide
Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215.69	Affects aromatase gene expression; no aromatase inhibition
Fenarimol	60168-88-9	$C_{17}H_{12}Cl_2N_2O$	331.2	Pyrimidine fungicide
4-nonylphenol	104-40-5	$C_{15}H_{24}O$	220.4	Affects AR/ER; no aromatase inhibition
Prochloraz	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	Conazole fungicide
Dibenz[a,h]anthracene	53-70-3	$C_{22}H_{14}$	278.35	Known non-aromatase inhibitor; Ah receptor agonist

2.2.1 Reference Chemical Formulation and Analysis

Reference chemical stock solutions will be prepared and analyzed by the CR and distributed to the laboratories. Reference chemicals will be formulated in buffer, absolute ethanol or dimethylsulfoxide (DMSO). The total volume of reference chemical formulation used in each assay should be no more than 1% of the total assay volume (i.e., 20 µL in a 2 mL assay) in order to minimize the potential of the solvent to inhibit the enzyme. Fresh dilutions of the stock solution will be prepared in the same solvent as the stock solution on the day of use such that the target concentration of inhibitor can be achieved by the addition of 20 µL of dilution to a 2 mL assay volume. Information on storage conditions for reference chemical stock solutions will be provided by the CR.

The reference chemicals will be numbered 1-10 by the CR and these same numeric designations will be used when the samples are coded prior to distribution to the assaying laboratories. This will ensure that, for example, Chemical 1 is always the same chemical in each laboratory. This is important for the proper balancing of the study as outlined in Table 4.

2.3 Control Substances

The known aromatase inhibitor, 4-hydroxyandrostenedione (4-OH ASDN), will be used as the positive control substance. A known aromatase non-inhibitor, lindane, will be used as the negative control substance. Table 2 contains identity and property information for these substances.

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Table 2. Control Substances

Test Substance	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Target Concentration in Assay (M)	Basis for Selection
4-OH ASDN	566-48-3	$C_{19}H_{26}O_3$	302.4	5×10^{-8}	Known aromatase inhibitor
Lindane	58-89-9	$C_6H_6Cl_6$	290.8	1×10^{-6}	Affects StAR and cholesterol metabolism; no aromatase activity

2.3.1 Control Substance Formulation and Analysis

Control substances stock solutions will be prepared and analyzed by the CR and distributed to the laboratories. Control substances will be formulated in buffer, ethanol or DMSO. The total volume of control substance formulation used in each assay should be no more than 1 % of the total assay volume (i.e., 20 µL in a 2 mL assay) in order to minimize the potential of the solvent to inhibit the enzyme. Fresh dilutions of the stock solution will be prepared in the same solvent as the stock solution on the day of use. Dilutions will be prepared such that the target concentration of control substance (Table 2) can be achieved by the addition of 20 µL of the dilution to a 2 mL assay volume. Information on storage conditions for control substance stock solution will be provided by the CR.

2.4 Human Placental Microsomes

Human placental microsomes (prepared in Task 6) will be supplied to each laboratory by Battelle and In Vitro Technologies. These samples should be treated as potentially infectious and appropriate precautions must be employed. The microsomes must be stored at approximately -70°C. The approximate protein content of the microsomes will be provided by the supplier.

Caution: Microsomes can be denaturated by detergents. Therefore, it is important to ensure that all glassware, etc. that is used in the preparation or usage of microsomes is free of detergent residue. New disposable test tubes, bottles, vials, pipets and pipet tips may be used directly in the assay. Durable labware that may have been exposed to detergents should be rinsed with water and/or buffer prior to use in the assay.

If the human placental microsomes are supplied in aliquots in excess of what is required to conduct a single experiment, they will be thawed, pooled, homogenized, divided into appropriate aliquots for conduct of a single experiment and refrozen as described below in order to minimize and standardize the number of freeze/thaw cycles each preparations undergoes. Microsomes will be thawed quickly in a 37 ± 1°C water bath and then will be immediately transferred to an ice bath. The microsomes will be pooled and rehomogenized using a Potter-Elvejhem homogenizer (about 5-10 passes). The pooled sample will be aliquoted into portions appropriate for use in single experiments (ca. 160 µL, dependant on the protein concentration of the preparation) and the samples will be flash frozen and stored

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at approximately -70°C for future use. Each tube will provide enough protein for a single experiment and any excess of thawed microsomal preparation will be discarded.

On the day of use, microsomes will be thawed quickly in a 37 ± 1°C water bath and then will be immediately transferred to an ice bath. The microsomes will be rehomogenized using a Potter-Elvejhem homogenizer (about 5-10 passes) or by vortexing about 5 seconds prior to use. The microsomes will be diluted in buffer (serial dilutions may be necessary) to an approximate protein concentration of 0.025 mg/mL. The addition of 1 mL of that microsomes dilution will result in a final approximate protein concentration of 0.0125 mg/mL in the assay tube. All microsomes samples must be kept on ice until they are placed in the water bath just prior to their addition to the aromatase assay. Microsomes are not to be left on ice for longer than approximately 1 h before proceeding with the assay. Appropriate documentation on time from thaw to use must be maintained.

Diluted microsomes must be used only on the day of preparation. Under no conditions should diluted microsomes be refrozen for later use in the assay.

2.5 Other Assay Components

2.5.1 Buffer

The assay buffer will be 0.1 M sodium phosphate buffer, pH 7.4. Sodium phosphate monobasic (MW: 137.99 g/mol) and sodium phosphate dibasic (MW: 141.96 g/mol) will be used in the preparation of the buffer. Solutions of each reagent at 0.1 M will be prepared in Milli-Q water and then the solutions will be combined to a final pH of 7.4. The assay buffer may be stored for up to one month in the refrigerator (ca. 2-8°C).

2.5.2 Propylene Glycol

Propylene glycol (MW: 76.1 g/mol) will be added to the assay directly as described below.

2.5.3 NADPH

NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form;MW: 833.4 g/mol)), is the required co-factor for CYP19. The final concentration in the assay will be 0.3 mM. Typically, a 6 mM stock solution will be prepared in assay buffer and then 100 µL of the stock will be added to the 2 mL assay volume. NADPH solution must be prepared fresh each day and kept on ice until use.

3.0 PROTEIN ASSAY

The protein concentration in the microsomes will be determined each day of microsome use in the aromatase assay by using a DC Protein Assay kit purchased from BioRad (Hercules, CA). QC standards (nominal protein concentrations of 10 and 100 µg/mL) will be prepared by the lead

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laboratory and distributed to each participating laboratory. Each of these QC standards will be run in duplicate with each run of the protein assay.

A 6-point standard curve will be prepared; target range will be from 5 to 250 µg protein/mL. The protein standards will be made from bovine serum albumin (BSA). To a 200 µL aliquot of unknown, QC or curve standard, 100 µL of BioRad DC Protein Kit Reagent A will be added and mixed. Next, 800 µL of BioRad DC Protein Kit Reagent B will be added to each sample and the samples will be vortex mixed. The samples will be allowed to sit at room temperature for at least 15 min to allow for color development. (The absorbances are stable for about 1 hour.) Each sample (unknown and standards) will be transferred to disposable polystyrene cuvettes and the absorbance (750 nm) will be measured using a spectrophotometer. The protein concentration of the microsomal sample will be determined by interpolation of the absorbance value using the curve developed from the protein standards.

4.0 AROMATASE ASSAY METHOD

The assays will be performed in 13x100 mm test tubes maintained at 37 ± 1°C in a shaking water bath. Each test tube will be uniquely identified by applying a label or writing directly on the test tube. Propylene glycol (100 µL), [^{3}H]ASDN, NADPH, and buffer (0.1 M sodium phosphate, pH 7.4) will be combined in the test tubes (total volume 1.0 mL). The final concentrations for the assay components are presented in Table 3. The tubes and the microsomal suspension will be placed at 37 ± 1°C in the water bath for approximately five minutes prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume will be 2.0 mL, and the tubes will be incubated for 15 min. The incubations will be stopped by the addition of methylene chloride (2.0 mL); the tubes will be vortex-mixed for ca. 5 s and placed on ice. The tubes will be then vortex-mixed an additional 20-25 s. The tubes will then be centrifuged using a Beckman GS-6 centrifuge with GH-3.8 rotor for 10 minutes at a setting of 1000 rpm. The methylene chloride layer will be removed and discarded; the aqueous layers are extracted again with methylene chloride (2.0 mL). This extraction procedure will be performed one additional time, each time discarding the methylene chloride layer. The aqueous layers will be transferred to vials and duplicate aliquots (0.5 mL) will be transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Packard, 10 mL) will be added to each counting vial and shaken to mix the solution.

Table 3. Optimized Aromatase Assay Conditions

Assay Factor (units)	Human Placental
Microsomal Protein (mg/mL)[a]	0.0125
NADPH (mM)[a]	0.3
[^{3}H]ASDN (nM)[a]	100
Incubation Time (min)	15

[a] Final concentrations

Analysis of the samples will be performed using liquid scintillation spectrometry (LSS). Radioactivity found in the aqueous fractions represents amount of formed ^3H_2O.

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5.0 DETERMINATION OF THE RESPONSE OF AROMATASE ACTIVITY TO REFERENCE CHEMICALS

This task is similar in structure to Task 5 except the test laboratories will use the microsomes prepared in laboratory 2 or in laboratory 4, according to the matrix shown below (Table 4). The inhibitor compounds will be tested, including the four inhibitor compounds in Task 5. The test is organized so that across the ten chemicals the allocation of microsomes sources to reference chemicals is approximately balanced.

For each chemical (1 to 10) laboratory 2 (Battelle) will supply two of laboratories RTI, Battelle and In Vitro Technologies (sometimes itself and sometimes not) and laboratory 4 (In Vitro Technologies) will supply the other two. The distribution of microsomal preparation will differ for different chemicals. Let M_2 denote the microsomal preparation from laboratory 2 and let M_4 denote the microsomal preparation from laboratory 4. The four laboratories will use microsomal preparations from the laboratories as shown in Table 4.

Table 4. Microsomes Distribution and Testing Matrix

Chemical	Laboratory 1 RTI	Laboratory 2 Battelle	Laboratory 3 WIL	Laboratory 4 In Vitro
Chemical 1	M_2	M_2	M_4	M_4
Chemical 2	M_2	M_2	M_4	M_4
Chemical 3	M_4	M_4	M_2	M_2
Chemical 4	M_2	M_4	M_2	M_4
Chemical 5	M_4	M_2	M_4	M_2
Chemical 6	M_4	M_4	M_2	M_2
Chemical 7	M_2	M_4	M_4	M_2
Chemical 8	M_2	M_4	M_4	M_2
Chemical 9	M_4	M_2	M_2	M_4
Chemical 10	M_4	M_2	M_2	M_4

Each test laboratory will analyze five chemicals with microsomal preparations made in laboratory 2 and five chemicals with microsomal preparation made in laboratory 4. Across the 10 chemicals the laboratories are approximately balanced for comparison of M_2 and M_4. Thus if there is no interaction between laboratory or microsomes source and chemical, the laboratory effects are excluded from the comparison between microsomal preparations.

Each replicate will test the response of aromatase activity to the presence of eight concentrations of a reference chemical. The reference chemicals must be coded prior to distribution to the assaying technicians in order that the replicates are conducted blind for reference chemical identity. This task will be conducted in three independent replicates. All three replicates for a given reference chemical must be conducted by the same technician. However, the same technician is not required to perform the three replicates for all ten reference chemicals. Each replicate for a given reference chemical must be conducted entirely independently of the other replicates for that reference chemical. Thus, it is recommended that if multiple replicates are conducted on a given day by a single technician, those replicates should use different reference chemicals. Each reference chemical will be tested at eight concentrations and there will be three (triplicate) repetitions for

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each concentration of a given replicate. A single replicate study of a given reference chemical is described in Table 5.

Four types of control samples will be included for each replicate. These include:

- Full enzyme (aromatase) activity controls (substrate, NADPH, propylene glycol, buffer, vehicle [used for preparation of reference chemical solutions] and microsomes)
- Background activity controls (all components that are in the full aromatase activity controls, except NADPH)
- Positive controls (all components that are in the full aromatase activity controls, except vehicle, and with the addition of 4-OH ASDN at 5×10^{-8} M)
- Negative controls (all components that are in the full aromatase activity controls, except vehicle, and with the addition of lindane at 1×10^{-6} M).

Four test tubes of each type of control are included with each replicate and are treated the same as the other samples. The controls sets will be split so that two tubes (of each control type) are run at the beginning and two at the end of each replicate set.

The assay will be conducted as described in Section 4.0 with the following modification. Reference chemical solution (or vehicle) will be added to the mixture of propylene glycol, substrate, NADPH and buffer in a volume not to exceed 20 µL prior to preincubation of that mixture. The volume of buffer used will be adjusted so the total incubation volume remains at 2 mL.

After completion of the first replicate, the data will be reviewed and, if necessary, the concentration of reference chemical used in the second and third replicates can be adjusted. The decision whether to adjust test concentrations rests with the Study Director. The decision should be based on the results from the first replicate with the following guidelines in mind:

- If insolubility is observed at the high concentration (10^{-3} M), then set the highest concentration for the second and third replicates at the highest concentration that appeared to be soluble (limited to 10^{-4} or 10^{-5} M). Do not use a concentration lower than 10^{-5} M for the highest concentration tested.
- If the highest concentration to be tested is lowered to 10^{-4} or 10^{-5} M, then add mid-log concentration(s) near the estimated IC_{50} based on the replicate one results in order to keep eight concentrations in the test set.
- The lowest concentration to be tested is 10^{-10} M.

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Table 5. Reference Chemical Study Design

Sample type	Repetitions (test tubes)	Description	Reference Chemical Concentration (M final)
Full Enzyme Activity Control	4	Complete assay[a] with reference chemical vehicle control	N/A
Background Activity Control	4	Complete assay with reference chemical vehicle control omitting NADPH	N/A
Positive Control	4	Complete assay with positive control chemical (4-OH ASDN) added	5×10^{-8}
Negative Control	4	Complete assay with negative control chemical (lindane) added	1×10^{-6}
Reference Chemical Concentration 1	3	Complete assay with Reference Chemical added	1×10^{-3}
Reference Chemical Concentration 2	3	Complete assay with Reference Chemical added	1×10^{-4}
Reference Chemical Concentration 3	3	Complete assay with Reference Chemical added	1×10^{-5}
Reference Chemical Concentration 4	3	Complete assay with Reference Chemical added	1×10^{-6}
Reference Chemical Concentration 5	3	Complete assay with Reference Chemical added	1×10^{-7}
Reference Chemical Concentration 6	3	Complete assay with Reference Chemical added	1×10^{-8}
Reference Chemical Concentration 7	3	Complete assay with Reference Chemical added	1×10^{-9}
Reference Chemical Concentration 8	3	Complete assay with Reference Chemical added	1×10^{-10}

[a] The complete assay contains buffer, propylene glycol, microsomal protein, [^{3}H]ASDN and NADPH

6.0 DATA ANALYSIS

6.1 Aromatase Activity and Percent of Control Calculations

Relevant data are entered into the latest version of the spreadsheet Aromatase_Master_Versionx.y.xls (where x and y denote version number designation) for calculation of aromatase activity and percent of control. The version of the spreadsheet used will be included in the reports. A working document detailing the use of this spreadsheet has been distributed previously.

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6.2 Statistical Analyses

6.2.1 Concentration Response Fits for the Reference Chemicals

For the reference chemicals, three independent replicates of the concentration response curve fit will be carried out.

For each replicate two repeat tubes of the full enzyme activity controls, the background activity controls and the positive and negative controls will be run prior to the to repetitions of the graded concentrations of the reference chemical and two repeat tubes of each control will be run following the repetition of the reference chemical. Three repetitions will be prepared for each concentration of the reference chemical.

For each repeat tube (full enzyme activity, background activity, positive, and negative controls and each reference chemical concentration) the Excel database spreadsheet will include total observed (uncorrected) disintegrations per minute (dpms) per tube and total aromatase activity per tube. The dpm and aromatase activity values are corrected for the background dpms, as measured by the average of the background activity control tubes. The aromatase activity is calculated as the corrected dpm, normalized by the specific activity of the [^{3}H]ASDN, the mg of protein of the aromatase, and the incubation time. The average (corrected) dpms and aromatase activity across the four background activity control repeat tubes must necessarily be equal to 0 within each replicate.

For each tube percent of control is determined by dividing the background corrected aromatase activity for that tube by the average background corrected aromatase activity for the four full enzyme activity control tubes and multiplying by 100.

Concentration response trend curves will be fitted to the percent of control activity values within each of the repeat tubes at each reference chemical concentration. Concentration is expressed on the log scale. In agreement with past convention, logarithms will be common logarithms (i.e. base 10). Let X denote the logarithm of the concentration of reference chemical (e.g. if concentration = 10^{-5} then X = -5).

Let

Y= percent of control activity in the inhibitor tube
X = logarithm (base 10) of the concentration
DAVG = average dpms across the repeat tubes with the same reference chemical concentration
T = top of plateau
B = bottom of plateau
β = slope of the concentration response curve (β will be negative)
μ = $\log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

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The following concentration response curve will be fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$Y = B + (T-B)/[1 + \{(T-B)/(50-B)-1\}\ 10^{(\mu-X)\beta}] + \varepsilon$$

where ε is the variation among repetitions, distributed with mean 0 and variance proportional to DAVG (based on Poisson distribution theory for radiation counts). The variance is approximately proportional to Y.

The response curve will be fitted by weighted least squares nonlinear regression analysis with weights equal to 1/Y. Model fits will be carried out using Prism software (Version 3 or higher).

Concentration response models will be fitted for each replicate test within each reference chemical. Based on the results of the fit within each replicate the extent of aromatase inhibition will be summarized as top (T), bottom (B), $\log_{10}IC_{50}$ (μ) and slope (β). The estimated T, B, $\log_{10}IC_{50}$ and β for a reference chemical will be a (weighted) means across the replicates. The estimated overall standard error will be based on the standard errors within each replicate and the replicate-to-replicate variability. The average value and standard errors of T, B, $\log_{10}IC_{50}$, or β and the replicate-to-replicate component of variation will be calculated based on one-way random effects analysis of variance model fit. For each reference chemical and replicate the estimated top (T), the within replicate standard error of T, bottom (B), the within replicate standard error of B, $\log_{10}IC_{50}$ (μ), the within replicate standard error of μ, the IC_{50}, the slope (β), the within replicate standard error of β, and the "Status" of each response curve will be displayed in a table. The "Status" of each response curve is indicated as:

- Complete curve – "inhibitor" – data are available up to at least 80% inhibition - Calculate IC_{50}.
- Incomplete curve – "presumed inhibitor" - Data are available up to at least 50% inhibition but not beyond 80% inhibition - Calculate IC_{50}.
- Incomplete curve - "equivocal" - Data are available to between 20% and 50% inhibition - Do not calculate IC_{50}.
- "No inhibition" - No data are available above 20% inhibition - Do not calculate IC_{50}.

6.2.2 Graphical and Analysis of Variance Comparisons Among Concentration Response Curve Fits

For each replicate the individual percent of control values will be plotted versus logarithm of the reference chemical concentration. The fitted concentration response curve will be superimposed on the plot. Individual plots will be prepared for each replicate.

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Additional plots will be prepared to compare the percent of control activity values across replicates. For each replicate the average percent of control values will be plotted versus logarithm of reference chemical concentration on the same plot. Plotting symbols will distinguish among replicates. The fitted concentration response curves for each replicate will be superimposed on the plots. On a separate plot the average percent of control values for each replicate will be plotted versus logarithm of reference chemical concentration. The average concentration response curve across replicates will be superimposed on the same plot. The average concentration response curve will be the unweighted average of the response curves within each replicate.

Top (T), bottom (B), slope (β) and $\log_{10}IC_{50}$ (μ) will be compared across replicates based on one-way random effects analysis of variance, treating the replicates as random effects. For each of T, B, β and μ, plots will be prepared that display the parameters within each replicate with associated 95% confidence intervals based on the within replicate standard error and the average across replicates with associated 95% confidence interval incorporating replicate-to replicate variation.

6.2.3 Graphical and Analysis of Variance Comparisons of Full Enzyme Activity, Background Activity, Positive and Negative Control Percent of Control Across Reference Chemicals and Replicates

Within each replicate of each reference chemical quadruplicate repetitions will be made of the full enzyme activity control, background activity control, and negative and positive control tubes. Half the repetitions will be carried out at the beginning of the replicate and half at the end. If the conditions are consistent throughout the replicate test, the control tubes at the beginning should be equivalent to those at the end.

To assess whether this is the case the control responses will be adjusted for background dpms, divided by the average of the (background adjusted) full enzyme activity control values, and expressed as percent of control. The average of the four background activity controls within a replicate must necessarily be 0 percent and the average of the four full enzyme activity controls within a replicate must necessarily be 100 percent. The full enzyme activity controls percent of control, the background activity controls percent of control, and the negative and positive controls percent of control values will be plotted across reference chemical and replicate within reference chemical, with plotting symbol distinguishing between beginning and end, and with reference line 0% (background activity control) or 100% (full enzyme activity control) respectively. These plots will display the extent of consistency across reference chemicals and replicates with respect to average value and variability and will provide comparisons of beginning versus end of each replicate. Additional plots will be prepared displaying the difference of the average of the first two percent of control values (i.e., those based on the "beginning" tubes) and the average of the last two percent of control values (i.e., those based on the "end" tubes) (end minus beginning) across reference chemicals and replicates within reference chemicals. Each plot will have a reference line of 0.

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Three-factor mixed effects analysis of variance models will be fitted, separately for the full enzyme activity control, the background activity control, and the positive and negative control tubes. The fixed effect factors in the analysis of variance will be

- Reference chemical
- Portion (beginning or end)
- Portion by reference chemical interaction.

The random effects will be

- Replicate nested within reference chemical
- Portion by replicate within reference chemical interaction.

The residual error variation corresponds to repetition within reference chemical, replicate and portion. The response will be percent of control. Since for the background activity and full enzyme activity controls the average of the repetitions within a reference chemical and replicate are constrained to be 0 and 100 respectively, by the way in which "percent of control" is defined, the variation associated with the reference chemical effect and the replication within reference chemical effect are both necessarily constrained to be 0.

If the daily replicates are in control the portion main effect and the portion by replicate within reference chemical interaction should be nonsignificant. If the portion by replicate within reference chemical interaction is significant, the nature of the effect will be assessed by comparing the portion effect within each replicate within reference chemical to the portion effect averaged across replicates within reference chemical. Simultaneity of inference will adjusted be for by Bonferroni's method.

6.2.4 Statistical Software

Concentration response curves will be fitted to the data using the non-linear regression analysis features in the PRISM statistical analysis package, Version 3 or higher. Supplemental statistical analyses and displays such as summary tables, graphical displays, analysis of variance, and multiple comparisons will be carried out using PRISM, the SAS statistical analysis system, Version 8 or higher, or other general purpose statistical packages (e.g. SPSS), as convenient.

6.2.5 Interlaboratory Statistical Analysis

The lead laboratory and each of the participating laboratories will carry out "intra-laboratory" statistical analyses based on their test data, according to this common statistical analysis plan, developed by the Data Coordination Center (Battelle). The Data Coordination Center will carry out the "inter-laboratory" statistical analysis. It will combine summary values developed in each of the intra-laboratory analyses to assess relationships among the laboratory results, the extent of laboratory-to-laboratory variation, and overall consensus estimates among the laboratories.

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7.0 RETENTION OF RECORDS

All records that remain the responsibility of the testing laboratory will be retained in the archives for the life of the contract.

8.0 QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES

Quality control (QC) and quality assurance (QA) procedures will follow those outlined in the Quality Assurance Project Plan (QAPP) that was prepared for this study. The study will be conducted in compliance with the Federal Register, 40 CFR Part 160. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practices Standards.

9.0 REPORTS

Interim data summaries, draft and final reports will be submitted as described in Section 9.5 of the QAPP.

The data to be reported in the interim data summaries will include (but is not limited to) the following information: assay date and run number, technician code, chemical code and log chemical concentration, background corrected aromatase activity (for each control and reference chemical repetition), percent of control activity, IC_{50}, slope and graphs of activity versus log chemical concentration.

In addition, draft and final reports will contain tables and graphs, as appropriate, containing the results of the intra- and inter-laboratory statistical analyses described in Section 6 of this document.

10.0 STUDY RECORDS TO BE MAINTAINED

- All records that document the conduct of the laboratory experiments and results obtained, as well as the equipment and chemicals used
- Protocol and any Amendments
- List of any Protocol Deviations
- List of Standard Operating Procedures
- QAPP and any Amendments
- List of any QAPP Deviations

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PROTOCOL AMENDMENT 1

STUDY NUMBER: **G608316**

STUDY TITLE: **Aromatase Assay Validation: Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories (WA 4-16, Task 7)**

PART TO BE CHANGED: The following paragraph is being added in to Section 2.2.1 entitled Reference Chemical Formulation and Analysis, page 5 after first sentence in the first paragraph.

Analysis of the reference chemical stock solutions will occur before the laboratories use the formulations in the assay. The analytical method used to analyze each of the reference chemicals in the stock solutions will be gas chromatography (4-OH ASDN and aminoglutethimide), gas chromatography with flame ionization detection (lindane, fenarimol, dicofol, atrazine, and dibenz[a,h]anthracene), HPLC (ketoconazole, econazole, and chrysin), HPLC with UV-Vis detection (prochloraz), and a combination of mass spectrometry and gas chromatography with flame ionization detection (4-nonylphenol). The chemistry procedures and results will be given to the laboratories in reports prepared and submitted to the laboratories by the CR.

REASON FOR CHANGE: The above paragraph is being added as per the Sponsor request.

PART TO BE CHANGED: The following sentence in the Section 6.2.1 entitled Concentration Response Fits for the Reference Chemicals, page 13, fourth paragraph from the top of the page is being changed.

The response curve will be fitted by weighted least squares nonlinear regression analysis, with weights equal to 1/Y.

CHANGE TO: The response curve will be fitted by non-weighted least squares nonlinear regression analysis.

REASON FOR CHANGE: The above sentence is being change per request of the Sponsor. The model of the response curve fitting was changed.

PART TO BE CHANGED: The following part of the Section 6.2.1 entitled Concentration Fits for the Reference Chemicals, page 12 and page 13, six paragraph is being changed.

Y= percent of control activity in the inhibitor tube
X = logarithm (base 10) of the concentration
DAVG =average dpms across the repeat tubes with the same reference chemical concentration
T = top of plateau
B = bottom of plateau
β = slope of the concentration response curve (β will be negative)
$\mu = \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

The following concentration response curve will be fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$Y = B + (T-B)/[1 + \{(T-B)/(50-B)-1\}\ 10^{(\mu-X)\beta}] + \varepsilon$$

where ε is the variation among repetitions, distributed with mean 0 and variance proportional to DAVG (based on Poisson distribution theory for radiation counts). The variance is approximately proportional to Y.

CHANGE TO:

Y= percent of control activity in the inhibitor tube
X = logarithm (base 10) of the concentration
T = top of plateau
B = bottom of plateau
H= Hill slope
$\mu = \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

The following concentration response curve will be fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$Y = B + (T-B)/1+10^{(X-\mu)*H}$$

REASON FOR CHANGE: The above paragraph is being changed as per request of the Sponsor. The model of the response curve fitting was changed.

EFFECTIVE DATE: November 15, 2005

APPROVED BY:

Bozena D. Lusiak, Study Director — 11-18-05 Date

Sponsor Representative — 11-18-05 Date

PROTOCOL AMENDMENT 2

STUDY NUMBER: **G608316**

STUDY TITLE: **Aromatase Assay Validation: Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories (WA 4-16, Task 7)**

PART TO BE CHANGED: Amendment 1, page 3, the equation in the sentence. The following concentration response curve will be fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$Y= B + (T-B)/1+10^{(X-\mu)*H}$$

CHANGE TO: The following concentration response curve will be fitted to relate percent of control activity to logarithm of concentration within each replicate:

$$Y= B + (T-B)/ [1+10^{(\mu -X)*H}]$$

REASON FOR CHANGE: The above equation is being changed as per request of the Sponsor. A transcription error occurred during preparation of the Amendment 1.

EFFECTIVE DATE: December 16, 2005

APPROVED BY:

Bozena D. Lusiak, Study Director — Date: 12-16-05

Jerry D. Johnson
Sponsor Representative — Date: 12-16-05

PROTOCOL AMENDMENT 3

STUDY NUMBER: **G608316**

STUDY TITLE: **Aromatase Assay Validation: Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories (WA 4-16, Task 7)**

PART TO BE CHANGED: The wrong CAS Number 104-40-5 given for 4-nonylphenol in Table 1, page 5.

CHANGE TO: The CAS Number for 4-nonylphenol is 84852-15-3.

EFFECTIVE DATE: March 20, 2006

APPROVED BY:

Bozena D. Lusiak

Bozena D. Lusiak, Study Director — 03-20-06 Date

Sponsor Representative — 3-20-06 Date

DEVIATION REPORT

G608316

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES (TASK 7)

Type of Deviation:	Protocol
Date of Deviation:	7-20-2005 and 8-2-2005
Nature of Deviation:	The protocol stated that the first replicate concentrations were to be $1x\ 10^{-3}$ to 1×10^{-10} M.
Cause of Deviation:	The concentration range used for the first replicate of aminoglutethimide and prochloraz was from 1×10^{-4} to 1×10^{-11} M. This was caused by technical error of the technician conducting the assay (wrong stock solutions concentration was taken to calculate required dilutions).
Impact of Deviation on the Study:	None.
Corrective Action:	None

Approved By: Bozena D. Lusiak Date: 02-02-06

Original: Study File
Copies: H. Flory

DEVIATION REPORT

G608316

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES (TASK 7)

Type of Deviation:	Protocol
Date of Deviation:	From 8-11-2005 through the Study
Nature of Deviation:	The protocol stated that the first replicate concentrations were to be $1x\ 10^{-3}$ to 1×10^{-10} M.
Cause of Deviation:	The concentration of stock solutions of dibenz[a,h]anthracene, chrysin and ketoconazole (send by the Sponsor) was 0.01 M. Using above stock solutions directly (without dilution) to the aromatase assay resulted in the final inhibitor concentration of $1x\ 10^{-4}$ M.
Impact of Deviation on the Study:	None.
Corrective Action:	None

Approved By: Bozena D. Lisiak Date: 02-02-06

Original: Study File
Copies : H. Flory

DEVIATION REPORT

G608316

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES (TASK 7)

Type of Deviation:	Protocol
Date of Deviation:	7-21-2005, 8-11-2005, and 8-25-2005
Nature of Deviation:	The protocol stated that the appropriate documentation on time from thaw to use of the microsomes must be maintained.
Cause of Deviation:	For the above three assays, the microsomes thaw and use time was not recorded.
Impact of Deviation on the Study:	None.
Corrective Action:	None

Approved By: Bozena D Sunial Date: 03-20-06

Original: Study File
Copies : H. Flory

DEVIATION REPORT

G608316

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES (TASK 7)

Type of Deviation:	Protocol
Date of Deviation:	10-4-2005
Nature of Deviation:	The expiration date for the dibenz[a,h]anthracene stock solution was 10-3-2005. This solution was used on 10-4-2005- the first day after expiration date.
Cause of Deviation:	At the day of experiment, the solution stability data were not available.
Impact of Deviation on the Study:	None.
Corrective Action:	None

Approved By: Brian D. Siniak Date: 05-11-06

Original: Study File
Copies : H. Flory

APPENDIX B

Spreadsheets – The Aromatase Activity Calculation Page of the Spreadsheet for Each Replicate. B-1

Assay Date	7/20/2005	Test Chemical ID	1	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0197	27372	1389442
2	0.0196	27887	1422806
3	0.0196	31446	1604388
4	0.0197	26735	1357107
5	0.0199	31913	1603668

Average DPM/g soln	1475482
SD	119623
CV	8.11
µCi/g soln	0.665

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.4	10		1040.00
Dilution A			100	10.40
Dilution B			10	1.04

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0516 g
Mass of dilution B used in substrate prep	4.5287 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.584958 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00752 µg/g soln.
µg/g soln.

a. µCi/g soln	0.665
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.584958 + 0.00752
= 0.592482 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.122 µCi/µg ASDN

713234 dpm/nmol

Assay Date	7/20/2005	Test Chemical ID	1	# Concentrations tested	8
Technician ID	TD	Replicate #	1	Microsome type	placental

Microsome ID 6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.512	0.299	0.136	0.072	0.027	0.015	0.000			
	0.514	0.306	0.138	0.072	0.032	0.019	0.000			
	0.513	0.305	0.138	0.069	0.028	0.015	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.263	0.037	0.059
	0.262	0.038	0.061
			0.063

Standard concentration (ng/mL) LE in 2-22-06 BJL	Volume of stock used	Final volume of Std	mg Protein per µL		Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200	0.0500	0.513	0.513	0.0482	m, b	0.098	-0.002
125	100	200	0.00013		200	0.0250	0.301	0.301	0.0275	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200	0.0100	0.137	0.137	0.0115	r^2, se_y	0.992	0.002
25	100	1000	0.00003		200	0.0050	0.071	0.071	0.0051	F, df	508	4
10	40	1000	0.00001		200	0.0020	0.029	0.029	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010	0.016	0.016	-0.0003			
			Blank	0.000								

Regression results are calculated using the function LINEST

r^2= 0.992
m= 0.098
b= -0.002

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.263	0.263	0.024	200	1	1	0.000	0.000	0.119
QC 100	0.262	0.262	0.024	200	1	1	0.000		
QC 100									
QC 10	0.037	0.037	0.002	200	1	1	0.000	0.000	0.009
QC 10	0.038	0.038	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.059	0.059	0.004	200	1	949	0.019	0.019	19.483
Microsomes	0.061	0.061	0.004	200	1	949	0.020		
Microsomes	0.063	0.063	0.004	200	1	949	0.020		

Assay Date 7/20/2005 | Test Chemical ID 1 | # Concentrations tested 8

Technician ID TD | Replicate # 1 | Microsome type placental | Microsome ID 6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.512	0.290	0.136	0.072	0.027	0.015	0.000			
	0.514	0.306	0.136	0.072	0.032	0.019	0.000			
	0.513	0.305	0.139	0.069	0.028	0.015	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.263	0.037	0.059
	0.262	0.038	0.061
			0.063

Standard concentration (mg/mL) LF in 2-27-06 LBC	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	$A_{adj.}$	Curve Output
250	200	200	0.00025	200	0.0500	0.513	0.513	0.0426
125	100	200	0.00013	200	0.0250	0.301	0.301	0.0246
50	200	1000	0.00005	200	0.0100	0.137	0.137	0.0108
25	100	1000	0.00003	200	0.0050	0.071	0.071	0.0053
10	40	1000	0.00001	200	0.0020	0.029	0.029	0.0017
5	20	1000	0.00001	200	0.0010	0.016	0.016	0.0006
				Blank	0.000			

Variables	Regression results	
m, b	0.084	-0.001
se_m, se_b	0.003	0.000
r^2, se_y	0.997	0.001
F, df	1089	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.997
m= 0.084
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.263	0.263	0.021	200	1	1	0.000	0.000	0.107
QC 100	0.262	0.262	0.021	200	1	1	0.000		
QC 100									
QC 10	0.037	0.037	0.002	200	1	1	0.000	0.000	0.012
QC 10	0.038	0.038	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.059	0.059	0.004	200	1	949	0.020	0.021	20.913
Microsomes	0.061	0.061	0.004	200	1	949	0.021		
Microsomes	0.063	0.063	0.005	200	1	949	0.021		

Assay Date	7/20/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Microsome Dilution Details		
Dilution A	0.1	mL microsome Stock used
	5	mL total volume
	50	dilution factor
Dilution B	2.5	mL microsome Dilution A used
	47.5	mL total volume
	19	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	950	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	20.91328
Protein Concentration (dilution added to assay, mg/mL):	0.022014

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	1.00E-07
5	1.00E-08
6	1.00E-09
7	1.00E-10
8	1.00E-11

Assay Date	7/20/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5612	11223.8	11173.2	22346.4	0.1	147548	15.15	22192	0.0311	1	0.011	15	0.0942
			0.5	2	5561	11122.6											
	2	2	0.5	1	5415	10829	10776.9	21553.8	0.1	147548	14.61	21399	0.0300	1	0.011	15	0.0909
			0.5	2	5362	10724.8											
	3	2	0.5	1	5187	10373.6	10444.2	20888.4	0.1	147548	14.16	20734	0.0291	1	0.011	15	0.0880
			0.5	2	5257	10514.8											
	4	2	0.5	1	5288	10575.8	10616.9	21233.8	0.1	147548	14.39	21079	0.0296	1	0.011	15	0.0895
			0.5	2	5329	10658											
Background control	1	2	0.5	1	45	90.06	84.76	169.52	0.1	147548	0.11	15	0.0000	1	0.011	15	0.0001
			0.5	2	40	79.46											
	2	2	0.5	1	36	71.74	71.54	143.08	0.1	147548	0.10	-11	0.0000	1	0.011	15	0.0000
			0.5	2	36	71.34											
	3	2	0.5	1	43	86.4	85.09	170.18	0.1	147548	0.12	16	0.0000	1	0.011	15	0.0001
			0.5	2	42	83.78											
	4	2	0.5	1	33	66.5	67.45	134.9	0.1	147548	0.09	-20	0.0000	1	0.011	15	-0.0001
			0.5	2	34	68.4											
Positive control	1	2	0.5	1	2482	4963.4	4982.8	9965.6	0.1	147548	6.75	9811	0.0138	1	0.011	15	0.0417
			0.5	2	2501	5002.2											
	2	2	0.5	1	2428	4855.8	4824.7	9649.4	0.1	147548	6.54	9495	0.0133	1	0.011	15	0.0403
			0.5	2	2397	4793.6											
	3	2	0.5	1	2350	4700.6	4685	9370	0.1	147548	6.35	9216	0.0129	1	0.011	15	0.0391
			0.5	2	2335	4669.4											
	4	2	0.5	1	2355	4709.4	4704.4	9408.8	0.1	147548	6.36	9254	0.0130	1	0.011	15	0.0393
			0.5	2	2350	4699.4											
Negative Control	1	2	0.5	1	5414	10828	10781.3	21562.6	0.1	147548	14.61	21408	0.0300	1	0.011	15	0.0909
			0.5	2	5367	10734.6											
	2	2	0.5	1	5467	10933.2	10942.1	21884.2	0.1	147548	14.83	21730	0.0305	1	0.011	15	0.0923
			0.5	2	5476	10951											
	3	2	0.5	1	5336	10672	10614.7	21229.4	0.1	147548	14.39	21075	0.0295	1	0.011	15	0.0895
			0.5	2	5279	10557.4											
	4	2	0.5	1	4777	9554.6	9393.8	18787.6	0.1	147548	12.73	18633	0.0261	1	0.011	15	0.0791
			0.5	2	4617	9233											
1	1-1	2	0.5	1	1945	3890.8	3921.7	7843.4	0.1	147548	5.32	7689	0.0108	1	0.011	15	0.0326
			0.5	2	1976	3952.6											
	1-2	2	0.5	1	2024	4048.8	4006.7	8013.4	0.1	147548	5.43	7859	0.0110	1	0.011	15	0.0334
			0.5	2	1982	3964.6											
	1-3	2	0.5	1	1979	3957.8	3969.1	7938.2	0.1	147548	5.38	7784	0.0109	1	0.011	15	0.0330
			0.5	2	1990	3980.4											
	2-1	2	0.5	1	4670	9339.2	9234.2	18468.4	0.1	147548	12.52	18314	0.0257	1	0.011	15	0.0778
			0.5	2	4565	9129.2											
	2-2	2	0.5	1	4625	9250.8	9245.6	18491.2	0.1	147548	12.53	18337	0.0257	1	0.011	15	0.0779
			0.5	2	4620	9240.4											
	2-3	2	0.5	1	4523	9045	9052.7	18105.4	0.1	147548	12.27	17951	0.0252	1	0.011	15	0.0762
			0.5	2	4530	9060.4											
	3-1	2	0.5	1	5272	10544.2	10527.3	21054.6	0.1	147548	14.27	20900	0.0293	1	0.011	15	0.0887
			0.5	2	5255	10510.4											
	3-2	2	0.5	1	5303	10605	10574.4	21148.8	0.1	147548	14.33	20994	0.0294	1	0.011	15	0.0891
			0.5	2	5272	10543.8											
	3-3	2	0.5	1	5188	10376.6	10356.5	20713	0.1	147548	14.04	20559	0.0288	1	0.011	15	0.0873
			0.5	2	5168	10336.4											
	4-1	2	0.5	1	5235	10470.6	10515.4	21030.8	0.1	147548	14.25	20876	0.0293	1	0.011	15	0.0886
			0.5	2	5280	10560.2											
	4-2	2	0.5	1	5248	10495	10504.5	21009	0.1	147548	14.24	20855	0.0292	1	0.011	15	0.0885
			0.5	2	5257	10514											
	4-3	2	0.5	1	5226	10452	10437.9	20875.8	0.1	147548	14.15	20721	0.0291	1	0.011	15	0.0880
			0.5	2	5212	10423.8											
	5-1	2	0.5	1	5299	10598.4	10580	21160	0.1	147548	14.34	21006	0.0295	1	0.011	15	0.0892
			0.5	2	5281	10561.6											
	5-2	2	0.5	1	5320	10639.6	10754.2	21508.4	0.1	147548	14.58	21354	0.0299	1	0.011	15	0.0907
			0.5	2	5434	10868.8											
	5-3	2	0.5	1	5229	10458.6	10464.7	20929.4	0.1	147548	14.18	20775	0.0291	1	0.011	15	0.0882
			0.5	2	5235	10470.8											
	6-1	2	0.5	1	5317	10633.4	10611.6	21223.2	0.1	147548	14.38	21069	0.0295	1	0.011	15	0.0895
			0.5	2	5295	10589.8											
	6-2	2	0.5	1	5266	10532	10579	21158	0.1	147548	14.34	21004	0.0294	1	0.011	15	0.0892
			0.5	2	5313	10626											
	6-3	2	0.5	1	5340	10680.6	10715.4	21430.8	0.1	147548	14.52	21276	0.0298	1	0.011	15	0.0903
			0.5	2	5375	10750.2											
	7-1	2	0.5	1	5280	10559.2	10648.5	21297	0.1	147548	14.43	21143	0.0296	1	0.011	15	0.0898
			0.5	2	5369	10737.8											
	7-2	2	0.5	1	5411	10821.8	10960.9	21921.8	0.1	147548	14.86	21767	0.0305	1	0.011	15	0.0924
			0.5	2	5550	11100											
	7-3	2	0.5	1	5483	10966.4	10939.7	21879.4	0.1	147548	14.83	21725	0.0305	1	0.011	15	0.0922
			0.5	2	5457	10913											
	8-1	2	0.5	1	5302	10603.6	10560.6	21121.2	0.1	147548	14.31	20967	0.0294	1	0.011	15	0.0890
			0.5	2	5259	10517.6											
	8-2	2	0.5	1	5365	10729	10869.7	21739.4	0.1	147548	14.73	21585	0.0303	1	0.011	15	0.0916
			0.5	2	5505	11010.4											
	8-3	2	0.5	1	5151	10301.4	10414.5	20829	0.1	147548	14.12	20675	0.0290	1	0.011	15	0.0878
			0.5	2	5264	10527.6											

Assay Date	7/20/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0925	0.0024
Full activity	End	0.0888	0.0010
Full activity	Overall	0.0907	0.0026
Background	Beginning	0.0000	7.93825E-05
Background	End	0.0000	0.000105923
Background	Overall	0.0000	7.69766E-05
Positive	Beginning	0.0410	0.0009
Positive	End	0.0392	0.0001
Positive	Overall	0.0401	0.0012
Negative	Beginning	0.0916	0.0010
Negative	End	0.0843	0.0073
Negative	Overall	0.0879	0.0060

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
1	1	1	1.00E-04	-4.00	0.0326
1	1	2	1.00E-04	-4.00	0.0334
1	1	3	1.00E-04	-4.00	0.0330
1	2	1	1.00E-05	-5.00	0.0778
1	2	2	1.00E-05	-5.00	0.0779
1	2	3	1.00E-05	-5.00	0.0762
1	3	1	1.00E-06	-6.00	0.0887
1	3	2	1.00E-06	-6.00	0.0891
1	3	3	1.00E-06	-6.00	0.0873
1	4	1	1.00E-07	-7.00	0.0886
1	4	2	1.00E-07	-7.00	0.0885
1	4	3	1.00E-07	-7.00	0.0880
1	5	1	1.00E-08	-8.00	0.0892
1	5	2	1.00E-08	-8.00	0.0907
1	5	3	1.00E-08	-8.00	0.0882
1	6	1	1.00E-09	-9.00	0.0895
1	6	2	1.00E-09	-9.00	0.0892
1	6	3	1.00E-09	-9.00	0.0903
1	7	1	1.00E-10	-10.00	0.0898
1	7	2	1.00E-10	-10.00	0.0924
1	7	3	1.00E-10	-10.00	0.0922
1	8	1	1.00E-11	-11.00	0.0890
1	8	2	1.00E-11	-11.00	0.0916
1	8	3	1.00E-11	-11.00	0.0878

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	36.01	36.81	36.46
2	-5.00	85.78	85.88	84.07
3	-6.00	97.89	98.33	96.29
4	-7.00	97.78	97.67	97.05
5	-8.00	98.38	100.01	97.30
6	-9.00	98.68	98.37	99.65
7	-10.00	99.02	101.95	101.75
8	-11.00	98.20	101.10	96.83

Assay Date	7/21/2005	Test Chemical ID	1	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0197	25917	1315584		
2	0.0197	28742	1458985		
3	0.0197	29720	1508629		
4	0.0198	31132	1572323		
5	0.0198	31869	1609545		
			Average DPM/g soln		1493013
			SD		114840
			CV		7.69
			µCi/g soln		0.673

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.3	10		1030.00
Dilution A			100	10.30
Dilution B			10	1.03

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6572 g
Mass of dilution B used in substrate prep	5.4312 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.579271 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00761 µg/g soln.
µg/g soln.

a. µCi/g soln 0.673
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.579271 + 0.00761
= 0.586884 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.146 µCi/µg ASDN

728592 dpm/nmol

Assay Date	7/21/2005	Test Chemical ID	1	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.520	0.294	0.122	0.053	0.017	0.008	0.000			
	0.516	0.296	0.124	0.053	0.023	0.008	0.000			
	0.509	0.296	0.122	0.057	0.019	0.008	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.255	0.031	0.065
	0.261	0.028	0.062
			0.060

Standard concentration (mg/mL) LF in 2-22-06 BRE	Volume of stock used	Final volume of Std	mg Protein per µL		Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200	0.0500	0.515	0.515	0.0484	m, b	0.095	-0.001
125	100	200	0.00013		200	0.0250	0.295	0.295	0.0275	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200	0.0100	0.123	0.123	0.0111	r^2, se_y	0.994	0.002
25	100	1000	0.00003		200	0.0050	0.054	0.054	0.0046	F, df	643	4
10	40	1000	0.00001		200	0.0020	0.020	0.020	0.0013	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010	0.008	0.008	0.0002			
				Blank	0.000							

Regression results are calculated using the function LINEST

r^2= 0.994
m= 0.095
b= -0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.255	0.255	0.024	200	1	1	0.000	0.000	0.120
QC 100	0.261	0.261	0.024	200	1	1	0.000		
QC 100									
QC 10	0.031	0.031	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.028	0.028	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.065	0.065	0.006	200	1	949	0.027	0.025	25.439
Microsomes	0.062	0.062	0.005	200	1	949	0.025		
Microsomes	0.060	0.060	0.005	200	1	949	0.024		

Assay Date	7/21/2005	Test Chemical ID	1	# Concentrations tested	8					
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305			

Standards:								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	250	125	50	25	10	5	0	25	100	11343-77
	0.520	0.294	0.122	0.053	0.017	0.008	0.000			
	0.516	0.296	0.124	0.053	0.023	0.008	0.000			
	0.509	0.296	0.122	0.057	0.019	0.008	0.000			

Samples:			
	QC 100	QC 10	Microsomes
	0.255	0.031	0.065
	0.261	0.028	0.062
			0.060

Standard concentration (mg/mL) LE w 2-22-06 BRL	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0500	0.515	0.515	0.0431	m, b	0.083	0.000
125	100	200	0.00013	200	0.0250	0.295	0.295	0.0248	se_m, se_b	0.001	0.000
50	200	1000	0.00005	200	0.0100	0.123	0.123	0.0105	r^2, se_y	0.999	0.000
25	100	1000	0.00003	200	0.0050	0.054	0.054	0.0048	F, df	3827	3
10	40	1000	0.00001	200	0.0020	0.020	0.020	0.0019	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.008	0.008	0.0010			

Regression results are calculated using the function LINEST

Blank	0.000			r^2=	0.999							
				m=	0.083							
				b=	0.000							

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.255	0.255	0.021	200	1	1	0.000	0.000	0.109
QC 100	0.261	0.261	0.022	200	1	1	0.000		
QC 100									
QC 10	0.031	0.031	0.003	200	1	1	0.000	0.000	0.014
QC 10	0.028	0.028	0.003	200	1	1	0.000		
QC 10									
Microsomes	0.065	0.065	0.006	200	1	949	0.027	0.026	26.038
Microsomes	0.062	0.062	0.005	200	1	949	0.026		
Microsomes	0.060	0.060	0.005	200	1	949	0.025		

Assay Date	7/21/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Microsome Dilution Details		
Dilution A	0.1	mL microsome Stock used
	5	mL total volume
	50	dilution factor
Dilution B	2.5	mL microsome Dilution A used
	47.5	mL total volume
	19	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	950	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	26.03845
Protein Concentration (dilution added to assay, mg/mL):	0.027409

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	2.50E-04
3	1.00E-04
4	5.00E-05
5	2.50E-05
6	1.00E-05
7	2.50E-06
8	1.00E-06

Assay Date	7/21/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5023	10045.2	9971.9	19943.8	0.1	149301	13.36	19764	0.0271	1	0.014	15	0.0660
			0.5	2	4949	9898.6											
	2	2	0.5	1	4906	9812.6	9823	19646	0.1	149301	13.16	19466	0.0267	1	0.014	15	0.0650
			0.5	2	4917	9833.4											
	3	2	0.5	1	4999	9997	9948.4	19896.8	0.1	149301	13.33	19717	0.0271	1	0.014	15	0.0658
			0.5	2	4950	9899.8											
	4	2	0.5	1	4777	9554.8	9535.9	19071.8	0.1	149301	12.77	18892	0.0259	1	0.014	15	0.0631
			0.5	2	4759	9517											
Background control	1	2	0.5	1	39	78.22	88.17	176.34	0.1	149301	0.12	-4	0.0000	1	0.014	15	0.0000
			0.5	2	49	98.12											
	2	2	0.5	1	51	102.18	95.26	190.52	0.1	149301	0.13	11	0.0000	1	0.014	15	0.0000
			0.5	2	44	88.34											
	3	2	0.5	1	48	95.8	94.16	188.32	0.1	149301	0.13	8	0.0000	1	0.014	15	0.0000
			0.5	2	46	92.52											
	4	2	0.5	1	41	81.26	82.39	164.78	0.1	149301	0.11	-15	0.0000	1	0.014	15	-0.0001
			0.5	2	42	83.52											
Positive control	1	2	0.5	1	2284	4567.2	4519.4	9038.8	0.1	149301	6.05	8859	0.0122	1	0.014	15	0.0296
			0.5	2	2236	4471.6											
	2	2	0.5	1	2260	4520.8	4508.1	9016.2	0.1	149301	6.04	8836	0.0121	1	0.014	15	0.0295
			0.5	2	2248	4495.4											
	3	2	0.5	1	2173	4346.4	4374.6	8749.2	0.1	149301	5.86	8569	0.0118	1	0.014	15	0.0286
			0.5	2	2201	4402.8											
	4	2	0.5	1	2231	4461.6	4434.4	8868.8	0.1	149301	5.94	8689	0.0119	1	0.014	15	0.0290
			0.5	2	2204	4407.2											
Negative Control	1	2	0.5	1	4695	9390.8	9409.7	18819.4	0.1	149301	12.60	18639	0.0256	1	0.014	15	0.0622
			0.5	2	4714	9428.6											
	2	2	0.5	1	4487	8973.4	9025.6	18051.2	0.1	149301	12.09	17871	0.0245	1	0.014	15	0.0597
			0.5	2	4539	9077.8											
	3	2	0.5	1	4575	9149	9072.2	18144.4	0.1	149301	12.15	17964	0.0247	1	0.014	15	0.0600
			0.5	2	4498	8995.4											
	4	2	0.5	1	4534	9067.2	9158.8	18317.6	0.1	149301	12.27	18138	0.0249	1	0.014	15	0.0605
			0.5	2	4625	9250.4											
1	1-1	2	0.5	1	343	685.66	678.85	1357.7	0.1	149301	0.91	1178	0.0016	1	0.014	15	0.0039
			0.5	2	336	672.04											
	1-2	2	0.5	1	347	694.28	698.45	1396.9	0.1	149301	0.94	1217	0.0017	1	0.014	15	0.0041
			0.5	2	351	702.62											
	1-3	2	0.5	1	357	714.6	704.5	1409	0.1	149301	0.94	1229	0.0017	1	0.014	15	0.0041
			0.5	2	347	694.4											
	2-1	2	0.5	1	1030	2059.42	2056.93	4113.86	0.1	149301	2.76	3934	0.0054	1	0.014	15	0.0131
			0.5	2	1027	2054.44											
	2-2	2	0.5	1	1061	2121.26	2107.83	4215.66	0.1	149301	2.82	4036	0.0055	1	0.014	15	0.0135
			0.5	2	1047	2094.4											
	2-3	2	0.5	1	1017	2033.26	2026.86	4053.72	0.1	149301	2.72	3874	0.0053	1	0.014	15	0.0129
			0.5	2	1010	2020.46											
	3-1	2	0.5	1	1905	3810.2	3833.9	7667.8	0.1	149301	5.14	7488	0.0103	1	0.014	15	0.0250
			0.5	2	1929	3857.6											
	3-2	2	0.5	1	1925	3850.6	3908.8	7817.6	0.1	149301	5.24	7638	0.0105	1	0.014	15	0.0255
			0.5	2	1984	3967											
	3-3	2	0.5	1	1975	3949	3904.2	7808.4	0.1	149301	5.23	7628	0.0105	1	0.014	15	0.0255
			0.5	2	1930	3859.4											
	4-1	2	0.5	1	2727	5453.6	5460.3	10920.6	0.1	149301	7.31	10741	0.0147	1	0.014	15	0.0359
			0.5	2	2734	5467											
	4-2	2	0.5	1	2779	5558.2	5612.2	11224.4	0.1	149301	7.52	11044	0.0152	1	0.014	15	0.0369
			0.5	2	2833	5666.2											
	4-3	2	0.5	1	2724	5447.8	5536.1	11072.2	0.1	149301	7.42	10892	0.0149	1	0.014	15	0.0364
			0.5	2	2812	5624.4											
	5-1	2	0.5	1	3500	7000.6	6994.7	13989.4	0.1	149301	9.37	13809	0.0190	1	0.014	15	0.0461
			0.5	2	3494	6988.8											
	5-2	2	0.5	1	3493	6985.6	7002.5	14005	0.1	149301	9.38	13825	0.0190	1	0.014	15	0.0462
			0.5	2	3510	7019.4											
	5-3	2	0.5	1	3519	7037.4	6998	13996	0.1	149301	9.37	13816	0.0190	1	0.014	15	0.0461
			0.5	2	3479	6958.6											
	6-1	2	0.5	1	4064	8127.6	8132.9	16265.8	0.1	149301	10.89	16086	0.0221	1	0.014	15	0.0537
			0.5	2	4069	8138.2											
	6-2	2	0.5	1	4172	8344.4	8341.3	16682.6	0.1	149301	11.17	16503	0.0227	1	0.014	15	0.0551
			0.5	2	4169	8338.2											
	6-3	2	0.5	1	4171	8342.6	8385.8	16771.6	0.1	149301	11.23	16592	0.0228	1	0.014	15	0.0554
			0.5	2	4215	8429											
	7-1	2	0.5	1	4515	9029	8902	17804	0.1	149301	11.92	17624	0.0242	1	0.014	15	0.0588
			0.5	2	4388	8775											
	7-2	2	0.5	1	4482	8964	8952.1	17904.2	0.1	149301	11.99	17724	0.0243	1	0.014	15	0.0592
			0.5	2	4470	8940.2											
	7-3	2	0.5	1	4377	8754.2	8646.6	17293.2	0.1	149301	11.58	17113	0.0235	1	0.014	15	0.0571
			0.5	2	4270	8539											
	8-1	2	0.5	1	4708	9415	9388.5	18777	0.1	149301	12.58	18597	0.0255	1	0.014	15	0.0621
			0.5	2	4681	9362											
	8-2	2	0.5	1	4396	8792.6	8875.3	17750.6	0.1	149301	11.89	17571	0.0241	1	0.014	15	0.0587
			0.5	2	4479	8958											
	8-3	2	0.5	1	4734	9468.2	9408.3	18816.6	0.1	149301	12.60	18637	0.0256	1	0.014	15	0.0622
			0.5	2	4674	9348.4											

Assay Date	7/21/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0655	0.0007
Full activity	End	0.0644	0.0019
Full activity	Overall	0.0650	0.0013
Background	Beginning	0.0000	3.3473E-05
Background	End	0.0000	5.55679E-05
Background	Overall	0.0000	3.97314E-05
Positive	Beginning	0.0295	0.0001
Positive	End	0.0288	0.0003
Positive	Overall	0.0292	0.0005
Negative	Beginning	0.0609	0.0018
Negative	End	0.0603	0.0004
Negative	Overall	0.0606	0.0011

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
1	1	1	1.00E-03	-3.00	0.0039
1	1	2	1.00E-03	-3.00	0.0041
1	1	3	1.00E-03	-3.00	0.0041
1	2	1	2.50E-04	-3.60	0.0131
1	2	2	2.50E-04	-3.60	0.0135
1	2	3	2.50E-04	-3.60	0.0129
1	3	1	1.00E-04	-4.00	0.0250
1	3	2	1.00E-04	-4.00	0.0255
1	3	3	1.00E-04	-4.00	0.0255
1	4	1	5.00E-05	-4.30	0.0359
1	4	2	5.00E-05	-4.30	0.0369
1	4	3	5.00E-05	-4.30	0.0364
1	5	1	2.50E-05	-4.60	0.0461
1	5	2	2.50E-05	-4.60	0.0462
1	5	3	2.50E-05	-4.60	0.0461
1	6	1	1.00E-05	-5.00	0.0537
1	6	2	1.00E-05	-5.00	0.0551
1	6	3	1.00E-05	-5.00	0.0554
1	7	1	2.50E-06	-5.60	0.0588
1	7	2	2.50E-06	-5.60	0.0592
1	7	3	2.50E-06	-5.60	0.0571
1	8	1	1.00E-06	-6.00	0.0621
1	8	2	1.00E-06	-6.00	0.0587
1	8	3	1.00E-06	-6.00	0.0622

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	6.05	6.25	6.32
2	-3.60	20.22	20.74	19.91
3	-4.00	38.48	39.25	39.20
4	-4.30	55.19	56.76	55.97
5	-4.60	70.96	71.04	71.00
6	-5.00	82.66	84.80	85.26
7	-5.60	90.57	91.08	87.94
8	-6.00	95.57	90.29	95.77

Assay Date	7/22/2005	Test Chemical ID	1	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0200	28002	1400100		
2	0.0200	30480	1524000		
3	0.0201	31088	1546667		
4	0.0200	33201	1660050		
5	0.0201	33881	1685622		
			Average DPM/g soln		1563288
			SD		114868
			CV		7.35
			µCi/g soln		0.704

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.9245 g
Mass of dilution B used in substrate prep	5.5847 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562719 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00797 µg/g soln.
µg/g soln.

a. µCi/g soln 0.704
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.562719 + 0.00797
= 0.570690 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.234 µCi/µg ASDN

784534 dpm/nmol

Assay Date 7/22/2005 | Test Chemical ID 1 | # Concentrations tested 8

Technician ID TD | Replicate # 3 | Microsome type placental | Microsome ID 6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.504	0.300	0.133	0.076	0.035	0.020	0.000			
	0.507	0.299	0.133	0.079	0.035	0.020	0.000			
	0.512	0.301	0.141	0.077	0.034	0.020	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.278	0.041	0.081							
	0.280	0.042	0.079							
			0.081							

Standard concentration (mg/mL) LE	Volume of stock used	Final volume of Std	mg Protein per µL	Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
M 8-22-06 BBL								
250	200	200	0.00025	200	0.0500	0.508	0.508	0.0482
125	100	200	0.00013	200	0.0250	0.300	0.300	0.0276
50	200	1000	0.00005	200	0.0100	0.136	0.136	0.0112
25	100	1000	0.00003	200	0.0050	0.077	0.077	0.0053
10	40	1000	0.00001	200	0.0020	0.035	0.035	0.0011
5	20	1000	0.00001	200	0.0010	0.020	0.020	-0.0004
		Blank	0.000					

Variables		Regression results	
m, b	0.100	-0.002
se_m, se_b	0.004	0.001
r^2, se_y	0.992	0.002
F, df	520	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

r^2= 0.992
m= 0.100
b= -0.002

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.278	0.278	0.025	200	1	1	0.000	0.000	0.127
QC 100	0.280	0.280	0.026	200	1	1	0.000		
QC 100									
QC 10	0.041	0.041	0.002	200	1	1	0.000	0.000	0.009
QC 10	0.042	0.042	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.081	0.081	0.006	200	1	949	0.027	0.027	26.823
Microsomes	0.079	0.079	0.006	200	1	949	0.026		
Microsomes	0.081	0.081	0.006	200	1	949	0.027		

Assay Date 7/22/2005 | Test Chemical ID 1 | # Concentrations tested 8

Technician ID TD | Replicate # 3 | Microsome type placental | Microsome ID 6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.504	0.300	0.133	0.076	0.035	0.020	0.000			
	0.507	0.299	0.133	0.079	0.035	0.020	0.000			
	0.512	0.301	0.141	0.077	0.034	0.020	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.278	0.041	0.081
	0.280	0.042	0.079
			0.081

Standard concentration (mg/mL) LE 11-22-06 BJC	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.508	0.508	0.0426
125	100	200	0.00013	200	0.0250	0.300	0.300	0.0247
50	200	1000	0.00005	200	0.0100	0.136	0.136	0.0105
25	100	1000	0.00003	200	0.0050	0.077	0.077	0.0055
10	40	1000	0.00001	200	0.0020	0.035	0.035	0.0018
5	20	1000	0.00001	200	0.0010	0.020	0.020	0.0005
		Blank	0.000					

r^2= 0.998
m= 0.086
b= -0.001

Variables	Regression results	
m, b	0.086	-0.001
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.001
F, df	1377	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.278	0.278	0.023	200	1	1	0.000	0.000	0.114
QC 100	0.280	0.280	0.023	200	1	1	0.000		
QC 100									
QC 10	0.041	0.041	0.002	200	1	1	0.000	0.000	0.012
QC 10	0.042	0.042	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.081	0.081	0.006	200	1	949	0.028	0.027	27.261
Microsomes	0.079	0.079	0.006	200	1	949	0.027		
Microsomes	0.081	0.081	0.006	200	1	949	0.027		

Assay Date	7/22/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	3

Microsome Dilution Details		
Dilution A	0.1	mL microsome Stock used
	5	mL total volume
	50	dilution factor
Dilution B	2.5	mL microsome Dilution A used
	47.5	mL total volume
	19	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	950	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	27.26115
Protein Concentration (dilution added to assay, mg/mL):	0.028696

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	2.50E-04
3	1.00E-04
4	5.00E-05
5	2.50E-05
6	1.00E-05
7	2.50E-06
8	1.00E-06

Assay Date	7/22/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5456	10911.8	10959	21918	0.1	156329	14.02	21643	0.0276	1	0.014	15	0.0641
			0.5	2	5503	11006.2											
	2	2	0.5	1	5721	11441	11523.9	23047.8	0.1	156329	14.74	22773	0.0290	1	0.014	15	0.0674
			0.5	2	5803	11606.8											
	3	2	0.5	1	5571	11141.8	11100.5	22201	0.1	156329	14.20	21926	0.0279	1	0.014	15	0.0649
			0.5	2	5530	11059.2											
	4	2	0.5	1	5331	10661.8	10578.1	21156.2	0.1	156329	13.53	20881	0.0266	1	0.014	15	0.0618
			0.5	2	5247	10494.4											
Background control	1	2	0.5	1	70	139.84	140.08	280.16	0.1	156329	0.18	5	0.0000	1	0.014	15	0.0000
			0.5	2	70	140.32											
	2	2	0.5	1	66	132.38	129.2	258.4	0.1	156329	0.17	-17	0.0000	1	0.014	15	0.0000
			0.5	2	63	126.02											
	3	2	0.5	1	70	139.46	135.79	271.58	0.1	156329	0.17	-3	0.0000	1	0.014	15	0.0000
			0.5	2	66	132.12											
	4	2	0.5	1	78	156	144.98	289.96	0.1	156329	0.19	15	0.0000	1	0.014	15	0.0000
			0.5	2	67	133.96											
Positive control	1	2	0.5	1	2440	4880.2	4889.3	9778.6	0.1	156329	6.26	9504	0.0121	1	0.014	15	0.0281
			0.5	2	2449	4898.4											
	2	2	0.5	1	2495	4989.8	5078.8	10157.6	0.1	156329	6.50	9883	0.0126	1	0.014	15	0.0293
			0.5	2	2584	5167.8											
	3	2	0.5	1	2459	4918.2	4914.1	9828.2	0.1	156329	6.29	9553	0.0122	1	0.014	15	0.0283
			0.5	2	2455	4910											
	4	2	0.5	1	2413	4826.2	4808.7	9617.4	0.1	156329	6.15	9342	0.0119	1	0.014	15	0.0277
			0.5	2	2396	4791.2											
Negative Control	1	2	0.5	1	5049	10098.6	10137.5	20275	0.1	156329	12.97	20000	0.0255	1	0.014	15	0.0592
			0.5	2	5088	10176.4											
	2	2	0.5	1	5011	10022.8	10170.4	20340.8	0.1	156329	13.01	20066	0.0256	1	0.014	15	0.0594
			0.5	2	5159	10318											
	3	2	0.5	1	4993	9985.6	9972.2	19944.4	0.1	156329	12.76	19669	0.0251	1	0.014	15	0.0582
			0.5	2	4979	9958.8											
	4	2	0.5	1	4791	9582.6	9593.7	19187.4	0.1	156329	12.27	18912	0.0241	1	0.014	15	0.0560
			0.5	2	4802	9604.8											
1	1-1	2	0.5	1	357	713.76	704.74	1409.48	0.1	156329	0.90	1134	0.0014	1	0.014	15	0.0034
			0.5	2	348	695.72											
	1-2	2	0.5	1	336	671.62	671.15	1342.3	0.1	156329	0.86	1067	0.0014	1	0.014	15	0.0032
			0.5	2	335	670.68											
	1-3	2	0.5	1	315	629.66	630.82	1261.64	0.1	156329	0.81	987	0.0013	1	0.014	15	0.0029
			0.5	2	316	631.98											
	2-1	2	0.5	1	1124	2248.8	2261.6	4523.2	0.1	156329	2.89	4248	0.0054	1	0.014	15	0.0126
			0.5	2	1137	2274.4											
	2-2	2	0.5	1	1127	2253.2	2228.2	4456.4	0.1	156329	2.85	4181	0.0053	1	0.014	15	0.0124
			0.5	2	1102	2203.2											
	2-3	2	0.5	1	1125	2249.2	2230.8	4461.6	0.1	156329	2.85	4187	0.0053	1	0.014	15	0.0124
			0.5	2	1106	2212.4											
	3-1	2	0.5	1	2189	4377.6	4335.3	8670.6	0.1	156329	5.55	8396	0.0107	1	0.014	15	0.0249
			0.5	2	2147	4293											
	3-2	2	0.5	1	2154	4308.8	4297.3	8594.6	0.1	156329	5.50	8320	0.0106	1	0.014	15	0.0246
			0.5	2	2143	4285.8											
	3-3	2	0.5	1	2145	4290.2	4276.8	8553.6	0.1	156329	5.47	8279	0.0106	1	0.014	15	0.0245
			0.5	2	2132	4263.4											
	4-1	2	0.5	1	3039	6078.8	6102.4	12204.8	0.1	156329	7.81	11930	0.0152	1	0.014	15	0.0353
			0.5	2	3063	6126											
	4-2	2	0.5	1	2979	5957.6	5954.3	11908.6	0.1	156329	7.62	11634	0.0148	1	0.014	15	0.0345
			0.5	2	2976	5951											
	4-3	2	0.5	1	3006	6012.8	6027.1	12054.2	0.1	156329	7.71	11779	0.0150	1	0.014	15	0.0349
			0.5	2	3021	6041.4											
	5-1	2	0.5	1	3861	7721.4	7787.5	15575	0.1	156329	9.96	15300	0.0195	1	0.014	15	0.0453
			0.5	2	3927	7853.6											
	5-2	2	0.5	1	3852	7703.8	7685.3	15370.6	0.1	156329	9.83	15096	0.0192	1	0.014	15	0.0447
			0.5	2	3833	7666.8											
	5-3	2	0.5	1	3797	7593.8	7632.7	15265.4	0.1	156329	9.76	14990	0.0191	1	0.014	15	0.0444
			0.5	2	3836	7671.6											
	6-1	2	0.5	1	4714	9427	9498.8	18997.6	0.1	156329	12.15	18723	0.0239	1	0.014	15	0.0554
			0.5	2	4785	9570.6											
	6-2	2	0.5	1	4626	9251	9232.4	18464.8	0.1	156329	11.81	18190	0.0232	1	0.014	15	0.0539
			0.5	2	4607	9213.8											
	6-3	2	0.5	1	4649	9297	9174.1	18348.2	0.1	156329	11.74	18073	0.0230	1	0.014	15	0.0535
			0.5	2	4526	9051.2											
	7-1	2	0.5	1	5217	10433.4	10371.7	20743.4	0.1	156329	13.27	20468	0.0261	1	0.014	15	0.0606
			0.5	2	5155	10310											
	7-2	2	0.5	1	5100	10200.4	10150.5	20301	0.1	156329	12.99	20026	0.0255	1	0.014	15	0.0593
			0.5	2	5050	10100.6											
	7-3	2	0.5	1	5132	10264.4	10127.3	20254.6	0.1	156329	12.96	19980	0.0255	1	0.014	15	0.0592
			0.5	2	4995	9990.2											
	8-1	2	0.5	1	5192	10383.8	10413.4	20826.8	0.1	156329	13.32	20552	0.0262	1	0.014	15	0.0609
			0.5	2	5222	10443											
	8-2	2	0.5	1	5428	10855	10782	21564	0.1	156329	13.79	21289	0.0271	1	0.014	15	0.0630
			0.5	2	5355	10709											
	8-3	2	0.5	1	5429	10858.6	10694.2	21388.4	0.1	156329	13.68	21113	0.0269	1	0.014	15	0.0625
			0.5	2	5265	10529.8											

Assay Date	7/22/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0658	0.0024
Full activity	End	0.0634	0.0022
Full activity	Overall	0.0646	0.0023
Background	Beginning	0.0000	4.55638E-05
Background	End	0.0000	3.84864E-05
Background	Overall	0.0000	3.96441E-05
Positive	Beginning	0.0287	0.0008
Positive	End	0.0280	0.0004
Positive	Overall	0.0283	0.0007
Negative	Beginning	0.0593	0.0001
Negative	End	0.0571	0.0016
Negative	Overall	0.0582	0.0016

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
1	1	1	1.00E-03	-3.00	0.0034
1	1	2	1.00E-03	-3.00	0.0032
1	1	3	1.00E-03	-3.00	0.0029
1	2	1	2.50E-04	-3.60	0.0126
1	2	2	2.50E-04	-3.60	0.0124
1	2	3	2.50E-04	-3.60	0.0124
1	3	1	1.00E-04	-4.00	0.0249
1	3	2	1.00E-04	-4.00	0.0246
1	3	3	1.00E-04	-4.00	0.0245
1	4	1	5.00E-05	-4.30	0.0353
1	4	2	5.00E-05	-4.30	0.0345
1	4	3	5.00E-05	-4.30	0.0349
1	5	1	2.50E-05	-4.60	0.0453
1	5	2	2.50E-05	-4.60	0.0447
1	5	3	2.50E-05	-4.60	0.0444
1	6	1	1.00E-05	-5.00	0.0554
1	6	2	1.00E-05	-5.00	0.0539
1	6	3	1.00E-05	-5.00	0.0535
1	7	1	2.50E-06	-5.60	0.0606
1	7	2	2.50E-06	-5.60	0.0593
1	7	3	2.50E-06	-5.60	0.0592
1	8	1	1.00E-06	-6.00	0.0609
1	8	2	1.00E-06	-6.00	0.0630
1	8	3	1.00E-06	-6.00	0.0625

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	5.20	4.89	4.52
2	-3.60	19.48	19.18	19.20
3	-4.00	38.50	38.15	37.97
4	-4.30	54.71	53.35	54.02
5	-4.60	70.16	69.23	68.75
6	-5.00	85.86	83.42	82.88
7	-5.60	93.87	91.84	91.63
8	-6.00	94.25	97.63	96.82

Assay Date	8/11/2005	Test Chemical ID	2	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0196	27556	1405918		
2	0.0201	29579	1471592		
3	0.0201	32484	1616119		
4	0.0200	32849	1642450		
5	0.0202	33174	1642277		
			Average DPM/g soln		1555671
			SD		109750
			CV		7.05
			µCi/g soln		0.701

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.1	10		1010.00
Dilution A			100	10.10
Dilution B			10	1.01

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6058 g
Mass of dilution B used in substrate prep	5.4221 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.570106 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00793 µg/g soln.
µg/g soln.

a. µCi/g soln 0.701
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.570106 + 0.00793
= 0.578038 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.212 µCi/µg ASDN

770787 dpm/nmol

Assay Date	8/11/2005	Test Chemical ID	2	# Concentrations tested	8					
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.506	0.297	0.136	0.062	0.024	0.012	0.000			
	0.499	0.295	0.137	0.063	0.025	0.011	0.000			
	0.504	0.297	0.123	0.060	0.025	0.013	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.257	0.029	0.064							
	0.263	0.028	0.064							
			0.063							

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.503	0.503	0.0481
125	100	200	0.00013	200	0.0250	0.296	0.296	0.0278
50	200	1000	0.00005	200	0.0100	0.132	0.132	0.0116
25	100	1000	0.00003	200	0.0050	0.061	0.061	0.0047
10	40	1000	0.00001	200	0.0020	0.025	0.025	0.0011
5	20	1000	0.00001	200	0.0010	0.012	0.012	-0.0002
			Blank	0.000				

Variables	Regression results	
m, b	0.098	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.991	0.002
F, df	443	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

r^2= 0.991
m= 0.098
b= -0.001

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.257	0.257	0.024	200	1	1	0.000	0.000	0.121
QC 100	0.263	0.263	0.024	200	1	1	0.000		
QC 100									
QC 10	0.029	0.029	0.002	200	1	1	0.000	0.000	0.007
QC 10	0.028	0.028	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.064	0.064	0.005	200	1	949	0.023	0.023	23.281
Microsomes	0.064	0.064	0.005	200	1	949	0.024		
Microsomes	0.063	0.063	0.005	200	1	949	0.023		

Assay Date	8/11/2005	Test Chemical ID	2	# Concentrations tested	8					
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.506	0.297	0.136	0.062	0.024	0.012	0.000			
	0.499	0.295	0.137	0.063	0.025	0.011	0.000			
	0.504	0.297	0.123	0.060	0.025	0.013	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.257	0.029	0.064							
	0.263	0.028	0.064							
			0.063							

Standard concentration (pg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.503	0.503	0.0421
125	100	200	0.00013	200	0.0250	0.296	0.296	0.0247
50	200	1000	0.00005	200	0.0100	0.132	0.132	0.0108
25	100	1000	0.00003	200	0.0050	0.061	0.061	0.0049
10	40	1000	0.00001	200	0.0020	0.025	0.025	0.0018
5	20	1000	0.00001	200	0.0010	0.012	0.012	0.0008
			Blank	0.000				

Variables	Regression results	
m, b	0.084	0.000
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.001
F, df	1257	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.998
m= 0.084
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.257	0.257	0.021	200	1	1	0.000	0.000	0.108
QC 100	0.263	0.263	0.022	200	1	1	0.000		
QC 100									
QC 10	0.029	0.029	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.028	0.028	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.064	0.064	0.005	200	1	949	0.024	0.024	24.234
Microsomes	0.064	0.064	0.005	200	1	949	0.025		
Microsomes	0.063	0.063	0.005	200	1	949	0.024		

Assay Date	8/11/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Microsome Dilution Details		
Dilution A	0.1	mL microsome Stock used
	5	mL total volume
	50	dilution factor
Dilution B	2.5	mL microsome Dilution A used
	47.5	mL total volume
	19	dilution factor
Dilution C (if applicable)	[illegible]	mL microsome Dilution B used
	[illegible]	mL total volume
	NA	dilution factor
	950	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	24.2345
Protein Concentration (dilution added to assay, mg/mL):	0.02551

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	2.50E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	8/11/2005	Test Chemical ID		2 # Concentrations tested		8 Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5246	10491.4	10508.8	21017.6	0.1	155567	13.51	20756	0.0269		0.013	15	0.0704
			0.5	2	5263	10526.2											
	2	2	0.5	1	5106	10211.8	10140.4	20280.8	0.1	155567	13.04	20019	0.0260		0.013	15	0.0679
			0.5	2	5035	10069											
	3	2	0.5	1	5009	10017	9964.1	19928.2	0.1	155567	12.81	19667	0.0255		0.013	15	0.0667
			0.5	2	4956	9911.2											
	4	2	0.5	1	4979	9958.8	9879.3	19758.6	0.1	155567	12.70	19497	0.0253		0.013	15	0.0661
			0.5	2	4900	9799.8											
Background control	1	2	0.5	1	65	130.08	129.86	259.72	0.1	155567	0.17	-2	0.0000		0.013	15	0.0000
			0.5	2	65	129.64											
	2	2	0.5	1	68	131.14	137.15	274.3	0.1	155567	0.18	13	0.0000		0.013	15	0.0000
			0.5	2	72	143.16											
	3	2	0.5	1	63	125.92	129.73	259.46	0.1	155567	0.17	-2	0.0000		0.013	15	0.0000
			0.5	2	67	133.54											
	4	2	0.5	1	62	124.1	126.13	252.26	0.1	155567	0.16	-9	0.0000		0.013	15	0.0000
			0.5	2	64	128.16											
Positive control	1	2	0.5	1	2310	4619	4585.8	9171.6	0.1	155567	5.90	8810	0.0116		0.013	15	0.0302
			0.5	2	2276	4552.6											
	2	2	0.5	1	2329	4658.6	4587.1	9174.2	0.1	155567	5.90	8813	0.0116		0.013	15	0.0302
			0.5	2	2258	4515.6											
	3	2	0.5	1	2173	4345.8	4369.5	8739	0.1	155567	5.62	8478	0.0110		0.013	15	0.0287
			0.5	2	2197	4393.2											
	4	2	0.5	1	2226	4452	4390.1	8780.2	0.1	155567	5.64	8519	0.0111		0.013	15	0.0289
			0.5	2	2164	4328.2											
Negative Control	1	2	0.5	1	5343	10686	10577.4	21154.8	0.1	155567	13.60	20893	0.0271		0.013	15	0.0708
			0.5	2	5234	10468.8											
	2	2	0.5	1	5253	10505.2	10606.8	21213.6	0.1	155567	13.64	20952	0.0272		0.013	15	0.0710
			0.5	2	5354	10708.4											
	3	2	0.5	1	5072	10143.4	10078.3	20156.6	0.1	155567	12.96	19895	0.0258		0.013	15	0.0675
			0.5	2	5007	10013.2											
	4	2	0.5	1	5004	10007	10082.7	20165.4	0.1	155567	12.96	19904	0.0258		0.013	15	0.0675
			0.5	2	5079	10158.4											
2	1-1	2	0.5	1	114	228.88	229.16	458.32	0.1	155567	0.29	197	0.0003		0.013	15	0.0007
			0.5	2	115	229.44											
	1-2	2	0.5	1	90	180.92	181.62	363.24	0.1	155567	0.23	102	0.0001		0.013	15	0.0003
			0.5	2	91	182.32											
	1-3	2	0.5	1	87	173.64	171.3	342.6	0.1	155567	0.22	81	0.0001		0.013	15	0.0003
			0.5	2	84	168.96											
	2-1	2	0.5	1	279	558.54	548.17	1096.34	0.1	155567	0.70	835	0.0011		0.013	15	0.0028
			0.5	2	269	537.8											
	2-2	2	0.5	1	268	535.16	534.05	1068.1	0.1	155567	0.69	807	0.0010		0.013	15	0.0027
			0.5	2	266	532.92											
	2-3	2	0.5	1	262	524.86	532.79	1065.58	0.1	155567	0.68	804	0.0010		0.013	15	0.0027
			0.5	2	270	540.72											
	3-1	2	0.5	1	1565	3129.4	3127.9	6255.8	0.1	155567	4.02	5994	0.0078		0.013	15	0.0203
			0.5	2	1563	3126.4											
	3-2	2	0.5	1	1631	3262.8	3227.7	6455.4	0.1	155567	4.15	6194	0.0080		0.013	15	0.0210
			0.5	2	1596	3192.6											
	3-3	2	0.5	1	1543	3085	3118.2	6236.4	0.1	155567	4.01	5979	0.0078		0.013	15	0.0203
			0.5	2	1576	3151.4											
	4-1	2	0.5	1	3328	6656.6	6648.3	13296.6	0.1	155567	8.55	13035	0.0169		0.013	15	0.0442
			0.5	2	3321	6641											
	4-2	2	0.5	1	3192	6384.8	6394.8	12789.6	0.1	155567	8.22	12528	0.0163		0.013	15	0.0425
			0.5	2	3202	6404.8											
	4-3	2	0.5	1	3245	6489.6	6458.9	12917.8	0.1	155567	8.30	12656	0.0164		0.013	15	0.0429
			0.5	2	3214	6428.2											
	5-1	2	0.5	1	3967	7973.4	8033.8	16067.6	0.1	155567	10.33	15806	0.0205		0.013	15	0.0536
			0.5	2	4047	8094.2											
	5-2	2	0.5	1	4021	8042.8	8032.1	16064.2	0.1	155567	10.33	15803	0.0205		0.013	15	0.0536
			0.5	2	4011	8021.4											
	5-3	2	0.5	1	3840	7679.2	7713.8	15427.6	0.1	155567	9.92	15166	0.0197		0.013	15	0.0514
			0.5	2	3874	7748.4											
	6-1	2	0.5	1	5136	10271	10176.8	20353.6	0.1	155567	13.08	20092	0.0261		0.013	15	0.0681
			0.5	2	5041	10082.6											
	6-2	2	0.5	1	5001	10002.6	9975.4	19950.8	0.1	155567	12.82	19689	0.0255		0.013	15	0.0668
			0.5	2	4974	9948.2											
	6-3	2	0.5	1	4754	9507.4	9579.9	19159.8	0.1	155567	12.32	18898	0.0245		0.013	15	0.0641
			0.5	2	4826	9652.4											
	7-1	2	0.5	1	5180	10360.8	10246.3	20492.6	0.1	155567	13.17	20231	0.0262		0.013	15	0.0686
			0.5	2	5066	10132											
	7-2	2	0.5	1	5138	10276.8	10132.8	20265.6	0.1	155567	13.03	20004	0.0260		0.013	15	0.0678
			0.5	2	4994	9988.8											
	7-3	2	0.5	1	5040	10080.2	10157.5	20315	0.1	155567	13.06	20064	0.0260		0.013	15	0.0680
			0.5	2	5117	10234.8											
	8-1	2	0.5	1	5133	10266	10196.5	20393	0.1	155567	13.11	20132	0.0261		0.013	15	0.0683
			0.5	2	5064	10127											
	8-2	2	0.5	1	5012	10024.8	10112	20224	0.1	155567	13.00	19963	0.0259		0.013	15	0.0677
			0.5	2	5100	10199.2											
	8-3	2	0.5	1	5015	10029.8	9960.1	19920.2	0.1	155567	12.80	19659	0.0255		0.013	15	0.0667
			0.5	2	4945	9890.4											

Assay Date	8/11/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0691	0.0018
Full activity	End	0.0664	0.0004
Full activity	Overall	0.0678	0.0019
Background	Beginning	0.0000	3.49548E-05
Background	End	0.0000	1.72616E-05
Background	Overall	0.0000	3.13526E-05
Positive	Beginning	0.0302	0.0000
Positive	End	0.0288	0.0001
Positive	Overall	0.0295	0.0008
Negative	Beginning	0.0709	0.0001
Negative	End	0.0675	0.0000
Negative	Overall	0.0692	0.0020

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
2	1	1	1.00E-04	-4.00	0.0007
2	1	2	1.00E-04	-4.00	0.0003
2	1	3	1.00E-04	-4.00	0.0003
2	2	1	1.00E-05	-5.00	0.0028
2	2	2	1.00E-05	-5.00	0.0027
2	2	3	1.00E-05	-5.00	0.0027
2	3	1	1.00E-06	-6.00	0.0203
2	3	2	1.00E-06	-6.00	0.0210
2	3	3	1.00E-06	-6.00	0.0203
2	4	1	2.50E-07	-6.60	0.0442
2	4	2	2.50E-07	-6.60	0.0425
2	4	3	2.50E-07	-6.60	0.0429
2	5	1	1.00E-07	-7.00	0.0536
2	5	2	1.00E-07	-7.00	0.0536
2	5	3	1.00E-07	-7.00	0.0514
2	6	1	1.00E-08	-8.00	0.0681
2	6	2	1.00E-08	-8.00	0.0668
2	6	3	1.00E-08	-8.00	0.0641
2	7	1	1.00E-09	-9.00	0.0686
2	7	2	1.00E-09	-9.00	0.0678
2	7	3	1.00E-09	-9.00	0.0680
2	8	1	1.00E-10	-10.00	0.0683
2	8	2	1.00E-10	-10.00	0.0677
2	8	3	1.00E-10	-10.00	0.0667

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.99	0.51	0.41
2	-5.00	4.18	4.04	4.02
3	-6.00	29.99	30.99	29.90
4	-6.60	65.23	62.69	63.33
5	-7.00	79.09	79.07	75.89
6	-8.00	100.54	98.52	94.56
7	-9.00	101.23	100.10	100.34
8	-10.00	100.73	99.89	98.37

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0195	28586	1465949	
2	0.0197	30466	1546497	
3	0.0197	31439	1595888	
4	0.0197	32189	1633959	
5	0.0196	33210	1694388	
			Average DPM/g soln	1587336
			SD	86761
			CV	5.47
			µCi/g soln	0.715

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.4	10		1040.00
Dilution A			100	10.40
Dilution B			10	1.04

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6304 g
Mass of dilution B used in substrate prep	5.429 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.586285 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00809 µg/g soln.
µg/g soln.

a. µCi/g soln 0.715
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.586285 + 0.00809
= 0.594379 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.203 µCi/µg ASDN

764854 dpm/nmol

QC: RG 1-30-06

Assay Date	8/12/2005	Test Chemical ID	2	# Concentrations tested	8						
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305				
								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID	
Standards:	250	125	50	25	10	5	0	25	100	11343-77	
	0.511	0.302	0.130	0.061	0.023	0.012	0.000				
	0.507	0.300	0.127	0.065	0.023	0.013	0.000				
	0.501	0.297	0.128	0.063	0.024	0.011	0.000				
Samples:	QC 100	QC 10	Microsomes								
	0.264	0.032	0.067								
	0.261	0.032	0.069								
			0.070								

Standard concentration (pg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL		mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	Used 200	0.0500	0.506	0.506	0.0480	m, b	0.097	-0.001
125	100	200	0.00013	200	0.0250	0.300	0.300	0.0279	se_m, se_b	0.005	0.001
50	200	1000	0.00005	200	0.0100	0.128	0.128	0.0112	r^2, se_y	0.991	0.002
25	100	1000	0.00003	200	0.0050	0.063	0.063	0.0049	F, df	448	4
10	40	1000	0.00001	200	0.0020	0.023	0.023	0.0010	SS_{reg}, SS_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.012	0.012	-0.0001			

Regression results are calculated using the function LINEST

Blank 0.000

r^2= 0.991
m= 0.097
b= -0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.264	0.264	0.024	200	1	1	0.000	0.000	0.122
QC 100	0.261	0.261	0.024	200	1	1	0.000		
QC 100									
QC 10	0.032	0.032	0.002	200	1	1	0.000	0.000	0.009
QC 10	0.032	0.032	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.067	0.067	0.005	200	1	949	0.025	0.026	25.541
Microsomes	0.069	0.069	0.005	200	1	949	0.026		
Microsomes	0.070	0.070	0.006	200	1	949	0.026		

Assay Date	8/12/2005	Test Chemical ID	2	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.511	0.302	0.130	0.061	0.023	0.012	0.000			
	0.507	0.300	0.127	0.065	0.023	0.013	0.000			
	0.501	0.297	0.128	0.063	0.024	0.011	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.264	0.032	0.067
	0.261	0.032	0.069
			0.070

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
LE 2-22-06 KBK											
250	200	200	0.00025	200	0.0500	0.506	0.506	0.0420	m, b	0.083	0.000
125	100	200	0.00013	200	0.0250	0.300	0.300	0.0248	se_m, se_b	0.001	0.000
50	200	1000	0.00005	200	0.0100	0.128	0.128	0.0105	r^2, se_y	0.999	0.000
25	100	1000	0.00003	200	0.0050	0.063	0.063	0.0051	F, df	3114	3
10	40	1000	0.00001	200	0.0020	0.023	0.023	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.012	0.012	0.0008			

Blank 0.000

r^2= 0.999
m= 0.083
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.264	0.264	0.022	200	1	1	0.000	0.000	0.108
QC 100	0.261	0.261	0.022	200	1	1	0.000		
QC 100									
QC 10	0.032	0.032	0.002	200	1	1	0.000	0.000	0.012
QC 10	0.032	0.032	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.067	0.067	0.005	200	1	949	0.026	0.026	26.169
Microsomes	0.069	0.069	0.006	200	1	949	0.026		
Microsomes	0.070	0.070	0.006	200	1	949	0.027		

QC: WE
1-30-06

G608316

Assay Date	8/12/2005	Test Chemical ID		# Concentrations tested	2	Microsome type	8	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Microsome Dilution Details		
Dilution A	0.1	mL microsome Stock used
	5	mL total volume
	50	dilution factor
Dilution B	2.5	mL microsome Dilution A used
	47.5	mL total volume
	19	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	950	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	26.1691
Protein Concentration (dilution added to assay, mg/mL):	0.027546

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	5.00E-07
5	2.50E-07
6	1.00E-07
7	1.00E-08
8	1.00E-09

Battelle Study No. G608316

B-28

G608316

Battelle Study No. G608316

B-29

Assay Date	8/12/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	19034	10088.8	10064.7	20129.4	0.1	158734	12.68	19795	0.0256	1	0.014	15	0.0625
			0.5	2	19030	10060.6											
	2	2	0.5	1	8990	9868.6	9781.1	19562.2	0.1	158734	12.32	19198	0.0251	1	0.014	15	0.0607
			0.5	2	18851	9702.6											
	3	2	0.5	1	18678	9357.8	9307.6	18615.2	0.1	158734	11.73	18251	0.0239	1	0.014	15	0.0577
			0.5	2	18629	9257.4											
	4	2	0.5	1	18284	8567.4	8731.3	17462.6	0.1	158734	11.00	17098	0.0224	1	0.014	15	0.0541
			0.5	2	18448	8895.2											
Background control	1	2	0.5	1	182	164.64	158.97	317.94	0.1	158734	0.20	-46	-0.0001	1	0.014	15	-0.0001
			0.5	2	197	153.3											
	2	2	0.5	1	127	253.8	238.37	476.74	0.1	158734	0.30	112	0.0001	1	0.014	15	0.0004
			0.5	2	111	222.94											
	3	2	0.5	1	183	165.76	164.59	329.18	0.1	158734	0.21	-35	0.0000	1	0.014	15	-0.0001
			0.5	2	82	163.42											
	4	2	0.5	1	81	162.52	168.87	333.74	0.1	158734	0.21	-31	0.0000	1	0.014	15	-0.0001
			0.5	2	88	171.22											
Positive control	1	2	0.5	1	2134	4268.8	4273.3	8546.6	0.1	158734	5.38	8182	0.0107	1	0.014	15	0.0269
			0.5	2	2138	4277.8											
	2	2	0.5	1	2206	4411.4	4479.8	8959.6	0.1	158734	5.64	8565	0.0112	1	0.014	15	0.0272
			0.5	2	2274	4548.2											
	3	2	0.5	1	2192	4384.6	4392.1	8784.2	0.1	158734	5.53	8420	0.0110	1	0.014	15	0.0266
			0.5	2	2200	4399.6											
	4	2	0.5	1	2154	4307	4374.4	8748.8	0.1	158734	5.51	8384	0.0110	1	0.014	15	0.0265
			0.5	2	2221	4441.8											
Negative Control	1	2	0.5	1	4842	9683.8	9648.7	19297.4	0.1	158734	12.16	18933	0.0248	1	0.014	15	0.0596
			0.5	2	4807	9613.6											
	2	2	0.5	1	4789	9577.2	9461.3	18922.6	0.1	158734	11.93	18556	0.0243	1	0.014	15	0.0587
			0.5	2	4673	9345.4											
	3	2	0.5	1	4752	9503.2	9448.4	18896.8	0.1	158734	11.90	18532	0.0242	1	0.014	15	0.0586
			0.5	2	4697	9393.6											
	4	2	0.5	1	4836	9671.4	9662	18324	0.1	158734	12.17	18960	0.0248	1	0.014	15	0.0600
			0.5	2	4826	9652.6											
2	1-1	2	0.5	1	124	248.6	241.37	482.74	0.1	158734	0.30	118	0.0002	1	0.014	15	0.0004
			0.5	2	117	234.14											
	1-2	2	0.5	1	82	164.12	167.74	335.48	0.1	158734	0.21	-29	0.0000	1	0.014	15	-0.0001
			0.5	2	88	171.36											
	1-3	2	0.5	1	80	159.04	168.29	336.58	0.1	158734	0.21	-28	0.0000	1	0.014	15	-0.0001
			0.5	2	89	177.54											
	2-1	2	0.5	1	290	579.96	578.12	1156.24	0.1	158734	0.73	792	0.0010	1	0.014	15	0.0025
			0.5	2	288	576.28											
	2-2	2	0.5	1	262	523.32	529.33	1058.66	0.1	158734	0.67	894	0.0009	1	0.014	15	0.0022
			0.5	2	268	535.34											
	2-3	2	0.5	1	289	577	579.48	1158.96	0.1	158734	0.73	795	0.0010	1	0.014	15	0.0025
			0.5	2	291	581.96											
	3-1	2	0.5	1	1588	3175.2	3180.1	6360.2	0.1	158734	4.01	5996	0.0078	1	0.014	15	0.0190
			0.5	2	1593	3185											
	3-2	2	0.5	1	1562	3104.8	3123.2	6246.4	0.1	158734	3.94	5882	0.0077	1	0.014	15	0.0186
			0.5	2	1571	3141.6											
	3-3	2	0.5	1	1571	3142.6	3104.9	6209.8	0.1	158734	3.91	5845	0.0076	1	0.014	15	0.0185
			0.5	2	1534	3067.2											
	4-1	2	0.5	1	2300	4600.2	4618.9	9237.8	0.1	158734	5.82	8873	0.0116	1	0.014	15	0.0281
			0.5	2	2319	4637.6											
	4-2	2	0.5	1	2274	4547.8	4484.7	8969.4	0.1	158734	5.65	8605	0.0113	1	0.014	15	0.0272
			0.5	2	2211	4421.6											
	4-3	2	0.5	1	2258	4516	4554.6	9109.2	0.1	158734	5.74	8745	0.0114	1	0.014	15	0.0277
			0.5	2	2297	4593.2											
	5-1	2	0.5	1	3024	6048.5	6128.9	12257.8	0.1	158734	7.72	11893	0.0155	1	0.014	15	0.0376
			0.5	2	3105	6209											
	5-2	2	0.5	1	3117	6234.4	6208.5	12417	0.1	158734	7.82	12053	0.0158	1	0.014	15	0.0381
			0.5	2	3091	6182.6											
	5-3	2	0.5	1	3037	6074.6	6044.5	12089	0.1	158734	7.62	11725	0.0153	1	0.014	15	0.0371
			0.5	2	3007	6014.4											
	6-1	2	0.5	1	3900	7800.8	7768.4	15536.8	0.1	158734	9.79	15172	0.0198	1	0.014	15	0.0480
			0.5	2	3868	7736											
	6-2	2	0.5	1	3870	7740	7804.3	15608.6	0.1	158734	9.83	15244	0.0199	1	0.014	15	0.0482
			0.5	2	3934	7868.6											
	6-3	2	0.5	1	3755	7509.8	7518	15036	0.1	158734	9.47	14672	0.0192	1	0.014	15	0.0464
			0.5	2	3753	7506.2											
	7-1	2	0.5	1	4473	8946.4	9009	18018	0.1	158734	11.35	17654	0.0231	1	0.014	15	0.0559
			0.5	2	4536	9071.6											
	7-2	2	0.5	1	4468	8932.6	9033.4	18066.8	0.1	158734	11.38	17702	0.0231	1	0.014	15	0.0560
			0.5	2	4567	9134.2											
	7-3	2	0.5	1	4469	8937	8988.4	17976.8	0.1	158734	11.33	17612	0.0230	1	0.014	15	0.0557
			0.5	2	4520	9039.8											
	8-1	2	0.5	1	4718	9435	9439.6	18879.2	0.1	158734	11.89	18515	0.0242	1	0.014	15	0.0586
			0.5	2	4722	9444.2											
	8-2	2	0.5	1	4672	9343.8	9349.4	18698.8	0.1	158734	11.78	18334	0.0240	1	0.014	15	0.0580
			0.5	2	4678	9355											
	8-3	2	0.5	1	4660	9319.6	9322.5	18645	0.1	158734	11.75	18281	0.0239	1	0.014	15	0.0578
			0.5	2	4663	9325.4											

QC: WT 2-14-06

G608316

Assay Date	8/12/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type	placental	Microsome ID 6-041305	Technician ID TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0616	0.0013
Full activity	End	0.0559	0.0026
Full activity	Overall	0.0588	0.0037
Background	Beginning	0.0001	0.000355304
Background	End	-0.0001	1.02027E-05
Background	Overall	0.0000	0.000237908
Positive	Beginning	0.0265	0.0009
Positive	End	0.0266	0.0001
Positive	Overall	0.0266	0.0005
Negative	Beginning	0.0593	0.0008
Negative	End	0.0593	0.0010
Negative	Overall	0.0593	0.0007

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
2	1	1	1.00E-04	-4.00	0.0004
2	1	2	1.00E-04	-4.00	-0.0001
2	1	3	1.00E-04	-4.00	-0.0001
2	2	1	1.00E-05	-5.00	0.0025
2	2	2	1.00E-05	-5.00	0.0022
2	2	3	1.00E-05	-5.00	0.0025
2	3	1	1.00E-06	-6.00	0.0190
2	3	2	1.00E-06	-6.00	0.0186
2	3	3	1.00E-06	-6.00	0.0185
2	4	1	5.00E-07	-6.30	0.0281
2	4	2	5.00E-07	-6.30	0.0272
2	4	3	5.00E-07	-6.30	0.0277
2	5	1	2.50E-07	-6.60	0.0376
2	5	2	2.50E-07	-6.60	0.0381
2	5	3	2.50E-07	-6.60	0.0371
2	6	1	1.00E-07	-7.00	0.0480
2	6	2	1.00E-07	-7.00	0.0482
2	6	3	1.00E-07	-7.00	0.0464
2	7	1	1.00E-08	-8.00	0.0559
2	7	2	1.00E-08	-8.00	0.0560
2	7	3	1.00E-08	-8.00	0.0557
2	8	1	1.00E-09	-9.00	0.0586
2	8	2	1.00E-09	-9.00	0.0580
2	8	3	1.00E-09	-9.00	0.0578

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.64	-0.16	-0.15
2	-5.00	4.26	3.74	4.28
3	-6.00	32.27	31.66	31.46
4	-6.30	47.76	46.32	47.07
5	-6.60	64.02	64.88	63.11
6	-7.00	81.67	82.06	78.97
7	-8.00	95.02	95.29	94.80
8	-9.00	99.66	98.69	98.40

Assay Date	8/15/2005	Test Chemical ID	2	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0197	28853	1464619
2	0.0197	30434	1544873
3	0.0202	32071	1587673
4	0.0201	32855	1634577
5	0.0199	32137	1614925
		Average DPM/g soln	1569333
		SD	67519
		CV	4.30
		µCi/g soln	0.707

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.5	10		1050.00
Dilution A			100	10.50
Dilution B			10	1.05

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6012 g
Mass of dilution B used in substrate prep	5.4232 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.593088 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00800 µg/g soln.
µg/g soln.

a. µCi/g soln	0.707
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.593088 + 0.00800
= 0.601091 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.176 µCi/µg ASDN

747736 dpm/nmol

Assay Date	8/15/2005	Test Chemical ID	2	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

Standards:								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	250	125	50	25	10	5	0	25	100	11343-77
	0.516	0.294	0.132	0.064	0.023	0.012	0.000			
	0.514	0.296	0.140	0.060	0.024	0.015	0.000			
	0.510	0.302	0.131	0.064	0.026	0.012	0.000			

Samples:			
	QC 100	QC 10	Microsomes
	0.264	0.028	0.066
	0.260	0.028	0.067
			0.066

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.513	0.513	0.0483
125	100	200	0.00013	200	0.0250	0.298	0.298	0.0274
50	200	1000	0.00005	200	0.0100	0.134	0.134	0.0116
25	100	1000	0.00003	200	0.0050	0.063	0.063	0.0047
10	40	1000	0.00001	200	0.0020	0.024	0.024	0.0010
5	20	1000	0.00001	200	0.0010	0.013	0.013	-0.0001
			Blank	0.000				

Variables	Regression results	
m, b	0.097	-0.001
se_m, se_b	0.004	0.001
r^2, se_y	0.992	0.002
F, df	526	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.992
m= 0.097
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.264	0.264	0.024	200	1	1	0.000	0.000	0.120
QC 100	0.260	0.260	0.024	200	1	1	0.000		
QC 100									
QC 10	0.028	0.028	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.028	0.028	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.066	0.066	0.005	200	1	949	0.024	0.024	24.065
Microsomes	0.067	0.067	0.005	200	1	949	0.024		
Microsomes	0.066	0.066	0.005	200	1	949	0.024		

Assay Date 8/15/2005 | Test Chemical ID 2 | # Concentrations tested 8

Technician ID TD | Replicate # 3 | Microsome type placental | Microsome ID 6-041305

Standards:	250	125	50	25	10	5	0
	0.516	0.294	0.132	0.064	0.023	0.012	0.000
	0.514	0.296	0.140	0.060	0.024	0.015	0.000
	0.510	0.302	0.131	0.064	0.026	0.012	0.000

Protein stock (mg BSA): 25 | Total volume of stock (mL): 100 | Protein stock ID: 11343-77

Samples:	QC 100	QC 10	Microsomes
	0.264	0.028	0.066
	0.260	0.028	0.067
			0.066

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL
250	200	200	0.00025
125	100	200	0.00013
50	200	1000	0.00005
25	100	1000	0.00003
10	40	1000	0.00001
5	20	1000	0.00001

LE 3-23-06

	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
	200	0.0500	0.513	0.513	0.0427
	200	0.0250	0.298	0.298	0.0246
	200	0.0100	0.134	0.134	0.0109
	200	0.0050	0.063	0.063	0.0049
	200	0.0020	0.024	0.024	0.0017
	200	0.0010	0.013	0.013	0.0008
Blank	0.000				

r^2= 0.997
m= 0.084
b= 0.000

Variables	Regression results	
m, b	0.084	0.000
se_m, se_b	0.003	0.000
r^2, se_y	0.997	0.001
F, df	1010	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)
QC 100	0.264	0.264	0.022	200	1	1
QC 100	0.260	0.260	0.021	200	1	1
QC 100						
QC 10	0.028	0.028	0.002	200	1	1
QC 10	0.028	0.028	0.002	200	1	1
QC 10						
Microsomes	0.066	0.066	0.005	200	1	949
Microsomes	0.067	0.067	0.005	200	1	949
Microsomes	0.066	0.066	0.005	200	1	949

mg protein/µL Prep.	average mg/µL	mg/mL
0.000	0.000	0.108
0.000		
0.000	0.000	0.010
0.000		
0.025	0.025	24.869
0.025		
0.025		

Assay Date	8/15/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	3

Microsome Dilution Details		
Dilution A	0.1	mL microsome Stock used
	5	mL total volume
	50	dilution factor
Dilution B	2.5	mL microsome Dilution A used
	47.5	mL total volume
	19	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	950	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	24.86909
Protein Concentration (dilution added to assay, mg/mL):	0.026178

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	5.00E-07
5	2.50E-07
6	1.00E-07
7	1.00E-08
8	1.00E-09

Assay Date	8/15/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3967	7933.2	7881.2	15762.4	0.1	156933	10.04	15489	0.0207	1	0.013	15	0.0528
			0.5	2	3915	7829.2											
	2	2	0.5	1	3889	7778.8	7723	15446	0.1	156933	9.84	15173	0.0203	1	0.013	15	0.0517
			0.5	2	3834	7667.2											
	3	2	0.5	1	3638	7276.8	7276.7	14553.4	0.1	156933	9.27	14280	0.0191	1	0.013	15	0.0486
			0.5	2	3638	7276.6											
	4	2	0.5	1	3384	6768.4	6825.3	13650.6	0.1	156933	8.70	13377	0.0179	1	0.013	15	0.0456
			0.5	2	3441	6882.2											
Background control	1	2	0.5	1	72	144.64	135.55	271.1	0.1	156933	0.17	-2	0.0000	1	0.013	15	0.0000
			0.5	2	63	126.46											
	2	2	0.5	1	74	148.02	135.5	271	0.1	156933	0.17	-2	0.0000	1	0.013	15	0.0000
			0.5	2	61	122.98											
	3	2	0.5	1	71	141.34	141.83	283.66	0.1	156933	0.18	10	0.0000	1	0.013	15	0.0000
			0.5	2	71	142.32											
	4	2	0.5	1	69	138.6	133.68	267.36	0.1	156933	0.17	-6	0.0000	1	0.013	15	0.0000
			0.5	2	64	128.86											
Positive control	1	2	0.5	1	2023	4045	4024.7	8049.4	0.1	156933	5.13	7776	0.0104	1	0.013	15	0.0265
			0.5	2	2002	4004.4											
	2	2	0.5	1	1803	3606.4	3593.1	7186.2	0.1	156933	4.58	6913	0.0092	1	0.013	15	0.0235
			0.5	2	1790	3579.8											
	3	2	0.5	1	1876	3751.6	3784.9	7569.8	0.1	156933	4.82	7297	0.0098	1	0.013	15	0.0249
			0.5	2	1909	3818.2											
	4	2	0.5	1	1960	3919.4	3902.1	7804.2	0.1	156933	4.97	7531	0.0101	1	0.013	15	0.0256
			0.5	2	1942	3884.8											
Negative Control	1	2	0.5	1	3705	7409.6	7418	14836	0.1	156933	9.45	14563	0.0195	1	0.013	15	0.0496
			0.5	2	3713	7426.4											
	2	2	0.5	1	3694	7388.4	7422.5	14845	0.1	156933	9.46	14572	0.0195	1	0.013	15	0.0496
			0.5	2	3728	7456.6											
	3	2	0.5	1	3616	7231.4	7238.8	14477.6	0.1	156933	9.23	14204	0.0190	1	0.013	15	0.0484
			0.5	2	3623	7246.2											
	4	2	0.5	1	3643	7286.4	7245	14490	0.1	156933	9.23	14217	0.0190	1	0.013	15	0.0484
			0.5	2	3602	7203.6											
2	1-1	2	0.5	1	88	175.18	158.09	316.18	0.1	156933	0.20	43	0.0001	1	0.013	15	0.0001
			0.5	2	71	141											
	1-2	2	0.5	1	83	165.36	162.85	325.7	0.1	156933	0.21	52	0.0001	1	0.013	15	0.0002
			0.5	2	80	160.34											
	1-3	2	0.5	1	84	168.56	162.99	325.98	0.1	156933	0.21	53	0.0001	1	0.013	15	0.0002
			0.5	2	79	157.42											
	2-1	2	0.5	1	247	493.9	511.99	1023.98	0.1	156933	0.65	751	0.0010	1	0.013	15	0.0026
			0.5	2	265	530.08											
	2-2	2	0.5	1	262	523.92	531.4	1062.8	0.1	156933	0.68	790	0.0011	1	0.013	15	0.0027
			0.5	2	269	538.88											
	2-3	2	0.5	1	245	489.42	516.32	1032.64	0.1	156933	0.66	759	0.0010	1	0.013	15	0.0026
			0.5	2	272	543.22											
	3-1	2	0.5	1	1338	2676.4	2678	5356	0.1	156933	3.41	5083	0.0068	1	0.013	15	0.0173
			0.5	2	1340	2679.6											
	3-2	2	0.5	1	1364	2727.8	2766	5532	0.1	156933	3.53	5259	0.0070	1	0.013	15	0.0179
			0.5	2	1402	2804.2											
	3-3	2	0.5	1	1368	2736.6	2730.9	5461.8	0.1	156933	3.48	5189	0.0069	1	0.013	15	0.0177
			0.5	2	1363	2725.2											
	4-1	2	0.5	1	1921	3842.6	3833.4	7666.8	0.1	156933	4.89	7394	0.0099	1	0.013	15	0.0252
			0.5	2	1912	3824.2											
	4-2	2	0.5	1	1954	3908	3893.6	7787.2	0.1	156933	4.96	7514	0.0100	1	0.013	15	0.0256
			0.5	2	1940	3879.2											
	4-3	2	0.5	1	1924	3848.6	3879.5	7759	0.1	156933	4.94	7486	0.0100	1	0.013	15	0.0255
			0.5	2	1955	3910.4											
	5-1	2	0.5	1	2568	5130.4	5156.7	10313.4	0.1	156933	6.57	10040	0.0134	1	0.013	15	0.0342
			0.5	2	2592	5183											
	5-2	2	0.5	1	2379	4757.2	4737.4	9474.8	0.1	156933	6.04	9202	0.0123	1	0.013	15	0.0313
			0.5	2	2359	4717.6											
	5-3	2	0.5	1	2518	5036.6	5142.4	10284.8	0.1	156933	6.55	10012	0.0134	1	0.013	15	0.0341
			0.5	2	2624	5248.2											
	6-1	2	0.5	1	3041	6081.2	6134.2	12268.4	0.1	156933	7.82	11995	0.0160	1	0.013	15	0.0409
			0.5	2	3094	6187.2											
	6-2	2	0.5	1	3075	6150.6	6204.7	12409.4	0.1	156933	7.91	12136	0.0162	1	0.013	15	0.0413
			0.5	2	3129	6258.8											
	6-3	2	0.5	1	3043	6085.4	6115.6	12231.2	0.1	156933	7.79	11958	0.0160	1	0.013	15	0.0407
			0.5	2	3073	6145.8											
	7-1	2	0.5	1	3574	7148.8	7105	14210	0.1	156933	9.05	13937	0.0186	1	0.013	15	0.0475
			0.5	2	3531	7061.2											
	7-2	2	0.5	1	3527	7053.6	7101.7	14203.4	0.1	156933	9.05	13930	0.0186	1	0.013	15	0.0474
			0.5	2	3575	7149.8											
	7-3	2	0.5	1	3072	6143.2	6159.1	12318.2	0.1	156933	7.85	12045	0.0161	1	0.013	15	0.0410
			0.5	2	3088	6175											
	8-1	2	0.5	1	3635	7270.8	7295.4	14590.8	0.1	156933	9.30	14318	0.0191	1	0.013	15	0.0488
			0.5	2	3660	7320											
	8-2	2	0.5	1	3606	7212.8	7221.3	14442.6	0.1	156933	9.20	14169	0.0189	1	0.013	15	0.0483
			0.5	2	3615	7229.8											
	8-3	2	0.5	1	3466	6932.6	7009.3	14018.6	0.1	156933	8.93	13745	0.0184	1	0.013	15	0.0468
			0.5	2	3543	7086											

Assay Date	8/15/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0522	0.0008
Full activity	End	0.0471	0.0022
Full activity	Overall	0.0497	0.0032
Background	Beginning	0.0000	2.40829E-07
Background	End	0.0000	3.92552E-05
Background	Overall	0.0000	2.4302E-05
Positive	Beginning	0.0250	0.0021
Positive	End	0.0252	0.0006
Positive	Overall	0.0251	0.0013
Negative	Beginning	0.0496	0.0000
Negative	End	0.0484	0.0000
Negative	Overall	0.0490	0.0007

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
2	1	1	1.00E-04	-4.00	0.0001
2	1	2	1.00E-04	-4.00	0.0002
2	1	3	1.00E-04	-4.00	0.0002
2	2	1	1.00E-05	-5.00	0.0026
2	2	2	1.00E-05	-5.00	0.0027
2	2	3	1.00E-05	-5.00	0.0026
2	3	1	1.00E-06	-6.00	0.0173
2	3	2	1.00E-06	-6.00	0.0179
2	3	3	1.00E-06	-6.00	0.0177
2	4	1	5.00E-07	-6.30	0.0252
2	4	2	5.00E-07	-6.30	0.0256
2	4	3	5.00E-07	-6.30	0.0255
2	5	1	2.50E-07	-6.60	0.0342
2	5	2	2.50E-07	-6.60	0.0313
2	5	3	2.50E-07	-6.60	0.0341
2	6	1	1.00E-07	-7.00	0.0409
2	6	2	1.00E-07	-7.00	0.0413
2	6	3	1.00E-07	-7.00	0.0407
2	7	1	1.00E-08	-8.00	0.0475
2	7	2	1.00E-08	-8.00	0.0474
2	7	3	1.00E-08	-8.00	0.0410
2	8	1	1.00E-09	-9.00	0.0488
2	8	2	1.00E-09	-9.00	0.0483
2	8	3	1.00E-09	-9.00	0.0468

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.29	0.36	0.36
2	-5.00	5.15	5.42	5.21
3	-6.00	34.86	36.07	35.59
4	-6.30	50.71	51.54	51.34
5	-6.60	68.86	63.11	68.67
6	-7.00	82.27	83.24	82.02
7	-8.00	95.59	95.54	82.61
8	-9.00	98.20	97.18	94.28

Assay Date	8/2/2005	Test Chemical ID	3	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0195	27757	1423436
2	0.0197	28885	1466244
3	0.0196	30594	1560918
4	0.0196	32460	1656122
5	0.0197	33073	1678832
		Average DPM/g soln	1557111
		SD	112650
		CV	7.23
		µCi/g soln	0.701

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.4	10		1040.00
Dilution A			100	10.40
Dilution B			10	1.04

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6521 g
Mass of dilution B used in substrate prep	5.4353 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.585646 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00794 µg/g soln.
µg/g soln.

a. µCi/g soln	0.701
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.585646 + 0.00794
= 0.593586 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.182 µCi/µg ASDN

751292 dpm/nmol

Assay Date	8/2/2005	Test Chemical ID	3	# Concentrations tested	8	
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID BAA

Standards:								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	250	125	50	25	10	5	0	25	100	11343-77
	0.522	0.307	0.142	0.069	0.032	0.017	0.000			
	0.523	0.305	0.140	0.067	0.031	0.016	0.000			
	0.521	0.309	0.137	0.067	0.030	0.016	0.000			

Samples:			
	QC 100	QC 10	Microsomes
	0.261	0.037	0.069
	0.265	0.034	0.069
			0.068

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL		Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200	0.0500	0.522	0.522	0.0482	m, b	0.096	-0.002
125	100	200	0.00013		200	0.0250	0.307	0.307	0.0276	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200	0.0100	0.140	0.140	0.0116	r^2, se_y	0.992	0.002
25	100	1000	0.00003		200	0.0050	0.068	0.068	0.0047	F, df	482	4
10	40	1000	0.00001		200	0.0020	0.031	0.031	0.0012	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010	0.016	0.016	-0.0002			
				Blank	0.000							

r^2= 0.992
m= 0.096
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.261	0.261	0.023	200	1	1	0.000	0.000	0.117
QC 100	0.265	0.265	0.024	200	1	1	0.000		
QC 100									
QC 10	0.037	0.037	0.002	200	1	1	0.000	0.000	0.008
QC 10	0.034	0.034	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.069	0.069	0.005	200	120	42000	0.008	0.008	8.397
Microsomes	0.069	0.069	0.005	200	120	42000	0.008		
Microsomes	0.068	0.068	0.005	200	120	42000	0.008		

Assay Date 8/2/2005 | Test Chemical ID 3 | # Concentrations tested 8

Technician ID TD | Replicate # 1 | Microsome type placental | Microsome ID BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.522	0.307	0.142	0.069	0.032	0.017	0.000			
	0.523	0.305	0.140	0.067	0.031	0.016	0.000			
	0.521	0.309	0.137	0.067	0.030	0.016	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.261	0.037	0.069
	0.265	0.034	0.069
			0.068

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.522	0.522	0.0424
125	100	200	0.00013	200	0.0250	0.307	0.307	0.0247
50	200	1000	0.00005	200	0.0100	0.140	0.140	0.0109
25	100	1000	0.00003	200	0.0050	0.068	0.068	0.0049
10	40	1000	0.00001	200	0.0020	0.031	0.031	0.0019
5	20	1000	0.00001	200	0.0010	0.016	0.016	0.0007
			Blank	0.000				

r^2= 0.998
m= 0.082
b= -0.001

Variables	Regression results	
m, b	0.082	-0.001
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.001
F, df	1205	3
SS_{reg}, SS_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.261	0.261	0.021	200	1	1	0.000	0.000	0.105
QC 100	0.265	0.265	0.021	200	1	1	0.000		
QC 100									
QC 10	0.037	0.037	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.034	0.034	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.069	0.069	0.005	200	120	42000	0.009	0.009	8.767
Microsomes	0.069	0.069	0.005	200	120	42000	0.009		
Microsomes	0.068	0.068	0.005	200	120	42000	0.009		

Assay Date	8/2/2005	Test Chemical ID		# Concentrations tested	3	Microsome type	8	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	1

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	42	mL total volume
	350	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	350	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.767
Protein Concentration (dilution added to assay, mg/mL):	0.025049

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	1.00E-07
5	1.00E-08
6	1.00E-09
7	1.00E-10
8	1.00E-11

Assay Date	8/2/2005	Test Chemical ID	3	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4308	8616.8	8668.3	17336.6	0.1	155711	11.13	17060	0.0227	1	0.013	15	0.0604
			0.5	2	4360	8719.8											
	2	2	0.5	1	4291	8581.2	8654.8	17309.6	0.1	155711	11.12	17033	0.0227	1	0.013	15	0.0603
			0.5	2	4364	8728.4											
	3	2	0.5	1	4236	8471	8411	16822	0.1	155711	10.80	16546	0.0220	1	0.013	15	0.0586
			0.5	2	4176	8351											
	4	2	0.5	1	4119	8237.4	8192.3	16384.6	0.1	155711	10.52	16108	0.0214	1	0.013	15	0.0571
			0.5	2	4074	8147.2											
Background control	1	2	0.5	1	69	138.92	140.07	280.14	0.1	155711	0.18	4	0.0000	1	0.013	15	0.0000
			0.5	2	71	141.22											
	2	2	0.5	1	75	150.76	145.61	291.22	0.1	155711	0.19	15	0.0000	1	0.013	15	0.0001
			0.5	2	70	140.46											
	3	2	0.5	1	59	117.06	124.41	248.82	0.1	155711	0.16	-28	0.0000	1	0.013	15	-0.0001
			0.5	2	66	131.76											
	4	2	0.5	1	71	142.5	142.57	285.14	0.1	155711	0.18	9	0.0000	1	0.013	15	0.0000
			0.5	2	71	142.64											
Positive control	1	2	0.5	1	1899	3798.6	3874.5	7749	0.1	155711	4.98	7473	0.0099	1	0.013	15	0.0265
			0.5	2	1975	3950.4											
	2	2	0.5	1	2029	4057.2	4015.9	8031.8	0.1	155711	5.16	7755	0.0103	1	0.013	15	0.0275
			0.5	2	1987	3974.6											
	3	2	0.5	1	1905	3809.2	3836.9	7673.8	0.1	155711	4.93	7397	0.0098	1	0.013	15	0.0262
			0.5	2	1932	3864.6											
	4	2	0.5	1	1908	3815.8	3813.8	7627.6	0.1	155711	4.90	7351	0.0098	1	0.013	15	0.0260
			0.5	2	1906	3811.8											
Negative Control	1	2	0.5	1	4170	8340.4	8411.6	16823.2	0.1	155711	10.80	16547	0.0220	1	0.013	15	0.0586
			0.5	2	4241	8482.8											
	2	2	0.5	1	4348	8695.6	8715	17430	0.1	155711	11.19	17154	0.0228	1	0.013	15	0.0608
			0.5	2	4367	8734.4											
	3	2	0.5	1	4058	8116.4	8135.8	16271.6	0.1	155711	10.45	15995	0.0213	1	0.013	15	0.0567
			0.5	2	4078	8155.2											
	4	2	0.5	1	4011	8021	8052.4	16104.8	0.1	155711	10.34	15828	0.0211	1	0.013	15	0.0561
			0.5	2	4042	8083.8											
3	1-1	2	0.5	1	86	171.48	162.58	325.16	0.1	155711	0.21	49	0.0001	1	0.013	15	0.0002
			0.5	2	77	153.68											
	1-2	2	0.5	1	67	134.86	138	276	0.1	155711	0.18	0	0.0000	1	0.013	15	0.0000
			0.5	2	71	141.14											
	1-3	2	0.5	1	69	138.32	131.63	263.26	0.1	155711	0.17	-13	0.0000	1	0.013	15	0.0000
			0.5	2	62	124.94											
	2-1	2	0.5	1	93	186.52	179.32	358.64	0.1	155711	0.23	82	0.0001	1	0.013	15	0.0003
			0.5	2	86	172.12											
	2-2	2	0.5	1	79	158.66	153.13	306.26	0.1	155711	0.20	30	0.0000	1	0.013	15	0.0001
			0.5	2	74	147.6											
	2-3	2	0.5	1	80	159.36	150.81	301.62	0.1	155711	0.19	25	0.0000	1	0.013	15	0.0001
			0.5	2	71	142.26											
	3-1	2	0.5	1	175	349.26	340.84	681.68	0.1	155711	0.44	405	0.0005	1	0.013	15	0.0014
			0.5	2	166	332.42											
	3-2	2	0.5	1	158	316.1	319.97	639.94	0.1	155711	0.41	364	0.0005	1	0.013	15	0.0013
			0.5	2	162	323.84											
	3-3	2	0.5	1	163	325.1	327.97	655.94	0.1	155711	0.42	380	0.0005	1	0.013	15	0.0013
			0.5	2	165	330.84											
	4-1	2	0.5	1	843	1685	1674.53	3349.06	0.1	155711	2.15	3073	0.0041	1	0.013	15	0.0109
			0.5	2	832	1664.06											
	4-2	2	0.5	1	855	1710.02	1716.21	3432.42	0.1	155711	2.20	3156	0.0042	1	0.013	15	0.0112
			0.5	2	861	1722.4											
	4-3	2	0.5	1	800	1599.38	1584.54	3169.08	0.1	155711	2.04	2893	0.0039	1	0.013	15	0.0102
			0.5	2	785	1569.7											
	5-1	2	0.5	1	2797	5593.2	5569.6	11139.2	0.1	155711	7.15	10863	0.0145	1	0.013	15	0.0385
			0.5	2	2773	5546											
	5-2	2	0.5	1	2861	5722.2	5721.4	11442.8	0.1	155711	7.35	11166	0.0149	1	0.013	15	0.0396
			0.5	2	2860	5720.6											
	5-3	2	0.5	1	2759	5518.2	5616.1	11232.2	0.1	155711	7.21	10956	0.0146	1	0.013	15	0.0388
			0.5	2	2857	5714											
	6-1	2	0.5	1	3868	7735.8	7878.5	15757	0.1	155711	10.12	15481	0.0206	1	0.013	15	0.0548
			0.5	2	4011	8021.4											
	6-2	2	0.5	1	4043	8085.2	7956.3	15912.6	0.1	155711	10.22	15636	0.0208	1	0.013	15	0.0554
			0.5	2	3914	7827.4											
	6-3	2	0.5	1	4051	8101.2	8179.9	16359.8	0.1	155711	10.51	16083	0.0214	1	0.013	15	0.0570
			0.5	2	4129	8258.6											
	7-1	2	0.5	1	4056	8111.8	8136	16272	0.1	155711	10.45	15996	0.0213	1	0.013	15	0.0567
			0.5	2	4080	8160.2											
	7-2	2	0.5	1	4047	8094.6	8067.7	16135.4	0.1	155711	10.36	15859	0.0211	1	0.013	15	0.0562
			0.5	2	4020	8040.8											
	7-3	2	0.5	1	4100	8199.6	8289.4	16578.8	0.1	155711	10.65	16302	0.0217	1	0.013	15	0.0578
			0.5	2	4190	8379.2											
	8-1	2	0.5	1	4126	8252.8	8197.3	16394.6	0.1	155711	10.53	16118	0.0215	1	0.013	15	0.0571
			0.5	2	4071	8141.8											
	8-2	2	0.5	1	4106	8212.6	8187.5	16375	0.1	155711	10.52	16099	0.0214	1	0.013	15	0.0570
			0.5	2	4081	8162.4											
	8-3	2	0.5	1	4117	8234.4	8236	16472	0.1	155711	10.58	16196	0.0216	1	0.013	15	0.0574
			0.5	2	4119	8237.6											

Assay Date	8/2/2005	Test Chemical ID	3	# Concentrations tested	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0604	0.0001
Full activity	End	0.0578	0.0011
Full activity	Overall	0.0591	0.0016
Background	Beginning	0.0000	2.7755E-05
Background	End	0.0000	9.09804E-05
Background	Overall	0.0000	6.69235E-05
Positive	Beginning	0.0270	0.0007
Positive	End	0.0261	0.0001
Positive	Overall	0.0265	0.0006
Negative	Beginning	0.0597	0.0015
Negative	End	0.0564	0.0004
Negative	Overall	0.0580	0.0021

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
3	1	1	1.00E-04	-4.00	0.0002
3	1	2	1.00E-04	-4.00	0.0000
3	1	3	1.00E-04	-4.00	0.0000
3	2	1	1.00E-05	-5.00	0.0003
3	2	2	1.00E-05	-5.00	0.0001
3	2	3	1.00E-05	-5.00	0.0001
3	3	1	1.00E-06	-6.00	0.0014
3	3	2	1.00E-06	-6.00	0.0013
3	3	3	1.00E-06	-6.00	0.0013
3	4	1	1.00E-07	-7.00	0.0109
3	4	2	1.00E-07	-7.00	0.0112
3	4	3	1.00E-07	-7.00	0.0102
3	5	1	1.00E-08	-8.00	0.0385
3	5	2	1.00E-08	-8.00	0.0396
3	5	3	1.00E-08	-8.00	0.0388
3	6	1	1.00E-09	-9.00	0.0548
3	6	2	1.00E-09	-9.00	0.0554
3	6	3	1.00E-09	-9.00	0.0570
3	7	1	1.00E-10	-10.00	0.0567
3	7	2	1.00E-10	-10.00	0.0562
3	7	3	1.00E-10	-10.00	0.0578
3	8	1	1.00E-11	-11.00	0.0571
3	8	2	1.00E-11	-11.00	0.0570
3	8	3	1.00E-11	-11.00	0.0574

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.29	0.00	-0.08
2	-5.00	0.49	0.18	0.15
3	-6.00	2.43	2.18	2.27
4	-7.00	18.41	18.91	17.34
5	-8.00	65.10	66.92	65.66
6	-9.00	92.77	93.70	96.38
7	-10.00	95.86	95.04	97.70
8	-11.00	96.59	96.48	97.06

Assay Date	8/3/2005	Test Chemical ID	3	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	28261	1420151		
2	0.0201	30187	1501841		
3	0.0202	30907	1530050		
4	0.0198	31935	1612879		
5	0.0201	33304	1656915		
			Average DPM/g soln	1544367	
			SD	93279	
			CV	6.04	
			µCi/g soln	0.696	

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.7	10		1070.00
Dilution A			100	10.70
Dilution B			10	1.07

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.5672 g
Mass of dilution B used in substrate prep	5.439 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.6083 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00787 µg/g soln.
µg/g soln.

a. µCi/g soln 0.696
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.608300 + 0.00787
= 0.616175 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.129 µCi/µg ASDN

717826 dpm/nmol

Assay Date 8/3/2005 | Test Chemical ID 3 | # Concentrations tested 8

Technician ID TD | Replicate # 2 | Microsome type placental | Microsome ID BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.521	0.308	0.137	0.073	0.029	0.017	0.000			
	0.526	0.311	0.138	0.079	0.031	0.017	0.000			
	0.520	0.308	0.138	0.073	0.030	0.017	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.267	0.037	0.070							
	0.268	0.036	0.074							
			0.074							

Standard concentration (pg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0500	0.522	0.522	0.0481	m, b	0.096	-0.002
125	100	200	0.00013	200	0.0250	0.309	0.309	0.0277	se_m, se_b	0.004	0.001
50	200	1000	0.00005	200	0.0100	0.138	0.138	0.0113	r^2, se_y	0.992	0.002
25	100	1000	0.00003	200	0.0050	0.075	0.075	0.0052	F, df	473	4
10	40	1000	0.00001	200	0.0020	0.030	0.030	0.0009	SS_{reg}, SS_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.017	0.017	-0.0003			
			Blank	0.000							

r^2= 0.992
m= 0.096
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.267	0.267	0.024	200	1	1	0.000	0.000	0.118
QC 100	0.268	0.268	0.024	200	1	1	0.000		
QC 100									
QC 10	0.037	0.037	0.002	200	1	1	0.000	0.000	0.008
QC 10	0.036	0.036	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.070	0.070	0.005	200	120	42000	0.008	0.009	8.850
Microsomes	0.074	0.074	0.005	200	120	42000	0.009		
Microsomes	0.074	0.074	0.005	200	120	42000	0.009		

Assay Date	8/3/2005	Test Chemical ID	3	# Concentrations tested	6		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.521	0.308	0.137	0.073	0.029	0.017	0.000			
	0.526	0.311	0.138	0.079	0.031	0.017	0.000			
	0.520	0.308	0.138	0.073	0.030	0.017	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.267	0.037	0.070
	0.268	0.036	0.074
			0.074

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.522	0.522	0.0423
125	100	200	0.00013	200	0.0250	0.309	0.309	0.0247
50	200	1000	0.00005	200	0.0100	0.138	0.138	0.0106
25	100	1000	0.00003	200	0.0050	0.075	0.075	0.0054
10	40	1000	0.00001	200	0.0020	0.030	0.030	0.0017
5	20	1000	0.00001	200	0.0010	0.017	0.017	0.0006
		Blank	0.000					

Variables	Regression results	
m, b	0.082	-0.001
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.001
F, df	1322	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.998
m= 0.082
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.267	0.267	0.021	200	1	1	0.000	0.000	0.106
QC 100	0.268	0.268	0.021	200	1	1	0.000		
QC 100									
QC 10	0.037	0.037	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.036	0.036	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.070	0.070	0.005	200	120	42000	0.009	0.009	9.159
Microsomes	0.074	0.074	0.005	200	120	42000	0.009		
Microsomes	0.074	0.074	0.005	200	120	42000	0.009		

Assay Date	8/3/2005	Test Chemical ID		3	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate # 2

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	42	mL total volume
	350	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	350	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	9.159
Protein Concentration (dilution added to assay, mg/mL):	0.026169

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	2.50E-08
5	1.00E-08
6	2.50E-09
7	1.00E-09
8	1.00E-10

Assay Date	8/3/2005	Test Chemical ID	3	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	2834	5668.8	6205.6	12411.2	0.1	154437	8.04	12104	0.0169	1	0.013	15	0.0430
			0.5	2	3371	6742.4											
	2	2	0.5	1	3442	6883.6	6812.7	13625.4	0.1	154437	8.82	13318	0.0186	1	0.013	15	0.0473
			0.5	2	3371	6741.8											
	3	2	0.5	1	3224	6448	6354.3	12708.6	0.1	154437	8.23	12401	0.0173	1	0.013	15	0.0440
			0.5	2	3130	6260.6											
	4	2	0.5	1	3081	6161.4	6249.7	12499.4	0.1	154437	8.09	12192	0.0170	1	0.013	15	0.0433
			0.5	2	3169	6338											
Background control	1	2	0.5	1	66	132.82	136.91	273.82	0.1	154437	0.18	-33	0.0000	1	0.013	15	-0.0001
			0.5	2	71	141											
	2	2	0.5	1	90	179.86	172.57	345.14	0.1	154437	0.22	38	0.0001	1	0.013	15	0.0001
			0.5	2	83	165.28											
	3	2	0.5	1	72	143.12	153.58	307.16	0.1	154437	0.20	0	0.0000	1	0.013	15	0.0000
			0.5	2	82	164.04											
	4	2	0.5	1	78	156.1	151.25	302.5	0.1	154437	0.20	-5	0.0000	1	0.013	15	0.0000
			0.5	2	73	146.4											
Positive control	1	2	0.5	1	1743	3486.6	3451.7	6903.4	0.1	154437	4.47	6596	0.0092	1	0.013	15	0.0234
			0.5	2	1708	3416.8											
	2	2	0.5	1	1690	3380	3321.2	6642.4	0.1	154437	4.30	6335	0.0088	1	0.013	15	0.0225
			0.5	2	1631	3262.4											
	3	2	0.5	1	1797	3594.4	3541.4	7082.8	0.1	154437	4.59	6776	0.0094	1	0.013	15	0.0240
			0.5	2	1744	3488.4											
	4	2	0.5	1	1737	3474.4	3490.6	6981.2	0.1	154437	4.52	6674	0.0093	1	0.013	15	0.0237
			0.5	2	1753	3506.8											
Negative Control	1	2	0.5	1	3081	6161.4	6180.6	12361.2	0.1	154437	8.00	12054	0.0168	1	0.013	15	0.0428
			0.5	2	3100	6199.8											
	2	2	0.5	1	3168	6335.8	6403.7	12807.4	0.1	154437	8.29	12500	0.0174	1	0.013	15	0.0444
			0.5	2	3236	6471.6											
	3	2	0.5	1	3223	6446.2	6425.7	12851.4	0.1	154437	8.32	12544	0.0175	1	0.013	15	0.0445
			0.5	2	3203	6405.2											
	4	2	0.5	1	3097	6193.8	6165	12330	0.1	154437	7.98	12023	0.0167	1	0.013	15	0.0427
			0.5	2	3068	6136.2											
3	1-1	2	0.5	1	78	151.7	163.12	326.24	0.1	154437	0.21	19	0.0000	1	0.013	15	0.0001
			0.5	2	87	174.54											
	1-2	2	0.5	1	76	151.52	149.57	299.14	0.1	154437	0.19	-8	0.0000	1	0.013	15	0.0000
			0.5	2	74	147.62											
	1-3	2	0.5	1	85	169.88	161.12	322.24	0.1	154437	0.21	15	0.0000	1	0.013	15	0.0001
			0.5	2	76	152.36											
	2-1	2	0.5	1	160	320.02	316.04	632.08	0.1	154437	0.41	325	0.0005	1	0.013	15	0.0012
			0.5	2	156	312.06											
	2-2	2	0.5	1	151	301.56	304.59	609.18	0.1	154437	0.39	302	0.0004	1	0.013	15	0.0011
			0.5	2	154	307.62											
	2-3	2	0.5	1	155	309.4	315.13	630.26	0.1	154437	0.41	323	0.0005	1	0.013	15	0.0011
			0.5	2	160	320.86											
	3-1	2	0.5	1	782	1563.78	1540.24	3080.48	0.1	154437	1.99	2773	0.0039	1	0.013	15	0.0098
			0.5	2	758	1516.7											
	3-2	2	0.5	1	773	1546.28	1557.8	3115.6	0.1	154437	2.02	2808	0.0039	1	0.013	15	0.0100
			0.5	2	785	1569.32											
	3-3	2	0.5	1	750	1500.28	1502.51	3005.02	0.1	154437	1.95	2698	0.0038	1	0.013	15	0.0096
			0.5	2	752	1504.74											
	4-1	2	0.5	1	1572	3144.6	3130.4	6260.8	0.1	154437	4.05	5954	0.0083	1	0.013	15	0.0211
			0.5	2	1558	3116.2											
	4-2	2	0.5	1	1640	3279.2	3279.4	6558.8	0.1	154437	4.25	6252	0.0087	1	0.013	15	0.0222
			0.5	2	1640	3279.6											
	4-3	2	0.5	1	1669	3337.6	3367.9	6735.8	0.1	154437	4.36	6429	0.0090	1	0.013	15	0.0228
			0.5	2	1699	3398.2											
	5-1	2	0.5	1	2310	4619.6	4639.1	9278.2	0.1	154437	6.01	8971	0.0125	1	0.013	15	0.0318
			0.5	2	2329	4658.6											
	5-2	2	0.5	1	2307	4614	4607.7	9215.4	0.1	154437	5.97	8908	0.0124	1	0.013	15	0.0316
			0.5	2	2301	4601.4											
	5-3	2	0.5	1	2337	4674	4653.3	9306.6	0.1	154437	6.03	8999	0.0125	1	0.013	15	0.0319
			0.5	2	2316	4632.6											
	6-1	2	0.5	1	2851	5702	5721	11442	0.1	154437	7.41	11135	0.0155	1	0.013	15	0.0395
			0.5	2	2870	5740											
	6-2	2	0.5	1	2892	5783.2	5738.5	11477	0.1	154437	7.43	11170	0.0156	1	0.013	15	0.0396
			0.5	2	2847	5693.8											
	6-3	2	0.5	1	2746	5492.2	5597.2	11194.4	0.1	154437	7.25	10887	0.0152	1	0.013	15	0.0386
			0.5	2	2851	5702.2											
	7-1	2	0.5	1	3111	6221.6	6147.6	12295.2	0.1	154437	7.96	11988	0.0167	1	0.013	15	0.0425
			0.5	2	3037	6073.6											
	7-2	2	0.5	1	3065	6130.6	6111.7	12223.4	0.1	154437	7.91	11916	0.0166	1	0.013	15	0.0423
			0.5	2	3046	6092.8											
	7-3	2	0.5	1	3047	6094	6052.4	12104.8	0.1	154437	7.84	11798	0.0164	1	0.013	15	0.0419
			0.5	2	3005	6010.8											
	8-1	2	0.5	1	3167	6334.8	6356.3	12712.6	0.1	154437	8.23	12405	0.0173	1	0.013	15	0.0440
			0.5	2	3189	6377.8											
	8-2	2	0.5	1	3172	6343.8	6347	12694	0.1	154437	8.22	12387	0.0173	1	0.013	15	0.0440
			0.5	2	3175	6350.2											
	8-3	2	0.5	1	3145	6289.4	6294.3	12588.6	0.1	154437	8.15	12281	0.0171	1	0.013	15	0.0436
			0.5	2	3150	6299.2											

Assay Date	8/3/2005	Test Chemical ID	3	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0451	0.0030
Full activity	End	0.0436	0.0005
Full activity	Overall	0.0444	0.0020
Background	Beginning	0.0000	0.000178981
Background	End	0.0000	1.16945E-05
Background	Overall	0.0000	0.000103992
Positive	Beginning	0.0229	0.0007
Positive	End	0.0239	0.0003
Positive	Overall	0.0234	0.0007
Negative	Beginning	0.0436	0.0011
Negative	End	0.0436	0.0013
Negative	Overall	0.0436	0.0010

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
3	1	1	1.00E-05	-5.00	0.0001
3	1	2	1.00E-05	-5.00	0.0000
3	1	3	1.00E-05	-5.00	0.0001
3	2	1	1.00E-06	-6.00	0.0012
3	2	2	1.00E-06	-6.00	0.0011
3	2	3	1.00E-06	-6.00	0.0011
3	3	1	1.00E-07	-7.00	0.0098
3	3	2	1.00E-07	-7.00	0.0100
3	3	3	1.00E-07	-7.00	0.0096
3	4	1	2.50E-08	-7.60	0.0211
3	4	2	2.50E-08	-7.60	0.0222
3	4	3	2.50E-08	-7.60	0.0228
3	5	1	1.00E-08	-8.00	0.0318
3	5	2	1.00E-08	-8.00	0.0316
3	5	3	1.00E-08	-8.00	0.0319
3	6	1	2.50E-09	-8.60	0.0395
3	6	2	2.50E-09	-8.60	0.0396
3	6	3	2.50E-09	-8.60	0.0386
3	7	1	1.00E-09	-9.00	0.0425
3	7	2	1.00E-09	-9.00	0.0423
3	7	3	1.00E-09	-9.00	0.0419
3	8	1	1.00E-10	-10.00	0.0440
3	8	2	1.00E-10	-10.00	0.0440
3	8	3	1.00E-10	-10.00	0.0436

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	0.15	-0.06	0.12
2	-6.00	2.60	2.42	2.58
3	-7.00	22.18	22.46	21.58
4	-7.60	47.61	50.00	51.41
5	-8.00	71.75	71.24	71.97
6	-8.60	89.05	89.33	87.07
7	-9.00	95.87	95.30	94.35
8	-10.00	99.21	99.06	98.22

Assay Date	8/4/2005	Test Chemical ID	3	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	27100	1361809		
2	0.0197	28659	1454772		
3	0.0197	30373	1541777		
4	0.0199	32299	1623065		
5	0.0199	32650	1640704		
			Average DPM/g soln		1524425
			SD		117097
			CV		7.68
			µCi/g soln		0.687

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.5	10		1050.00
Dilution A			100	10.50
Dilution B			10	1.05

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6101 g
Mass of dilution B used in substrate prep	5.4085 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.590933 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00777 µg/g soln.
µg/g soln.

a. µCi/g soln 0.687
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0. 590933 + 0.00777
= 0.598706 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.147 µCi/µg ASDN

729231 dpm/nmol

Assay Date	8/4/2005	Test Chemical ID	3	# Concentrations tested	8				
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	BAA		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.593	0.299	0.135	0.065	0.026	0.012	0.000			
	0.595	0.300	0.132	0.068	0.025	0.014	0.000			
	0.595	0.306	0.134	0.068	0.027	0.014	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.252	0.034	0.066							
	0.258	0.029	0.057							
			0.066							

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL		Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200	0.0500	0.595	0.595	0.0498	m, b	0.085	-0.001
125	100	200	0.00013		200	0.0250	0.302	0.302	0.0250	se_m, se_b	0.001	0.000
50	200	1000	0.00005		200	0.0100	0.134	0.134	0.0108	r^2, se_y	0.999	0.000
25	100	1000	0.00003		200	0.0050	0.067	0.067	0.0051	F, df	7646	4
10	40	1000	0.00001		200	0.0020	0.026	0.026	0.0017	SS_{reg}, SS_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010	0.013	0.013	0.0006			
				Blank	0.000							

Regression results are calculated using the function LINEST

r^2= 0.999
m= 0.085
b= -0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.252	0.252	0.021	200	1	1	0.000	0.000	0.105
QC 100	0.258	0.258	0.021	200	1	1	0.000		
QC 100									
QC 10	0.034	0.034	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.029	0.029	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.066	0.066	0.005	200	120	42000	0.009	0.009	8.884
Microsomes	0.067	0.067	0.005	200	120	42000	0.009		
Microsomes	0.066	0.066	0.005	200	120	42000	0.009		

Assay Date	8/4/2005	Test Chemical ID	3	# Concentrations tested	8					
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	BAA			

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.593	0.299	0.135	0.065	0.026	0.012	0.000	25	100	11343-77
	0.595	0.300	0.132	0.068	0.025	0.014	0.000			
	0.595	0.306	0.134	0.068	0.027	0.014	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.252	0.034	0.066
	0.258	0.029	0.067
			0.066

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0500	0.595	0.595	0.0491	m, b	0.083	0.000
125	100	200	0.00013	200	0.0250	0.302	0.302	0.0247	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.134	0.134	0.0107	r^2, se_y	0.998	0.001
25	100	1000	0.00003	200	0.0050	0.067	0.067	0.0052	F, df	1456	3
10	40	1000	0.00001	200	0.0020	0.026	0.026	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.013	0.013	0.0007			

Regression results are calculated using the function LINEST

Blank	0.000	r^2=	0.998	
		m=	0.083	
		b=	0.000	

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.252	0.252	0.021	200	1	1	0.000	0.000	0.104
QC 100	0.258	0.258	0.021	200	1	1	0.000		
QC 100									
QC 10	0.034	0.034	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.029	0.029	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.066	0.066	0.005	200	120	42000	0.009	0.009	8.934
Microsomes	0.067	0.067	0.005	200	120	42000	0.009		
Microsomes	0.066	0.066	0.005	200	120	42000	0.009		

Assay Date	8/4/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	3

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	42	mL total volume
	350	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	350	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.933539
Protein Concentration (dilution added to assay, mg/mL):	0.025524

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	2.50E-08
5	1.00E-08
6	2.50E-09
7	1.00E-09
8	1.00E-10

Assay Date	8/4/2005	Test Chemical ID	3	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	2696	5791	6399.4	12798.8	0.1	152443	8.40	12526	0.0172	1	0.013	15	0.0449
			0.5	2	3504	7007.8											
	2	2	0.5	1	2931	5861.6	5863.9	11727.8	0.1	152443	7.69	11455	0.0157	1	0.013	15	0.0410
			0.5	2	2933	5866.2											
	3	2	0.5	1	2753	5505.4	5454.1	10908.2	0.1	152443	7.16	10635	0.0146	1	0.013	15	0.0381
			0.5	2	2701	5402.8											
	4	2	0.5	1	2521	5041.4	5075.4	10150.8	0.1	152443	6.66	9878	0.0135	1	0.013	15	0.0354
			0.5	2	2555	5109.4											
Background control	1	2	0.5	1	70	140.94	143.18	286.36	0.1	152443	0.19	13	0.0000	1	0.013	15	0.0000
			0.5	2	73	145.42											
	2	2	0.5	1	70	140.18	145.6	291.2	0.1	152443	0.19	18	0.0000	1	0.013	15	0.0001
			0.5	2	76	151.02											
	3	2	0.5	1	67	133.72	131.71	263.42	0.1	152443	0.17	-10	0.0000	1	0.013	15	0.0000
			0.5	2	65	129.7											
	4	2	0.5	1	60	120.14	125.48	250.96	0.1	152443	0.16	-22	0.0000	1	0.013	15	-0.0001
			0.5	2	65	130.82											
Positive control	1	2	0.5	1	1553	3106.6	3156	6312	0.1	152443	4.14	6039	0.0083	1	0.013	15	0.0216
			0.5	2	1603	3205.4											
	2	2	0.5	1	1475	2950.6	2989.4	5978.8	0.1	152443	3.92	5706	0.0078	1	0.013	15	0.0204
			0.5	2	1514	3028.2											
	3	2	0.5	1	1690	3380.2	3308	6616	0.1	152443	4.34	6343	0.0087	1	0.013	15	0.0227
			0.5	2	1618	3235.8											
	4	2	0.5	1	1684	3368.6	3397.2	6794.4	0.1	152443	4.46	6521	0.0089	1	0.013	15	0.0234
			0.5	2	1713	3425.8											
Negative Control	1	2	0.5	1	2963	5965.6	5926.7	11853.4	0.1	152443	7.78	11580	0.0159	1	0.013	15	0.0415
			0.5	2	2944	5887.8											
	2	2	0.5	1	2939	5877	5744.9	11489.8	0.1	152443	7.54	11217	0.0154	1	0.013	15	0.0402
			0.5	2	2806	5612.8											
	3	2	0.5	1	2786	5571	5638.9	11277.8	0.1	152443	7.40	11005	0.0151	1	0.013	15	0.0394
			0.5	2	2853	5706.8											
	4	2	0.5	1	2785	5570.2	5507.2	11014.4	0.1	152443	7.23	10741	0.0147	1	0.013	15	0.0385
			0.5	2	2722	5444.2											
3	1-1	2	0.5	1	61	122.8	133.95	267.9	0.1	152443	0.18	-5	0.0000	1	0.013	15	0.0000
			0.5	2	73	145.1											
	1-2	2	0.5	1	79	157.76	140.93	281.86	0.1	152443	0.18	9	0.0000	1	0.013	15	0.0000
			0.5	2	62	124.1											
	1-3	2	0.5	1	73	146.88	148.89	297.78	0.1	152443	0.20	25	0.0000	1	0.013	15	0.0001
			0.5	2	75	150.9											
	2-1	2	0.5	1	168	335.2	316.69	633.38	0.1	152443	0.42	360	0.0005	1	0.013	15	0.0013
			0.5	2	149	298.18											
	2-2	2	0.5	1	157	314.36	311.82	623.64	0.1	152443	0.41	351	0.0005	1	0.013	15	0.0013
			0.5	2	155	309.28											
	2-3	2	0.5	1	154	308.04	316.29	632.58	0.1	152443	0.41	360	0.0005	1	0.013	15	0.0013
			0.5	2	162	324.54											
	3-1	2	0.5	1	754	1508.1	1504.5	3009	0.1	152443	1.97	2736	0.0038	1	0.013	15	0.0098
			0.5	2	750	1500.9											
	3-2	2	0.5	1	735	1470.86	1469.77	2939.54	0.1	152443	1.93	2667	0.0037	1	0.013	15	0.0096
			0.5	2	734	1468.68											
	3-3	2	0.5	1	740	1480.16	1481.84	2963.68	0.1	152443	1.94	2691	0.0037	1	0.013	15	0.0096
			0.5	2	742	1483.52											
	4-1	2	0.5	1	1644	3288.8	3282.9	6565.8	0.1	152443	4.31	6293	0.0086	1	0.013	15	0.0225
			0.5	2	1639	3277											
	4-2	2	0.5	1	1566	3132.8	3142.6	6285.2	0.1	152443	4.12	6012	0.0082	1	0.013	15	0.0215
			0.5	2	1576	3152.4											
	4-3	2	0.5	1	1552	3103.4	3150.7	6301.4	0.1	152443	4.13	6028	0.0083	1	0.013	15	0.0216
			0.5	2	1599	3198											
	5-1	2	0.5	1	2166	4332	4344.6	8689.2	0.1	152443	5.70	8416	0.0115	1	0.013	15	0.0301
			0.5	2	2179	4357.2											
	5-2	2	0.5	1	2098	4196.4	4139.5	8279	0.1	152443	5.43	8006	0.0110	1	0.013	15	0.0287
			0.5	2	2041	4082.6											
	5-3	2	0.5	1	2107	4213.8	4234	8468	0.1	152443	5.55	8195	0.0112	1	0.013	15	0.0294
			0.5	2	2127	4254.2											
	6-1	2	0.5	1	2498	4995.8	4978.1	9956.2	0.1	152443	6.53	9683	0.0133	1	0.013	15	0.0347
			0.5	2	2480	4960.4											
	6-2	2	0.5	1	2548	5095.6	5104.6	10209.2	0.1	152443	6.70	9936	0.0136	1	0.013	15	0.0356
			0.5	2	2557	5113.6											
	6-3	2	0.5	1	2619	5237.4	5228.5	10457	0.1	152443	6.86	10184	0.0140	1	0.013	15	0.0365
			0.5	2	2610	5219.6											
	7-1	2	0.5	1	2676	5351.4	5337.4	10674.8	0.1	152443	7.00	10402	0.0143	1	0.013	15	0.0373
			0.5	2	2662	5323.4											
	7-2	2	0.5	1	2742	5484.6	5495.4	10990.8	0.1	152443	7.21	10718	0.0147	1	0.013	15	0.0384
			0.5	2	2753	5506.2											
	7-3	2	0.5	1	2653	5306	5366	10732	0.1	152443	7.04	10459	0.0143	1	0.013	15	0.0375
			0.5	2	2713	5426											
	8-1	2	0.5	1	2822	5644.6	5707.2	11414.4	0.1	152443	7.49	11141	0.0153	1	0.013	15	0.0399
			0.5	2	2885	5769.8											
	8-2	2	0.5	1	2715	5430.6	5392.5	10785	0.1	152443	7.07	10512	0.0144	1	0.013	15	0.0377
			0.5	2	2677	5354.4											
	8-3	2	0.5	1	2769	5537.6	5512.2	11024.4	0.1	152443	7.23	10751	0.0147	1	0.013	15	0.0385
			0.5	2	2743	5486.8											

Assay Date	8/4/2005	Test Chemical ID	3	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0429	0.0027
Full activity	End	0.0367	0.0019
Full activity	Overall	0.0398	0.0041
Background	Beginning	0.0001	1.2258E-05
Background	End	-0.0001	3.15567E-05
Background	Overall	0.0000	6.8186E-05
Positive	Beginning	0.0210	0.0008
Positive	End	0.0230	0.0005
Positive	Overall	0.0220	0.0013
Negative	Beginning	0.0408	0.0009
Negative	End	0.0389	0.0007
Negative	Overall	0.0399	0.0013

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
3	1	1	1.00E-05	-5.00	0.0000
3	1	2	1.00E-05	-5.00	0.0000
3	1	3	1.00E-05	-5.00	0.0001
3	2	1	1.00E-06	-6.00	0.0013
3	2	2	1.00E-06	-6.00	0.0013
3	2	3	1.00E-06	-6.00	0.0013
3	3	1	1.00E-07	-7.00	0.0098
3	3	2	1.00E-07	-7.00	0.0096
3	3	3	1.00E-07	-7.00	0.0096
3	4	1	2.50E-08	-7.60	0.0225
3	4	2	2.50E-08	-7.60	0.0215
3	4	3	2.50E-08	-7.60	0.0216
3	5	1	1.00E-08	-8.00	0.0301
3	5	2	1.00E-08	-8.00	0.0287
3	5	3	1.00E-08	-8.00	0.0294
3	6	1	2.50E-09	-8.60	0.0347
3	6	2	2.50E-09	-8.60	0.0356
3	6	3	2.50E-09	-8.60	0.0365
3	7	1	1.00E-09	-9.00	0.0373
3	7	2	1.00E-09	-9.00	0.0384
3	7	3	1.00E-09	-9.00	0.0375
3	8	1	1.00E-10	-10.00	0.0399
3	8	2	1.00E-10	-10.00	0.0377
3	8	3	1.00E-10	-10.00	0.0385

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	-0.05	0.08	0.22
2	-6.00	3.24	3.15	3.23
3	-7.00	24.60	23.97	24.19
4	-7.60	56.57	54.05	54.20
5	-8.00	75.66	71.97	73.67
6	-8.60	87.05	89.33	91.55
7	-9.00	93.51	96.35	94.03
8	-10.00	100.16	94.50	96.66

Assay Date	9/27/2005	Test Chemical ID	4	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0200	23152	1157600		
2	0.0200	24412	1220600		
3	0.0201	24678	1227761		
4	0.0202	25136	1244356		
5	0.0202	26154	1294752		
			Average DPM/g soln		1229014
			SD		49319
			CV		4.01
			µCi/g soln		0.554

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.5	10		1050.00
Dilution A			100	10.50
Dilution B			10	1.05

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6518 g
Mass of dilution B used in substrate prep	5.4298 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.590697 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00627 µg/g soln.
µg/g soln.

a. µCi/g soln	0.554
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0. 590697 + 0.00627
= 0.596964 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 0.927 µCi/µg ASDN

589633 dpm/nmol

Assay Date 9/27/2005 | Test Chemical ID 4 | # Concentrations tested 6

Technician ID TD | Replicate # 1 | Microsome type placental | Microsome ID Lot # BAA

Standards:	250	125	50	25	10	5	0
	0.447	0.273	0.121	0.058	0.025	0.012	0.000
	0.449	0.269	0.120	0.057	0.025	0.013	0.000
	0.450	0.266	0.114	0.062	0.024	0.013	0.000

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25	100	11343-77

Samples:	QC 100	QC 10	Microsomes
	0.235	0.028	0.068
	0.231	0.027	0.065
			0.067

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.449	0.449	0.0479
125	100	200	0.00013	200	0.0250	0.269	0.269	0.0281
50	200	1000	0.00005	200	0.0100	0.118	0.118	0.0114
25	100	1000	0.00003	200	0.0050	0.059	0.059	0.0049
10	40	1000	0.00001	200	0.0020	0.025	0.025	0.0011
5	20	1000	0.00001	200	0.0010	0.013	0.013	-0.0003

LE 3-23-06 µg/µL

Variables	Regression results	
m, b	0.110	-0.002
se_m, se_b	0.006	0.001
r^2, se_y	0.990	0.002
F, df	396	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank 0.000

r^2= 0.990
m= 0.110
b= -0.002

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.235	0.235	0.024	200	1	1	0.000	0.000	0.120
QC 100	0.231	0.231	0.024	200	1	1	0.000		
QC 100									
QC 10	0.028	0.028	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.027	0.027	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.068	0.068	0.006	200	120	42000	0.010	0.010	9.951
Microsomes	0.065	0.065	0.005	200	120	42000	0.010		
Microsomes	0.067	0.067	0.006	200	120	42000	0.010		

Assay Date 9/27/2005 | Test Chemical ID 4 | # Concentrations tested 8

Technician ID TD | Replicate # 1 | Microsome type placental | Microsome ID Lot # BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.447	0.273	0.121	0.058	0.025	0.012	0.000			
	0.449	0.269	0.120	0.057	0.025	0.013	0.000			
	0.450	0.266	0.114	0.062	0.024	0.013	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.235	0.028	0.068
	0.231	0.027	0.065
			0.067

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.449	0.449	0.0416
125	100	200	0.00013	200	0.0250	0.269	0.269	0.0247
50	200	1000	0.00005	200	0.0100	0.118	0.118	0.0106
25	100	1000	0.00003	200	0.0050	0.059	0.059	0.0051
10	40	1000	0.00001	200	0.0020	0.025	0.025	0.0019
5	20	1000	0.00001	200	0.0010	0.013	0.013	0.0007
			Blank	0.000				

r^2= 0.999
m= 0.094
b= 0.000

Variables	Regression results	
m, b	0.094	0.000
se_m, se_b	0.002	0.000
r^2, se_y	0.999	0.000
F, df	2109	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.235	0.235	0.022	200	1	1	0.000	0.000	0.107
QC 100	0.231	0.231	0.021	200	1	1	0.000		
QC 100									
QC 10	0.028	0.028	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.027	0.027	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.068	0.068	0.006	200	120	42000	0.010	0.010	10.106
Microsomes	0.065	0.065	0.006	200	120	42000	0.010		
Microsomes	0.067	0.067	0.006	200	120	42000	0.010		

Assay Date	9/27/2005	Test Chemical ID		4	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate # 1

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	42	mL total volume
	350	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	350	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	10.10593
Protein Concentration (dilution added to assay, mg/mL):	0.028874

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Battelle Study No. G608316

Assay Date	9/27/2005	Test Chemical ID		4 # Concentrations tested		8 Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	2580	5159.8	5159.9	10319.8	0.1	122901	8.40	9535	0.0162	1	0.014	15	0.0373
			0.5	2	2580	5160											
	2	2	0.5	1	2457	4914.4	4973.9	9947.8	0.1	122901	8.09	9183	0.0155	1	0.014	15	0.0359
			0.5	2	2517	5033.4											
	3	2	0.5	1	2441	4882.2	4884	9768	0.1	122901	7.95	8984	0.0152	1	0.014	15	0.0352
			0.5	2	2443	4885.8											
	4	2	0.5	1	2529	5058.8	5005.5	10011	0.1	122901	8.15	9227	0.0156	1	0.014	15	0.0361
			0.5	2	2476	4952.2											
Background control	1	2	0.5	1	220	440.76	435.01	870.02	0.1	122901	0.71	86	0.0001	1	0.014	15	0.0003
			0.5	2	215	429.26											
	2	2	0.5	1	149	298.3	308.43	616.86	0.1	122901	0.50	-167	-0.0003	1	0.014	15	-0.0007
			0.5	2	159	318.56											
	3	2	0.5	1	256	511.34	466.48	932.96	0.1	122901	0.76	149	0.0003	1	0.014	15	0.0006
			0.5	2	211	421.62											
	4	2	0.5	1	178	355.24	358.76	717.52	0.1	122901	0.58	-67	-0.0001	1	0.014	15	-0.0003
			0.5	2	181	362.28											
Positive control	1	2	0.5	1	1497	2994.2	2965.6	5931.2	0.1	122901	4.83	5147	0.0087	1	0.014	15	0.0202
			0.5	2	1469	2937											
	2	2	0.5	1	1537	3073.4	3080	6160	0.1	122901	5.01	5376	0.0091	1	0.014	15	0.0210
			0.5	2	1543	3086.6											
	3	2	0.5	1	1457	2914	2856.4	5712.8	0.1	122901	4.65	4928	0.0084	1	0.014	15	0.0193
			0.5	2	1399	2798.8											
	4	2	0.5	1	1392	2783.8	2796.2	5592.4	0.1	122901	4.55	4808	0.0082	1	0.014	15	0.0188
			0.5	2	1404	2808.6											
Negative Control	1	2	0.5	1	2287	4573.2	4636.8	9273.6	0.1	122901	7.55	8489	0.0144	1	0.014	15	0.0332
			0.5	2	2350	4700.4											
	2	2	0.5	1	2414	4828.6	4787.2	9574.4	0.1	122901	7.79	8790	0.0149	1	0.014	15	0.0344
			0.5	2	2373	4745.8											
	3	2	0.5	1	2285	4570	4651.9	9303.8	0.1	122901	7.57	8519	0.0144	1	0.014	15	0.0334
			0.5	2	2367	4733.8											
	4	2	0.5	1	2336	4672.2	4646.3	9292.6	0.1	122901	7.56	8508	0.0144	1	0.014	15	0.0333
			0.5	2	2310	4620.4											
4	1-1	2	0.5	1	305	609.9	567.98	1135.96	0.1	122901	0.92	362	0.0006	1	0.014	15	0.0014
			0.5	2	263	526.06											
	1-2	2	0.5	1	162	323.56	319.38	638.76	0.1	122901	0.52	-146	-0.0002	1	0.014	15	-0.0006
			0.5	2	158	315.2											
	1-3	2	0.5	1	190	379.34	369.24	738.48	0.1	122901	0.60	-46	-0.0001	1	0.014	15	-0.0002
			0.5	2	180	359.14											
	2-1	2	0.5	1	611	1221.78	1208.01	2416.02	0.1	122901	1.97	1632	0.0028	1	0.014	15	0.0064
			0.5	2	597	1194.24											
	2-2	2	0.5	1	562	1123.14	1105.79	2211.58	0.1	122901	1.80	1427	0.0024	1	0.014	15	0.0056
			0.5	2	544	1088.44											
	2-3	2	0.5	1	609	1217.14	1180.51	2361.02	0.1	122901	1.92	1577	0.0027	1	0.014	15	0.0062
			0.5	2	572	1143.88											
	3-1	2	0.5	1	2392	4783.8	4836.7	9673.4	0.1	122901	7.87	8889	0.0151	1	0.014	15	0.0348
			0.5	2	2445	4889.6											
	3-2	2	0.5	1	2423	4846.2	4838.2	9676.4	0.1	122901	7.87	8892	0.0151	1	0.014	15	0.0348
			0.5	2	2415	4830.2											
	3-3	2	0.5	1	2438	4876.4	4850.6	9701.2	0.1	122901	7.89	8917	0.0151	1	0.014	15	0.0349
			0.5	2	2412	4824.8											
	4-1	2	0.5	1	2486	4971.8	5075.6	10151.2	0.1	122901	8.26	9367	0.0159	1	0.014	15	0.0367
			0.5	2	2590	5179.4											
	4-2	2	0.5	1	2516	5032.8	5007	10014	0.1	122901	8.15	9230	0.0157	1	0.014	15	0.0361
			0.5	2	2491	4981.2											
	4-3	2	0.5	1	2507	5013	4946.6	9893.2	0.1	122901	8.05	9109	0.0154	1	0.014	15	0.0357
			0.5	2	2440	4880.2											
	5-1	2	0.5	1	2679	5357.6	5339.1	10678.2	0.1	122901	8.69	9894	0.0168	1	0.014	15	0.0387
			0.5	2	2660	5320.6											
	5-2	2	0.5	1	2548	5096.4	5101.6	10203.2	0.1	122901	8.30	9419	0.0160	1	0.014	15	0.0369
			0.5	2	2553	5106.8											
	5-3	2	0.5	1	2679	5358	5301.4	10602.8	0.1	122901	8.63	9818	0.0167	1	0.014	15	0.0384
			0.5	2	2622	5244.8											
	6-1	2	0.5	1	2328	4656.2	4691.4	9382.8	0.1	122901	7.63	8598	0.0146	1	0.014	15	0.0337
			0.5	2	2363	4726.6											
	6-2	2	0.5	1	2318	4636.6	4687.4	9374.8	0.1	122901	7.63	8590	0.0146	1	0.014	15	0.0336
			0.5	2	2369	4738.2											
	6-3	2	0.5	1	2435	4870.6	4829.1	9658.2	0.1	122901	7.86	8874	0.0150	1	0.014	15	0.0347
			0.5	2	2394	4787.6											
	7-1	2	0.5	1	2029	4057.2	4108.8	8217.6	0.1	122901	6.69	7433	0.0126	1	0.014	15	0.0291
			0.5	2	2080	4160.4											
	7-2	2	0.5	1	2394	4787.8	4846.5	9693	0.1	122901	7.89	8909	0.0151	1	0.014	15	0.0349
			0.5	2	2453	4905.2											
	7-3	2	0.5	1	2319	4637	4708.6	9417.2	0.1	122901	7.66	8633	0.0146	1	0.014	15	0.0338
			0.5	2	2390	4780.2											
	8-1	2	0.5	1	2307	4613.6	4766.5	9533	0.1	122901	7.76	8749	0.0148	1	0.014	15	0.0343
			0.5	2	2460	4919.4											
	8-2	2	0.5	1	2337	4673	4674	9348	0.1	122901	7.61	8564	0.0145	1	0.014	15	0.0335
			0.5	2	2338	4675											
	8-3	2	0.5	1	2423	4845.4	4847	9694	0.1	122901	7.89	8910	0.0151	1	0.014	15	0.0349
			0.5	2	2424	4848.6											

Assay Date	9/27/2005	Test Chemical ID	4	# Concentrations tested	Microsome 8 type	placental	Microsome ID Lot # BAA	Technician ID TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0366	0.0010
Full activity	End	0.0357	0.0007
Full activity	Overall	0.0361	0.0009
Background	Beginning	-0.0002	0.000700969
Background	End	0.0002	0.000596527
Background	Overall	0.0000	0.000562672
Positive	Beginning	0.0206	0.0006
Positive	End	0.0191	0.0003
Positive	Overall	0.0198	0.0010
Negative	Beginning	0.0338	0.0008
Negative	End	0.0333	0.0000
Negative	Overall	0.0336	0.0006

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
4	1	1	1.00E-03	-3.00	0.0014
4	1	2	1.00E-03	-3.00	-0.0006
4	1	3	1.00E-03	-3.00	-0.0002
4	2	1	1.00E-04	-4.00	0.0064
4	2	2	1.00E-04	-4.00	0.0056
4	2	3	1.00E-04	-4.00	0.0062
4	3	1	1.00E-05	-5.00	0.0348
4	3	2	1.00E-05	-5.00	0.0348
4	3	3	1.00E-05	-5.00	0.0349
4	4	1	1.00E-06	-6.00	0.0367
4	4	2	1.00E-06	-6.00	0.0361
4	4	3	1.00E-06	-6.00	0.0357
4	5	1	1.00E-07	-7.00	0.0387
4	5	2	1.00E-07	-7.00	0.0369
4	5	3	1.00E-07	-7.00	0.0384
4	6	1	1.00E-08	-8.00	0.0337
4	6	2	1.00E-08	-8.00	0.0336
4	6	3	1.00E-08	-8.00	0.0347
4	7	1	1.00E-09	-9.00	0.0291
4	7	2	1.00E-09	-9.00	0.0349
4	7	3	1.00E-09	-9.00	0.0338
4	8	1	1.00E-10	-10.00	0.0343
4	8	2	1.00E-10	-10.00	0.0335
4	8	3	1.00E-10	-10.00	0.0349

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	3.81	-1.58	-0.50
2	-4.00	17.68	15.47	17.09
3	-5.00	96.33	96.37	96.64
4	-6.00	101.51	100.03	98.72
5	-7.00	107.22	102.08	106.41
6	-8.00	93.18	93.10	96.17
7	-9.00	80.56	96.55	93.56
8	-10.00	94.81	92.81	96.56

Assay Date	9/28/2005	Test Chemical ID	4	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	Lot # BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0197	21619	1097386		
2	0.0200	22947	1147350		
3	0.0198	22666	1144747		
4	0.0198	23552	1189495		
5	0.0198	23889	1206515		
			Average DPM/g soln		1157099
			SD		42734
			CV		3.69
			µCi/g soln		0.521

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.2	10		1020.00
Dilution A			100	10.20
Dilution B			10	1.02

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6061 g
Mass of dilution B used in substrate prep	5.4283 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.576391 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00590 µg/g soln.
µg/g soln.

a. µCi/g soln 0.521
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.576391 + 0.00590
= 0.582291 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 0.895 µCi/µg ASDN

569119 dpm/nmol

Assay Date	9/28/2005	Test Chemical ID	4	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	Lot # BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.451	0.280	0.128	0.066	0.027	0.014	0.000			
	0.489	0.286	0.134	0.070	0.026	0.013	0.000			
	0.493	0.289	0.131	0.068	0.025	0.031	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.247	0.027	0.070
	0.250	0.027	0.071
			0.068

Standard concentration (µg/mL)	Volume of stock used (µL)	Final volume of Std (µL)	mg Protein per µL	Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0500	0.477	0.477	0.0480	m, b	0.105	-0.002
125	100	200	0.00013	200	0.0250	0.285	0.285	0.0278	se_m, se_b	0.005	0.001
50	200	1000	0.00005	200	0.0100	0.131	0.131	0.0117	r^2, se_y	0.990	0.092
25	100	1000	0.00003	200	0.0050	0.067	0.067	0.0050	F, df	400	4
10	40	1000	0.00001	200	0.0020	0.026	0.026	0.0006	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.019	0.019	-0.0001			

Regression results are calculated using the function LINEST

Blank	0.000		r^2=	0.990									
			m=	0.105									
			b=	-0.002									

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.247	0.247	0.024	200	1	1		0.000	0.000	0.120
QC 100	0.250	0.250	0.024	200	1	1		0.000		
QC 100										
QC 10	0.027	0.027	0.001	200	1	1		0.000	0.000	0.005
QC 10	0.027	0.027	0.001	200	1	1		0.000		
QC 10										
Microsomes	0.070	0.070	0.005	200	120	42000		0.009	0.009	9.373
Microsomes	0.071	0.071	0.005	200	120	42000		0.009		
Microsomes	0.068	0.068	0.005	200	120	42000		0.009		

Assay Date	9/28/2005	Test Chemical ID	4	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	Lot # BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.451	0.280	0.128	0.066	0.027	0.014	0.000			
	0.489	0.286	0.134	0.070	0.026	0.013	0.000			
	0.493	0.289	0.131	0.066	0.025	0.031	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.247	0.027	0.070
	0.250	0.027	0.071
			0.068

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.477	0.477	0.0418
125	100	200	0.00013	200	0.0250	0.285	0.285	0.0246
50	200	1000	0.00005	200	0.0100	0.131	0.131	0.0109
25	100	1000	0.00003	200	0.0050	0.067	0.067	0.0052
10	40	1000	0.00001	200	0.0020	0.026	0.026	0.0015
5	20	1000	0.00001	200	0.0010	0.019	0.019	0.0009
	Blank		0.000					

Variables	Regression results	
m, b	0.089	-0.001
se_m, se_b	0.003	0.000
r^2, se_y	0.997	0.001
F, df	978	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.997
m= 0.089
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.247	0.247	0.021	200	1	1	0.000	0.000	0.106
QC 100	0.250	0.250	0.021	200	1	1	0.000		
QC 100									
QC 10	0.027	0.027	0.002	200	1	1	0.000	0.000	0.009
QC 10	0.027	0.027	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.070	0.070	0.005	200	120	42000	0.009	0.009	9.618
Microsomes	0.071	0.071	0.005	200	120	42000	0.010		
Microsomes	0.068	0.068	0.005	200	120	42000	0.009		

Assay Date	9/28/2005	Test Chemical ID		4	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate # 2

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	42	mL total volume
	350	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	350	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	9.617817
Protein Concentration (dilution added to assay, mg/mL):	0.027479

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	5.00E-05
4	2.50E-05
5	1.00E-05
6	1.00E-06
7	1.00E-07
8	1.00E-08

Assay Date	9/28/2005	Test Chemical ID	4	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed						
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (in/fal)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3077	6154.6	6190.7	12381.4	0.1	115710	10.70	12073	0.0212	1	0.014	15	0.0515
			0.5	2	3113	6226.8											
	2	2	0.5	1	2998	5985	6058.6	12117.2	0.1	115710	10.47	11809	0.0207	1	0.014	15	0.0503
			0.5	2	3061	6122.2											
	3	2	0.5	1	2911	5822.8	5781.8	11563.6	0.1	115710	9.99	11255	0.0198	1	0.014	15	0.0480
			0.5	2	2870	5740.8											
	4	2	0.5	1	2751	5502.8	5449.5	10899	0.1	115710	9.42	10591	0.0186	1	0.014	15	0.0451
			0.5	2	2698	5396.4											
Background control	1	2	0.5	1	100	199.98	188.38	376.76	0.1	115710	0.33	68	0.0001	1	0.014	15	0.0003
			0.5	2	88	176.78											
	2	2	0.5	1	56	112.72	111.7	223.4	0.1	115710	0.19	-85	-0.0001	1	0.014	15	-0.0004
			0.5	2	55	110.68											
	3	2	0.5	1	114	228.86	187.63	375.26	0.1	115710	0.32	67	0.0001	1	0.014	15	0.0003
			0.5	2	73	146.4											
	4	2	0.5	1	63	125.2	129.07	258.14	0.1	115710	0.22	-50	-0.0001	1	0.014	15	-0.0002
			0.5	2	66	132.94											
Positive control	1	2	0.5	1	1475	2952	2961.6	5923.2	0.1	115710	5.12	5615	0.0099	1	0.014	15	0.0239
			0.5	2	1486	2971.2											
	2	2	0.5	1	1439	2877	2888.8	5777.6	0.1	115710	4.99	5469	0.0096	1	0.014	15	0.0233
			0.5	2	1450	2900.6											
	3	2	0.5	1	1370	2740.2	2733.9	5467.8	0.1	115710	4.73	5159	0.0091	1	0.014	15	0.0220
			0.5	2	1364	2727.6											
	4	2	0.5	1	1409	2818.4	2855.9	5711.8	0.1	115710	4.94	5403	0.0095	1	0.014	15	0.0230
			0.5	2	1447	2893.4											
Negative Control	1	2	0.5	1	2780	5559.4	5554.3	11108.6	0.1	115710	9.60	10800	0.0190	1	0.014	15	0.0460
			0.5	2	2775	5549.2											
	2	2	0.5	1	2844	5687.8	5718.5	11437	0.1	115710	9.88	11129	0.0196	1	0.014	15	0.0474
			0.5	2	2875	5749.2											
	3	2	0.5	1	2759	5518	5465	10930	0.1	115710	9.45	10622	0.0187	1	0.014	15	0.0453
			0.5	2	2706	5412											
	4	2	0.5	1	2668	5336.2	5341.9	10683.8	0.1	115710	9.23	10375	0.0182	1	0.014	15	0.0442
			0.5	2	2674	5347.6											
4	1-1	2	0.5	1	124	248.76	240.56	481.12	0.1	115710	0.42	173	0.0003	1	0.014	15	0.0007
			0.5	2	116	232.36											
	1-2	2	0.5	1	80	159.48	154.67	309.74	0.1	115710	0.27	-1	0.0000	1	0.014	15	0.0000
			0.5	2	75	150.26											
	1-3	2	0.5	1	54	108.9	110.43	220.86	0.1	115710	0.19	-86	-0.0002	1	0.014	15	-0.0004
			0.5	2	56	111.96											
	2-1	2	0.5	1	423	845.54	839.68	1679.36	0.1	115710	1.45	1371	0.0024	1	0.014	15	0.0058
			0.5	2	417	833.82											
	2-2	2	0.5	1	395	789.02	789.61	1579.22	0.1	115710	1.36	1271	0.0022	1	0.014	15	0.0054
			0.5	2	395	790.2											
	2-3	2	0.5	1	450	900.42	903.04	1806.08	0.1	115710	1.56	1498	0.0026	1	0.014	15	0.0064
			0.5	2	453	905.66											
	3-1	2	0.5	1	840	1679.48	1678.12	3356.24	0.1	115710	2.90	3048	0.0054	1	0.014	15	0.0130
			0.5	2	838	1676.76											
	3-2	2	0.5	1	897	1794.3	1752.48	3504.96	0.1	115710	3.03	3197	0.0056	1	0.014	15	0.0136
			0.5	2	855	1710.66											
	3-3	2	0.5	1	850	1700.84	1680.91	3361.82	0.1	115710	2.91	3053	0.0054	1	0.014	15	0.0130
			0.5	2	830	1660.98											
	4-1	2	0.5	1	1372	2743.8	2772.4	5544.8	0.1	115710	4.79	5236	0.0092	1	0.014	15	0.0223
			0.5	2	1401	2801											
	4-2	2	0.5	1	1398	2795	2791	5582	0.1	115710	4.82	5274	0.0093	1	0.014	15	0.0225
			0.5	2	1394	2787											
	4-3	2	0.5	1	1387	2774.4	2805.3	5610.6	0.1	115710	4.85	5302	0.0093	1	0.014	15	0.0226
			0.5	2	1418	2836.2											
	5-1	2	0.5	1	2476	4952.8	4954.6	9909.2	0.1	115710	8.56	9601	0.0169	1	0.014	15	0.0409
			0.5	2	2478	4956.4											
	5-2	2	0.5	1	2500	5000.4	5065.8	10131.6	0.1	115710	8.76	9823	0.0173	1	0.014	15	0.0419
			0.5	2	2566	5131.2											
	5-3	2	0.5	1	2437	4873.6	4846.6	9693.2	0.1	115710	8.38	9385	0.0165	1	0.014	15	0.0400
			0.5	2	2410	4819.6											
	6-1	2	0.5	1	2790	5579.2	5531.6	11063.2	0.1	115710	8.56	10755	0.0189	1	0.014	15	0.0458
			0.5	2	2742	5484											
	6-2	2	0.5	1	2805	5610.4	5618.3	11236.6	0.1	115710	9.71	10928	0.0192	1	0.014	15	0.0466
			0.5	2	2813	5626.2											
	6-3	2	0.5	1	2835	5669.6	5637.9	11275.8	0.1	115710	9.74	10967	0.0193	1	0.014	15	0.0468
			0.5	2	2803	5606.2											
	7-1	2	0.5	1	2557	5113.6	5184.5	10369	0.1	115710	8.96	10061	0.0177	1	0.014	15	0.0429
			0.5	2	2628	5255.4											
	7-2	2	0.5	1	2755	5509.8	5499.8	10999.6	0.1	115710	9.51	10691	0.0188	1	0.014	15	0.0456
			0.5	2	2745	5489.8											
	7-3	2	0.5	1	2693	5385.8	5369.6	10739.2	0.1	115710	9.28	10431	0.0183	1	0.014	15	0.0445
			0.5	2	2677	5353.4											
	8-1	2	0.5	1	2670	5339.2	5344.6	10689.2	0.1	115710	9.24	10381	0.0182	1	0.014	15	0.0443
			0.5	2	2675	5350											
	8-2	2	0.5	1	2680	5359.8	5368.1	10736.2	0.1	115710	9.28	10428	0.0183	1	0.014	15	0.0445
			0.5	2	2688	5376.4											
	8-3	2	0.5	1	2835	5670.8	5610.9	11221.8	0.1	115710	9.70	10913	0.0192	1	0.014	15	0.0465
			0.5	2	2776	5551											

Assay Date	9/28/2005	Test Chemical ID	4	# Concentrations tested	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0509	0.0008
Full activity	End	0.0466	0.0020
Full activity	Overall	0.0487	0.0028
Background	Beginning	0.0000	0.000462268
Background	End	0.0000	0.000353031
Background	Overall	0.0000	0.000338301
Positive	Beginning	0.0236	0.0004
Positive	End	0.0225	0.0007
Positive	Overall	0.0231	0.0008
Negative	Beginning	0.0467	0.0010
Negative	End	0.0448	0.0007
Negative	Overall	0.0457	0.0014

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
4	1	1	1.00E-03	-3.00	0.0007
4	1	2	1.00E-03	-3.00	0.0000
4	1	3	1.00E-03	-3.00	-0.0004
4	2	1	1.00E-04	-4.00	0.0058
4	2	2	1.00E-04	-4.00	0.0054
4	2	3	1.00E-04	-4.00	0.0064
4	3	1	5.00E-05	-4.30	0.0130
4	3	2	5.00E-05	-4.30	0.0136
4	3	3	5.00E-05	-4.30	0.0130
4	4	1	2.50E-05	-4.60	0.0223
4	4	2	2.50E-05	-4.60	0.0225
4	4	3	2.50E-05	-4.60	0.0226
4	5	1	1.00E-05	-5.00	0.0409
4	5	2	1.00E-05	-5.00	0.0419
4	5	3	1.00E-05	-5.00	0.0400
4	6	1	1.00E-06	-6.00	0.0458
4	6	2	1.00E-06	-6.00	0.0466
4	6	3	1.00E-06	-6.00	0.0468
4	7	1	1.00E-07	-7.00	0.0429
4	7	2	1.00E-07	-7.00	0.0456
4	7	3	1.00E-07	-7.00	0.0445
4	8	1	1.00E-08	-8.00	0.0443
4	8	2	1.00E-08	-8.00	0.0445
4	8	3	1.00E-08	-8.00	0.0465

Percent of control values				
		Replicate		
Level	Log[test substance]	1	2	3
1	-3.00	1.51	0.01	-0.77
2	-4.00	11.99	11.12	13.10
3	-4.30	26.66	27.96	26.71
4	-4.60	45.81	46.13	46.38
5	-5.00	83.98	85.93	82.09
6	-6.00	94.08	95.59	95.94
7	-7.00	88.00	93.52	91.24
8	-8.00	90.81	91.22	95.46

Assay Date	9/29/2005	Test Chemical ID	4	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	Lot# BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0198	23443	1183990		
2	0.0198	23157	1169545		
3	0.0197	24176	1227208		
4	0.0201	25497	1268507		
5	0.0199	25354	1274070		
			Average DPM/g soln		1224664
			SD		47598
			CV		3.89
			µCi/g soln		0.552

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.2	10		1020.00
Dilution A			100	10.20
Dilution B			10	1.02

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.595 g
Mass of dilution B used in substrate prep	5.4129 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.57542 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00624 µg/g soln.
µg/g soln.

a. µCi/g soln 0.552
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.575420 + 0.00624
= 0.581665 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 0.948 µCi/µg ASDN

603000 dpm/nmol

Assay Date	9/29/2005	Test Chemical ID	4	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	Lot# BAA

	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.469	0.282	0.126	0.067	0.031	0.013	0.000	25	100	11343-77
	0.467	0.277	0.133	0.071	0.030	0.017	0.000			
	0.478	0.283	0.134	0.068	0.029	0.018	0.000			

	QC 100	QC 10	Microsomes
Samples:	0.245	0.035	0.072
	0.245	0.033	0.074
			0.072

Standard concentration (mg/mL) LE in 5-10-06 BAL	Volume of stock used	Final volume of Std	mg Protein per µL	Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.471	0.471	0.0480
125	100	200	0.00013	200	0.0250	0.280	0.280	0.0277
50	200	1000	0.00005	200	0.0100	0.131	0.131	0.0118
25	100	1000	0.00003	200	0.0050	0.068	0.068	0.0051
10	40	1000	0.00001	200	0.0020	0.030	0.030	0.0010
5	20	1000	0.00001	200	0.0010	0.016	0.016	-0.0005
			Blank	0.000				

Variables	Regression results	
m, b	0.107	-0.002
se_m, se_b	0.005	0.001
r^2, se_y	0.990	0.002
F, df	409	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.990
m= 0.107
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.245	0.245	0.024	200	1	1	0.000	0.000	0.119
QC 100	0.245	0.245	0.024	200	1	1	0.000		
QC 100									
QC 10	0.035	0.035	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.033	0.033	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.072	0.072	0.005	200	120	42000	0.010	0.010	9.705
Microsomes	0.074	0.074	0.006	200	120	42000	0.010		
Microsomes	0.072	0.072	0.005	200	120	42000	0.010		

Assay Date	9/29/2005	Test Chemical ID	4	# Concentrations tested	8						
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	Lot# BAA				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.469	0.282	0.126	0.067	0.031	0.013	0.000			
	0.467	0.277	0.133	0.071	0.030	0.017	0.000			
	0.478	0.283	0.134	0.068	0.029	0.018	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.245	0.035	0.072
	0.245	0.033	0.074
			0.072

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0500	0.471	0.471	0.0419	m, b	0.091	-0.001
125	100	200	0.00013	200	0.0250	0.280	0.280	0.0245	se_m, se_b	0.003	0.000
50	200	1000	0.00005	200	0.0100	0.131	0.131	0.0110	r^2, se_y	0.996	0.001
25	100	1000	0.00003	200	0.0050	0.068	0.068	0.0052	F, df	753	3
10	40	1000	0.00001	200	0.0020	0.030	0.030	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.016	0.016	0.0005			

Regression results are calculated using the function LINEST

Blank	0.000	r^2=	0.996		
		m=	0.091		
		b=	-0.001		

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.245	0.245	0.021	200	1	1	0.000	0.000	0.107
QC 100	0.245	0.245	0.021	200	1	1	0.000		
QC 100									
QC 10	0.035	0.035	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.033	0.033	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.072	0.072	0.006	200	120	42000	0.010	0.010	9.897
Microsomes	0.074	0.074	0.006	200	120	42000	0.010		
Microsomes	0.072	0.072	0.006	200	120	42000	0.010		

Assay Date	9/29/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot# BAA	Technician ID	TD	Replicate #	3

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	42	mL total volume
	350	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	350	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	9.89717
Protein Concentration (dilution added to assay, mg/mL):	0.028278

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	5.00E-05
4	2.50E-05
5	1.00E-05
6	1.00E-06
7	1.00E-07
8	1.00E-08

Assay Date	9/29/2005	Test Chemical ID	4	# Concentrations tested	6	Microsome type	placental	Microsome ID	Lot# BAA	Technician ID	TD	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq.	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	2570	5139.6	5343.6	10687.2	0.1	122466	8.73	10409	0.0173	1	0.014	15	0.0407
			0.5	2	2774	5547.6											
	2	2	0.5	1	2832	5663.2	5878.3	11756.6	0.1	122466	9.60	11478	0.0190	1	0.014	15	0.0449
			0.5	2	2947	5893.4											
	3	2	0.5	1	2714	5427.4	5346.8	10693.6	0.1	122466	8.73	10415	0.0173	1	0.014	15	0.0407
			0.5	2	2633	5266.2											
	4	2	0.5	1	2735	5469.2	5351.4	10702.8	0.1	122466	8.74	10424	0.0173	1	0.014	15	0.0408
			0.5	2	2617	5233.6											
Background control	1	2	0.5	1	89	178.96	162.79	325.58	0.1	122466	0.27	47	0.0001	1	0.014	15	0.0002
			0.5	2	73	146.62											
	2	2	0.5	1	41	82.42	84.8	169.6	0.1	122466	0.14	-109	-0.0002	1	0.014	15	-0.0004
			0.5	2	44	87.18											
	3	2	0.5	1	87	174.96	166.44	332.88	0.1	122466	0.27	54	0.0001	1	0.014	15	0.0002
			0.5	2	79	157.92											
	4	2	0.5	1	85	169.66	143.09	286.18	0.1	122466	0.23	8	0.0000	1	0.014	15	0.0000
			0.5	2	58	116.52											
Positive control	1	2	0.5	1	1452	2904.2	2945.8	5891.6	0.1	122466	4.81	5613	0.0093	1	0.014	15	0.0219
			0.5	2	1494	2987.4											
	2	2	0.5	1	1324	2647.8	2672	5344	0.1	122466	4.36	5085	0.0084	1	0.014	15	0.0198
			0.5	2	1348	2696.2											
	3	2	0.5	1	1343	2686.4	2658.3	5316.6	0.1	122466	4.34	5038	0.0084	1	0.014	15	0.0197
			0.5	2	1315	2630.2											
	4	2	0.5	1	1294	2588.6	2579.9	5159.8	0.1	122466	4.21	4881	0.0081	1	0.014	15	0.0191
			0.5	2	1286	2571.2											
Negative Control	1	2	0.5	1	2741	5482	5502	11004	0.1	122466	8.99	10725	0.0178	1	0.014	15	0.0419
			0.5	2	2761	5522											
	2	2	0.5	1	2858	5716.2	5691.8	11383.6	0.1	122466	9.30	11105	0.0184	1	0.014	15	0.0434
			0.5	2	2834	5667.4											
	3	2	0.5	1	2541	5082.6	5087	10174	0.1	122466	8.31	9895	0.0164	1	0.014	15	0.0387
			0.5	2	2546	5091.4											
	4	2	0.5	1	2635	5269.8	5281.3	10562.6	0.1	122466	8.62	10284	0.0171	1	0.014	15	0.0402
			0.5	2	2646	5292.8											
4	1-1	2	0.5	1	80	160.3	154.81	309.62	0.1	122466	0.25	31	0.0001	1	0.014	15	0.0001
			0.5	2	75	149.32											
	1-2	2	0.5	1	80	160.82	156.05	312.1	0.1	122466	0.25	34	0.0001	1	0.014	15	0.0001
			0.5	2	76	151.28											
	1-3	2	0.5	1	86	172.24	160.99	321.96	0.1	122466	0.26	43	0.0001	1	0.014	15	0.0002
			0.5	2	75	149.74											
	2-1	2	0.5	1	424	848.1	830.55	1661.1	0.1	122466	1.36	1383	0.0023	1	0.014	15	0.0054
			0.5	2	407	813											
	2-2	2	0.5	1	431	862.92	881.71	1763.42	0.1	122466	1.44	1485	0.0025	1	0.014	15	0.0058
			0.5	2	450	900.5											
	2-3	2	0.5	1	480	958.34	959.82	1919.64	0.1	122466	1.57	1641	0.0027	1	0.014	15	0.0064
			0.5	2	480	960.3											
	3-1	2	0.5	1	942	1884.88	1879.12	3758.24	0.1	122466	3.07	3460	0.0058	1	0.014	15	0.0136
			0.5	2	937	1873.36											
	3-2	2	0.5	1	929	1857.72	1858.71	3717.42	0.1	122466	3.04	3439	0.0057	1	0.014	15	0.0134
			0.5	2	930	1859.7											
	3-3	2	0.5	1	983	1965.96	1935.61	3871.22	0.1	122466	3.16	3593	0.0060	1	0.014	15	0.0140
			0.5	2	953	1905.26											
	4-1	2	0.5	1	1314	2627.8	2648.2	5296.4	0.1	122466	4.32	5018	0.0083	1	0.014	15	0.0196
			0.5	2	1334	2668.6											
	4-2	2	0.5	1	1396	2792.2	2716.1	5432.2	0.1	122466	4.44	5154	0.0085	1	0.014	15	0.0201
			0.5	2	1320	2640											
	4-3	2	0.5	1	1383	2765.4	2809.8	5619.6	0.1	122466	4.59	5341	0.0089	1	0.014	15	0.0209
			0.5	2	1427	2854.2											
	5-1	2	0.5	1	2386	4772.4	4817.4	9634.8	0.1	122466	7.87	9356	0.0155	1	0.014	15	0.0366
			0.5	2	2431	4862.4											
	5-2	2	0.5	1	2403	4805.8	4714.8	9429.6	0.1	122466	7.70	9151	0.0152	1	0.014	15	0.0358
			0.5	2	2312	4623.8											
	5-3	2	0.5	1	2454	4907.8	4871.5	9743	0.1	122466	7.96	9464	0.0157	1	0.014	15	0.0370
			0.5	2	2418	4835.2											
	6-1	2	0.5	1	2732	5463.2	5448.5	10897.2	0.1	122466	8.90	10619	0.0176	1	0.014	15	0.0415
			0.5	2	2717	5434											
	6-2	2	0.5	1	2692	5384.6	5433.8	10867.6	0.1	122466	8.87	10589	0.0176	1	0.014	15	0.0414
			0.5	2	2742	5483											
	6-3	2	0.5	1	2657	5314.4	5290.3	10580.6	0.1	122466	8.64	10302	0.0171	1	0.014	15	0.0403
			0.5	2	2633	5266.2											
	7-1	2	0.5	1	2591	5182.6	5241.6	10483.2	0.1	122466	8.56	10205	0.0169	1	0.014	15	0.0399
			0.5	2	2650	5300.4											
	7-2	2	0.5	1	2650	5300.4	5301.7	10603.4	0.1	122466	8.66	10325	0.0171	1	0.014	15	0.0404
			0.5	2	2652	5303											
	7-3	2	0.5	1	2796	5589	5637.4	11274.8	0.1	122466	9.21	10996	0.0182	1	0.014	15	0.0430
			0.5	2	2843	5685.8											
	8-1	2	0.5	1	2679	5357	5399.3	10798.6	0.1	122466	8.82	10520	0.0174	1	0.014	15	0.0411
			0.5	2	2721	5441.6											
	8-2	2	0.5	1	2691	5382.8	5393.6	10787.2	0.1	122466	8.81	10509	0.0174	1	0.014	15	0.0411
			0.5	2	2702	5404.4											
	8-3	2	0.5	1	2625	5250.8	5288	10576	0.1	122466	8.64	10297	0.0171	1	0.014	15	0.0403
			0.5	2	2663	5325.2											

Assay Date	9/29/2005	Test Chemical ID		4	# Concentrations tested	Microsome 8 type	placental	Microsome ID Lot# BAA		Technician ID TD		Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0428	0.0030
Full activity	End	0.0407	0.0000
Full activity	Overall	0.0418	0.0021
Background	Beginning	-0.0001	0.000431224
Background	End	0.0001	0.000129107
Background	Overall	0.0000	0.000295109
Positive	Beginning	0.0209	0.0015
Positive	End	0.0194	0.0004
Positive	Overall	0.0201	0.0012
Negative	Beginning	0.0427	0.0010
Negative	End	0.0394	0.0011
Negative	Overall	0.0411	0.0021

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
4	1	1	1.00E-03	-3.00	0.0001
4	1	2	1.00E-03	-3.00	0.0001
4	1	3	1.00E-03	-3.00	0.0002
4	2	1	1.00E-04	-4.00	0.0054
4	2	2	1.00E-04	-4.00	0.0058
4	2	3	1.00E-04	-4.00	0.0064
4	3	1	5.00E-05	-4.30	0.0136
4	3	2	5.00E-05	-4.30	0.0134
4	3	3	5.00E-05	-4.30	0.0140
4	4	1	2.50E-05	-4.60	0.0196
4	4	2	2.50E-05	-4.60	0.0201
4	4	3	2.50E-05	-4.60	0.0209
4	5	1	1.00E-05	-5.00	0.0366
4	5	2	1.00E-05	-5.00	0.0358
4	5	3	1.00E-05	-5.00	0.0370
4	6	1	1.00E-06	-6.00	0.0415
4	6	2	1.00E-06	-6.00	0.0414
4	6	3	1.00E-06	-6.00	0.0403
4	7	1	1.00E-07	-7.00	0.0399
4	7	2	1.00E-07	-7.00	0.0404
4	7	3	1.00E-07	-7.00	0.0430
4	8	1	1.00E-08	-8.00	0.0411
4	8	2	1.00E-08	-8.00	0.0411
4	8	3	1.00E-08	-8.00	0.0403

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.29	0.31	0.41
2	-4.00	12.94	13.90	15.36
3	-4.30	32.58	32.19	33.63
4	-4.60	46.98	48.25	50.00
5	-5.00	87.59	85.67	88.61
6	-6.00	99.41	99.13	96.45
7	-7.00	95.54	96.66	102.95
8	-8.00	98.49	98.38	96.40

Assay Date	9/30/2005	Test Chemical ID	5	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	23133	1168333	
2	0.0201	24600	1223881	
3	0.0202	25175	1246287	
4	0.0203	24852	1224236	
5	0.0198	23736	1198788	
			Average DPM/g soln	1212305
			SD	29780
			CV	2.46
			µCi/g soln	0.546

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.5696 g
Mass of dilution B used in substrate prep	5.42 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.566377 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00618 µg/g soln.
µg/g soln.

a. µCi/g soln	0.546
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.566377 + 0.00618
= 0.572559 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 0.954 µCi/µg ASDN

606408 dpm/nmol

Assay Date	9/30/2005	Test Chemical ID	5	# Concentrations tested	6	
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID 6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.481	0.294	0.133	0.070	0.034	0.020	0.000			
	0.487	0.277	0.137	0.075	0.031	0.017	0.000			
	0.493	0.279	0.127	0.077	0.034	0.021	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.266	0.403	0.077
	0.260	0.036	0.074
			0.076

Standard concentration (ng/mL)	Volume of stock used	Final volume of Std	mg Protein per µL		Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200	0.0500	0.487	0.487	0.0484	m, b	0.104	-0.002
125	100	200	0.00013		200	0.0250	0.283	0.283	0.0272	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200	0.0100	0.132	0.132	0.0114	r^2, se_y	0.993	0.002
25	100	1000	0.00003		200	0.0050	0.074	0.074	0.0053	F, df	598	4
10	40	1000	0.00001		200	0.0020	0.033	0.033	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010	0.019	0.019	-0.0004			
				Blank	0.000							

r^2= 0.993
m= 0.104
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.266	0.266	0.025	200	1	1	0.000	0.000	0.125
QC 100	0.260	0.260	0.025	200	1	1	0.000		
QC 100									
QC 10	0.403	0.403	0.040	200	1	1	0.000	0.000	0.103
QC 10	0.036	0.036	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.077	0.077	0.006	200	1	949	0.027	0.026	26.158
Microsomes	0.074	0.074	0.005	200	1	949	0.025		
Microsomes	0.076	0.076	0.006	200	1	949	0.026		

Assay Date 9/30/2005 | Test Chemical ID 5 | # Concentrations tested 8

Technician ID TD | Replicate # 1 | Microsome type placental | Microsome ID 6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.481	0.294	0.133	0.070	0.034	0.020	0.000			
	0.487	0.277	0.137	0.075	0.031	0.017	0.000			
	0.493	0.279	0.127	0.077	0.034	0.021	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.266	0.040	0.077
	0.260	0.036	0.074
			0.076

Standard concentration (ng/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.487	0.487	0.0432
125	100	200	0.00013	200	0.0250	0.283	0.283	0.0246
50	200	1000	0.00005	200	0.0100	0.132	0.132	0.0108
25	100	1000	0.00003	200	0.0050	0.074	0.074	0.0055
10	40	1000	0.00001	200	0.0020	0.033	0.033	0.0017
5	20	1000	0.00001	200	0.0010	0.019	0.019	0.0004
			Blank	0.000				

Variables	Regression results	
m, b	0.091	-0.001
se_m, se_b	0.003	0.000
r^2, se_y	0.996	0.001
F, df	841	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

r^2= 0.996
m= 0.091
b= -0.001

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.266	0.266	0.023	200	1	1	0.000	0.000	0.114
QC 100	0.260	0.260	0.022	200	1	1	0.000		
QC 100									
QC 10	0.040	0.040	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.036	0.036	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.077	0.077	0.006	200	1	949	0.027	0.027	26.670
Microsomes	0.074	0.074	0.005	200	1	949	0.026		
Microsomes	0.076	0.076	0.006	200	1	949	0.027		

Assay Date	9/30/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1
			5										

Microsome Dilution Details

Dilution A	0.1	mL microsome Stock used
	5	mL total volume
	50	dilution factor
Dilution B	2.5	mL microsome Dilution A used
	47.5	mL total volume
	19	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	950	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	26.67013
Protein Concentration (dilution added to assay, mg/mL):	0.028074

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	2.50E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Battelle Study No. G608316

B-77

Test Chemical ID	5	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
2	0.5	1	3508	7016.6	7089.8	14179.6	0.1	121231	11.70	13993	0.0231	1	0.014	15	0.0548
	0.5	2	3582	7163											
2	0.5	1	3602	7203.6	7195.7	14391.4	0.1	121231	11.87	14204	0.0234	1	0.014	15	0.0556
	0.5	2	3594	7187.8											
2	0.5	1	3325	6649.8	6694.7	13389.4	0.1	121231	11.04	13202	0.0218	1	0.014	15	0.0517
	0.5	2	3370	6739.6											
2	0.5	1	3303	6606.8	6605.9	13211.8	0.1	121231	10.90	13025	0.0215	1	0.014	15	0.0510
	0.5	2	3303	6605											
2	0.5	1	35	70.68	69.34	138.68	0.1	121231	0.11	-48	-0.0001	1	0.014	15	-0.0002
	0.5	2	34	68											
2	0.5	1	40	80.46	86.65	173.3	0.1	121231	0.14	-14	0.0000	1	0.014	15	-0.0001
	0.5	2	46	92.84											
2	0.5	1	73	145.98	123.84	247.68	0.1	121231	0.20	61	0.0001	1	0.014	15	0.0002
	0.5	2	51	101.7											
2	0.5	1	47	93.7	94.27	188.54	0.1	121231	0.16	1	0.0000	1	0.014	15	0.0000
	0.5	2	47	94.84											
2	0.5	1	1586	3172	3178.8	6357.6	0.1	121231	5.24	6171	0.0102	1	0.014	15	0.0242
	0.5	2	1593	3185.6											
2	0.5	1	1839	3677.4	3601.4	7202.8	0.1	121231	5.94	7016	0.0116	1	0.014	15	0.0275
	0.5	2	1763	3525.4											
2	0.5	1	1690	3380.8	3341.3	6682.6	0.1	121231	5.51	6496	0.0107	1	0.014	15	0.0254
	0.5	2	1651	3301.8											
2	0.5	1	1650	3299.2	3359.1	6718.2	0.1	121231	5.54	6531	0.0108	1	0.014	15	0.0256
	0.5	2	1710	3419											
2	0.5	1	3461	6921	7061.8	14123.6	0.1	121231	11.65	13937	0.0230	1	0.014	15	0.0546
	0.5	2	3601	7202.6											
2	0.5	1	3487	6973.4	6913.5	13827	0.1	121231	11.41	13640	0.0225	1	0.014	15	0.0534
	0.5	2	3427	6853.6											
2	0.5	1	3359	6717.4	6696.9	13393.8	0.1	121231	11.05	13207	0.0218	1	0.014	15	0.0517
	0.5	2	3338	6676.4											
2	0.5	1	3300	6600	6602.9	13205.8	0.1	121231	10.89	13019	0.0215	1	0.014	15	0.0510
	0.5	2	3303	6605.8											
2	0.5	1	3431	6861.8	6877.7	13755.4	0.1	121231	11.35	13568	0.0224	1	0.014	15	0.0531
	0.5	2	3447	6893.6											
2	0.5	1	3356	6711.4	6824.6	13649.2	0.1	121231	11.26	13462	0.0222	1	0.014	15	0.0527
	0.5	2	3469	6937.8											
2	0.5	1	3534	7067.8	7054.9	14109.8	0.1	121231	11.64	13923	0.0230	1	0.014	15	0.0545
	0.5	2	3521	7042											
2	0.5	1	3626	7251.8	7299.1	14598.2	0.1	121231	12.04	14411	0.0238	1	0.014	15	0.0564
	0.5	2	3673	7346.4											
2	0.5	1	3633	7266.2	7232.2	14464.4	0.1	121231	11.93	14277	0.0235	1	0.014	15	0.0559
	0.5	2	3599	7198.2											
2	0.5	1	3596	7192	7246.7	14493.4	0.1	121231	11.96	14306	0.0236	1	0.014	15	0.0560
	0.5	2	3651	7301.4											
2	0.5	1	3686	7372.8	7406.8	14813.6	0.1	121231	12.22	14627	0.0241	1	0.014	15	0.0573
	0.5	2	3720	7440.8											
2	0.5	1	3723	7445.2	7425	14850	0.1	121231	12.25	14663	0.0242	1	0.014	15	0.0574
	0.5	2	3702	7404.8											
2	0.5	1	3806	7611.4	7626	15252	0.1	121231	12.58	15065	0.0248	1	0.014	15	0.0590
	0.5	2	3820	7640.6											
2	0.5	1	3669	7337.8	7403.4	14806.8	0.1	121231	12.21	14620	0.0241	1	0.014	15	0.0573
	0.5	2	3735	7469											
2	0.5	1	3612	7224	7206.4	14412.8	0.1	121231	11.89	14226	0.0235	1	0.014	15	0.0557
	0.5	2	3594	7188.8											
2	0.5	1	3590	7179.2	7179.1	14358.2	0.1	121231	11.84	14171	0.0234	1	0.014	15	0.0555
	0.5	2	3590	7179											
2	0.5	1	3656	7312.4	7327.4	14654.8	0.1	121231	12.09	14468	0.0239	1	0.014	15	0.0567
	0.5	2	3671	7342.4											
2	0.5	1	3422	6844.8	6775.2	13550.4	0.1	121231	11.18	13363	0.0220	1	0.014	15	0.0523
	0.5	2	3353	6705.6											
2	0.5	1	3582	7163	7218.5	14437	0.1	121231	11.91	14250	0.0235	1	0.014	15	0.0558
	0.5	2	3637	7274											
2	0.5	1	3584	7167.6	7090.7	14181.4	0.1	121231	11.70	13994	0.0231	1	0.014	15	0.0548
	0.5	2	3507	7013.8											
2	0.5	1	3444	6888.2	6948.3	13896.6	0.1	121231	11.46	13710	0.0226	1	0.014	15	0.0537
	0.5	2	3504	7008.4											
2	0.5	1	3597	7194.8	7226.5	14453	0.1	121231	11.92	14266	0.0235	1	0.014	15	0.0559
	0.5	2	3629	7258.2											
2	0.5	1	3619	7237.8	7268.8	14537.6	0.1	121231	11.99	14351	0.0237	1	0.014	15	0.0562
	0.5	2	3650	7299.8											
2	0.5	1	3518	7036.4	7020.6	14041.2	0.1	121231	11.58	13854	0.0228	1	0.014	15	0.0543
	0.5	2	3502	7004.8											
2	0.5	1	3380	6760	6901.4	13802.8	0.1	121231	11.39	13616	0.0225	1	0.014	15	0.0533
	0.5	2	3521	7042.8											
2	0.5	1	3348	6696.6	6732.4	13464.8	0.1	121231	11.11	13278	0.0219	1	0.014	15	0.0520
	0.5	2	3384	6768.2											
2	0.5	1	3629	7257.8	7268.4	14536.8	0.1	121231	11.99	14350	0.0237	1	0.014	15	0.0562
	0.5	2	3640	7279											
2	0.5	1	3473	6946.8	6977	13954	0.1	121231	11.51	13767	0.0227	1	0.014	15	0.0539
	0.5	2	3504	7007.2											

Assay Date	9/30/2005	Test Chemical ID	5	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0552	0.0006
Full activity	End	0.0514	0.0005
Full activity	Overall	0.0533	0.0023
Background	Beginning	-0.0001	9.58637E-05
Background	End	0.0001	0.00016376
Background	Overall	0.0000	0.000178123
Positive	Beginning	0.0258	0.0023
Positive	End	0.0255	0.0001
Positive	Overall	0.0257	0.0014
Negative	Beginning	0.0540	0.0008
Negative	End	0.0513	0.0005
Negative	Overall	0.0527	0.0016

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
5	1	1	1.00E-04	-4.00	0.0531
5	1	2	1.00E-04	-4.00	0.0527
5	1	3	1.00E-04	-4.00	0.0545
5	2	1	1.00E-05	-5.00	0.0564
5	2	2	1.00E-05	-5.00	0.0559
5	2	3	1.00E-05	-5.00	0.0560
5	3	1	1.00E-06	-6.00	0.0573
5	3	2	1.00E-06	-6.00	0.0574
5	3	3	1.00E-06	-6.00	0.0590
5	4	1	2.50E-07	-6.60	0.0573
5	4	2	2.50E-07	-6.60	0.0557
5	4	3	2.50E-07	-6.60	0.0555
5	5	1	1.00E-07	-7.00	0.0567
5	5	2	1.00E-07	-7.00	0.0523
5	5	3	1.00E-07	-7.00	0.0558
5	6	1	1.00E-08	-8.00	0.0548
5	6	2	1.00E-08	-8.00	0.0537
5	6	3	1.00E-08	-8.00	0.0559
5	7	1	1.00E-09	-9.00	0.0562
5	7	2	1.00E-09	-9.00	0.0543
5	7	3	1.00E-09	-9.00	0.0533
5	8	1	1.00E-10	-10.00	0.0520
5	8	2	1.00E-10	-10.00	0.0562
5	8	3	1.00E-10	-10.00	0.0539

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	99.72	98.94	102.33
2	-5.00	105.92	104.93	105.15
3	-6.00	107.50	107.77	110.72
4	-6.60	107.45	104.55	104.15
5	-7.00	106.33	98.22	104.73
6	-8.00	102.85	100.76	104.85
7	-9.00	105.47	101.82	100.07
8	-10.00	97.59	105.47	101.18

Assay Date	10/3/2005	Test Chemical ID	5	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0201	21994	1094214		
2	0.0199	22397	1125477		
3	0.0202	22868	1132079		
4	0.0200	22676	1133800		
5	0.0199	22921	1151809		
			Average DPM/g soln		1127476
			SD		20998
			CV		1.86
			µCi/g soln		0.508

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.3	10		1030.00
Dilution A			100	10.30
Dilution B			10	1.03

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6534 g
Mass of dilution B used in substrate prep	5.4251 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.578848 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00575 µg/g soln.
µg/g soln.

a. µCi/g soln 0.508
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.578848 + 0.00575
= 0.584597 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 0.869 µCi/µg ASDN

552362 dpm/nmol

Assay Date	10/3/2005	Test Chemical ID	5	# Concentrations tested	8				
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.485	0.281	0.125	0.069	0.027	0.013	0.000			
	0.483	0.286	0.130	0.069	0.024	0.015	0.000			
	0.483	0.280	0.129	0.068	0.026	0.014	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.249	0.028	0.067
	0.253	0.030	0.069
			0.067

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL		Used		mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200		0.0500		0.484	0.484	0.0482	m, b	0.103	-0.002
125	100	200	0.00013		200		0.0250		0.283	0.283	0.0274	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200		0.0100		0.128	0.128	0.0115	r^2, se_y	0.992	0.002
25	100	1000	0.00003		200		0.0050		0.068	0.068	0.0053	F, df	502	4
10	40	1000	0.00001		200		0.0020		0.026	0.026	0.0009	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.014	0.014	-0.0003			
				Blank	0.000		r^2=	0.992						
							m=	0.103						
							b=	-0.002						

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.249	0.249	0.024	200	1	1	0.000	0.000	0.121
QC 100	0.253	0.253	0.024	200	1	1	0.000		
QC 100									
QC 10	0.028	0.028	0.001	200	1	1	0.000	0.000	0.006
QC 10	0.030	0.030	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.067	0.067	0.005	200	1	949	0.024	0.025	24.741
Microsomes	0.069	0.069	0.005	200	1	949	0.025		
Microsomes	0.067	0.067	0.005	200	1	949	0.024		

Assay Date	10/3/2005	Test Chemical ID	5	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.485	0.281	0.125	0.069	0.027	0.013	0.000			
	0.483	0.286	0.130	0.069	0.024	0.015	0.000			
	0.483	0.280	0.129	0.068	0.026	0.014	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.249	0.028	0.067
	0.253	0.030	0.069
			0.067

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0500	0.484	0.484	0.0426	m, b	0.089	-0.001
125	100	200	0.00013	200	0.0250	0.283	0.283	0.0246	se_m, se_b	0.003	0.000
50	200	1000	0.00005	200	0.0100	0.128	0.128	0.0108	r^2, se_y	0.997	0.001
25	100	1000	0.00003	200	0.0050	0.068	0.068	0.0054	F, df	900	3
10	40	1000	0.00001	200	0.0020	0.026	0.026	0.0016	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.014	0.014	0.0006			
			Blank	0.000							

Regression results are calculated using the function LINEST

r^2= 0.997
m= 0.089
b= -0.001

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.249	0.249	0.022	200	1	1	0.000	0.000	0.109
QC 100	0.253	0.253	0.022	200	1	1	0.000		
QC 100									
QC 10	0.028	0.028	0.002	200	1	1	0.000	0.000	0.010
QC 10	0.030	0.030	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.067	0.067	0.005	200	1	949	0.025	0.025	25.462
Microsomes	0.069	0.069	0.005	200	1	949	0.026		
Microsomes	0.067	0.067	0.005	200	1	949	0.025		

Assay Date	10/3/2005	Test Chemical ID		5	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate # 2

Microsome Dilution Details

Dilution A	0.1	mL microsome Stock used
	5	mL total volume
	50	dilution factor
Dilution B	2.5	mL microsome Dilution A used
	47.5	mL total volume
	19	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	950	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	25.46209
Protein Concentration (dilution added to assay, mg/mL):	0.026802

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	2.50E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	10/3/2005	Test Chemical ID	5	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3542	7083.8	7038.4	14076.8	0.1	112748	12.49	13751	0.0249	1	0.013	15	0.0619
			0.5	2	3497	6993											
	2	2	0.5	1	2882	5764.2	5787	11574	0.1	112748	10.27	11249	0.0204	1	0.013	15	0.0507
			0.5	2	2905	5809.8											
	3	2	0.5	1	2923	5845.8	5847.7	11695.4	0.1	112748	10.37	11370	0.0206	1	0.013	15	0.0512
			0.5	2	2925	5849.6											
	4	2	0.5	1	2903	5806.2	5839.2	11678.4	0.1	112748	10.36	11353	0.0206	1	0.013	15	0.0511
			0.5	2	2936	5872.2											
Background control	1	2	0.5	1	78	155.9	156.35	312.7	0.1	112748	0.26	-13	0.0000	1	0.013	15	-0.0001
			0.5	2	78	156.8											
	2	2	0.5	1	60	120.16	119.58	239.16	0.1	112748	0.21	-86	-0.0002	1	0.013	15	-0.0004
			0.5	2	60	119											
	3	2	0.5	1	136	271.38	260.06	520.12	0.1	112748	0.46	195	0.0004	1	0.013	15	0.0009
			0.5	2	124	248.74											
	4	2	0.5	1	56	111.06	114.71	229.42	0.1	112748	0.20	-96	-0.0002	1	0.013	15	-0.0004
			0.5	2	59	118.36											
Positive control	1	2	0.5	1	1708	3415.4	3410.3	6820.6	0.1	112748	6.05	6495	0.0118	1	0.013	15	0.0292
			0.5	2	1703	3405.2											
	2	2	0.5	1	1674	3347.4	3354	6708	0.1	112748	5.95	6383	0.0116	1	0.013	15	0.0287
			0.5	2	1680	3360.6											
	3	2	0.5	1	1633	3266	3255.7	6511.4	0.1	112748	5.78	6186	0.0112	1	0.013	15	0.0279
			0.5	2	1623	3245.4											
	4	2	0.5	1	1720	3439.6	3397.5	6795	0.1	112748	6.03	6470	0.0117	1	0.013	15	0.0291
			0.5	2	1678	3355.4											
Negative Control	1	2	0.5	1	3109	6217.2	6293.3	12586.6	0.1	112748	11.16	12261	0.0222	1	0.013	15	0.0552
			0.5	2	3185	6369.4											
	2	2	0.5	1	2812	5624.4	5623.5	11247	0.1	112748	9.98	10922	0.0198	1	0.013	15	0.0492
			0.5	2	2811	5622.6											
	3	2	0.5	1	2865	5729.8	5805.6	11611.2	0.1	112748	10.30	11286	0.0204	1	0.013	15	0.0508
			0.5	2	2941	5881.4											
	4	2	0.5	1	2978	5955.2	6003.3	12006.6	0.1	112748	10.65	11681	0.0211	1	0.013	15	0.0526
			0.5	2	3026	6051.4											
5	1-1	2	0.5	1	3277	6553.4	6580	13160	0.1	112748	11.67	12835	0.0232	1	0.013	15	0.0578
			0.5	2	3303	6606.6											
	1-2	2	0.5	1	3119	6238.4	6289	12578	0.1	112748	11.16	12253	0.0222	1	0.013	15	0.0552
			0.5	2	3170	6339.6											
	1-3	2	0.5	1	3283	6565.8	6638.4	13276.8	0.1	112748	11.78	12951	0.0234	1	0.013	15	0.0583
			0.5	2	3356	6711											
	2-1	2	0.5	1	3067	6134.8	6169.4	12338.8	0.1	112748	10.94	12013	0.0217	1	0.013	15	0.0541
			0.5	2	3102	6204											
	2-2	2	0.5	1	3277	6553.6	6511.6	13023.2	0.1	112748	11.55	12698	0.0230	1	0.013	15	0.0572
			0.5	2	3235	6469.6											
	2-3	2	0.5	1	3160	6320.4	6337.5	12675	0.1	112748	11.24	12350	0.0224	1	0.013	15	0.0556
			0.5	2	3177	6354.6											
	3-1	2	0.5	1	3118	6236.4	6260.4	12520.8	0.1	112748	11.11	12195	0.0221	1	0.013	15	0.0549
			0.5	2	3142	6284.4											
	3-2	2	0.5	1	3334	6668	6603.8	13207.6	0.1	112748	11.71	12882	0.0233	1	0.013	15	0.0580
			0.5	2	3270	6539.6											
	3-3	2	0.5	1	3268	6531.8	6485.8	12971.6	0.1	112748	11.50	12646	0.0229	1	0.013	15	0.0569
			0.5	2	3220	6439.8											
	4-1	2	0.5	1	3295	6590.2	6533.8	13067.6	0.1	112748	11.59	12742	0.0231	1	0.013	15	0.0574
			0.5	2	3239	6477.4											
	4-2	2	0.5	1	3327	6654.6	6662	13324	0.1	112748	11.82	12999	0.0235	1	0.013	15	0.0585
			0.5	2	3335	6669.4											
	4-3	2	0.5	1	3302	6604.2	6524.4	13048.8	0.1	112748	11.57	12723	0.0230	1	0.013	15	0.0573
			0.5	2	3222	6444.6											
	5-1	2	0.5	1	3152	6304.8	6326.7	12653.4	0.1	112748	11.22	12328	0.0223	1	0.013	15	0.0555
			0.5	2	3174	6348.6											
	5-2	2	0.5	1	3014	6028.2	6042.5	12085	0.1	112748	10.72	11760	0.0213	1	0.013	15	0.0530
			0.5	2	3028	6056.8											
	5-3	2	0.5	1	3343	6686	6670.1	13340.2	0.1	112748	11.83	13015	0.0236	1	0.013	15	0.0586
			0.5	2	3327	6654.2											
	6-1	2	0.5	1	3074	6147.4	6154.3	12308.6	0.1	112748	10.92	11983	0.0217	1	0.013	15	0.0540
			0.5	2	3081	6161.2											
	6-2	2	0.5	1	2917	5833.4	5794.2	11588.4	0.1	112748	10.28	11263	0.0204	1	0.013	15	0.0507
			0.5	2	2878	5755											
	6-3	2	0.5	1	3163	6326.4	6319.7	12639.4	0.1	112748	11.21	12314	0.0223	1	0.013	15	0.0555
			0.5	2	3157	6313											
	7-1	2	0.5	1	3278	6556.2	6572.7	13145.4	0.1	112748	11.66	12820	0.0232	1	0.013	15	0.0577
			0.5	2	3295	6589.2											
	7-2	2	0.5	1	3156	6309.2	6318.6	12637.2	0.1	112748	11.21	12312	0.0223	1	0.013	15	0.0554
			0.5	2	3164	6328											
	7-3	2	0.5	1	3062	6123.6	6173.8	12347.6	0.1	112748	10.95	12022	0.0218	1	0.013	15	0.0541
			0.5	2	3112	6224											
	8-1	2	0.5	1	2907	5813	5739	11478	0.1	112748	10.18	11153	0.0202	1	0.013	15	0.0502
			0.5	2	2833	5665											
	8-2	2	0.5	1	3082	6163	6230.6	12461.2	0.1	112748	11.05	12136	0.0220	1	0.013	15	0.0546
			0.5	2	3149	6298.2											
	8-3	2	0.5	1	3114	6228	6252.3	12504.6	0.1	112748	11.09	12179	0.0220	1	0.013	15	0.0548
			0.5	2	3138	6276.6											

Assay Date	10/3/2005	Test Chemical ID	5	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0563	0.0080
Full activity	End	0.0512	0.0001
Full activity	Overall	0.0537	0.0055
Background	Beginning	-0.0002	0.000234166
Background	End	0.0002	0.000925646
Background	Overall	0.0000	0.00060821
Positive	Beginning	0.0290	0.0004
Positive	End	0.0285	0.0009
Positive	Overall	0.0287	0.0006
Negative	Beginning	0.0522	0.0043
Negative	End	0.0517	0.0013
Negative	Overall	0.0520	0.0026

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
5	1	1	1.00E-04	-4.00	0.0578
5	1	2	1.00E-04	-4.00	0.0552
5	1	3	1.00E-04	-4.00	0.0583
5	2	1	1.00E-05	-5.00	0.0541
5	2	2	1.00E-05	-5.00	0.0572
5	2	3	1.00E-05	-5.00	0.0556
5	3	1	1.00E-06	-6.00	0.0549
5	3	2	1.00E-06	-6.00	0.0580
5	3	3	1.00E-06	-6.00	0.0569
5	4	1	2.50E-07	-6.60	0.0574
5	4	2	2.50E-07	-6.60	0.0585
5	4	3	2.50E-07	-6.60	0.0573
5	5	1	1.00E-07	-7.00	0.0555
5	5	2	1.00E-07	-7.00	0.0530
5	5	3	1.00E-07	-7.00	0.0586
5	6	1	1.00E-08	-8.00	0.0540
5	6	2	1.00E-08	-8.00	0.0507
5	6	3	1.00E-08	-8.00	0.0555
5	7	1	1.00E-09	-9.00	0.0577
5	7	2	1.00E-09	-9.00	0.0554
5	7	3	1.00E-09	-9.00	0.0541
5	8	1	1.00E-10	-10.00	0.0502
5	8	2	1.00E-10	-10.00	0.0546
5	8	3	1.00E-10	-10.00	0.0548

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	107.58	102.70	108.55
2	-5.00	100.69	106.43	103.51
3	-6.00	102.22	107.97	106.00
4	-6.60	106.80	108.95	106.64
5	-7.00	103.33	98.57	109.09
6	-8.00	100.44	94.40	103.21
7	-9.00	107.45	103.19	100.77
8	-10.00	93.48	101.72	102.08

Assay Date	10/4/2005	Test Chemical ID	5	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0203	21568	1062438		
2	0.0200	22858	1142900		
3	0.0201	22416	1115224		
4	0.0203	23921	1178374		
5	0.0201	23726	1180398		
			Average DPM/g soln		1135867
			SD		49136
			CV		4.33
			µCi/g soln		0.512

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.4	10		1040.00
Dilution A			100	10.40
Dilution B			10	1.04

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6019	g
Mass of dilution B used in substrate prep	5.4155	g
Concentration of nonradiolabeled ASDN in substrate soln.	0.586563	µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00579 µg/g soln.
µg/g soln.

a. µCi/g soln 0.512
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.586563 + 0.00579
= 0.592355 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 0.871 µCi/µg ASDN

549185 dpm/nmol

Assay Date	10/4/2005	Test Chemical ID	5	# Concentrations tested	8	
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID 6-041305

Standards:								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	250	125	50	25	10	5	0	25	100	11343-77
	0.469	0.279	0.127	0.066	0.027	0.014	0.000			
	0.477	0.278	0.129	0.060	0.028	0.016	0.000			
	0.480	0.276	0.125	0.063	0.023	0.015	0.000			

Samples:			
	QC 100	QC 10	Microsomes
	0.242	0.031	0.070
	0.237	0.029	0.067
			0.067

Standard concentration (µg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL		Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025		200	0.0500	0.475	0.475	0.0482
125	100	200	0.00013		200	0.0250	0.278	0.278	0.0275
50	200	1000	0.00005		200	0.0100	0.127	0.127	0.0116
25	100	1000	0.00003		200	0.0050	0.063	0.063	0.0049
10	40	1000	0.00001		200	0.0020	0.026	0.026	0.0010
5	20	1000	0.00001		200	0.0010	0.015	0.015	-0.0002
				Blank	0.000				

Variables	Regression results	
m, b	0.105	-0.002
se_m, se_b	0.005	0.001
r^2, se_y	0.992	0.002
F, df	512	4
SS_{reg}, SS_{resid}	0.002	0.000

r^2= 0.992
m= 0.105
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.242	0.242	0.024	200	1	1	0.000	0.000	0.117
QC 100	0.237	0.237	0.023	200	1	1	0.000		
QC 100									
QC 10	0.031	0.031	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.029	0.029	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.070	0.070	0.006	200	1	949	0.026	0.026	25.574
Microsomes	0.067	0.067	0.005	200	1	949	0.025		
Microsomes	0.067	0.067	0.005	200	1	949	0.025		

Assay Date	10/4/2005	Test Chemical ID	5	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.469	0.279	0.127	0.066	0.027	0.014	0.000			
	0.477	0.278	0.129	0.060	0.028	0.016	0.000			
	0.480	0.276	0.125	0.063	0.023	0.015	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.242	0.031	0.070
	0.237	0.029	0.067
			0.067

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.475	0.475	0.0426
125	100	200	0.00013	200	0.0250	0.278	0.278	0.0246
50	200	1000	0.00005	200	0.0100	0.127	0.127	0.0109
25	100	1000	0.00003	200	0.0050	0.063	0.063	0.0051
10	40	1000	0.00001	200	0.0020	0.026	0.026	0.0017
5	20	1000	0.00001	200	0.0010	0.015	0.015	0.0007
			Blank	0.000				

Variables	Regression results	
m, b	0.091	-0.001
se_m, se_b	0.003	0.000
r^2, se_y	0.997	0.001
F, df	1019	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.997
m= 0.091
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.242	0.242	0.021	200	1	1	0.000	0.000	0.106
QC 100	0.237	0.237	0.021	200	1	1	0.000		
QC 100									
QC 10	0.031	0.031	0.002	200	1	1	0.000	0.000	0.010
QC 10	0.029	0.029	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.070	0.070	0.006	200	1	949	0.027	0.026	26.181
Microsomes	0.067	0.067	0.005	200	1	949	0.026		
Microsomes	0.067	0.067	0.005	200	1	949	0.026		

Assay Date	10/4/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	3
			5										

Microsome Dilution Details

Dilution A	0.1	mL microsome Stock used
	5	mL total volume
	50	dilution factor
Dilution B	2.5	mL microsome Dilution A used
	47.5	mL total volume
	19	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	950	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	26.18086
Protein Concentration (dilution added to assay, mg/mL):	0.027559

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	2.50E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	10/4/2005	Test Chemical ID	5	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3420	6840.8	6797.4	13594.8	0.1	113587	11.97	13275	0.0242	1	0.014	15	0.0585
			0.5	2	3377	6754											
	2	2	0.5	1	3492	6983.8	7041.5	14083	0.1	113587	12.40	13763	0.0251	1	0.014	15	0.0606
			0.5	2	3550	7099.2											
	3	2	0.5	1	3305	6610.8	6567.1	13134.2	0.1	113587	11.56	12815	0.0233	1	0.014	15	0.0564
			0.5	2	3282	6523.4											
	4	2	0.5	1	3304	6608	6675.3	13350.6	0.1	113587	11.75	13031	0.0237	1	0.014	15	0.0574
			0.5	2	3371	6742.6											
Background control	1	2	0.5	1	78	156.48	155.37	310.74	0.1	113587	0.27	-9	0.0000	1	0.014	15	0.0000
			0.5	2	77	154.26											
	2	2	0.5	1	107	213.3	230.33	460.66	0.1	113587	0.41	141	0.0003	1	0.014	15	0.0006
			0.5	2	124	247.36											
	3	2	0.5	1	81	162.4	159.29	318.58	0.1	113587	0.28	-1	0.0000	1	0.014	15	0.0000
			0.5	2	78	156.18											
	4	2	0.5	1	46	91.18	94.07	188.14	0.1	113587	0.17	-131	-0.0002	1	0.014	15	-0.0006
			0.5	2	48	96.96											
Positive control	1	2	0.5	1	1826	3651.8	3637.7	7275.4	0.1	113587	6.41	6956	0.0127	1	0.014	15	0.0306
			0.5	2	1812	3623.6											
	2	2	0.5	1	1740	3479	3472.8	6945.6	0.1	113587	6.11	6626	0.0121	1	0.014	15	0.0292
			0.5	2	1733	3466.6											
	3	2	0.5	1	1695	3389.4	3383.6	6767.2	0.1	113587	5.96	6448	0.0117	1	0.014	15	0.0284
			0.5	2	1689	3377.8											
	4	2	0.5	1	1774	3547.4	3528.9	7057.8	0.1	113587	6.21	6738	0.0123	1	0.014	15	0.0297
			0.5	2	1755	3510.4											
Negative Control	1	2	0.5	1	3531	7062.8	7099.4	14198.8	0.1	113587	12.50	13879	0.0253	1	0.014	15	0.0611
			0.5	2	3568	7136											
	2	2	0.5	1	3348	6697.8	6776.1	13552.2	0.1	113587	11.93	13233	0.0241	1	0.014	15	0.0583
			0.5	2	3427	6854.4											
	3	2	0.5	1	3315	6629.4	6638.6	13277.2	0.1	113587	11.69	12958	0.0236	1	0.014	15	0.0571
			0.5	2	3324	6647.8											
	4	2	0.5	1	3263	6525.4	6563.7	13127.4	0.1	113587	11.56	12808	0.0233	1	0.014	15	0.0564
			0.5	2	3301	6602											
5	1-1	2	0.5	1	3430	6860.2	6829.4	13658.8	0.1	113587	12.03	13339	0.0243	1	0.014	15	0.0588
			0.5	2	3399	6798.6											
	1-2	2	0.5	1	3315	6629.6	6610.7	13221.4	0.1	113587	11.64	12902	0.0235	1	0.014	15	0.0568
			0.5	2	3296	6591.8											
	1-3	2	0.5	1	3413	6825.2	6809.3	13618.6	0.1	113587	11.99	13299	0.0242	1	0.014	15	0.0586
			0.5	2	3397	6793.4											
	2-1	2	0.5	1	3247	6494.4	6470.7	12941.4	0.1	113587	11.39	12622	0.0230	1	0.014	15	0.0556
			0.5	2	3224	6447											
	2-2	2	0.5	1	3296	6592.6	6538.6	13077.2	0.1	113587	11.51	12758	0.0232	1	0.014	15	0.0562
			0.5	2	3242	6484.4											
	2-3	2	0.5	1	3405	6810.4	6703.6	13407.2	0.1	113587	11.80	13088	0.0238	1	0.014	15	0.0576
			0.5	2	3298	6596.8											
	3-1	2	0.5	1	3474	6947	6883.9	13767.8	0.1	113587	12.12	13448	0.0245	1	0.014	15	0.0592
			0.5	2	3410	6820.6											
	3-2	2	0.5	1	3448	6895.6	6932.9	13865.8	0.1	113587	12.21	13546	0.0247	1	0.014	15	0.0597
			0.5	2	3485	6970.2											
	3-3	2	0.5	1	3259	6517.2	6548.2	13096.4	0.1	113587	11.53	12777	0.0233	1	0.014	15	0.0563
			0.5	2	3290	6579.2											
	4-1	2	0.5	1	3317	6634.4	6557.9	13115.8	0.1	113587	11.55	12796	0.0233	1	0.014	15	0.0564
			0.5	2	3241	6481.4											
	4-2	2	0.5	1	3378	6755.6	6796.5	13593	0.1	113587	11.97	13273	0.0242	1	0.014	15	0.0585
			0.5	2	3419	6837.4											
	4-3	2	0.5	1	3421	6842.2	6889.9	13779.8	0.1	113587	12.13	13460	0.0245	1	0.014	15	0.0593
			0.5	2	3469	6937.6											
	5-1	2	0.5	1	3279	6559	6629.1	13258.2	0.1	113587	11.67	12939	0.0236	1	0.014	15	0.0570
			0.5	2	3350	6700.2											
	5-2	2	0.5	1	3356	6712.6	6734.7	13469.4	0.1	113587	11.86	13150	0.0239	1	0.014	15	0.0579
			0.5	2	3378	6756.8											
	5-3	2	0.5	1	3374	6747.2	6768.7	13533.4	0.1	113587	11.91	13214	0.0241	1	0.014	15	0.0582
			0.5	2	3393	6786.2											
	6-1	2	0.5	1	3328	6656.2	6726.4	13452.8	0.1	113587	11.84	13133	0.0239	1	0.014	15	0.0578
			0.5	2	3398	6796.6											
	6-2	2	0.5	1	3354	6728.8	6749.3	13498.6	0.1	113587	11.86	13179	0.0240	1	0.014	15	0.0581
			0.5	2	3385	6769.8											
	6-3	2	0.5	1	3506	7011.5	6892.2	13784.4	0.1	113587	12.14	13465	0.0245	1	0.014	15	0.0593
			0.5	2	3386	6772.6											
	7-1	2	0.5	1	3276	6551	6621.5	13243	0.1	113587	11.66	12923	0.0235	1	0.014	15	0.0569
			0.5	2	3346	6692											
	7-2	2	0.5	1	3391	6781.6	6748.3	13496.6	0.1	113587	11.86	13177	0.0240	1	0.014	15	0.0580
			0.5	2	3358	6715											
	7-3	2	0.5	1	3257	6513.2	6519.4	13038.8	0.1	113587	11.48	12719	0.0232	1	0.014	15	0.0560
			0.5	2	3283	6525.6											
	8-1	2	0.5	1	3285	6530.2	6621.9	13243.8	0.1	113587	11.68	12924	0.0235	1	0.014	15	0.0569
			0.5	2	3357	6713.6											
	8-2	2	0.5	1	3227	6453.6	6526	13052	0.1	113587	11.49	12732	0.0232	1	0.014	15	0.0561
			0.5	2	3298	6598.4											
	8-3	2	0.5	1	2554	5108	5087.3	10174.6	0.1	113587	8.96	9355	0.0179	1	0.014	15	0.0434
			0.5	2	2533	5066.6											

Assay Date	10/4/2005	Test Chemical ID		5	# Concentrations tested	8	Microsome type	placental	Microsome ID 6-041305	Technician ID TD	Replicate # 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0596	0.0015
Full activity	End	0.0569	0.0007
Full activity	Overall	0.0582	0.0018
Background	Beginning	0.0003	0.000466955
Background	End	-0.0003	0.00040628
Background	Overall	0.0000	0.000490892
Positive	Beginning	0.0299	0.0010
Positive	End	0.0290	0.0009
Positive	Overall	0.0295	0.0009
Negative	Beginning	0.0597	0.0020
Negative	End	0.0567	0.0005
Negative	Overall	0.0582	0.0021

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
5	1	1	1.00E-04	-4.00	0.0588
5	1	2	1.00E-04	-4.00	0.0568
5	1	3	1.00E-04	-4.00	0.0586
5	2	1	1.00E-05	-5.00	0.0556
5	2	2	1.00E-05	-5.00	0.0562
5	2	3	1.00E-05	-5.00	0.0576
5	3	1	1.00E-06	-6.00	0.0592
5	3	2	1.00E-06	-6.00	0.0597
5	3	3	1.00E-06	-6.00	0.0563
5	4	1	2.50E-07	-6.60	0.0564
5	4	2	2.50E-07	-6.60	0.0585
5	4	3	2.50E-07	-6.60	0.0593
5	5	1	1.00E-07	-7.00	0.0570
5	5	2	1.00E-07	-7.00	0.0579
5	5	3	1.00E-07	-7.00	0.0582
5	6	1	1.00E-08	-8.00	0.0578
5	6	2	1.00E-08	-8.00	0.0581
5	6	3	1.00E-08	-8.00	0.0593
5	7	1	1.00E-09	-9.00	0.0569
5	7	2	1.00E-09	-9.00	0.0580
5	7	3	1.00E-09	-9.00	0.0560
5	8	1	1.00E-10	-10.00	0.0569
5	8	2	1.00E-10	-10.00	0.0561
5	8	3	1.00E-10	-10.00	0.0434

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	100.89	97.59	100.59
2	-5.00	95.47	96.49	98.99
3	-6.00	101.72	102.46	96.64
4	-6.60	96.79	100.40	101.81
5	-7.00	97.86	99.46	99.95
6	-8.00	99.34	99.68	101.84
7	-9.00	97.75	99.67	96.20
8	-10.00	97.75	96.30	74.54

Assay Date	8/5/2005	Test Chemical ID	6	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	28731	1443769		
2	0.0202	30965	1532921		
3	0.0202	31448	1556832		
4	0.0197	31376	1592690		
5	0.0198	31356	1583636		
			Average DPM/g soln		1541970
			SD		59697
			CV		3.87
			µCi/g soln		0.695

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.5	10		1050.00
Dilution A			100	10.50
Dilution B			10	1.05

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6205 g
Mass of dilution B used in substrate prep	5.4262 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.592226 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00786 µg/g soln.
µg/g soln.

a. µCi/g soln	0.695
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.592226 + 0.00786
= 0.600089 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.157 µCi/µg ASDN

735925 dpm/nmol

Assay Date 8/5/2005 | Test Chemical ID 6 | # Concentrations tested 8

Technician ID TD | Replicate # 1 | Microsome type placental | Microsome ID BAA

	250	125	50	25	10	5	0
Standards:	0.520	0.307	0.137	0.073	0.032	0.021	0.000
	0.522	0.305	0.136	0.077	0.032	0.019	0.000
	0.521	0.304	0.133	0.073	0.031	0.019	0.000

Protein stock (mg BSA): 25 | Total volume of stock (mL): 100 | Protein stock ID: 11343-77

	QC 100	QC 10	Microsomes
Samples:	0.269	0.037	0.076
	0.270	0.037	0.076
			0.082

Standard concentration (mg/mL) LE 11 3-20-06 RQL	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.521	0.521	0.0429
125	100	200	0.00013	200	0.0250	0.305	0.305	0.0248
50	200	1000	0.00005	200	0.0100	0.135	0.135	0.0105
25	100	1000	0.00003	200	0.0050	0.074	0.074	0.0053
10	40	1000	0.00001	200	0.0020	0.032	0.032	0.0017
5	20	1000	0.00001	200	0.0010	0.020	0.020	0.0007
			Blank	0.000				

Variables	Regression results	
m, b	0.084	-0.001
se_m, se_b	0.002	0.000
r^2, se_y	0.999	0.000
F, df	2308	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.999
m= 0.084
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.269	0.269	0.022	200	1	1	0.000	0.000	0.109
QC 100	0.270	0.270	0.022	200	1	1	0.000		
QC 100									
QC 10	0.037	0.037	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.037	0.037	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.076	0.076	0.005	200	120	42000	0.010	0.010	9.843
Microsomes	0.076	0.076	0.005	200	120	42000	0.010		
Microsomes	0.082	0.082	0.006	200	120	42000	0.010		

Assay Date	8/5/2005	Test Chemical ID		6	# Concentrations tested	8	
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.520	0.307	0.137	0.073	0.032	0.021	0.000			
	0.522	0.305	0.136	0.077	0.032	0.019	0.000			
	0.521	0.304	0.133	0.073	0.031	0.019	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.269	0.037	0.076
	0.270	0.037	0.076
			0.082

Standard concentration (ng/mL) LE m 05-22-06 EBM	Volume of stock used	Final volume of Std	mg Protein per µL		Used		mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200		0.0500		0.521	0.521	0.0483	m, b	0.097	-0.002
125	100	200	0.00013		200		0.0250		0.305	0.305	0.0275	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200		0.0100		0.135	0.135	0.0111	r^2, se_y	0.993	0.002
25	100	1000	0.00003		200		0.0050		0.074	0.074	0.0052	F, df	589	4
10	40	1000	0.00001		200		0.0020		0.032	0.032	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.020	0.020	-0.0001			
				Blank	0.000									
						r^2=	0.993							
						m=	0.097							
						b=	-0.002							

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.269	0.269	0.024	200	1	1	0.000	0.000	0.120
QC 100	0.270	0.270	0.024	200	1	1	0.000		
QC 100									
QC 10	0.037	0.037	0.002	200	1	1	0.000	0.000	0.008
QC 10	0.037	0.037	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.076	0.076	0.005	200	120	42000	0.009	0.010	9.652
Microsomes	0.076	0.076	0.005	200	120	42000	0.009		
Microsomes	0.082	0.082	0.006	200	120	42000	0.010		

Assay Date	8/5/2005	Test Chemical ID	6	# Concentrations tested	3	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background - Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3898	7796.6	7802.9	15605.8	0.1	154197	10.12	15315	0.0208	1	0.014	15	0.0493
			0.5	2	3905	7809.2											
	2	2	0.5	1	3862	7724.6	7622	15244	0.1	154197	9.89	14954	0.0203	1	0.014	15	0.0482
			0.5	2	3760	7519.4											
	3	2	0.5	1	3698	7395.6	7371.5	14743	0.1	154197	9.56	14453	0.0196	1	0.014	15	0.0466
			0.5	2	3674	7347.4											
	4	2	0.5	1	3717	7434.4	7365.5	14731	0.1	154197	9.55	14441	0.0196	1	0.014	15	0.0465
			0.5	2	3648	7296.6											
Background control	1	2	0.5	1	75	149.26	153.48	306.96	0.1	154197	0.20	18	0.0000	1	0.014	15	0.0001
			0.5	2	79	157.7											
	2	2	0.5	1	68	136.18	144.32	288.64	0.1	154197	0.19	-2	0.0000	1	0.014	15	0.0000
			0.5	2	76	152.46											
	3	2	0.5	1	62	124.02	147.14	294.28	0.1	154197	0.19	4	0.0000	1	0.014	15	0.0000
			0.5	2	85	170.26											
	4	2	0.5	1	63	125	136.03	272.06	0.1	154197	0.18	-18	0.0000	1	0.014	15	-0.0001
			0.5	2	74	147.06											
Positive control	1	2	0.5	1	1683	3365	3404.9	6809.8	0.1	154197	4.42	6519	0.0089	1	0.014	15	0.0310
			0.5	2	1722	3444.8											
	2	2	0.5	1	1786	3572.2	3524.3	7048.6	0.1	154197	4.57	6756	0.0092	1	0.014	15	0.0318
			0.5	2	1738	3476.4											
	3	2	0.5	1	1619	3238.2	3262.7	6525.4	0.1	154197	4.23	6235	0.0085	1	0.014	15	0.0291
			0.5	2	1644	3287.2											
	4	2	0.5	1	1645	3290.8	3323.1	6646.2	0.1	154197	4.31	6356	0.0086	1	0.014	15	0.0295
			0.5	2	1678	3355.4											
Negative Control	1	2	0.5	1	3929	7857.2	7914.4	15828.8	0.1	154197	10.14	15338	0.0208	1	0.014	15	0.0494
			0.5	2	3886	7771.6											
	2	2	0.5	1	3927	7853.6	7789.4	15578.8	0.1	154197	10.10	15288	0.0208	1	0.014	15	0.0492
			0.5	2	3863	7725.2											
	3	2	0.5	1	3643	7286	7372.8	14745.6	0.1	154197	9.56	14455	0.0196	1	0.014	15	0.0465
			0.5	2	3730	7459.6											
	4	2	0.5	1	3190	6379.2	6395.5	12791	0.1	154197	8.30	12501	0.0170	1	0.014	15	0.0403
			0.5	2	3206	6411.8											
8	1-1	2	0.5	1	117	234.46	238.44	476.88	0.1	154197	0.31	186	0.0003	1	0.014	15	0.0006
			0.5	2	121	242.42											
	1-2	2	0.5	1	115	229.34	223.96	447.92	0.1	154197	0.29	157	0.0002	1	0.014	15	0.0005
			0.5	2	109	218.58											
	1-3	2	0.5	1	125	250.08	242.78	485.56	0.1	154197	0.31	195	0.0003	1	0.014	15	0.0006
			0.5	2	118	235.48											
	2-1	2	0.5	1	305	609.7	619.69	1239.38	0.1	154197	0.80	949	0.0013	1	0.014	15	0.0031
			0.5	2	315	629.68											
	2-2	2	0.5	1	302	602.16	592.87	1185.74	0.1	154197	0.77	895	0.0012	1	0.014	15	0.0029
			0.5	2	291	582.58											
	2-3	2	0.5	1	288	576.32	579.72	1159.44	0.1	154197	0.75	869	0.0012	1	0.014	15	0.0028
			0.5	2	292	583.12											
	3-1	2	0.5	1	1327	2653.6	2645.1	5290.2	0.1	154197	3.43	5000	0.0068	1	0.014	15	0.0161
			0.5	2	1318	2636.6											
	3-2	2	0.5	1	1532	3064.2	3056.6	6113.2	0.1	154197	3.96	5823	0.0079	1	0.014	15	0.0189
			0.5	2	1525	3049											
	3-3	2	0.5	1	1559	3117.8	3074.8	6149.6	0.1	154197	3.99	5859	0.0080	1	0.014	15	0.0189
			0.5	2	1516	3031.8											
	4-1	2	0.5	1	3154	6308	6325.1	12650.2	0.1	154197	8.20	12360	0.0168	1	0.014	15	0.0398
			0.5	2	3171	6342.2											
	4-2	2	0.5	1	3236	6471.8	6393.9	12787.8	0.1	154197	8.29	12497	0.0170	1	0.014	15	0.0403
			0.5	2	3158	6316											
	4-3	2	0.5	1	3262	6524.6	6475.4	12950.8	0.1	154197	8.40	12660	0.0172	1	0.014	15	0.0408
			0.5	2	3213	6426.2											
	5-1	2	0.5	1	3646	7291.6	7392.1	14784.2	0.1	154197	9.57	14474	0.0197	1	0.014	15	0.0466
			0.5	2	3736	7472.6											
	5-2	2	0.5	1	3897	7794.6	7730.1	15460.2	0.1	154197	10.03	15170	0.0206	1	0.014	15	0.0489
			0.5	2	3833	7665.6											
	5-3	2	0.5	1	3770	7539.4	7529.6	15059.2	0.1	154197	9.77	14789	0.0201	1	0.014	15	0.0476
			0.5	2	3760	7519.8											
	6-1	2	0.5	1	3626	7251.4	7369.9	14739.8	0.1	154197	9.56	14449	0.0196	1	0.014	15	0.0465
			0.5	2	3744	7488.4											
	6-2	2	0.5	1	3656	7312	7226.5	14453	0.1	154197	9.37	14163	0.0192	1	0.014	15	0.0456
			0.5	2	3571	7141											
	6-3	2	0.5	1	3699	7397.8	7464.8	14929.6	0.1	154197	9.68	14639	0.0199	1	0.014	15	0.0472
			0.5	2	3768	7531.8											
	7-1	2	0.5	1	3697	7393.4	7352.6	14705.2	0.1	154197	9.54	14415	0.0196	1	0.014	15	0.0464
			0.5	2	3656	7311.8											
	7-2	2	0.5	1	3778	7556.4	7504.3	15008.6	0.1	154197	9.73	14718	0.0200	1	0.014	15	0.0474
			0.5	2	3726	7452.2											
	7-3	2	0.5	1	3738	7476.2	7553.8	15107.6	0.1	154197	9.80	14817	0.0201	1	0.014	15	0.0477
			0.5	2	3816	7631.4											
	8-1	2	0.5	1	3651	7301.6	7317.2	14634.4	0.1	154197	9.49	14344	0.0195	1	0.014	15	0.0462
			0.5	2	3666	7332.8											
	8-2	2	0.5	1	3731	7462.6	7546.1	15092.2	0.1	154197	9.79	14802	0.0201	1	0.014	15	0.0477
			0.5	2	3815	7629.6											
	8-3	2	0.5	1	3776	7552.8	7516.6	15033.2	0.1	154197	9.75	14743	0.0200	1	0.014	15	0.0475
			0.5	2	3740	7480.4											

Assay Date	8/5/2005	Test Chemical ID	6	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0488	0.0008
Full activity	End	0.0465	0.0000
Full activity	Overall	0.0476	0.0014
Background	Beginning	0.0000	4.17298E-05
Background	End	0.0000	5.06134E-05
Background	Overall	0.0000	4.66339E-05
Positive	Beginning	0.0214	0.0005
Positive	End	0.0203	0.0003
Positive	Overall	0.0208	0.0007
Negative	Beginning	0.0493	0.0001
Negative	End	0.0434	0.0045
Negative	Overall	0.0464	0.0043

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
6	1	1	1.00E-03	-3.00	0.0006
6	1	2	1.00E-03	-3.00	0.0005
6	1	3	1.00E-03	-3.00	0.0006
6	2	1	1.00E-04	-4.00	0.0031
6	2	2	1.00E-04	-4.00	0.0029
6	2	3	1.00E-04	-4.00	0.0028
6	3	1	1.00E-05	-5.00	0.0161
6	3	2	1.00E-05	-5.00	0.0188
6	3	3	1.00E-05	-5.00	0.0189
6	4	1	1.00E-06	-6.00	0.0398
6	4	2	1.00E-06	-6.00	0.0403
6	4	3	1.00E-06	-6.00	0.0408
6	5	1	1.00E-07	-7.00	0.0466
6	5	2	1.00E-07	-7.00	0.0489
6	5	3	1.00E-07	-7.00	0.0476
6	6	1	1.00E-08	-8.00	0.0465
6	6	2	1.00E-08	-8.00	0.0456
6	6	3	1.00E-08	-8.00	0.0472
6	7	1	1.00E-09	-9.00	0.0464
6	7	2	1.00E-09	-9.00	0.0474
6	7	3	1.00E-09	-9.00	0.0477
6	8	1	1.00E-10	-10.00	0.0462
6	8	2	1.00E-10	-10.00	0.0477
6	8	3	1.00E-10	-10.00	0.0475

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.26	1.06	1.32
2	-4.00	6.42	6.05	5.88
3	-5.00	33.80	39.37	39.61
4	-6.00	83.57	84.50	85.60
5	-7.00	97.86	102.56	99.85
6	-8.00	97.69	95.75	98.98
7	-9.00	97.46	99.51	100.18
8	-10.00	96.98	100.08	99.68

Assay Date	8/8/2005	Test Chemical ID	6	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0201	26601	1323433		
2	0.0197	27866	1414518		
3	0.0197	29571	1501066		
4	0.0196	31138	1588673		
5	0.0196	31699	1617296		
			Average DPM/g soln		1488997
			SD		121963
			CV		8.19
			µCi/g soln		0.671

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.7	10		1070.00
Dilution A			100	10.70
Dilution B			10	1.07

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.594 g
Mass of dilution B used in substrate prep	5.4289 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.605475 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00759 µg/g soln.
µg/g soln.

a. µCi/g soln 0.671
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.605475 + 0.00759
= 0.613067 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.094 µCi/µg ASDN

695599 dpm/nmol

Assay Date	8/8/2005	Test Chemical ID	6	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.519	0.298	0.133	0.076	0.027	0.014	0.000			
	0.521	0.298	0.135	0.071	0.028	0.014	0.000			
	0.520	0.298	0.135	0.069	0.026	0.016	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.261	0.034	0.070
	0.263	0.034	0.074
			0.070

Standard concentration (ng/mL) LE 3-30-06 BJR	Volume of stock used	Final volume of Std	mg Protein per µL		Used	mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200	0.0500		0.520	0.520	0.0485	m, b	0.096	-0.002
125	100	200	0.00013		200	0.0250		0.298	0.298	0.0271	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200	0.0100		0.134	0.134	0.0113	r^2, se_y	0.994	0.002
25	100	1000	0.00003		200	0.0050		0.072	0.072	0.0053	F, df	658	4
10	40	1000	0.00001		200	0.0020		0.027	0.027	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010		0.015	0.015	-0.0002			
				Blank	0.000								

Regression results are calculated using the function LINEST

r^2= 0.994
m= 0.096
b= -0.002

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL	
QC 100	0.261	0.261	0.024	200	1	1	0.000	0.000	0.118	
QC 100	0.263	0.263	0.024	200	1	1	0.000			
QC 100										
QC 10	0.034	0.034	0.002	200	1	1	0.000	0.000	0.008	
QC 10	0.034	0.034	0.002	200	1	1	0.000			
QC 10										
Microsomes	0.070	0.070	0.005	200	120	42000	0.009	0.009	9.183	
Microsomes	0.074	0.074	0.006	200	120	42000	0.010			
Microsomes	0.070	0.070	0.005	200	120	42000	0.009			

Assay Date	8/8/2005	Test Chemical ID	6	# Concentrations tested	8	
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID BAA

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.519	0.298	0.133	0.076	0.027	0.014	0.000	25	100	11343-77
	0.521	0.298	0.135	0.071	0.028	0.014	0.000			
	0.520	0.298	0.135	0.069	0.026	0.016	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.261	0.034	0.070
	0.263	0.034	0.074
			0.070

Standard concentration (mg/mL) LE JW 3-20-06 BJL	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0500	0.520	0.520	0.0435	m, b	0.085	-0.001
125	100	200	0.00013	200	0.0250	0.298	0.298	0.0246	se_m, se_b	0.003	0.000
50	200	1000	0.00005	200	0.0100	0.134	0.134	0.0107	r^2, se_y	0.997	0.001
25	100	1000	0.00003	200	0.0050	0.072	0.072	0.0055	F, df	946	3
10	40	1000	0.00001	200	0.0020	0.027	0.027	0.0016	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.015	0.015	0.0006			

Regression results are calculated using the function LINEST

Blank	0.000	r^2=	0.997	
		m=	0.085	
		b=	-0.001	

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.261	0.261	0.022	200	1	1	0.000	0.000	0.108
QC 100	0.263	0.263	0.022	200	1	1	0.000		
QC 100									
QC 10	0.034	0.034	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.034	0.034	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.070	0.070	0.005	200	120	42000	0.009	0.009	9.424
Microsomes	0.074	0.074	0.006	200	120	42000	0.010		
Microsomes	0.070	0.070	0.005	200	120	42000	0.009		

Assay Date	8/8/2005	Test Chemical ID	6	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed						
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4048	8095.4	8045.7	16091.4	0.1	148900	10.81	15813	0.0227	1	0.013	15	0.0563
			0.5	2	3998	7996											
	2	2	0.5	1	4112	8224.4	8178.6	16357.2	0.1	148900	10.99	16079	0.0231	1	0.013	15	0.0572
			0.5	2	4066	8132.8											
	3	2	0.5	1	3551	7101.8	7116.4	14232.8	0.1	148900	9.56	13955	0.0201	1	0.013	15	0.0497
			0.5	2	3566	7131											
	4	2	0.5	1	3435	6869.2	6903	13806	0.1	148900	9.27	13528	0.0194	1	0.013	15	0.0482
			0.5	2	3468	6936.8											
Background control	1	2	0.5	1	66	132.5	131	262	0.1	148900	0.18	-16	0.0000	1	0.013	15	-0.0001
			0.5	2	65	129.5											
	2	2	0.5	1	72	143.68	144.02	288.04	0.1	148900	0.19	10	0.0000	1	0.013	15	0.0000
			0.5	2	72	144.36											
	3	2	0.5	1	70	140.86	135.38	270.76	0.1	148900	0.18	-7	0.0000	1	0.013	15	0.0000
			0.5	2	65	129.9											
	4	2	0.5	1	73	146.92	145.96	291.92	0.1	148900	0.20	14	0.0000	1	0.013	15	0.0000
			0.5	2	73	145											
Positive control	1	2	0.5	1	1735	3469.4	3471.6	6943.2	0.1	148900	4.66	6665	0.0096	1	0.013	15	0.0237
			0.5	2	1737	3473.8											
	2	2	0.5	1	1727	3453.4	3429.9	6859.8	0.1	148900	4.61	6582	0.0095	1	0.013	15	0.0234
			0.5	2	1703	3406.4											
	3	2	0.5	1	1825	3649	3627	7254	0.1	148900	4.87	6976	0.0100	1	0.013	15	0.0248
			0.5	2	1803	3605											
	4	2	0.5	1	1746	3492	3453.6	6907.2	0.1	148900	4.64	6629	0.0095	1	0.013	15	0.0236
			0.5	2	1708	3415.2											
Negative Control	1	2	0.5	1	3832	7664	7689.7	15379.4	0.1	148900	10.33	15101	0.0217	1	0.013	15	0.0538
			0.5	2	3858	7715.4											
	2	2	0.5	1	3871	7742.8	7730	15460	0.1	148900	10.38	15182	0.0216	1	0.013	15	0.0540
			0.5	2	3859	7717.2											
	3	2	0.5	1	3677	7353.8	7333.1	14666.2	0.1	148900	9.85	14388	0.0207	1	0.013	15	0.0512
			0.5	2	3656	7312.4											
	4	2	0.5	1	3783	7565.8	7554.2	15108.4	0.1	148900	10.15	14830	0.0213	1	0.013	15	0.0528
			0.5	2	3771	7542.6											
6	1-1	2	0.5	1	122	243.16	246.96	493.92	0.1	148900	0.33	216	0.0003	1	0.013	15	0.0008
			0.5	2	125	250.76											
	1-2	2	0.5	1	130	260.18	266.37	532.74	0.1	148900	0.36	256	0.0004	1	0.013	15	0.0009
			0.5	2	136	272.56											
	1-3	2	0.5	1	130	260.88	259.65	519.3	0.1	148900	0.35	241	0.0003	1	0.013	15	0.0009
			0.5	2	129	258.42											
	2-1	2	0.5	1	304	608.88	614.22	1228.44	0.1	148900	0.83	950	0.0014	1	0.013	15	0.0034
			0.5	2	310	619.56											
	2-2	2	0.5	1	278	556.38	594.22	1188.44	0.1	148900	0.80	910	0.0013	1	0.013	15	0.0032
			0.5	2	316	632.06											
	2-3	2	0.5	1	287	574.12	573.03	1146.06	0.1	148900	0.77	868	0.0012	1	0.013	15	0.0031
			0.5	2	286	571.94											
	3-1	2	0.5	1	1450	2900	2857.2	5714.4	0.1	148900	3.84	5436	0.0078	1	0.013	15	0.0194
			0.5	2	1407	2814.4											
	3-2	2	0.5	1	1461	2922	2978.2	5956.4	0.1	148900	4.00	5678	0.0082	1	0.013	15	0.0202
			0.5	2	1517	3034.4											
	3-3	2	0.5	1	1445	2889.6	2916	5832	0.1	148900	3.92	5554	0.0080	1	0.013	15	0.0198
			0.5	2	1471	2942.4											
	4-1	2	0.5	1	2641	5281.6	5211.9	10423.8	0.1	148900	7.00	10146	0.0146	1	0.013	15	0.0361
			0.5	2	2571	5142.2											
	4-2	2	0.5	1	2576	5151.4	5114.5	10229	0.1	148900	6.87	9951	0.0143	1	0.013	15	0.0354
			0.5	2	2539	5077.6											
	4-3	2	0.5	1	2705	5409.2	5410.9	10821.8	0.1	148900	7.27	10544	0.0152	1	0.013	15	0.0375
			0.5	2	2706	5412.6											
	5-1	2	0.5	1	3020	6040.4	6009.5	12019	0.1	148900	8.07	11741	0.0169	1	0.013	15	0.0418
			0.5	2	2989	5978.6											
	5-2	2	0.5	1	3094	6188.6	6268.2	12536.4	0.1	148900	8.42	12258	0.0176	1	0.013	15	0.0436
			0.5	2	3174	6347.8											
	5-3	2	0.5	1	3071	6141.4	6143.8	12287.6	0.1	148900	8.25	12069	0.0173	1	0.013	15	0.0427
			0.5	2	3073	6146.2											
	6-1	2	0.5	1	3651	7301.6	7366.6	14733.2	0.1	148900	9.89	14455	0.0208	1	0.013	15	0.0515
			0.5	2	3716	7431.6											
	6-2	2	0.5	1	3569	7137	7102	14204	0.1	148900	9.54	13926	0.0200	1	0.013	15	0.0496
			0.5	2	3534	7067											
	6-3	2	0.5	1	3553	7105.4	7068.2	14136.4	0.1	148900	9.49	13858	0.0199	1	0.013	15	0.0493
			0.5	2	3516	7031											
	7-1	2	0.5	1	3613	7225.2	7222.8	14445.6	0.1	148900	9.70	14167	0.0204	1	0.013	15	0.0504
			0.5	2	3610	7220.4											
	7-2	2	0.5	1	3812	7624	7620.1	15240.2	0.1	148900	10.24	14962	0.0215	1	0.013	15	0.0533
			0.5	2	3808	7616.2											
	7-3	2	0.5	1	3658	7316.6	7286.1	14572.2	0.1	148900	9.79	14294	0.0205	1	0.013	15	0.0509
			0.5	2	3628	7255.6											
	8-1	2	0.5	1	3617	7234.2	7315.5	14631	0.1	148900	9.83	14353	0.0206	1	0.013	15	0.0511
			0.5	2	3698	7396.8											
	8-2	2	0.5	1	3658	7316.6	7436.8	14873.6	0.1	148900	9.99	14595	0.0210	1	0.013	15	0.0520
			0.5	2	3779	7557											
	8-3	2	0.5	1	3679	7357	7373.1	14746.2	0.1	148900	9.90	14468	0.0208	1	0.013	15	0.0515
			0.5	2	3695	7389.2											

Assay Date	8/8/2005	Test Chemical ID	6	# Concentrations tested	Microsome 8 type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0568	0.0007
Full activity	End	0.0489	0.0011
Full activity	Overall	0.0528	0.0046
Background	Beginning	0.0000	6.55433E-05
Background	End	0.0000	5.32603E-05
Background	Overall	0.0000	5.04602E-05
Positive	Beginning	0.0236	0.0002
Positive	End	0.0242	0.0009
Positive	Overall	0.0239	0.0006
Negative	Beginning	0.0539	0.0002
Negative	End	0.0520	0.0011
Negative	Overall	0.0530	0.0013

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
6	1	1	1.00E-03	-3.00	0.0008
6	1	2	1.00E-03	-3.00	0.0009
6	1	3	1.00E-03	-3.00	0.0009
6	2	1	1.00E-04	-4.00	0.0034
6	2	2	1.00E-04	-4.00	0.0032
6	2	3	1.00E-04	-4.00	0.0031
6	3	1	1.00E-05	-5.00	0.0194
6	3	2	1.00E-05	-5.00	0.0202
6	3	3	1.00E-05	-5.00	0.0198
6	4	1	2.50E-06	-5.60	0.0361
6	4	2	2.50E-06	-5.60	0.0354
6	4	3	2.50E-06	-5.60	0.0375
6	5	1	1.00E-06	-6.00	0.0418
6	5	2	1.00E-06	-6.00	0.0436
6	5	3	1.00E-06	-6.00	0.0427
6	6	1	1.00E-07	-7.00	0.0515
6	6	2	1.00E-07	-7.00	0.0496
6	6	3	1.00E-07	-7.00	0.0493
6	7	1	1.00E-08	-8.00	0.0504
6	7	2	1.00E-08	-8.00	0.0533
6	7	3	1.00E-08	-8.00	0.0509
6	8	1	1.00E-09	-9.00	0.0511
6	8	2	1.00E-09	-9.00	0.0520
6	8	3	1.00E-09	-9.00	0.0515

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.45	1.71	1.62
2	-4.00	6.40	6.13	5.85
3	-5.00	36.62	38.25	37.42
4	-5.60	68.35	67.04	71.03
5	-6.00	79.10	82.58	80.91
6	-7.00	97.38	93.82	93.36
7	-8.00	95.44	100.80	96.30
8	-9.00	96.69	98.33	97.47

Assay Date	8/9/2005	Test Chemical ID	6	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0197	33197	1685127		
2	0.0201	33066	1645075		
3	0.0201	31700	1577114		
4	0.0200	29260	1463000		
5	0.0199	26257	1319447		
			Average DPM/g soln		1537953
			SD		148351
			CV		9.65
			µCi/g soln		0.693

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.3	10		1030.00
Dilution A			100	10.30
Dilution B			10	1.03

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6131 g
Mass of dilution B used in substrate prep	5.4204 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.580771 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00784 µg/g soln.
µg/g soln.

a. µCi/g soln 0.693
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.580771 + 0.00784
= 0.588614 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.177 µCi/µg ASDN

748317 dpm/nmol

Assay Date	8/9/2005	Test Chemical ID	6	# Concentrations tested	8				
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	BAA		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.524	0.313	0.136	0.062	0.020	0.009	0.000			
	0.522	0.305	0.136	0.061	0.015	0.005	0.000			
	0.519	0.303	0.133	0.065	0.016	0.005	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.260	0.028	0.055
	0.261	0.026	0.058
			0.055

Standard concentration (pg/mL) LE in 3-20-06 book	Volume of stock used	Final volume of Std	mg Protein per µL	Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0500	0.522	0.522	0.0479	m, b	0.093	-0.001
125	100	200	0.00013	200	0.0250	0.307	0.307	0.0279	se_m, se_b	0.005	0.001
50	200	1000	0.00005	200	0.0100	0.135	0.135	0.0118	r^2, se_y	0.990	0.002
25	100	1000	0.00003	200	0.0050	0.063	0.063	0.0050	F, df	379	4
10	40	1000	0.00001	200	0.0020	0.017	0.017	0.0007	SS_{reg}, SS_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.006	0.006	-0.0003			
	Blank		0.000								

Regression results are calculated using the function LINEST

r^2= 0.990
m= 0.093
b= -0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.260	0.260	0.023	200	1	1	0.000	0.000	0.118
QC 100	0.261	0.261	0.024	200	1	1	0.000		
QC 100									
QC 10	0.028	0.028	0.002	200	1	1	0.000	0.000	0.008
QC 10	0.026	0.026	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.055	0.055	0.004	200	120	42000	0.007	0.008	7.650
Microsomes	0.058	0.058	0.005	200	120	42000	0.008		
Microsomes	0.055	0.055	0.004	200	120	42000	0.007		

Assay Date	8/9/2005	Test Chemical ID	6	# Concentrations tested	8					
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	BAA			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.524	0.313	0.136	0.062	0.020	0.009	0.000			
	0.522	0.305	0.136	0.061	0.015	0.005	0.000			
	0.519	0.303	0.133	0.065	0.016	0.005	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.260	0.028	0.055
	0.261	0.026	0.058
			0.055

Standard concentration (µg/mL) LE in 3-20-06 BAL	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.522	0.522	0.0416
125	100	200	0.00013	200	0.0250	0.307	0.307	0.0246
50	200	1000	0.00005	200	0.0100	0.135	0.135	0.0109
25	100	1000	0.00003	200	0.0050	0.063	0.063	0.0052
10	40	1000	0.00001	200	0.0020	0.017	0.017	0.0016
5	20	1000	0.00001	200	0.0010	0.006	0.006	0.0007
			Blank	0.000				

Variables	Regression results	
m, b	0.079	0.000
se_m, se_b	0.003	0.000
r^2, se_y	0.996	0.001
F, df	828	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

r^2= 0.996
m= 0.079
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.260	0.260	0.021	200	1	1	0.000	0.000	0.104
QC 100	0.261	0.261	0.021	200	1	1	0.000		
QC 100									
QC 10	0.028	0.028	0.002	200	1	1	0.000	0.000	0.012
QC 10	0.026	0.026	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.055	0.055	0.005	200	120	42000	0.008	0.008	8.156
Microsomes	0.058	0.058	0.005	200	120	42000	0.009		
Microsomes	0.055	0.055	0.005	200	120	42000	0.008		

Assay Date	8/9/2005	Test Chemical ID	6	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3618	7235.2	7181	14362	0.1	153795	9.34	14073	0.0188	1	0.012	15	0.0538
			0.5	2	3563	7126.8											
	2	2	0.5	1	3442	6883.6	6851.6	13703.2	0.1	153795	8.91	13414	0.0179	1	0.012	15	0.0513
			0.5	2	3410	6819.6											
	3	2	0.5	1	3011	6022.8	5976.8	11953.6	0.1	153795	7.77	11664	0.0156	1	0.012	15	0.0446
			0.5	2	2965	5930.8											
	4	2	0.5	1	3117	6233.8	6167.7	12335.4	0.1	153795	8.02	12046	0.0161	1	0.012	15	0.0461
			0.5	2	3051	6101.6											
Background control	1	2	0.5	1	69	137.46	140.59	281.18	0.1	153795	0.18	-8	0.0000	1	0.012	15	0.0000
			0.5	2	72	143.72											
	2	2	0.5	1	76	152.14	154.94	309.88	0.1	153795	0.20	21	0.0000	1	0.012	15	0.0001
			0.5	2	79	157.74											
	3	2	0.5	1	71	141.16	143.78	287.56	0.1	153795	0.19	-2	0.0000	1	0.012	15	0.0000
			0.5	2	73	146.4											
	4	2	0.5	1	66	131.06	138.95	277.9	0.1	153795	0.18	-11	0.0000	1	0.012	15	0.0000
			0.5	2	73	146.84											
Positive control	1	2	0.5	1	1546	3092.2	3101.1	6202.2	0.1	153795	4.03	5913	0.0079	1	0.012	15	0.0226
			0.5	2	1555	3110											
	2	2	0.5	1	1537	3073.2	3047.2	6094.4	0.1	153795	3.96	5805	0.0078	1	0.012	15	0.0222
			0.5	2	1511	3021.2											
	3	2	0.5	1	1503	3005.4	2973.4	5946.8	0.1	153795	3.87	5658	0.0076	1	0.012	15	0.0216
			0.5	2	1471	2941.4											
	4	2	0.5	1	1563	3126.4	3101.7	6203.4	0.1	153795	4.03	5914	0.0079	1	0.012	15	0.0226
			0.5	2	1539	3077											
Negative Control	1	2	0.5	1	3330	6660.6	6667.8	13335.6	0.1	153795	8.67	13046	0.0174	1	0.012	15	0.0499
			0.5	2	3338	6675											
	2	2	0.5	1	3422	6843.8	6730.5	13461	0.1	153795	8.75	13172	0.0176	1	0.012	15	0.0504
			0.5	2	3309	6617.2											
	3	2	0.5	1	3174	6348.8	6385.3	12770.6	0.1	153795	8.30	12481	0.0167	1	0.012	15	0.0477
			0.5	2	3211	6421.8											
	4	2	0.5	1	3096	6192.8	6224.9	12449.8	0.1	153795	8.10	12161	0.0163	1	0.012	15	0.0465
			0.5	2	3129	6257											
6	1-1	2	0.5	1	111	221.52	227.83	455.66	0.1	153795	0.30	167	0.0002	1	0.012	15	0.0006
			0.5	2	117	234.14											
	1-2	2	0.5	1	109	217.64	225.59	451.18	0.1	153795	0.29	162	0.0002	1	0.012	15	0.0006
			0.5	2	117	233.54											
	1-3	2	0.5	1	108	215.52	219.4	438.8	0.1	153795	0.29	150	0.0002	1	0.012	15	0.0006
			0.5	2	112	223.28											
	2-1	2	0.5	1	265	529.36	513.64	1027.28	0.1	153795	0.67	738	0.0010	1	0.012	15	0.0028
			0.5	2	249	497.92											
	2-2	2	0.5	1	247	494.94	506.88	1013.76	0.1	153795	0.66	725	0.0010	1	0.012	15	0.0028
			0.5	2	259	518.82											
	2-3	2	0.5	1	259	517.14	522.34	1044.68	0.1	153795	0.68	756	0.0010	1	0.012	15	0.0029
			0.5	2	264	527.54											
	3-1	2	0.5	1	1296	2591.2	2579	5158	0.1	153795	3.35	4869	0.0065	1	0.012	15	0.0186
			0.5	2	1283	2566.8											
	3-2	2	0.5	1	1298	2595	2590.3	5180.6	0.1	153795	3.37	4891	0.0065	1	0.012	15	0.0187
			0.5	2	1293	2585.6											
	3-3	2	0.5	1	1271	2542.4	2567.4	5134.8	0.1	153795	3.34	4846	0.0065	1	0.012	15	0.0185
			0.5	2	1296	2592.4											
	4-1	2	0.5	1	2303	4605.2	4641.7	9283.4	0.1	153795	6.04	8994	0.0120	1	0.012	15	0.0344
			0.5	2	2339	4678.2											
	4-2	2	0.5	1	2399	4798.2	4840.2	9680.4	0.1	153795	6.29	9391	0.0125	1	0.012	15	0.0359
			0.5	2	2441	4882.2											
	4-3	2	0.5	1	2191	4381	4400.1	8800.2	0.1	153795	5.72	8511	0.0114	1	0.012	15	0.0325
			0.5	2	2210	4419.2											
	5-1	2	0.5	1	2841	5682.4	5669.1	11338.2	0.1	153795	7.37	11049	0.0148	1	0.012	15	0.0422
			0.5	2	2828	5655.8											
	5-2	2	0.5	1	2883	5766.4	5772.5	11545	0.1	153795	7.51	11256	0.0150	1	0.012	15	0.0430
			0.5	2	2889	5778.6											
	5-3	2	0.5	1	2886	5771.2	5777.8	11555.6	0.1	153795	7.51	11266	0.0151	1	0.012	15	0.0431
			0.5	2	2892	5784.4											
	6-1	2	0.5	1	3146	6291.4	6335	12670	0.1	153795	8.24	12381	0.0165	1	0.012	15	0.0473
			0.5	2	3189	6378.6											
	6-2	2	0.5	1	3207	6414.2	6419.7	12839.4	0.1	153795	8.35	12550	0.0168	1	0.012	15	0.0480
			0.5	2	3213	6425.2											
	6-3	2	0.5	1	3180	6360	6365.8	12731.6	0.1	153795	8.28	12442	0.0166	1	0.012	15	0.0476
			0.5	2	3186	6371.6											
	7-1	2	0.5	1	3295	6589.2	6644	13288	0.1	153795	8.64	12999	0.0174	1	0.012	15	0.0497
			0.5	2	3349	6698.8											
	7-2	2	0.5	1	3101	6201.8	6265.8	12531.6	0.1	153795	8.15	12242	0.0164	1	0.012	15	0.0468
			0.5	2	3165	6329.8											
	7-3	2	0.5	1	3216	6432.8	6432.6	12865.2	0.1	153795	8.37	12576	0.0168	1	0.012	15	0.0481
			0.5	2	3216	6432.4											
	8-1	2	0.5	1	3224	6448.2	6431.1	12862.2	0.1	153795	8.36	12573	0.0168	1	0.012	15	0.0481
			0.5	2	3207	6414											
	8-2	2	0.5	1	3341	6682	6626.6	13253.2	0.1	153795	8.62	12964	0.0173	1	0.012	15	0.0496
			0.5	2	3286	6571.2											
	8-3	2	0.5	1	3244	6487.4	6503.5	13007	0.1	153795	8.46	12718	0.0170	1	0.012	15	0.0486
			0.5	2	3260	6519.6											

Assay Date	8/9/2005	Test Chemical ID	6	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0525	0.0018
Full activity	End	0.0453	0.0010
Full activity	Overall	0.0489	0.0043
Background	Beginning	0.0000	7.75882E-05
Background	End	0.0000	2.6115E-05
Background	Overall	0.0000	5.50659E-05
Positive	Beginning	0.0224	0.0003
Positive	End	0.0221	0.0007
Positive	Overall	0.0223	0.0005
Negative	Beginning	0.0501	0.0003
Negative	End	0.0471	0.0009
Negative	Overall	0.0486	0.0018

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
6	1	1	1.00E-03	-3.00	0.0006
6	1	2	1.00E-03	-3.00	0.0006
6	1	3	1.00E-03	-3.00	0.0006
6	2	1	1.00E-04	-4.00	0.0028
6	2	2	1.00E-04	-4.00	0.0028
6	2	3	1.00E-04	-4.00	0.0029
6	3	1	1.00E-05	-5.00	0.0186
6	3	2	1.00E-05	-5.00	0.0187
6	3	3	1.00E-05	-5.00	0.0185
6	4	1	2.50E-06	-5.60	0.0344
6	4	2	2.50E-06	-5.60	0.0359
6	4	3	2.50E-06	-5.60	0.0325
6	5	1	1.00E-06	-6.00	0.0422
6	5	2	1.00E-06	-6.00	0.0430
6	5	3	1.00E-06	-6.00	0.0431
6	6	1	1.00E-07	-7.00	0.0473
6	6	2	1.00E-07	-7.00	0.0480
6	6	3	1.00E-07	-7.00	0.0476
6	7	1	1.00E-08	-8.00	0.0497
6	7	2	1.00E-08	-8.00	0.0468
6	7	3	1.00E-08	-8.00	0.0481
6	8	1	1.00E-09	-9.00	0.0481
6	8	2	1.00E-09	-9.00	0.0496
6	8	3	1.00E-09	-9.00	0.0486

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.30	1.27	1.17
2	-4.00	5.77	5.66	5.90
3	-5.00	38.04	38.22	37.86
4	-5.60	70.27	73.37	66.50
5	-6.00	86.32	87.94	88.02
6	-7.00	96.73	98.05	97.21
7	-8.00	101.56	95.65	98.25
8	-9.00	98.23	101.29	99.36

Assay Date	8/17/2005	Test Chemical ID	7	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	Lot # BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0200	28939	1446950		
2	0.0200	30872	1543600		
3	0.0197	31400	1593909		
4	0.0200	33124	1656200		
5	0.0200	33109	1655450		
			Average DPM/g soln		1579222
			SD		87631
			CV		5.55
			µCi/g soln		0.711

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.2	10		1020.00
Dilution A			100	10.20
Dilution B			10	1.02

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6405 g
Mass of dilution B used in substrate prep	5.4322 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.574747 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00805 µg/g soln.
µg/g soln.

a. µCi/g soln	0.711
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.574747 + 0.00805
= 0.582799 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.221 µCi/µg ASDN

776063 dpm/nmol

Assay Date	8/17/2005	Test Chemical ID	7	# Concentrations tested	8						
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	Lot # BAA				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.501	0.302	0.127	0.066	0.023	0.013	0.000			
	0.506	0.299	0.126	0.064	0.024	0.012	0.000			
	0.509	0.296	0.123	0.062	0.022	0.013	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.257	0.029	0.064
	0.255	0.027	0.066
			0.064

Standard concentration (mg/mL) LE in 3-30-06 lab book	Volume of stock used	Final volume of Std	mg Protein per µL		Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200	0.0500	0.506	0.506	0.0481	m, b	0.097	-0.001
125	100	200	0.00013		200	0.0250	0.299	0.299	0.0279	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200	0.0100	0.126	0.126	0.0110	r^2, se_y	0.992	0.002
25	100	1000	0.00003		200	0.0050	0.064	0.064	0.0050	F, df	475	4
10	40	1000	0.00001		200	0.0020	0.023	0.023	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010	0.013	0.013	0.0000			
				Blank	0.000							

r^2= 0.992
m= 0.097
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.257	0.257	0.024	200	1	1	0.000	0.000	0.119
QC 100	0.255	0.255	0.024	200	1	1	0.000		
QC 100									
QC 10	0.029	0.029	0.002	200	1	1	0.000	0.000	0.007
QC 10	0.027	0.027	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.064	0.064	0.005	200	120	42000	0.009	0.009	8.868
Microsomes	0.066	0.066	0.005	200	120	42000	0.009		
Microsomes	0.064	0.064	0.005	200	120	42000	0.009		

Assay Date	8/17/2005	Test Chemical ID	7	# Concentrations tested	8
Technician ID	TD	Replicate #	1	Microsome type	placental

Microsome ID: Lot # BAA

Standards:	250	125	50	25	10	5	0
	0.501	0.302	0.127	0.066	0.023	0.013	0.000
	0.508	0.299	0.126	0.064	0.024	0.012	0.000
	0.509	0.296	0.123	0.062	0.022	0.013	0.000

Protein stock (mg BSA): 25 | Total volume of stock (mL): 100 | Protein stock ID: 11343-77

Samples:	QC 100	QC 10	Microsomes
	0.257	0.029	0.064
	0.255	0.027	0.066
			0.064

Standard concentration (mg/mL) LE in 3-20-06 BRL	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.506	0.506	0.0422
125	100	200	0.00013	200	0.0250	0.299	0.299	0.0248
50	200	1000	0.00005	200	0.0100	0.126	0.126	0.0103
25	100	1000	0.00003	200	0.0050	0.064	0.064	0.0052
10	40	1000	0.00001	200	0.0020	0.023	0.023	0.0018
5	20	1000	0.00001	200	0.0010	0.013	0.013	0.0009
		Blank	0.000					

r^2= 0.999
m= 0.084
b= 0.000

Variables	Regression results	
m, b	0.084	0.000
se_m, se_b	0.001	0.000
r^2, se_y	0.999	0.000
F, df	4806	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.257	0.257	0.021	200	1	1	0.000	0.000	0.106
QC 100	0.255	0.255	0.021	200	1	1	0.000		
QC 100									
QC 10	0.029	0.029	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.027	0.027	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.064	0.064	0.005	200	120	42000	0.009	0.009	9.176
Microsomes	0.066	0.066	0.005	200	120	42000	0.009		
Microsomes	0.064	0.064	0.005	200	120	42000	0.009		

Assay Date	8/17/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4029	8058.8	7982.1	15964.2	0.1	157922	10.11	15685	0.0202	1	0.013	15	0.0514
			0.5	2	3953	7905.4											
	2	2	0.5	1	4080	8160.6	8075.9	16151.8	0.1	157922	10.23	15872	0.0205	1	0.013	15	0.0520
			0.5	2	3996	7991.2											
	3	2	0.5	1	1638	3276.8	3304.2	6608.4	0.1	157922	4.18	6329	0.0082	1	0.013	15	0.0207
			0.5	2	1666	3331.6											
	4	2	0.5	1	1775	3550	3538.2	7076.4	0.1	157922	4.48	6797	0.0088	1	0.013	15	0.0223
			0.5	2	1763	3526.4											
Background control	1	2	0.5	1	71	141.52	139.64	279.28	0.1	157922	0.18	0	0.0000	1	0.013	15	0.0000
			0.5	2	69	137.76											
	2	2	0.5	1	73	145.78	144.06	288.12	0.1	157922	0.18	8	0.0000	1	0.013	15	0.0000
			0.5	2	71	142.34											
	3	2	0.5	1	67	134.18	137.6	275.2	0.1	157922	0.17	-4	0.0000	1	0.013	15	0.0000
			0.5	2	71	141.02											
	4	2	0.5	1	75	150.28	138.05	276.1	0.1	157922	0.17	-4	0.0000	1	0.013	15	0.0000
			0.5	2	63	125.82											
Positive control	1	2	0.5	1	1912	3823	3827.3	7654.6	0.1	157922	4.85	7375	0.0095	1	0.013	15	0.0242
			0.5	2	1916	3831.6											
	2	2	0.5	1	1888	3775.8	3776.6	7553.2	0.1	157922	4.78	7274	0.0094	1	0.013	15	0.0238
			0.5	2	1889	3777.4											
	3	2	0.5	1	1115	2229	2214.1	4428.2	0.1	157922	2.80	4149	0.0053	1	0.013	15	0.0135
			0.5	2	1100	2199.2											
	4	2	0.5	1	1044	2087.14	2089.13	4178.26	0.1	157922	2.65	3899	0.0050	1	0.013	15	0.0128
			0.5	2	1046	2091.12											
Negative Control	1	2	0.5	1	3742	7484	7461.2	14922.4	0.1	157922	9.45	14643	0.0189	1	0.013	15	0.0480
			0.5	2	3719	7438.4											
	2	2	0.5	1	4080	8160.6	8181.6	16363.2	0.1	157922	10.36	16084	0.0207	1	0.013	15	0.0527
			0.5	2	4101	8202.6											
	3	2	0.5	1	1814	3628.4	3577.5	7155	0.1	157922	4.53	6875	0.0089	1	0.013	15	0.0225
			0.5	2	1763	3526.6											
	4	2	0.5	1	1650	3299.6	3286.6	6573.2	0.1	157922	4.16	6294	0.0081	1	0.013	15	0.0206
			0.5	2	1637	3273.6											
7	1-1	2	0.5	1	60	119.22	119.44	238.88	0.1	157922	0.15	-41	-0.0001	1	0.013	15	-0.0001
			0.5	2	60	119.66											
	1-2	2	0.5	1	61	122.46	117.65	235.3	0.1	157922	0.15	-44	-0.0001	1	0.013	15	-0.0001
			0.5	2	56	112.84											
	1-3	2	0.5	1	63	125.72	125.43	250.86	0.1	157922	0.16	-29	0.0000	1	0.013	15	-0.0001
			0.5	2	63	125.14											
	2-1	2	0.5	1	67	133.62	118.58	237.16	0.1	157922	0.15	-43	-0.0001	1	0.013	15	-0.0001
			0.5	2	52	103.54											
	2-2	2	0.5	1	54	108.42	102.77	205.54	0.1	157922	0.13	-74	-0.0001	1	0.013	15	-0.0002
			0.5	2	49	97.12											
	2-3	2	0.5	1	48	95.62	106.97	213.94	0.1	157922	0.14	-66	-0.0001	1	0.013	15	-0.0002
			0.5	2	59	118.32											
	3-1	2	0.5	1	78	151.82	145.65	291.3	0.1	157922	0.18	12	0.0000	1	0.013	15	0.0000
			0.5	2	70	139.48											
	3-2	2	0.5	1	47	94.12	97.9	195.8	0.1	157922	0.12	-84	-0.0001	1	0.013	15	-0.0003
			0.5	2	51	101.68											
	3-3	2	0.5	1	59	118	119	238	0.1	157922	0.15	-42	-0.0001	1	0.013	15	-0.0001
			0.5	2	60	120											
	4-1	2	0.5	1	59	117.14	117.6	235.2	0.1	157922	0.15	-44	-0.0001	1	0.013	15	-0.0001
			0.5	2	59	118.06											
	4-2	2	0.5	1	66	131.32	143.66	287.32	0.1	157922	0.18	8	0.0000	1	0.013	15	0.0000
			0.5	2	78	156											
	4-3	2	0.5	1	60	119.96	122.8	245.6	0.1	157922	0.16	-34	0.0000	1	0.013	15	-0.0001
			0.5	2	63	125.64											
	5-1	2	0.5	1	100	200.14	212.13	424.26	0.1	157922	0.27	145	0.0002	1	0.013	15	0.0005
			0.5	2	112	224.12											
	5-2	2	0.5	1	92	183.9	209.51	419.02	0.1	157922	0.27	139	0.0002	1	0.013	15	0.0005
			0.5	2	118	235.12											
	5-3	2	0.5	1	103	206.22	209.83	419.66	0.1	157922	0.27	140	0.0002	1	0.013	15	0.0005
			0.5	2	107	213.44											
	6-1	2	0.5	1	437	874.26	876.04	1752.08	0.1	157922	1.11	1472	0.0019	1	0.013	15	0.0048
			0.5	2	439	877.82											
	6-2	2	0.5	1	401	801.48	790.2	1580.4	0.1	157922	1.00	1301	0.0017	1	0.013	15	0.0043
			0.5	2	389	778.92											
	6-3	2	0.5	1	409	818.46	809.65	1619.3	0.1	157922	1.03	1340	0.0017	1	0.013	15	0.0044
			0.5	2	400	800.84											
	7-1	2	0.5	1	1188	2376.6	2412.2	4824.4	0.1	157922	3.05	4545	0.0059	1	0.013	15	0.0149
			0.5	2	1224	2447.8											
	7-2	2	0.5	1	1260	2520.2	2543.2	5086.4	0.1	157922	3.22	4807	0.0062	1	0.013	15	0.0157
			0.5	2	1283	2566.2											
	7-3	2	0.5	1	1308	2616	2601.7	5203.4	0.1	157922	3.29	4924	0.0063	1	0.013	15	0.0161
			0.5	2	1294	2587.4											
	8-1	2	0.5	1	1680	3359.4	3324.1	6648.2	0.1	157922	4.21	6369	0.0082	1	0.013	15	0.0209
			0.5	2	1644	3288.8											
	8-2	2	0.5	1	1681	3361.8	3371.2	6742.4	0.1	157922	4.27	6463	0.0083	1	0.013	15	0.0212
			0.5	2	1690	3380.6											
	8-3	2	0.5	1	1476	2951	2945.6	5891.2	0.1	157922	3.73	5612	0.0072	1	0.013	15	0.0184
			0.5	2	1470	2940.2											

Assay Date	8/17/2005	Test Chemical ID	7	# Concentrations tested	Microsome 8 type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0517	0.0004
Full activity	End	0.0215	0.0011
Full activity	Overall	0.0366	0.0174
Background	Beginning	0.0000	2.04805E-05
Background	End	0.0000	2.08512E-06
Background	Overall	0.0000	1.93172E-05
Positive	Beginning	0.0240	0.0002
Positive	End	0.0132	0.0006
Positive	Overall	0.0186	0.0063
Negative	Beginning	0.0503	0.0033
Negative	End	0.0216	0.0013
Negative	Overall	0.0360	0.0167

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
7	1	1	1.00E-03	-3.00	-0.0001
7	1	2	1.00E-03	-3.00	-0.0001
7	1	3	1.00E-03	-3.00	-0.0001
7	2	1	1.00E-04	-4.00	-0.0001
7	2	2	1.00E-04	-4.00	-0.0002
7	2	3	1.00E-04	-4.00	-0.0002
7	3	1	1.00E-05	-5.00	0.0000
7	3	2	1.00E-05	-5.00	-0.0003
7	3	3	1.00E-05	-5.00	-0.0001
7	4	1	1.00E-06	-6.00	-0.0001
7	4	2	1.00E-06	-6.00	0.0000
7	4	3	1.00E-06	-6.00	-0.0001
7	5	1	1.00E-07	-7.00	0.0005
7	5	2	1.00E-07	-7.00	0.0005
7	5	3	1.00E-07	-7.00	0.0005
7	6	1	1.00E-08	-8.00	0.0048
7	6	2	1.00E-08	-8.00	0.0043
7	6	3	1.00E-08	-8.00	0.0044
7	7	1	1.00E-09	-9.00	0.0149
7	7	2	1.00E-09	-9.00	0.0157
7	7	3	1.00E-09	-9.00	0.0161
7	8	1	1.00E-10	-10.00	0.0209
7	8	2	1.00E-10	-10.00	0.0212
7	8	3	1.00E-10	-10.00	0.0184

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	-0.37	-0.40	-0.26
2	-4.00	-0.38	-0.66	-0.59
3	-5.00	0.10	-0.75	-0.37
4	-6.00	-0.40	0.07	-0.31
5	-7.00	1.29	1.25	1.25
6	-8.00	13.18	11.64	11.99
7	-9.00	40.68	43.03	44.08
8	-10.00	57.01	57.86	50.24

Assay Date	8/18/2005	Test Chemical ID	7	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	Lot # BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0195	26367	1352154		
2	0.0200	27703	1385150		
3	0.0200	28337	1416850		
4	0.0200	28751	1437550		
5	0.0198	28573	1443081		
			Average DPM/g soln	1406957	
			SD	38137	
			CV	2.71	
			µCi/g soln	0.634	

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.7	10		1070.00
Dilution A			100	10.70
Dilution B			10	1.07

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6248 g
Mass of dilution B used in substrate prep	5.4237 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.602959 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00717 µg/g soln.
µg/g soln.

a. µCi/g soln 0.634
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.602959 + 0.00717
= 0.610133 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.039 µCi/µg ASDN

660434 dpm/nmol

Assay Date	8/18/2005	Test Chemical ID	7	# Concentrations tested	8				
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	Lot # BAA		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.514	0.307	0.129	0.064	0.027	0.009	0.000			
	0.509	0.300	0.128	0.065	0.023	0.013	0.000			
	0.471	0.300	0.128	0.064	0.025	0.012	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.262	0.027	0.064							
	0.261	0.029	0.062							
			0.060							

Standard concentration (mg/mL) LE in 3-20-06 BSL	Volume of stock used	Final volume of Std	mg Protein per µL		Used		mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200		0.0500		0.498	0.498	0.0477	m, b	0.099	-0.001
125	100	200	0.00013		200		0.0250		0.302	0.302	0.0284	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200		0.0100		0.128	0.128	0.0112	r^2, se_y	0.988	0.002
25	100	1000	0.00003		200		0.0050		0.064	0.064	0.0049	F, df	341	4
10	40	1000	0.00001		200		0.0020		0.025	0.025	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.011	0.011	-0.0003			
				Blank	0.000									

Regression results are calculated using the function LINEST

r^2= 0.988
m= 0.099
b= -0.001

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.262	0.262	0.024	200	1	1	0.000	0.000	0.122
QC 100	0.261	0.261	0.024	200	1	1	0.000		
QC 100									
QC 10	0.027	0.027	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.029	0.029	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.064	0.064	0.005	200	120	42000	0.009	0.008	8.200
Microsomes	0.062	0.062	0.005	200	120	42000	0.008		
Microsomes	0.060	0.060	0.004	200	120	42000	0.008		

Assay Date	8/18/2005	Test Chemical ID	7	# Concentrations tested	8	
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID Lot # BAA

Standards:	250	125	50	25	10	5	0
	0.514	0.307	0.129	0.064	0.027	0.009	0.000
	0.509	0.300	0.128	0.065	0.023	0.013	0.000
	0.471	0.300	0.128	0.064	0.025	0.012	0.000

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25	100	11343-77

Samples:	QC 100	QC 10	Microsomes
	0.262	0.027	0.064
	0.261	0.029	0.062
			0.060

Standard concentration (mg/mL) LE in 3-20-06 BDL	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.498	0.498	0.0410
125	100	200	0.00013	200	0.0250	0.302	0.302	0.0248
50	200	1000	0.00005	200	0.0100	0.128	0.128	0.0104
25	100	1000	0.00003	200	0.0050	0.064	0.064	0.0051
10	40	1000	0.00001	200	0.0020	0.025	0.025	0.0019
5	20	1000	0.00001	200	0.0010	0.011	0.011	0.0007
			Blank	0.000				

Variables	Regression results	
m, b	0.083	0.000
se_m, se_b	0.001	0.000
r^2, se_y	0.999	0.000
F, df	3683	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.999
m= 0.083
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.262	0.262	0.022	200	1	1	0.000	0.000	0.107
QC 100	0.261	0.261	0.021	200	1	1	0.000		
QC 100									
QC 10	0.027	0.027	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.029	0.029	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.064	0.064	0.005	200	120	42000	0.009	0.009	8.636
Microsomes	0.062	0.062	0.005	200	120	42000	0.009		
Microsomes	0.060	0.060	0.005	200	120	42000	0.008		

Assay Date	8/18/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3377	6754.4	6700.3	13400.6	0.1	140696	9.52	13081	0.0198	1	0.012	15	0.0535
			0.5	2	3323	6646.2											
	2	2	0.5	1	3102	6203	6210.1	12420.2	0.1	140696	8.83	12101	0.0183	1	0.012	15	0.0495
			0.5	2	3109	6217.2											
	3	2	0.5	1	2902	5804	5924.9	11849.8	0.1	140696	8.42	11531	0.0175	1	0.012	15	0.0472
			0.5	2	3023	6045.8											
	4	2	0.5	1	2971	5942	5967.9	11935.8	0.1	140696	8.48	11617	0.0176	1	0.012	15	0.0475
			0.5	2	2997	5993.8											
Background control	1	2	0.5	1	80	160.62	163.34	326.68	0.1	140696	0.23	7	0.0000	1	0.012	15	0.0000
			0.5	2	83	166.06											
	2	2	0.5	1	79	158.34	157.98	315.96	0.1	140696	0.22	-3	0.0000	1	0.012	15	0.0000
			0.5	2	79	157.62											
	3	2	0.5	1	81	162.24	164.03	328.06	0.1	140696	0.23	9	0.0000	1	0.012	15	0.0000
			0.5	2	83	165.82											
	4	2	0.5	1	76	152.04	153.22	306.44	0.1	140696	0.22	-13	0.0000	1	0.012	15	-0.0001
			0.5	2	77	154.4											
Positive control	1	2	0.5	1	1611	3221.6	3159.4	6318.8	0.1	140696	4.49	6000	0.0091	1	0.012	15	0.0245
			0.5	2	1549	3097.2											
	2	2	0.5	1	1586	3171	3191.8	6383.6	0.1	140696	4.54	6064	0.0092	1	0.012	15	0.0248
			0.5	2	1606	3212.6											
	3	2	0.5	1	1449	2897.2	2871.9	5743.8	0.1	140696	4.08	5425	0.0082	1	0.012	15	0.0222
			0.5	2	1423	2846.6											
	4	2	0.5	1	1531	3061.4	3079.8	6159.6	0.1	140696	4.38	5840	0.0088	1	0.012	15	0.0239
			0.5	2	1549	3098.2											
Negative Control	1	2	0.5	1	3105	6210.4	6325.2	12650.4	0.1	140696	8.99	12331	0.0187	1	0.012	15	0.0504
			0.5	2	3220	6440											
	2	2	0.5	1	3103	6205	6276.7	12553.4	0.1	140696	8.92	12234	0.0185	1	0.012	15	0.0501
			0.5	2	3174	6348.4											
	3	2	0.5	1	2949	5898	5907.5	11815	0.1	140696	8.40	11496	0.0174	1	0.012	15	0.0470
			0.5	2	2959	5917											
	4	2	0.5	1	3059	6117	6200.7	12401.4	0.1	140696	8.81	12092	0.0183	1	0.012	15	0.0494
			0.5	2	3142	6284.4											
7	1-1	2	0.5	1	73	145.3	136.26	272.52	0.1	140696	0.19	-47	-0.0001	1	0.012	15	-0.0002
			0.5	2	64	127.22											
	1-2	2	0.5	1	87	174.18	164.96	329.92	0.1	140696	0.23	11	0.0000	1	0.012	15	0.0000
			0.5	2	78	155.74											
	1-3	2	0.5	1	69	138.46	135.95	271.9	0.1	140696	0.19	-47	-0.0001	1	0.012	15	-0.0002
			0.5	2	67	133.44											
	2-1	2	0.5	1	69	137	137.58	275.16	0.1	140696	0.20	-44	-0.0001	1	0.012	15	-0.0002
			0.5	2	69	138.16											
	2-2	2	0.5	1	94	188.92	179.97	359.94	0.1	140696	0.26	41	0.0001	1	0.012	15	0.0002
			0.5	2	86	171.02											
	2-3	2	0.5	1	77	154.76	146.58	293.16	0.1	140696	0.21	-26	0.0000	1	0.012	15	-0.0001
			0.5	2	69	138.4											
	3-1	2	0.5	1	66	132.46	139.79	279.58	0.1	140696	0.20	-40	-0.0001	1	0.012	15	-0.0002
			0.5	2	74	147.12											
	3-2	2	0.5	1	70	139.54	143.84	287.68	0.1	140696	0.20	-32	0.0000	1	0.012	15	-0.0001
			0.5	2	74	148.14											
	3-3	2	0.5	1	63	126.34	130.69	261.38	0.1	140696	0.19	-58	-0.0001	1	0.012	15	-0.0002
			0.5	2	68	135.04											
	4-1	2	0.5	1	119	237.14	249.08	498.16	0.1	140696	0.35	179	0.0003	1	0.012	15	0.0007
			0.5	2	131	261.02											
	4-2	2	0.5	1	142	283.64	274.8	549.6	0.1	140696	0.39	230	0.0003	1	0.012	15	0.0009
			0.5	2	133	265.96											
	4-3	2	0.5	1	127	254.2	248.66	497.32	0.1	140696	0.35	178	0.0003	1	0.012	15	0.0007
			0.5	2	122	243.12											
	5-1	2	0.5	1	581	1161.14	1130.2	2260.4	0.1	140696	1.61	1941	0.0029	1	0.012	15	0.0079
			0.5	2	550	1099.26											
	5-2	2	0.5	1	581	1161.24	1152.65	2305.3	0.1	140696	1.64	1986	0.0030	1	0.012	15	0.0081
			0.5	2	572	1144.06											
	5-3	2	0.5	1	565	1130.52	1143.03	2286.06	0.1	140696	1.62	1967	0.0030	1	0.012	15	0.0080
			0.5	2	578	1155.54											
	6-1	2	0.5	1	1458	2916.2	2920.3	5840.6	0.1	140696	4.15	5521	0.0084	1	0.012	15	0.0226
			0.5	2	1462	2924.4											
	6-2	2	0.5	1	1441	2881.4	2858	5716	0.1	140696	4.06	5397	0.0082	1	0.012	15	0.0221
			0.5	2	1417	2834.6											
	6-3	2	0.5	1	1389	2777.6	2794.1	5588.2	0.1	140696	3.97	5269	0.0080	1	0.012	15	0.0216
			0.5	2	1405	2810.6											
	7-1	2	0.5	1	1975	3949.8	3996.3	7992.6	0.1	140696	5.68	7673	0.0116	1	0.012	15	0.0314
			0.5	2	2021	4042.8											
	7-2	2	0.5	1	2046	4092.4	4087.8	8175.6	0.1	140696	5.81	7856	0.0119	1	0.012	15	0.0321
			0.5	2	2042	4083.2											
	7-3	2	0.5	1	2102	4204.6	4244.7	8489.4	0.1	140696	6.03	8170	0.0124	1	0.012	15	0.0334
			0.5	2	2142	4284.8											
	8-1	2	0.5	1	2570	5140.4	5219.4	10438.8	0.1	140696	7.42	10120	0.0153	1	0.012	15	0.0414
			0.5	2	2649	5298.4											
	8-2	2	0.5	1	2810	5620.8	5605.4	11210.8	0.1	140696	7.97	10892	0.0165	1	0.012	15	0.0446
			0.5	2	2795	5590											
	8-3	2	0.5	1	2969	5977.4	5811.6	11623.2	0.1	140696	8.26	11304	0.0171	1	0.012	15	0.0462
			0.5	2	2823	5645.8											

Assay Date	8/18/2005	Test Chemical ID	7	# Concentrations tested	Microsome 8 type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0515	0.0028
Full activity	End	0.0473	0.0002
Full activity	Overall	0.0494	0.0029
Background	Beginning	0.0000	3.10109E-05
Background	End	0.0000	6.25425E-05
Background	Overall	0.0000	4.14346E-05
Positive	Beginning	0.0247	0.0002
Positive	End	0.0230	0.0012
Positive	Overall	0.0239	0.0012
Negative	Beginning	0.0502	0.0003
Negative	End	0.0482	0.0017
Negative	Overall	0.0492	0.0015

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
7	1	1	1.00E-04	-4.00	-0.0002
7	1	2	1.00E-04	-4.00	0.0000
7	1	3	1.00E-04	-4.00	-0.0002
7	2	1	1.00E-05	-5.00	-0.0002
7	2	2	1.00E-05	-5.00	0.0002
7	2	3	1.00E-05	-5.00	-0.0001
7	3	1	1.00E-06	-6.00	-0.0002
7	3	2	1.00E-06	-6.00	-0.0001
7	3	3	1.00E-06	-6.00	-0.0002
7	4	1	1.00E-07	-7.00	0.0007
7	4	2	1.00E-07	-7.00	0.0009
7	4	3	1.00E-07	-7.00	0.0007
7	5	1	1.00E-08	-8.00	0.0079
7	5	2	1.00E-08	-8.00	0.0081
7	5	3	1.00E-08	-8.00	0.0080
7	6	1	2.50E-09	-8.60	0.0226
7	6	2	2.50E-09	-8.60	0.0221
7	6	3	2.50E-09	-8.60	0.0216
7	7	1	1.00E-09	-9.00	0.0314
7	7	2	1.00E-09	-9.00	0.0321
7	7	3	1.00E-09	-9.00	0.0334
7	8	1	1.00E-10	-10.00	0.0414
7	8	2	1.00E-10	-10.00	0.0446
7	8	3	1.00E-10	-10.00	0.0462

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	-0.39	0.09	-0.39
2	-5.00	-0.37	0.34	-0.22
3	-6.00	-0.33	-0.26	-0.48
4	-7.00	1.48	1.91	1.47
5	-8.00	16.07	16.44	16.28
6	-8.60	45.70	44.67	43.61
7	-9.00	63.51	65.02	67.62
8	-10.00	83.75	90.14	93.56

Assay Date	8/19/2005	Test Chemical ID	7	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	Lot # BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	28031	1408593	
2	0.0197	29189	1481675	
3	0.0197	30900	1568528	
4	0.0198	32090	1620707	
5	0.0199	32514	1633869	
			Average DPM/g soln	1542674
			SD	95881
			CV	6.22
			µCi/g soln	0.695

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.6	10		1060.00
Dilution A			100	10.60
Dilution B			10	1.06

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.5939 g
Mass of dilution B used in substrate prep	5.3954 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.596121 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00787 µg/g soln.
µg/g soln.

a. µCi/g soln 0.695
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.596121 + 0.00787
= 0.603987 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.151 µCi/µg ASDN

731509 dpm/nmol

Assay Date	8/19/2005	Test Chemical ID	7	# Concentrations tested	8	
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID / Lot # BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.501	0.299	0.130	0.065	0.027	0.013	0.000			
	0.502	0.298	0.129	0.067	0.026	0.015	0.000			
	0.504	0.305	0.132	0.067	0.029	0.013	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.254	0.031	0.067
	0.253	0.032	0.068
			0.065

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL
	LE 3-20-06 BSL		
250	200	200	0.00025
125	100	200	0.00013
50	200	1000	0.00005
25	100	1000	0.00003
10	40	1000	0.00001
5	20	1000	0.00001

		Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
		200	0.0500	0.502	0.502	0.0479	m, b	0.099	-0.002
		200	0.0250	0.301	0.301	0.0281	se_m, se_b	0.005	0.001
		200	0.0100	0.130	0.130	0.0113	r^2, se_y	0.990	0.002
		200	0.0050	0.066	0.066	0.0049	F, df	405	4
		200	0.0020	0.027	0.027	0.0011	SS_{reg}, SS_{resid}	0.002	0.000
		200	0.0010	0.013	0.013	-0.0003			
Blank		0.000							

Regression results are calculated using the function LINEST

r^2= 0.990
m= 0.099
b= -0.002

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.254	0.254	0.023	200	1	1	0.000	0.000	0.117
QC 100	0.253	0.253	0.023	200	1	1	0.000		
QC 100									
QC 10	0.031	0.031	0.001	200	1	1	0.000	0.000	0.008
QC 10	0.032	0.032	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.067	0.067	0.005	200	120	42000	0.009	0.009	8.680
Microsomes	0.068	0.068	0.005	200	120	42000	0.009		
Microsomes	0.065	0.065	0.005	200	120	42000	0.008		

Assay Date	8/19/2005	Test Chemical ID	7	# Concentrations tested	8						
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	Lot # BAA				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.501	0.299	0.130	0.065	0.027	0.013	0.000			
	0.502	0.298	0.129	0.067	0.026	0.015	0.000			
	0.504	0.305	0.132	0.067	0.029	0.013	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.254	0.031	0.067
	0.253	0.032	0.068
			0.065

Standard concentration (ng/mL) LE M 3-20-06 BSL	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.502	0.502	0.0416
125	100	200	0.00013	200	0.0250	0.301	0.301	0.0248
50	200	1000	0.00005	200	0.0100	0.130	0.130	0.0105
25	100	1000	0.00003	200	0.0050	0.066	0.066	0.0051
10	40	1000	0.00001	200	0.0020	0.027	0.027	0.0019
5	20	1000	0.00001	200	0.0010	0.013	0.013	0.0007
			Blank	0.000				

Variables	Regression results	
m, b	0.084	0.000
se_m, se_b	0.002	0.000
r^2, se_y	0.999	0.000
F, df	2648	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

r^2= 0.999
m= 0.084
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.254	0.254	0.021	200	1	1	0.000	0.000	0.104
QC 100	0.253	0.253	0.021	200	1	1	0.000		
QC 100									
QC 10	0.031	0.031	0.002	200	1	1	0.000	0.000	0.011
QC 10	0.032	0.032	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.067	0.067	0.005	200	120	42000	0.009	0.009	9.026
Microsomes	0.068	0.068	0.005	200	120	42000	0.009		
Microsomes	0.065	0.065	0.005	200	120	42000	0.009		

Assay Date	8/19/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3500	6999	7099.1	14198.2	0.1	154267	9.20	13914	0.0190	1	0.013	15	0.0492
			0.5	2	3600	7199.2											
	2	2	0.5	1	3274	6548.8	6520.5	13041	0.1	154267	8.45	12757	0.0174	1	0.013	15	0.0451
			0.5	2	3246	6492.2											
	3	2	0.5	1	2557	5114	5183.5	10367	0.1	154267	6.72	10083	0.0138	1	0.013	15	0.0356
			0.5	2	2627	5253											
	4	2	0.5	1	3284	6567.4	6560.7	13121.4	0.1	154267	8.51	12837	0.0175	1	0.013	15	0.0454
			0.5	2	3277	6554											
Background control	1	2	0.5	1	63	125.62	128.63	257.26	0.1	154267	0.17	-27	0.0000	1	0.013	15	-0.0001
			0.5	2	66	131.64											
	2	2	0.5	1	66	131.96	136.55	273.1	0.1	154267	0.18	-11	0.0000	1	0.013	15	0.0000
			0.5	2	71	141.14											
	3	2	0.5	1	78	156.46	163.96	327.92	0.1	154267	0.21	44	0.0001	1	0.013	15	0.0002
			0.5	2	86	171.46											
	4	2	0.5	1	72	144.44	139.1	278.2	0.1	154267	0.18	-6	0.0000	1	0.013	15	0.0000
			0.5	2	67	133.76											
Positive control	1	2	0.5	1	1658	3315.6	3328.6	6657.2	0.1	154267	4.32	6373	0.0087	1	0.013	15	0.0225
			0.5	2	1671	3341.6											
	2	2	0.5	1	1611	3222.8	3205.8	6411.6	0.1	154267	4.16	6127	0.0084	1	0.013	15	0.0217
			0.5	2	1594	3188.8											
	3	2	0.5	1	1677	3354.8	3355.3	6710.6	0.1	154267	4.35	6426	0.0088	1	0.013	15	0.0227
			0.5	2	1678	3355.8											
	4	2	0.5	1	1639	3278.6	3305.5	6611	0.1	154267	4.29	6327	0.0086	1	0.013	15	0.0224
			0.5	2	1666	3332.4											
Negative Control	1	2	0.5	1	3178	6355.6	6327.7	12655.4	0.1	154267	8.20	12371	0.0169	1	0.013	15	0.0437
			0.5	2	3150	6299.8											
	2	2	0.5	1	3225	6450.6	6444.2	12888.4	0.1	154267	8.35	12604	0.0172	1	0.013	15	0.0445
			0.5	2	3219	6437.8											
	3	2	0.5	1	3007	6014.4	5985.5	11971	0.1	154267	7.76	11687	0.0160	1	0.013	15	0.0413
			0.5	2	2978	5956.6											
	4	2	0.5	1	3289	6577.2	6656.9	13313.8	0.1	154267	8.63	13030	0.0178	1	0.013	15	0.0460
			0.5	2	3368	6736.6											
7	1-1	2	0.5	1	63	126.66	135.52	271.04	0.1	154267	0.18	-13	0.0000	1	0.013	15	0.0000
			0.5	2	72	144.38											
	1-2	2	0.5	1	59	118.5	115.08	230.16	0.1	154267	0.15	-54	-0.0001	1	0.013	15	-0.0002
			0.5	2	56	111.66											
	1-3	2	0.5	1	63	126.48	118.67	237.34	0.1	154267	0.15	-47	-0.0001	1	0.013	15	-0.0002
			0.5	2	55	110.86											
	2-1	2	0.5	1	58	116.3	119.74	239.48	0.1	154267	0.16	-45	-0.0001	1	0.013	15	-0.0002
			0.5	2	62	123.18											
	2-2	2	0.5	1	57	114.34	107.27	214.54	0.1	154267	0.14	-70	-0.0001	1	0.013	15	-0.0002
			0.5	2	50	100.2											
	2-3	2	0.5	1	78	155.72	151.95	303.9	0.1	154267	0.20	20	0.0000	1	0.013	15	0.0001
			0.5	2	74	148.18											
	3-1	2	0.5	1	67	133.74	137.31	274.62	0.1	154267	0.18	-10	0.0000	1	0.013	15	0.0000
			0.5	2	70	140.88											
	3-2	2	0.5	1	67	134.58	121.95	243.9	0.1	154267	0.16	-40	-0.0001	1	0.013	15	-0.0001
			0.5	2	55	109.32											
	3-3	2	0.5	1	66	131.7	133.13	266.26	0.1	154267	0.17	-18	0.0000	1	0.013	15	-0.0001
			0.5	2	67	134.56											
	4-1	2	0.5	1	140	279.68	283.64	567.28	0.1	154267	0.37	283	0.0004	1	0.013	15	0.0010
			0.5	2	144	287.4											
	4-2	2	0.5	1	141	281.78	275.34	550.68	0.1	154267	0.36	267	0.0004	1	0.013	15	0.0009
			0.5	2	134	268.9											
	4-3	2	0.5	1	130	259.14	252.83	505.66	0.1	154267	0.33	222	0.0003	1	0.013	15	0.0008
			0.5	2	123	246.52											
	5-1	2	0.5	1	592	1183.42	1192.44	2384.88	0.1	154267	1.55	2101	0.0029	1	0.013	15	0.0074
			0.5	2	601	1201.46											
	5-2	2	0.5	1	573	1145.12	1123.69	2247.38	0.1	154267	1.46	1963	0.0027	1	0.013	15	0.0069
			0.5	2	551	1102.26											
	5-3	2	0.5	1	576	1151.08	1134.3	2268.6	0.1	154267	1.47	1984	0.0027	1	0.013	15	0.0070
			0.5	2	559	1117.52											
	6-1	2	0.5	1	1428	2856.2	2936.4	5872.8	0.1	154267	3.81	5589	0.0076	1	0.013	15	0.0197
			0.5	2	1508	3016.6											
	6-2	2	0.5	1	1353	2705.4	2732.5	5465	0.1	154267	3.54	5181	0.0071	1	0.013	15	0.0183
			0.5	2	1380	2759.6											
	6-3	2	0.5	1	1492	2984.2	2967.7	5935.4	0.1	154267	3.85	5651	0.0077	1	0.013	15	0.0200
			0.5	2	1476	2951.2											
	7-1	2	0.5	1	2201	4402.2	4445.4	8890.8	0.1	154267	5.76	8607	0.0116	1	0.013	15	0.0304
			0.5	2	2244	4488.6											
	7-2	2	0.5	1	2188	4376.4	4381.7	8763.4	0.1	154267	5.68	8479	0.0116	1	0.013	15	0.0300
			0.5	2	2194	4387											
	7-3	2	0.5	1	2180	4360.2	4375.1	8750.2	0.1	154267	5.67	8466	0.0116	1	0.013	15	0.0299
			0.5	2	2195	4390											
	8-1	2	0.5	1	2952	5903.2	5972.9	11945.8	0.1	154267	7.74	11662	0.0159	1	0.013	15	0.0412
			0.5	2	3021	6042.6											
	8-2	2	0.5	1	3155	6310	6370.4	12740.8	0.1	154267	8.26	12457	0.0170	1	0.013	15	0.0440
			0.5	2	3215	6430.8											
	8-3	2	0.5	1	3248	6496.2	6120.2	12240.4	0.1	154267	7.93	11956	0.0163	1	0.013	15	0.0423
			0.5	2	2872	5744.2											

Assay Date	8/19/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0471	0.0029
Full activity	End	0.0405	0.0069
Full activity	Overall	0.0438	0.0058
Background	Beginning	-0.0001	3.95802E-05
Background	End	0.0001	0.000124238
Background	Overall	0.0000	0.000107889
Positive	Beginning	0.0221	0.0006
Positive	End	0.0225	0.0002
Positive	Overall	0.0223	0.0005
Negative	Beginning	0.0441	0.0006
Negative	End	0.0437	0.0034
Negative	Overall	0.0439	0.0020

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
7	1	1	1.00E-04	-4.00	0.0000
7	1	2	1.00E-04	-4.00	-0.0002
7	1	3	1.00E-04	-4.00	-0.0002
7	2	1	1.00E-05	-5.00	-0.0002
7	2	2	1.00E-05	-5.00	-0.0002
7	2	3	1.00E-05	-5.00	0.0001
7	3	1	1.00E-06	-6.00	0.0000
7	3	2	1.00E-06	-6.00	-0.0001
7	3	3	1.00E-06	-6.00	-0.0001
7	4	1	1.00E-07	-7.00	0.0010
7	4	2	1.00E-07	-7.00	0.0009
7	4	3	1.00E-07	-7.00	0.0008
7	5	1	1.00E-08	-8.00	0.0074
7	5	2	1.00E-08	-8.00	0.0069
7	5	3	1.00E-08	-8.00	0.0070
7	6	1	2.50E-09	-8.60	0.0197
7	6	2	2.50E-09	-8.60	0.0183
7	6	3	2.50E-09	-8.60	0.0200
7	7	1	1.00E-09	-9.00	0.0304
7	7	2	1.00E-09	-9.00	0.0300
7	7	3	1.00E-09	-9.00	0.0299
7	8	1	1.00E-10	-10.00	0.0412
7	8	2	1.00E-10	-10.00	0.0440
7	8	3	1.00E-10	-10.00	0.0423

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	-0.11	-0.44	-0.38
2	-5.00	-0.36	-0.56	0.16
3	-6.00	-0.08	-0.32	-0.14
4	-7.00	2.28	2.15	1.79
5	-8.00	16.94	15.84	16.01
6	-8.60	45.08	41.79	45.58
7	-9.00	69.42	68.39	68.29
8	-10.00	94.06	100.48	96.44

Assay Date	8/23/2005	Test Chemical ID	8	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	Lot # BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0197	27786	1410457	
2	0.0196	30460	1554082	
3	0.0196	32282	1647041	
4	0.0196	32375	1651786	
5	0.0196	33533	1710867	
			Average DPM/g soln	1594846
			SD	117355
			CV	7.36
			µCi/g soln	0.718

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.5	10		1050.00
Dilution A			100	10.50
Dilution B			10	1.05

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6059 g
Mass of dilution B used in substrate prep	5.4191 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.59235 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00813 µg/g soln.
µg/g soln.

a. µCi/g soln 0.718
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.592350 + 0.00813
= 0.600482 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.196 µCi/µg ASDN

760662 dpm/nmol

Assay Date	8/23/2005	Test Chemical ID	8	# Concentrations tested	8	
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID Lot # BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.479	0.272	0.113	0.063	0.031	0.019	0.000			
	0.478	0.279	0.118	0.063	0.030	0.017	0.000			
	0.487	0.291	0.116	0.065	0.029	0.016	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.232	0.027	0.061							
	0.234	0.027	0.063							
			0.064							

Standard concentration (pg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL
	LE 3-20-06 BQL		
250	200	200	0.00025
125	100	200	0.00013
50	200	1000	0.00005
25	100	1000	0.00003
10	40	1000	0.00001
5	20	1000	0.00001

	Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
Blank	0.000				
	200	0.0500	0.481	0.481	0.0485
	200	0.0250	0.280	0.280	0.0276
	200	0.0100	0.116	0.116	0.0104
	200	0.0050	0.064	0.064	0.0050
	200	0.0020	0.030	0.030	0.0014
	200	0.0010	0.017	0.017	0.0001

r^2= 0.994
m= 0.104
b= -0.002

Variables	Regression results	
m, b	0.104	-0.002
se_m, se_b	0.004	0.001
r^2, se_y	0.994	0.002
F, df	715	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)
QC 100	0.232	0.232	0.022	200	1	1
QC 100	0.234	0.234	0.023	200	1	1
QC 100						
QC 10	0.027	0.027	0.001	200	1	1
QC 10	0.027	0.027	0.001	200	1	1
QC 10						
Microsomes	0.061	0.061	0.005	200	120	42000
Microsomes	0.063	0.063	0.005	200	120	42000
Microsomes	0.064	0.064	0.005	200	120	42000

mg protein/µL Prep.	average mg/µL	mg/mL
0.000	0.000	0.113
0.000		
0.000	0.000	0.006
0.000		
0.008	0.008	8.477
0.009		
0.009		

Assay Date	8/23/2005	Test Chemical ID		8	# Concentrations tested		8		
Technician ID	TD	Replicate #		1	Microsome type	placental	Microsome ID	Lot # BAA	

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.479	0.272	0.113	0.063	0.031	0.019	0.000			
	0.478	0.279	0.118	0.063	0.030	0.017	0.000			
	0.487	0.291	0.118	0.065	0.029	0.016	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.232	0.027	0.061							
	0.234	0.027	0.063							
			0.064							

Standard concentration (mg/mL) LE 1w 3-20-06 BBC	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.481	0.481	0.0434
125	100	200	0.00013	200	0.0250	0.280	0.280	0.0250
50	200	1000	0.00005	200	0.0100	0.116	0.116	0.0099
25	100	1000	0.00003	200	0.0050	0.064	0.064	0.0051
10	40	1000	0.00001	200	0.0020	0.030	0.030	0.0020
5	20	1000	0.00001	200	0.0010	0.017	0.017	0.0009
			Blank	0.000				

Variables	Regression results	
m, b	0.092	-0.001
se_m, se_b	0.001	0.000
r^2, se_y	1.000	0.000
F, df	29925	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 1.000
m= 0.092
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.232	0.232	0.021	200	1	1	0.000	0.000	0.103
QC 100	0.234	0.234	0.021	200	1	1	0.000		
QC 100									
QC 10	0.027	0.027	0.002	200	1	1	0.000	0.000	0.009
QC 10	0.027	0.027	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.061	0.061	0.005	200	120	42000	0.009	0.009	8.803
Microsomes	0.063	0.063	0.005	200	120	42000	0.009		
Microsomes	0.064	0.064	0.005	200	120	42000	0.009		

Assay Date	8/23/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3681	7361.6	7335.5	14671	0.1	159485	9.20	14330	0.0188	1	0.013	15	0.0499
			0.5	2	3655	7309.4											
	2	2	0.5	1	3599	7198.6	7263.7	14527.4	0.1	159485	9.11	14186	0.0187	1	0.013	15	0.0494
			0.5	2	3664	7328.8											
	3	2	0.5	1	3352	6703.6	6670.7	13341.4	0.1	159485	8.37	13000	0.0171	1	0.013	15	0.0453
			0.5	2	3319	6637.8											
	4	2	0.5	1	3398	6796	6751.7	13503.4	0.1	159485	8.47	13162	0.0173	1	0.013	15	0.0459
			0.5	2	3354	6707.4											
Background control	1	2	0.5	1	111	221.8	214.49	428.98	0.1	159485	0.27	88	0.0001	1	0.013	15	0.0003
			0.5	2	104	207.18											
	2	2	0.5	1	67	133.44	144.04	288.08	0.1	159485	0.18	-53	-0.0001	1	0.013	15	-0.0002
			0.5	2	77	154.64											
	3	2	0.5	1	94	187.32	188.73	377.46	0.1	159485	0.24	36	0.0000	1	0.013	15	0.0001
			0.5	2	95	190.14											
	4	2	0.5	1	67	133.96	134.8	269.6	0.1	159485	0.17	-71	-0.0001	1	0.013	15	-0.0002
			0.5	2	68	135.64											
Positive control	1	2	0.5	1	1534	3068.2	3110	6220	0.1	159485	3.90	5879	0.0077	1	0.013	15	0.0205
			0.5	2	1576	3151.8											
	2	2	0.5	1	1710	3420.2	3440.1	6880.2	0.1	159485	4.31	6539	0.0086	1	0.013	15	0.0228
			0.5	2	1730	3460											
	3	2	0.5	1	1538	3075.6	3079.3	6158.6	0.1	159485	3.86	5818	0.0076	1	0.013	15	0.0203
			0.5	2	1542	3083											
	4	2	0.5	1	1578	3155.8	3123	6246	0.1	159485	3.92	5905	0.0078	1	0.013	15	0.0206
			0.5	2	1545	3090.2											
Negative Control	1	2	0.5	1	3781	7562.8	7579.5	15159	0.1	159485	9.50	14818	0.0195	1	0.013	15	0.0516
			0.5	2	3798	7596.2											
	2	2	0.5	1	3429	6857.8	6872.3	13744.6	0.1	159485	8.62	13404	0.0176	1	0.013	15	0.0467
			0.5	2	3443	6886.8											
	3	2	0.5	1	3277	6554.8	6610.8	13221.6	0.1	159485	8.29	12881	0.0169	1	0.013	15	0.0449
			0.5	2	3333	6666.8											
	4	2	0.5	1	3134	6267.2	6296	12592	0.1	159485	7.90	12251	0.0161	1	0.013	15	0.0427
			0.5	2	3162	6324.8											
8	1-1	2	0.5	1	799	1598.2	1574.1	3148.2	0.1	159485	1.97	2807	0.0037	1	0.013	15	0.0098
			0.5	2	775	1550											
	1-2	2	0.5	1	767	1534.72	1556.32	3112.64	0.1	159485	1.95	2772	0.0036	1	0.013	15	0.0097
			0.5	2	789	1577.92											
	1-3	2	0.5	1	927	1853.72	1888.59	3777.18	0.1	159485	2.37	3436	0.0045	1	0.013	15	0.0120
			0.5	2	962	1923.46											
	2-1	2	0.5	1	928	1855.58	1879.47	3758.94	0.1	159485	2.36	3418	0.0045	1	0.013	15	0.0119
			0.5	2	952	1903.36											
	2-2	2	0.5	1	946	1891.18	1898.4	3796.8	0.1	159485	2.38	3456	0.0045	1	0.013	15	0.0120
			0.5	2	953	1905.62											
	2-3	2	0.5	1	879	1757.04	1797.39	3594.78	0.1	159485	2.25	3254	0.0043	1	0.013	15	0.0113
			0.5	2	919	1837.74											
	3-1	2	0.5	1	2509	5018	5061.3	10122.6	0.1	159485	6.35	9782	0.0129	1	0.013	15	0.0341
			0.5	2	2552	5104.6											
	3-2	2	0.5	1	2754	5508.8	5403.4	10806.8	0.1	159485	6.78	10466	0.0138	1	0.013	15	0.0365
			0.5	2	2649	5298											
	3-3	2	0.5	1	2657	5313.2	5383.9	10767.8	0.1	159485	6.75	10427	0.0137	1	0.013	15	0.0363
			0.5	2	2727	5454.6											
	4-1	2	0.5	1	2999	5998.6	6067.9	12135.8	0.1	159485	7.61	11795	0.0155	1	0.013	15	0.0411
			0.5	2	3069	6137.2											
	4-2	2	0.5	1	3049	6097	6111.9	12223.8	0.1	159485	7.66	11883	0.0156	1	0.013	15	0.0414
			0.5	2	3063	6126.8											
	4-3	2	0.5	1	3093	6186	6169.3	12338.6	0.1	159485	7.74	11998	0.0158	1	0.013	15	0.0418
			0.5	2	3076	6152.6											
	5-1	2	0.5	1	3216	6432.6	6472.5	12945	0.1	159485	8.12	12604	0.0166	1	0.013	15	0.0439
			0.5	2	3256	6512.4											
	5-2	2	0.5	1	3220	6440.6	6367.1	12734.2	0.1	159485	7.98	12393	0.0163	1	0.013	15	0.0432
			0.5	2	3147	6293.8											
	5-3	2	0.5	1	3138	6276.4	6226.2	12452.4	0.1	159485	7.81	12111	0.0159	1	0.013	15	0.0422
			0.5	2	3088	6176											
	6-1	2	0.5	1	2999	5998.8	6007.8	12015.6	0.1	159485	7.53	11675	0.0153	1	0.013	15	0.0407
			0.5	2	3008	6016.8											
	6-2	2	0.5	1	3249	6497.2	6526.3	13052.6	0.1	159485	8.18	12712	0.0167	1	0.013	15	0.0443
			0.5	2	3278	6555.4											
	6-3	2	0.5	1	3115	6229.4	6232.6	12465.2	0.1	159485	7.82	12124	0.0159	1	0.013	15	0.0422
			0.5	2	3118	6235.8											
	7-1	2	0.5	1	3075	6149.2	6233.4	12466.8	0.1	159485	7.82	12126	0.0159	1	0.013	15	0.0423
			0.5	2	3159	6317.6											
	7-2	2	0.5	1	3290	6580.8	6594.3	13188.6	0.1	159485	8.27	12848	0.0169	1	0.013	15	0.0448
			0.5	2	3304	6607.8											
	7-3	2	0.5	1	3346	6692	6630.2	13260.4	0.1	159485	8.31	12919	0.0170	1	0.013	15	0.0450
			0.5	2	3284	6568.4											
	8-1	2	0.5	1	3286	6572.8	6544.6	13089.2	0.1	159485	8.21	12748	0.0168	1	0.013	15	0.0444
			0.5	2	3258	6516.4											
	8-2	2	0.5	1	3144	6288.4	6346.2	12692.4	0.1	159485	7.96	12351	0.0162	1	0.013	15	0.0430
			0.5	2	3202	6404											
	8-3	2	0.5	1	3093	6185	6123.1	12246.2	0.1	159485	7.68	11905	0.0157	1	0.013	15	0.0415
			0.5	2	3031	6061.2											

Assay Date	8/23/2005	Test Chemical ID	8	# Concentrations tested	Microsome 8 type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0497	0.0004
Full activity	End	0.0456	0.0004
Full activity	Overall	0.0476	0.0024
Background	Beginning	0.0001	0.000347166
Background	End	-0.0001	0.000265758
Background	Overall	0.0000	0.00026206
Positive	Beginning	0.0216	0.0016
Positive	End	0.0204	0.0002
Positive	Overall	0.0210	0.0012
Negative	Beginning	0.0492	0.0035
Negative	End	0.0438	0.0016
Negative	Overall	0.0465	0.0038

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
8	1	1	1.00E-04	-4.00	0.0098
8	1	2	1.00E-04	-4.00	0.0097
8	1	3	1.00E-04	-4.00	0.0120
8	2	1	1.00E-05	-5.00	0.0119
8	2	2	1.00E-05	-5.00	0.0120
8	2	3	1.00E-05	-5.00	0.0113
8	3	1	1.00E-06	-6.00	0.0341
8	3	2	1.00E-06	-6.00	0.0365
8	3	3	1.00E-06	-6.00	0.0363
8	4	1	2.50E-07	-6.60	0.0411
8	4	2	2.50E-07	-6.60	0.0414
8	4	3	2.50E-07	-6.60	0.0418
8	5	1	1.00E-07	-7.00	0.0439
8	5	2	1.00E-07	-7.00	0.0432
8	5	3	1.00E-07	-7.00	0.0422
8	6	1	1.00E-08	-8.00	0.0407
8	6	2	1.00E-08	-8.00	0.0443
8	6	3	1.00E-08	-8.00	0.0422
8	7	1	1.00E-09	-9.00	0.0423
8	7	2	1.00E-09	-9.00	0.0448
8	7	3	1.00E-09	-9.00	0.0450
8	8	1	1.00E-10	-10.00	0.0444
8	8	2	1.00E-10	-10.00	0.0430
8	8	3	1.00E-10	-10.00	0.0415

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	20.54	20.28	25.14
2	-5.00	25.00	25.28	23.80
3	-6.00	71.56	76.56	76.28
4	-6.60	86.28	86.93	87.77
5	-7.00	92.20	90.66	88.60
6	-8.00	85.40	92.99	88.69
7	-9.00	88.71	93.99	94.51
8	-10.00	93.26	90.36	87.09

Assay Date	8/24/2005	Test Chemical ID	8	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	Lot # BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0202	29644	1467525		
2	0.0198	29780	1504040		
3	0.0198	31159	1573687		
4	0.0202	33254	1646238		
5	0.0198	32855	1659343		
			Average DPM/g soln		1570167
			SD		84646
			CV		5.39
			µCi/g soln		0.707

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.8	10		1080.00
Dilution A			100	10.80
Dilution B			10	1.08

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6528 g
Mass of dilution B used in substrate prep	5.4219 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.606627 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00801 µg/g soln.
µg/g soln.

a. µCi/g soln 0.707
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.606627 + 0.00801
= 0.614634 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.151 µCi/µg ASDN

731648 dpm/nmol

Assay Date	8/24/2005	Test Chemical ID	8	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	Lot # BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.478	0.285	0.123	0.065	0.028	0.017	0.000			
	0.476	0.283	0.124	0.066	0.028	0.015	0.000			
	0.471	0.283	0.121	0.065	0.028	0.017	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.242	0.030	0.068
	0.244	0.031	0.069
			0.065

Standard concentration (µg/mL) LE in 3-20-06 BBL	Volume of stock used	Final volume of Std	mg Protein per µL		Used		mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200		0.0500		0.475	0.475	0.0481	m, b	0.105	-0.002
125	100	200	0.00013		200		0.0250		0.284	0.284	0.0279	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200		0.0100		0.123	0.123	0.0111	r^2, se_y	0.991	0.002
25	100	1000	0.00003		200		0.0050		0.065	0.065	0.0050	F, df	460	4
10	40	1000	0.00001		200		0.0020		0.028	0.028	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.016	0.016	-0.0001			
				Blank	0.000		r^2=	0.991						
							m=	0.105						
							b=	-0.002						

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.242	0.242	0.024	200	1	1	0.000	0.000	0.118
QC 100	0.244	0.244	0.024	200	1	1	0.000		
QC 100									
QC 10	0.030	0.030	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.031	0.031	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.068	0.068	0.005	200	120	42000	0.009	0.009	9.133
Microsomes	0.069	0.069	0.005	200	120	42000	0.009		
Microsomes	0.065	0.065	0.005	200	120	42000	0.009		

Assay Date	8/24/2005	Test Chemical ID		8	# Concentrations tested		8		
Technician ID	TD	Replicate #		2	Microsome type	placental	Microsome ID	Lot # BAA	

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.478	0.285	0.123	0.065	0.028	0.017	0.000			
	0.476	0.283	0.124	0.066	0.028	0.015	0.000			
	0.471	0.283	0.121	0.065	0.028	0.017	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.242	0.030	0.068
	0.244	0.031	0.069
			0.065

Standard concentration (mg/mL) LE m 3-20-06 BSL	Volume of stock used	Final volume of Std	mg Protein per µL		µL Standard Used		mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200		0.0500		0.475	0.475	0.0421	m, b	0.090	-0.001
125	100	200	0.00013		200		0.0250		0.284	0.284	0.0248	se_m, se_b	0.001	0.000
50	200	1000	0.00005		200		0.0100		0.123	0.123	0.0104	r^2, se_y	0.999	0.000
25	100	1000	0.00003		200		0.0050		0.065	0.065	0.0052	F, df	4238	3
10	40	1000	0.00001		200		0.0020		0.028	0.028	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001		200		0.0010		0.016	0.016	0.0008			
				Blank	0.000		r^2=	0.999						
							m=	0.090						
							b=	-0.001						

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.242	0.242	0.021	200	1	1	0.000	0.000	0.106
QC 100	0.244	0.244	0.021	200	1	1	0.000		
QC 100									
QC 10	0.030	0.030	0.002	200	1	1	0.000	0.000	0.010
QC 10	0.031	0.031	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.068	0.068	0.005	200	120	42000	0.010	0.009	9.405
Microsomes	0.069	0.069	0.005	200	120	42000	0.010		
Microsomes	0.065	0.065	0.005	200	120	42000	0.009		

Assay Date	8/24/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3336	6670.8	6720.5	13441	0.1	157017	8.56	13144	0.0180	1	0.013	15	0.0446
			0.5	2	3385	6770.2											
	2	2	0.5	1	3195	6389	6401.9	12803.8	0.1	157017	8.15	12506	0.0171	1	0.013	15	0.0424
			0.5	2	3207	6414.8											
	3	2	0.5	1	3243	6486.6	6446	12892	0.1	157017	8.21	12595	0.0172	1	0.013	15	0.0427
			0.5	2	3203	6405.4											
	4	2	0.5	1	2738	5476	5467.1	10934.2	0.1	157017	6.96	10637	0.0145	1	0.013	15	0.0361
			0.5	2	2729	5458.2											
Background control	1	2	0.5	1	78	156.86	149.46	298.92	0.1	157017	0.19	2	0.0000	1	0.013	15	0.0000
			0.5	2	71	142.06											
	2	2	0.5	1	71	142.36	145.33	290.66	0.1	157017	0.19	-7	0.0000	1	0.013	15	0.0000
			0.5	2	74	148.3											
	3	2	0.5	1	73	145.02	139.05	278.1	0.1	157017	0.18	-19	0.0000	1	0.013	15	-0.0001
			0.5	2	67	133.08											
	4	2	0.5	1	77	154.44	160.94	321.88	0.1	157017	0.20	24	0.0000	1	0.013	15	0.0001
			0.5	2	84	167.44											
Positive control	1	2	0.5	1	1515	3029.4	3020.5	6041	0.1	157017	3.85	5744	0.0079	1	0.013	15	0.0195
			0.5	2	1506	3011.6											
	2	2	0.5	1	1569	3137.6	3137.4	6274.8	0.1	157017	4.00	5977	0.0082	1	0.013	15	0.0203
			0.5	2	1569	3137.2											
	3	2	0.5	1	1554	3107.2	3088	6176	0.1	157017	3.93	5879	0.0080	1	0.013	15	0.0199
			0.5	2	1534	3068.8											
	4	2	0.5	1	1478	2955	2977.9	5955.8	0.1	157017	3.79	5658	0.0077	1	0.013	15	0.0192
			0.5	2	1500	3000.8											
Negative Control	1	2	0.5	1	3042	6084.6	6122.9	12245.8	0.1	157017	7.80	11948	0.0163	1	0.013	15	0.0405
			0.5	2	3081	6161.2											
	2	2	0.5	1	3405	6810.8	6764.8	13529.6	0.1	157017	8.62	13232	0.0181	1	0.013	15	0.0449
			0.5	2	3359	6718.8											
	3	2	0.5	1	3123	6245	6211.1	12422.2	0.1	157017	7.91	12125	0.0166	1	0.013	15	0.0411
			0.5	2	3089	6177.2											
	4	2	0.5	1	2932	5863.2	5813.4	11626.8	0.1	157017	7.40	11329	0.0155	1	0.013	15	0.0384
			0.5	2	2882	5763.6											
8	1-1	2	0.5	1	673	1346.16	1340.17	2680.34	0.1	157017	1.71	2383	0.0033	1	0.013	15	0.0081
			0.5	2	667	1334.18											
	1-2	2	0.5	1	591	1181.74	1194.86	2389.72	0.1	157017	1.52	2092	0.0029	1	0.013	15	0.0071
			0.5	2	604	1207.98											
	1-3	2	0.5	1	726	1452.2	1418.19	2836.38	0.1	157017	1.81	2539	0.0035	1	0.013	15	0.0086
			0.5	2	692	1384.18											
	2-1	2	0.5	1	913	1825.46	1842.63	3685.26	0.1	157017	2.35	3388	0.0046	1	0.013	15	0.0115
			0.5	2	930	1859.8											
	2-2	2	0.5	1	883	1765.3	1780.56	3561.12	0.1	157017	2.27	3284	0.0045	1	0.013	15	0.0111
			0.5	2	898	1795.82											
	2-3	2	0.5	1	911	1822.26	1824.3	3648.6	0.1	157017	2.32	3351	0.0046	1	0.013	15	0.0114
			0.5	2	913	1826.34											
	3-1	2	0.5	1	1239	2478.2	2446.9	4893.8	0.1	157017	3.12	4596	0.0063	1	0.013	15	0.0156
			0.5	2	1208	2415.6											
	3-2	2	0.5	1	1270	2540	2580.9	5161.8	0.1	157017	3.29	4864	0.0066	1	0.013	15	0.0165
			0.5	2	1311	2621.8											
	3-3	2	0.5	1	1295	2589.6	2602	5204	0.1	157017	3.31	4907	0.0067	1	0.013	15	0.0166
			0.5	2	1307	2614.4											
	4-1	2	0.5	1	1662	3324.8	3268.5	6537	0.1	157017	4.16	6240	0.0085	1	0.013	15	0.0212
			0.5	2	1606	3212.2											
	4-2	2	0.5	1	1657	3313	3384.7	6769.4	0.1	157017	4.31	6472	0.0088	1	0.013	15	0.0219
			0.5	2	1728	3456.4											
	4-3	2	0.5	1	1702	3404	3435.6	6871.2	0.1	157017	4.38	6574	0.0090	1	0.013	15	0.0223
			0.5	2	1734	3467.2											
	5-1	2	0.5	1	2366	4732.2	4716.8	9433.6	0.1	157017	6.01	9136	0.0125	1	0.013	15	0.0310
			0.5	2	2351	4701.4											
	5-2	2	0.5	1	2361	4721.8	4702	9404	0.1	157017	5.99	9107	0.0124	1	0.013	15	0.0309
			0.5	2	2341	4682.2											
	5-3	2	0.5	1	2459	4918.6	4927.1	9854.2	0.1	157017	6.28	9557	0.0131	1	0.013	15	0.0324
			0.5	2	2468	4935.6											
	6-1	2	0.5	1	3096	6192.8	6151	12302	0.1	157017	7.83	12005	0.0164	1	0.013	15	0.0407
			0.5	2	3055	6109.2											
	6-2	2	0.5	1	2794	5588.8	5572.9	11145.8	0.1	157017	7.10	10848	0.0148	1	0.013	15	0.0368
			0.5	2	2779	5557											
	6-3	2	0.5	1	2770	5540.8	5570.4	11140.8	0.1	157017	7.10	10843	0.0148	1	0.013	15	0.0368
			0.5	2	2800	5600											
	7-1	2	0.5	1	2962	5923.6	5942.6	11885.2	0.1	157017	7.57	11588	0.0158	1	0.013	15	0.0393
			0.5	2	2981	5961.6											
	7-2	2	0.5	1	3029	6058	6074.2	12148.4	0.1	157017	7.74	11851	0.0162	1	0.013	15	0.0402
			0.5	2	3045	6090.4											
	7-3	2	0.5	1	2920	5839.2	5848	11696	0.1	157017	7.45	11399	0.0156	1	0.013	15	0.0387
			0.5	2	2928	5856.8											
	8-1	2	0.5	1	2433	4866.4	4852.2	9704.4	0.1	157017	6.18	9407	0.0129	1	0.013	15	0.0319
			0.5	2	2419	4838											
	8-2	2	0.5	1	3178	6355.2	6389.2	12778.4	0.1	157017	8.14	12481	0.0171	1	0.013	15	0.0423
			0.5	2	3212	6423.2											
	8-3	2	0.5	1	3248	6496.6	6537.9	13075.8	0.1	157017	8.33	12778	0.0175	1	0.013	15	0.0433
			0.5	2	3290	6579.2											

Assay Date	8/24/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0435	0.0015
Full activity	End	0.0394	0.0047
Full activity	Overall	0.0414	0.0037
Background	Beginning	0.0000	1.98049E-05
Background	End	0.0000	0.000104971
Background	Overall	0.0000	6.25085E-05
Positive	Beginning	0.0199	0.0006
Positive	End	0.0196	0.0005
Positive	Overall	0.0197	0.0005
Negative	Beginning	0.0427	0.0031
Negative	End	0.0398	0.0019
Negative	Overall	0.0412	0.0027

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
8	1	1	1.00E-04	-4.00	0.0081
8	1	2	1.00E-04	-4.00	0.0071
8	1	3	1.00E-04	-4.00	0.0086
8	2	1	1.00E-05	-5.00	0.0115
8	2	2	1.00E-05	-5.00	0.0111
8	2	3	1.00E-05	-5.00	0.0114
8	3	1	5.00E-06	-5.30	0.0156
8	3	2	5.00E-06	-5.30	0.0165
8	3	3	5.00E-06	-5.30	0.0166
8	4	1	2.50E-06	-5.60	0.0212
8	4	2	2.50E-06	-5.60	0.0219
8	4	3	2.50E-06	-5.60	0.0223
8	5	1	1.00E-06	-6.00	0.0310
8	5	2	1.00E-06	-6.00	0.0309
8	5	3	1.00E-06	-6.00	0.0324
8	6	1	1.00E-07	-7.00	0.0407
8	6	2	1.00E-07	-7.00	0.0368
8	6	3	1.00E-07	-7.00	0.0368
8	7	1	1.00E-08	-8.00	0.0393
8	7	2	1.00E-08	-8.00	0.0402
8	7	3	1.00E-08	-8.00	0.0387
8	8	1	1.00E-09	-9.00	0.0319
8	8	2	1.00E-09	-9.00	0.0423
8	8	3	1.00E-09	-9.00	0.0433

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	19.50	17.12	20.78
2	-5.00	27.72	26.71	27.42
3	-5.30	37.61	39.81	40.15
4	-5.60	51.06	52.96	53.79
5	-6.00	74.76	74.52	78.20
6	-7.00	98.23	88.77	88.73
7	-8.00	94.82	96.98	93.28
8	-9.00	76.98	102.13	104.57

Assay Date	8/25/2005	Test Chemical ID	8	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	Lot # BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	28171	1401542	
2	0.0199	29942	1504623	
3	0.0203	32890	1620197	
4	0.0201	32791	1631393	
5	0.0202	34140	1690099	
			Average DPM/g soln	1569571
			SD	115503
			CV	7.36
			µCi/g soln	0.707

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.9	10		1090.00
Dilution A			100	10.90
Dilution B			10	1.09

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.5505 g
Mass of dilution B used in substrate prep	5.3643 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.612228 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00800 µg/g soln.
µg/g soln.

a. µCi/g soln 0.707
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.612228 + 0.00800
= 0.620232 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.140 µCi/µg ASDN

724769 dpm/nmol

Assay Date	8/25/2005	Test Chemical ID	8	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	Lot # BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.492	0.287	0.127	0.065	0.028	0.016	0.000			
	0.487	0.286	0.127	0.067	0.029	0.016	0.000			
	0.499	0.286	0.121	0.066	0.029	0.015	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.248	0.029	0.069							
	0.249	0.029	0.067							
			0.068							

Standard concentration (mg/mL) CE in 3-20-06 NBL	Volume of stock used	Final volume of Std	mg Protein per µL
250	200	200	0.00025
125	100	200	0.00013
50	200	1000	0.00005
25	100	1000	0.00003
10	40	1000	0.00001
5	20	1000	0.00001

			mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
	Used									
	200		0.0500		0.492	0.492	0.0484	m, b	0.102	-0.002
	200		0.0250		0.286	0.286	0.0274	se_m, se_b	0.004	0.001
	200		0.0100		0.125	0.125	0.0110	r^2, se_y	0.994	0.002
	200		0.0050		0.066	0.066	0.0050	F, df	646	4
	200		0.0020		0.029	0.029	0.0012	SS_{reg}, SS_{resid}	0.002	0.000
	200		0.0010		0.016	0.016	-0.0001			
Blank	0.000		r^2=	0.994						
			m=	0.102						
			b=	-0.002						

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.248	0.248	0.024	200	1	1	0.000	0.000	0.118
QC 100	0.249	0.249	0.024	200	1	1	0.000		
QC 100									
QC 10	0.029	0.029	0.001	200	1	1	0.000	0.000	0.006
QC 10	0.029	0.029	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.069	0.069	0.005	200	120	42000	0.009	0.009	9.127
Microsomes	0.067	0.067	0.005	200	120	42000	0.009		
Microsomes	0.068	0.068	0.005	200	120	42000	0.009		

Assay Date	8/25/2005	Test Chemical ID	8	# Concentrations tested	8				
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	Lot # BAA		

Standards:								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	250	125	50	25	10	5	0	25	100	11343-77
	0.492	0.287	0.127	0.065	0.028	0.016	0.000			
	0.487	0.286	0.127	0.067	0.029	0.016	0.000			
	0.499	0.285	0.121	0.066	0.029	0.015	0.000			

Samples:			
	QC 100	QC 10	Microsomes
	0.248	0.029	0.069
	0.249	0.029	0.067
			0.068

Standard concentration (mg/mL) LE in 3-20-06 158L	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0500	0.492	0.492	0.0432	m, b	0.089	-0.001
125	100	200	0.00013	200	0.0250	0.286	0.286	0.0248	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.125	0.125	0.0104	r^2, se_y	0.999	0.000
25	100	1000	0.00003	200	0.0050	0.066	0.066	0.0052	F, df	3151	3
10	40	1000	0.00001	200	0.0020	0.029	0.029	0.0019	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.016	0.016	0.0007			
		Blank	0.000								

r^2= 0.999
m= 0.089
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.248	0.248	0.021	200	1	1	0.000	0.000	0.107
QC 100	0.249	0.249	0.022	200	1	1	0.000		
QC 100									
QC 10	0.029	0.029	0.002	200	1	1	0.000	0.000	0.010
QC 10	0.029	0.029	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.069	0.069	0.005	200	120	42000	0.010	0.009	9.380
Microsomes	0.067	0.067	0.005	200	120	42000	0.009		
Microsomes	0.068	0.068	0.005	200	120	42000	0.009		

Assay Date	8/25/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (nitial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogan formed/mg protein/min
Full activity control	1	2	0.5	1	3961	7921	8021.2	16042.4	0.1	156957	10.22	15695	0.0217	1	0.013	15	0.0539
			0.5	2	4061	8121.4											
	2	2	0.5	1	3891	7781	7803.8	15607.6	0.1	156957	9.94	15260	0.0211	1	0.013	15	0.0524
			0.5	2	3913	7826.6											
	3	2	0.5	1	3351	6702.2	6668.9	13337.8	0.1	156957	8.50	12991	0.0179	1	0.013	15	0.0446
			0.5	2	3318	6635.6											
	4	2	0.5	1	3664	7327.8	7253.1	14506.2	0.1	156957	9.24	14159	0.0195	1	0.013	15	0.0486
			0.5	2	3589	7178.4											
Background control	1	2	0.5	1	97	194.5	182.94	365.88	0.1	156957	0.23	19	0.0000	1	0.013	15	0.0001
			0.5	2	86	171.38											
	2	2	0.5	1	74	148.38	152.54	305.08	0.1	156957	0.19	-42	-0.0001	1	0.013	15	-0.0001
			0.5	2	78	156.7											
	3	2	0.5	1	97	193.24	181.95	363.9	0.1	156957	0.23	17	0.0000	1	0.013	15	0.0001
			0.5	2	85	170.66											
	4	2	0.5	1	102	204.22	177.01	354.02	0.1	156957	0.23	7	0.0000	1	0.013	15	0.0000
			0.5	2	75	149.8											
Positive control	1	2	0.5	1	1781	3562	3542.8	7085.6	0.1	156957	4.51	6738	0.0093	1	0.013	15	0.0231
			0.5	2	1762	3523.6											
	2	2	0.5	1	1790	3580.6	3576.1	7152.2	0.1	156957	4.56	6805	0.0094	1	0.013	15	0.0234
			0.5	2	1786	3571.6											
	3	2	0.5	1	1693	3385.8	3389.3	6778.6	0.1	156957	4.32	6431	0.0089	1	0.013	15	0.0221
			0.5	2	1696	3392.8											
	4	2	0.5	1	1707	3414.8	3435.9	6871.8	0.1	156957	4.38	6525	0.0090	1	0.013	15	0.0224
			0.5	2	1729	3457											
Negative Control	1	2	0.5	1	3683	7365.2	7410.7	14821.4	0.1	156957	9.44	14474	0.0200	1	0.013	15	0.0497
			0.5	2	3728	7456.2											
	2	2	0.5	1	3447	6893.8	6944.4	13888.8	0.1	156957	8.85	13542	0.0187	1	0.013	15	0.0465
			0.5	2	3498	6995											
	3	2	0.5	1	2809	5618.2	5558.8	11117.6	0.1	156957	7.08	10770	0.0149	1	0.013	15	0.0370
			0.5	2	2750	5499.4											
	4	2	0.5	1	3488	6975.4	6998.2	13996.4	0.1	156957	8.92	13649	0.0188	1	0.013	15	0.0468
			0.5	2	3511	7021											
8	1-1	2	0.5	1	815	1629.32	1613.89	3227.78	0.1	156957	2.06	2881	0.0040	1	0.013	15	0.0099
			0.5	2	799	1598.46											
	1-2	2	0.5	1	835	1670.94	1683.64	3367.28	0.1	156957	2.15	3020	0.0042	1	0.013	15	0.0104
			0.5	2	848	1696.34											
	1-3	2	0.5	1	845	1690.68	1681.21	3362.42	0.1	156957	2.14	3015	0.0042	1	0.013	15	0.0103
			0.5	2	836	1671.54											
	2-1	2	0.5	1	1105	2212.4	2199.3	4398.6	0.1	156957	2.80	4051	0.0056	1	0.013	15	0.0139
			0.5	2	1093	2186.2											
	2-2	2	0.5	1	1066	2132.08	2120.53	4241.06	0.1	156957	2.70	3894	0.0054	1	0.013	15	0.0134
			0.5	2	1054	2108.98											
	2-3	2	0.5	1	1035	2069.98	2030.24	4060.48	0.1	156957	2.59	3713	0.0051	1	0.013	15	0.0127
			0.5	2	995	1990.5											
	3-1	2	0.5	1	1527	3054.4	3077.2	6154.4	0.1	156957	3.92	5807	0.0080	1	0.013	15	0.0199
			0.5	2	1550	3100											
	3-2	2	0.5	1	1535	3069	3070.8	6141.6	0.1	156957	3.91	5794	0.0080	1	0.013	15	0.0199
			0.5	2	1536	3072.6											
	3-3	2	0.5	1	1564	3128	3055.6	6111.2	0.1	156957	3.89	5764	0.0080	1	0.013	15	0.0198
			0.5	2	1492	2983.2											
	4-1	2	0.5	1	2014	4028	4084.7	8169.4	0.1	156957	5.20	7822	0.0108	1	0.013	15	0.0268
			0.5	2	2071	4141.4											
	4-2	2	0.5	1	2062	4123.4	4153.9	8307.8	0.1	156957	5.29	7961	0.0110	1	0.013	15	0.0273
			0.5	2	2092	4184.4											
	4-3	2	0.5	1	2111	4221.8	4235	8470	0.1	156957	5.40	8123	0.0112	1	0.013	15	0.0279
			0.5	2	2124	4248.2											
	5-1	2	0.5	1	2703	5405	5422.1	10844.2	0.1	156957	6.91	10497	0.0145	1	0.013	15	0.0360
			0.5	2	2720	5439.2											
	5-2	2	0.5	1	2569	5137.6	5160.4	10320.8	0.1	156957	6.58	9974	0.0138	1	0.013	15	0.0342
			0.5	2	2592	5183.2											
	5-3	2	0.5	1	2665	5329	5278.5	10557	0.1	156957	6.73	10210	0.0141	1	0.013	15	0.0350
			0.5	2	2614	5228											
	6-1	2	0.5	1	3319	6637.8	6724.9	13449.8	0.1	156957	8.57	13103	0.0181	1	0.013	15	0.0450
			0.5	2	3406	6812											
	6-2	2	0.5	1	2129	4258.8	4291.5	8583	0.1	156957	5.47	8236	0.0114	1	0.013	15	0.0283
			0.5	2	2162	4324.2											
	6-3	2	0.5	1	3372	6743.6	6748.8	13497.6	0.1	156957	8.60	13150	0.0181	1	0.013	15	0.0451
			0.5	2	3377	6754											
	7-1	2	0.5	1	3522	7043.8	7051.7	14103.4	0.1	156957	8.99	13756	0.0190	1	0.013	15	0.0472
			0.5	2	3530	7059.6											
	7-2	2	0.5	1	3282	6563.8	6572.7	13145.4	0.1	156957	8.36	12798	0.0177	1	0.013	15	0.0439
			0.5	2	3291	6581.6											
	7-3	2	0.5	1	3564	7128.8	7131.2	14262.4	0.1	156957	9.09	13915	0.0192	1	0.013	15	0.0478
			0.5	2	3567	7133.6											
	8-1	2	0.5	1	3443	6885.2	6989.4	13978.8	0.1	156957	8.91	13632	0.0188	1	0.013	15	0.0466
			0.5	2	3547	7093.6											
	8-2	2	0.5	1	3473	6945.2	7054.4	14108.8	0.1	156957	8.99	13762	0.0190	1	0.013	15	0.0472
			0.5	2	3582	7163.6											
	8-3	2	0.5	1	3415	6830.4	6814.6	13629.2	0.1	156957	8.68	13282	0.0183	1	0.013	15	0.0456
			0.5	2	3399	6798.8											

Assay Date	8/25/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	placental	Microsome ID	Lot # BAA	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0531	0.0011
Full activity	End	0.0466	0.0028
Full activity	Overall	0.0499	0.0042
Background	Beginning	0.0000	0.000147563
Background	End	0.0000	2.39789E-05
Background	Overall	0.0000	9.80554E-05
Positive	Beginning	0.0232	0.0002
Positive	End	0.0222	0.0002
Positive	Overall	0.0227	0.0006
Negative	Beginning	0.0481	0.0023
Negative	End	0.0419	0.0070
Negative	Overall	0.0450	0.0055

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
8	1	1	1.00E-04	-4.00	0.0099
8	1	2	1.00E-04	-4.00	0.0104
8	1	3	1.00E-04	-4.00	0.0103
8	2	1	1.00E-05	-5.00	0.0139
8	2	2	1.00E-05	-5.00	0.0134
8	2	3	1.00E-05	-5.00	0.0127
8	3	1	5.00E-06	-5.30	0.0199
8	3	2	5.00E-06	-5.30	0.0199
8	3	3	5.00E-06	-5.30	0.0198
8	4	1	2.50E-06	-5.60	0.0268
8	4	2	2.50E-06	-5.60	0.0273
8	4	3	2.50E-06	-5.60	0.0279
8	5	1	1.00E-06	-6.00	0.0360
8	5	2	1.00E-06	-6.00	0.0342
8	5	3	1.00E-06	-6.00	0.0350
8	6	1	1.00E-07	-7.00	0.0450
8	6	2	1.00E-07	-7.00	0.0283
8	6	3	1.00E-07	-7.00	0.0451
8	7	1	1.00E-08	-8.00	0.0472
8	7	2	1.00E-08	-8.00	0.0439
8	7	3	1.00E-08	-8.00	0.0478
8	8	1	1.00E-09	-9.00	0.0468
8	8	2	1.00E-09	-9.00	0.0472
8	8	3	1.00E-09	-9.00	0.0456

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	19.83	20.79	20.76
2	-5.00	27.89	26.81	25.56
3	-5.30	39.98	39.89	39.68
4	-5.60	53.85	54.80	55.92
5	-6.00	72.26	68.66	70.28
6	-7.00	90.20	56.70	90.53
7	-8.00	94.70	88.10	95.79
8	-9.00	93.84	94.74	91.43

Assay Date	8/29/2005	Test Chemical ID	9	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0198	27462	1386970		
2	0.0199	28832	1448844		
3	0.0199	29576	1486231		
4	0.0200	30848	1542400		
5	0.0200	30961	1548050		
			Average DPM/g soln		1482499
			SD		67375
			CV		4.54
			µCi/g soln		0.668

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.8	10		1080.00
Dilution A			100	10.80
Dilution B			10	1.08

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6145 g
Mass of dilution B used in substrate prep	5.4095 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.607651 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00756 µg/g soln.
µg/g soln.

a. µCi/g soln 0.668
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.607651 + 0.00756
= 0.615210 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.085 µCi/µg ASDN

690150 dpm/nmol

Assay Date	8/29/2005	Test Chemical ID	9	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.482	0.275	0.127	0.065	0.029	0.017	0.000	25	100	11343-77
	0.475	0.280	0.123	0.067	0.029	0.018	0.000			
	0.475	0.275	0.126	0.067	0.029	0.017	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.237	0.031	0.066
	0.236	0.031	0.066
			0.067

Standard concentration (pg/mL) LE in 3-20-06 LBL	Volume of stock used	Final volume of Std	mg Protein per µL		Used		mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200		0.0500		0.477	0.477	0.0484	m, b	0.106	-0.002
125	100	200	0.00013		200		0.0250		0.277	0.277	0.0273	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200		0.0100		0.125	0.125	0.0113	r^2, se_y	0.994	0.002
25	100	1000	0.00003		200		0.0050		0.066	0.066	0.0051	F, df	642	4
10	40	1000	0.00001		200		0.0020		0.029	0.029	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.017	0.017	-0.0002			
				Blank	0.000									
						r^2=	0.994							
						m=	0.106							
						b=	-0.002							

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.237	0.237	0.023	200	1	1	0.000	0.000	0.115
QC 100	0.236	0.236	0.023	200	1	1	0.000		
QC 100									
QC 10	0.031	0.031	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.031	0.031	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.066	0.066	0.005	200	1	949	0.024	0.024	23.818
Microsomes	0.066	0.066	0.005	200	1	949	0.024		
Microsomes	0.067	0.067	0.005	200	1	949	0.024		

Assay Date	8/29/2005	Test Chemical ID	9	# Concentrations tested	8				
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.482	0.275	0.127	0.065	0.029	0.017	0.000			
	0.475	0.280	0.123	0.067	0.029	0.018	0.000			
	0.475	0.275	0.126	0.067	0.029	0.017	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.237	0.031	0.066							
	0.236	0.031	0.066							
			0.067							

Standard concentration (mg/mL) IF W 3-10-06 KBC	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.477	0.477	0.0433
125	100	200	0.00013	200	0.0250	0.277	0.277	0.0247
50	200	1000	0.00005	200	0.0100	0.125	0.125	0.0107
25	100	1000	0.00003	200	0.0050	0.066	0.066	0.0052
10	40	1000	0.00001	200	0.0020	0.029	0.029	0.0018
5	20	1000	0.00001	200	0.0010	0.017	0.017	0.0006
			Blank	0.000				

r^2= 0.998
m= 0.093
b= -0.001

Variables	Regression results	
m, b	0.093	-0.001
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.001
F, df	1530	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.237	0.237	0.021	200	1	1	0.000	0.000	0.105
QC 100	0.236	0.236	0.021	200	1	1	0.000		
QC 100									
QC 10	0.031	0.031	0.002	200	1	1	0.000	0.000	0.010
QC 10	0.031	0.031	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.066	0.066	0.005	200	1	949	0.024	0.025	24.613
Microsomes	0.066	0.066	0.005	200	1	949	0.025		
Microsomes	0.067	0.067	0.005	200	1	949	0.025		

Assay Date	8/29/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3778	7555.6	7708.4	15416.8	0.1	148250	10.40	15109	0.0219	1	0.013	15	0.0563
			0.5	2	3931	7861.2											
	2	2	0.5	1	3511	7022.2	7036.1	14072.2	0.1	148250	9.49	13764	0.0199	1	0.013	15	0.0513
			0.5	2	3525	7050											
	3	2	0.5	1	3363	6726.4	6722.2	13444.4	0.1	148250	9.07	13136	0.0190	1	0.013	15	0.0490
			0.5	2	3359	6718											
	4	2	0.5	1	3197	6394.4	6445.5	12891	0.1	148250	8.70	12583	0.0182	1	0.013	15	0.0469
			0.5	2	3248	6496.6											
Background control	1	2	0.5	1	72	143.16	147.16	294.32	0.1	148250	0.20	-14	0.0000	1	0.013	15	-0.0001
			0.5	2	76	151.16											
	2	2	0.5	1	91	181.26	174.19	348.38	0.1	148250	0.23	40	0.0001	1	0.013	15	0.0002
			0.5	2	84	167.12											
	3	2	0.5	1	72	143.2	139.57	279.14	0.1	148250	0.19	-29	0.0000	1	0.013	15	-0.0001
			0.5	2	68	135.94											
	4	2	0.5	1	73	145.18	155.16	310.32	0.1	148250	0.21	2	0.0000	1	0.013	15	0.0000
			0.5	2	83	165.14											
Positive control	1	2	0.5	1	1920	3839.2	3817.4	7634.6	0.1	148250	5.15	7327	0.0106	1	0.013	15	0.0273
			0.5	2	1898	3795.6											
	2	2	0.5	1	1741	3481.6	3469.4	6938.8	0.1	148250	4.68	6631	0.0096	1	0.013	15	0.0247
			0.5	2	1729	3457.2											
	3	2	0.5	1	1868	3735.8	3718.1	7436.2	0.1	148250	5.02	7128	0.0103	1	0.013	15	0.0266
			0.5	2	1850	3700.4											
	4	2	0.5	1	1793	3586.2	3631.6	7263.2	0.1	148250	4.90	6955	0.0101	1	0.013	15	0.0259
			0.5	2	1839	3677											
Negative Control	1	2	0.5	1	3616	7231	7294.4	14588.8	0.1	148250	9.84	14281	0.0207	1	0.013	15	0.0532
			0.5	2	3679	7357.8											
	2	2	0.5	1	3403	6805.4	6761.6	13523.2	0.1	148250	9.12	13215	0.0191	1	0.013	15	0.0493
			0.5	2	3359	6717.8											
	3	2	0.5	1	3368	6736.6	6762.1	13524.2	0.1	148250	9.12	13216	0.0191	1	0.013	15	0.0493
			0.5	2	3394	6787.6											
	4	2	0.5	1	3314	6628.4	6650.1	13300.2	0.1	148250	8.97	12992	0.0188	1	0.013	15	0.0484
			0.5	2	3336	6671.8											
9	1-1	2	0.5	1	167	334.04	328.66	657.32	0.1	148250	0.44	349	0.0005	1	0.013	15	0.0013
			0.5	2	162	323.28											
	1-2	2	0.5	1	162	324.88	333.38	666.76	0.1	148250	0.45	359	0.0005	1	0.013	15	0.0013
			0.5	2	171	341.88											
	1-3	2	0.5	1	186	371.08	405.33	810.66	0.1	148250	0.55	503	0.0007	1	0.013	15	0.0019
			0.5	2	220	439.58											
	2-1	2	0.5	1	760	1520.72	1519.12	3038.24	0.1	148250	2.05	2730	0.0040	1	0.013	15	0.0102
			0.5	2	759	1517.52											
	2-2	2	0.5	1	772	1543	1529.55	3059.1	0.1	148250	2.06	2751	0.0040	1	0.013	15	0.0103
			0.5	2	758	1516.1											
	2-3	2	0.5	1	808	1615.08	1613.5	3227	0.1	148250	2.18	2919	0.0042	1	0.013	15	0.0109
			0.5	2	806	1611.92											
	3-1	2	0.5	1	2179	4357.8	4369.8	8739.6	0.1	148250	5.90	8432	0.0122	1	0.013	15	0.0314
			0.5	2	2191	4381.8											
	3-2	2	0.5	1	2125	4250	4227.4	8454.8	0.1	148250	5.70	8147	0.0118	1	0.013	15	0.0304
			0.5	2	2102	4204.8											
	3-3	2	0.5	1	1914	3828.4	3886.8	7773.6	0.1	148250	5.24	7466	0.0108	1	0.013	15	0.0278
			0.5	2	1973	3945.2											
	4-1	2	0.5	1	3124	6248.6	6233.6	12467.2	0.1	148250	8.41	12189	0.0176	1	0.013	15	0.0453
			0.5	2	3109	6218.6											
	4-2	2	0.5	1	3269	6538.2	6583.6	13167.2	0.1	148250	8.88	12859	0.0186	1	0.013	15	0.0479
			0.5	2	3315	6629											
	4-3	2	0.5	1	3150	6300.2	6277	12554	0.1	148250	8.47	12246	0.0177	1	0.013	15	0.0457
			0.5	2	3127	6253.8											
	5-1	2	0.5	1	3289	6577.8	6646.2	13292.4	0.1	148250	8.97	12984	0.0188	1	0.013	15	0.0484
			0.5	2	3357	6714.6											
	5-2	2	0.5	1	3414	6827.6	6876.8	13751.6	0.1	148250	9.28	13444	0.0195	1	0.013	15	0.0501
			0.5	2	3462	6924											
	5-3	2	0.5	1	3599	7197.6	7158.9	14317.8	0.1	148250	9.66	14010	0.0203	1	0.013	15	0.0522
			0.5	2	3560	7120.2											
	6-1	2	0.5	1	3385	6770.2	6800.9	13601.8	0.1	148250	9.17	13294	0.0193	1	0.013	15	0.0496
			0.5	2	3416	6831.6											
	6-2	2	0.5	1	3365	6730	6700.4	13400.8	0.1	148250	9.04	13093	0.0190	1	0.013	15	0.0488
			0.5	2	3335	6670.8											
	6-3	2	0.5	1	3400	6800.2	6849.4	13698.8	0.1	148250	9.24	13391	0.0194	1	0.013	15	0.0499
			0.5	2	3449	6898.8											
	7-1	2	0.5	1	3326	6652.8	6629.3	13258.6	0.1	148250	8.94	12951	0.0188	1	0.013	15	0.0483
			0.5	2	3303	6605.8											
	7-2	2	0.5	1	3399	6798	6719	13438	0.1	148250	9.06	13130	0.0190	1	0.013	15	0.0490
			0.5	2	3320	6640											
	7-3	2	0.5	1	3324	6648.8	6724.3	13448.6	0.1	148250	9.07	13141	0.0190	1	0.013	15	0.0490
			0.5	2	3400	6799.8											
	8-1	2	0.5	1	3502	7003.8	7030.1	14060.2	0.1	148250	9.48	13752	0.0199	1	0.013	15	0.0513
			0.5	2	3528	7056.4											
	8-2	2	0.5	1	3519	7038	7010.7	14021.4	0.1	148250	9.46	13713	0.0199	1	0.013	15	0.0511
			0.5	2	3492	6983.4											
	8-3	2	0.5	1	3222	6444.4	6493.2	12986.4	0.1	148250	8.76	12678	0.0184	1	0.013	15	0.0473
			0.5	2	3271	6542											

Assay Date	8/29/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0538	0.0035
Full activity	End	0.0479	0.0015
Full activity	Overall	0.0509	0.0041
Background	Beginning	0.0000	0.000142522
Background	End	0.0000	8.2202E-05
Background	Overall	0.0000	0.000110936
Positive	Beginning	0.0260	0.0018
Positive	End	0.0263	0.0005
Positive	Overall	0.0261	0.0011
Negative	Beginning	0.0513	0.0028
Negative	End	0.0489	0.0006
Negative	Overall	0.0501	0.0022

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
9	1	1	1.00E-03	-3.00	0.0013
9	1	2	1.00E-03	-3.00	0.0013
9	1	3	1.00E-03	-3.00	0.0019
9	2	1	1.00E-04	-4.00	0.0102
9	2	2	1.00E-04	-4.00	0.0103
9	2	3	1.00E-04	-4.00	0.0109
9	3	1	1.00E-05	-5.00	0.0314
9	3	2	1.00E-05	-5.00	0.0304
9	3	3	1.00E-05	-5.00	0.0278
9	4	1	1.00E-06	-6.00	0.0453
9	4	2	1.00E-06	-6.00	0.0479
9	4	3	1.00E-06	-6.00	0.0457
9	5	1	1.00E-07	-7.00	0.0484
9	5	2	1.00E-07	-7.00	0.0501
9	5	3	1.00E-07	-7.00	0.0522
9	6	1	1.00E-08	-8.00	0.0496
9	6	2	1.00E-08	-8.00	0.0488
9	6	3	1.00E-08	-8.00	0.0499
9	7	1	1.00E-09	-9.00	0.0483
9	7	2	1.00E-09	-9.00	0.0490
9	7	3	1.00E-09	-9.00	0.0490
9	8	1	1.00E-10	-10.00	0.0513
9	8	2	1.00E-10	-10.00	0.0511
9	8	3	1.00E-10	-10.00	0.0473

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.56	2.63	3.68
2	-4.00	20.00	20.16	21.39
3	-5.00	61.78	59.69	54.70
4	-6.00	89.09	94.22	89.73
5	-7.00	95.14	98.50	102.65
6	-8.00	97.40	95.93	98.11
7	-9.00	94.89	96.20	96.28
8	-10.00	100.76	100.48	92.89

Assay Date	8/30/2005	Test Chemical ID	9	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0200	28685	1434250		
2	0.0199	28432	1428744		
3	0.0202	30867	1528069		
4	0.0200	31224	1561200		
5	0.0200	31719	1585950		
			Average DPM/g soln		1507643
			SD		72507
			CV		4.81
			µCi/g soln		0.679

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.9	10		1090.00
Dilution A			100	10.90
Dilution B			10	1.09

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.5811 g
Mass of dilution B used in substrate prep	5.4006 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.614403 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00769 µg/g soln.
µg/g soln.

a. µCi/g soln	0.679
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.614403 + 0.00769
= 0.622090 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.092 µCi/µg ASDN

694093 dpm/nmol

Assay Date	8/30/2005	Test Chemical ID	9	# Concentrations tested	8	
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID 6-041305

Standards:	250	125	50	25	10	5	0
	0.496	0.281	0.128	0.069	0.030	0.018	0.000
	0.492	0.283	0.127	0.068	0.030	0.019	0.000
	0.491	0.281	0.127	0.069	0.031	0.019	0.000

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25	100	11343-77

Samples:	QC 100	QC 10	Microsomes
	0.250	0.032	0.072
	0.247	0.034	0.073
			0.070

Standard concentration (mg/mL) LE 2-23-06	Volume of stock used	Final volume of Std	mg Protein per µL
250	200	200	0.00025
125	100	200	0.00013
50	200	1000	0.00005
25	100	1000	0.00003
10	40	1000	0.00001
5	20	1000	0.00001

		Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
	Blank	0.000				
		200	0.0500	0.493	0.493	0.0487
		200	0.0250	0.281	0.281	0.0270
		200	0.0100	0.127	0.127	0.0111
		200	0.0050	0.069	0.069	0.0051
		200	0.0020	0.031	0.031	0.0012
		200	0.0010	0.018	0.018	0.0000

r^2= 0.995
m= 0.103
b= -0.002

Variables	Regression results	
m, b	0.103	-0.002
se_m, se_b	0.004	0.001
r^2, se_y	0.995	0.001
F, df	837	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.250	0.250	0.024	200	1	1	0.000	0.000	0.118
QC 100	0.247	0.247	0.023	200	1	1	0.000		
QC 100									
QC 10	0.032	0.032	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.034	0.034	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.072	0.072	0.005	200	1	949	0.026	0.026	25.692
Microsomes	0.073	0.073	0.006	200	1	949	0.026		
Microsomes	0.070	0.070	0.005	200	1	949	0.025		

Assay Date 8/30/2005 | Test Chemical ID 9 | # Concentrations tested 8

Technician ID TD | Replicate # 2 | Microsome type placental | Microsome ID 6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.496	0.281	0.128	0.069	0.030	0.018	0.000			
	0.492	0.283	0.127	0.068	0.030	0.019	0.000			
	0.491	0.281	0.127	0.069	0.031	0.019	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.250	0.032	0.072
	0.247	0.034	0.073
			0.070

Standard concentration (mg/mL) LE in 3-20-06 BGL	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.493	0.493	0.0440
125	100	200	0.00013	200	0.0250	0.281	0.281	0.0247
50	200	1000	0.00005	200	0.0100	0.127	0.127	0.0106
25	100	1000	0.00003	200	0.0050	0.069	0.069	0.0053
10	40	1000	0.00001	200	0.0020	0.031	0.031	0.0018
5	20	1000	0.00001	200	0.0010	0.018	0.018	0.0007
			Blank	0.000				

r^2= 0.998
m= 0.091
b= -0.001

Variables	Regression results	
m, b	0.091	-0.001
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.000
F, df	1706	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.250	0.250	0.022	200	1	1	0.000	0.000	0.108
QC 100	0.247	0.247	0.022	200	1	1	0.000		
QC 100									
QC 10	0.032	0.032	0.002	200	1	1	0.000	0.000	0.010
QC 10	0.034	0.034	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.072	0.072	0.006	200	1	949	0.027	0.026	26.219
Microsomes	0.073	0.073	0.006	200	1	949	0.027		
Microsomes	0.070	0.070	0.005	200	1	949	0.026		

Assay Date	8/30/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3562	7124.4	7127.2	14254.4	0.1	150764	9.45	13946	0.0201	1	0.014	15	0.0485
			0.5	2	3565	7130											
	2	2	0.5	1	3467	6933.8	6916.8	13833.6	0.1	150764	9.18	13525	0.0195	1	0.014	15	0.0471
			0.5	2	3450	6899.8											
	3	2	0.5	1	3220	6439	6495.6	12991.2	0.1	150764	8.62	12683	0.0183	1	0.014	15	0.0441
			0.5	2	3276	6552.2											
	4	2	0.5	1	3289	6577	6582.6	13165.2	0.1	150764	8.73	12857	0.0185	1	0.014	15	0.0447
			0.5	2	3294	6588.2											
Background control	1	2	0.5	1	73	145.7	145.27	290.54	0.1	150764	0.19	-18	0.0000	1	0.014	15	-0.0001
			0.5	2	72	144.84											
	2	2	0.5	1	80	159.64	161.28	322.56	0.1	150764	0.21	14	0.0000	1	0.014	15	0.0001
			0.5	2	81	162.92											
	3	2	0.5	1	85	170.5	167.94	335.88	0.1	150764	0.22	28	0.0000	1	0.014	15	0.0001
			0.5	2	83	165.38											
	4	2	0.5	1	67	134.88	141.72	283.44	0.1	150764	0.19	-25	0.0000	1	0.014	15	-0.0001
			0.5	2	74	148.56											
Positive control	1	2	0.5	1	1824	3648	3652.6	7305.2	0.1	150764	4.85	6997	0.0101	1	0.014	15	0.0244
			0.5	2	1829	3657.2											
	2	2	0.5	1	1916	3832.2	3814	7628	0.1	150764	5.06	7320	0.0105	1	0.014	15	0.0255
			0.5	2	1898	3795.8											
	3	2	0.5	1	1812	3623	3564.1	7128.2	0.1	150764	4.73	6820	0.0098	1	0.014	15	0.0237
			0.5	2	1753	3505.2											
	4	2	0.5	1	1808	3616.4	3580.3	7160.6	0.1	150764	4.75	6852	0.0099	1	0.014	15	0.0238
			0.5	2	1772	3544.2											
Negative Control	1	2	0.5	1	3569	7138.8	7087.2	14174.4	0.1	150764	9.40	13866	0.0200	1	0.014	15	0.0483
			0.5	2	3518	7035.6											
	2	2	0.5	1	3403	6805.8	6770.7	13541.4	0.1	150764	8.98	13233	0.0191	1	0.014	15	0.0461
			0.5	2	3368	6735.6											
	3	2	0.5	1	3254	6508.2	6557.5	13115	0.1	150764	8.70	12807	0.0185	1	0.014	15	0.0446
			0.5	2	3303	6606.8											
	4	2	0.5	1	3383	6765.2	6737.3	13474.6	0.1	150764	8.94	13166	0.0190	1	0.014	15	0.0458
			0.5	2	3355	6709.4											
9	1-1	2	0.5	1	179	358.88	356.37	712.74	0.1	150764	0.47	405	0.0006	1	0.014	15	0.0014
			0.5	2	177	353.86											
	1-2	2	0.5	1	165	330.74	324.72	649.44	0.1	150764	0.43	341	0.0005	1	0.014	15	0.0012
			0.5	2	159	318.7											
	1-3	2	0.5	1	168	336.04	336	672	0.1	150764	0.45	364	0.0005	1	0.014	15	0.0013
			0.5	2	168	336.96											
	2-1	2	0.5	1	960	1919.28	1878.64	3757.28	0.1	150764	2.49	3449	0.0050	1	0.014	15	0.0120
			0.5	2	919	1838											
	2-2	2	0.5	1	799	1597.86	1584.31	3168.62	0.1	150764	2.10	2861	0.0041	1	0.014	15	0.0100
			0.5	2	785	1570.76											
	2-3	2	0.5	1	771	1542.46	1526.69	3053.38	0.1	150764	2.03	2745	0.0040	1	0.014	15	0.0096
			0.5	2	755	1510.92											
	3-1	2	0.5	1	1231	2502.8	2463.8	4927.6	0.1	150764	3.27	4619	0.0067	1	0.014	15	0.0161
			0.5	2	1212	2424.8											
	3-2	2	0.5	1	1543	3086.2	3106	6212	0.1	150764	4.12	5904	0.0085	1	0.014	15	0.0205
			0.5	2	1563	3125.8											
	3-3	2	0.5	1	1103	2206.8	2185.84	4371.68	0.1	150764	2.90	4064	0.0059	1	0.014	15	0.0141
			0.5	2	1082	2164.88											
	4-1	2	0.5	1	2130	4259.8	4274.9	8549.8	0.1	150764	5.67	8242	0.0119	1	0.014	15	0.0287
			0.5	2	2145	4290											
	4-2	2	0.5	1	2062	4124.6	4152.2	8304.4	0.1	150764	5.51	7996	0.0115	1	0.014	15	0.0278
			0.5	2	2090	4179.8											
	4-3	2	0.5	1	2162	4324.8	4368	8736	0.1	150764	5.79	8428	0.0121	1	0.014	15	0.0293
			0.5	2	2206	4411.2											
	5-1	2	0.5	1	3117	6233.4	6287.8	12575.6	0.1	150764	8.34	12267	0.0177	1	0.014	15	0.0427
			0.5	2	3171	6342.2											
	5-2	2	0.5	1	3121	6241.6	6261.3	12522.6	0.1	150764	8.31	12214	0.0176	1	0.014	15	0.0425
			0.5	2	3141	6281											
	5-3	2	0.5	1	3193	6386.6	6403.8	12807.6	0.1	150764	8.50	12499	0.0180	1	0.014	15	0.0435
			0.5	2	3211	6421											
	6-1	2	0.5	1	3239	6477.4	6539.1	13078.2	0.1	150764	8.67	12770	0.0184	1	0.014	15	0.0444
			0.5	2	3300	6600.8											
	6-2	2	0.5	1	3279	6557.6	6635	13270	0.1	150764	8.80	12962	0.0187	1	0.014	15	0.0451
			0.5	2	3356	6712.4											
	6-3	2	0.5	1	3277	6553.6	6606.2	13212.4	0.1	150764	8.76	12904	0.0186	1	0.014	15	0.0449
			0.5	2	3329	6658.8											
	7-1	2	0.5	1	3316	6632.6	6706.4	13412.8	0.1	150764	8.90	13105	0.0189	1	0.014	15	0.0456
			0.5	2	3390	6780.2											
	7-2	2	0.5	1	3319	6638.6	6596.9	13193.8	0.1	150764	8.75	12886	0.0186	1	0.014	15	0.0448
			0.5	2	3278	6555.2											
	7-3	2	0.5	1	3378	6756.8	6701.6	13403.2	0.1	150764	8.89	13095	0.0189	1	0.014	15	0.0456
			0.5	2	3323	6646.4											
	8-1	2	0.5	1	3145	6290.2	6431.6	12863.2	0.1	150764	8.53	12555	0.0181	1	0.014	15	0.0437
			0.5	2	3287	6573											
	8-2	2	0.5	1	3255	6509.2	6568.6	13137.2	0.1	150764	8.71	12829	0.0185	1	0.014	15	0.0446
			0.5	2	3314	6628											
	8-3	2	0.5	1	3243	6485	6571.3	13142.6	0.1	150764	8.72	12834	0.0185	1	0.014	15	0.0447
			0.5	2	3329	6657.6											

Assay Date	8/30/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0478	0.0010
Full activity	End	0.0444	0.0004
Full activity	Overall	0.0461	0.0020
Background	Beginning	0.0000	7.8795E-05
Background	End	0.0000	0.000129045
Background	Overall	0.0000	8.75182E-05
Positive	Beginning	0.0249	0.0008
Positive	End	0.0238	0.0001
Positive	Overall	0.0244	0.0008
Negative	Beginning	0.0472	0.0016
Negative	End	0.0452	0.0009
Negative	Overall	0.0462	0.0015

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
9	1	1	1.00E-03	-3.00	0.0014
9	1	2	1.00E-03	-3.00	0.0012
9	1	3	1.00E-03	-3.00	0.0013
9	2	1	1.00E-04	-4.00	0.0120
9	2	2	1.00E-04	-4.00	0.0100
9	2	3	1.00E-04	-4.00	0.0096
9	3	1	5.00E-05	-4.30	0.0161
9	3	2	5.00E-05	-4.30	0.0205
9	3	3	5.00E-05	-4.30	0.0141
9	4	1	1.00E-05	-5.00	0.0287
9	4	2	1.00E-05	-5.00	0.0278
9	4	3	1.00E-05	-5.00	0.0293
9	5	1	1.00E-06	-6.00	0.0427
9	5	2	1.00E-06	-6.00	0.0425
9	5	3	1.00E-06	-6.00	0.0435
9	6	1	1.00E-07	-7.00	0.0444
9	6	2	1.00E-07	-7.00	0.0451
9	6	3	1.00E-07	-7.00	0.0449
9	7	1	1.00E-08	-8.00	0.0456
9	7	2	1.00E-08	-8.00	0.0448
9	7	3	1.00E-08	-8.00	0.0456
9	8	1	1.00E-09	-9.00	0.0437
9	8	2	1.00E-09	-9.00	0.0446
9	8	3	1.00E-09	-9.00	0.0447

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	3.05	2.58	2.75
2	-4.00	26.03	21.58	20.71
3	-4.30	34.86	44.55	30.66
4	-5.00	62.19	60.34	63.59
5	-6.00	92.56	92.16	94.31
6	-7.00	96.36	97.80	97.37
7	-8.00	98.88	97.23	98.81
8	-9.00	94.73	96.80	96.84

Assay Date	8/31/2005	Test Chemical ID	9	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0202	28973	1434307
2	0.0201	30971	1540846
3	0.0200	31184	1559200
4	0.0203	32435	1597783
5	0.0198	32146	1623535
		Average DPM/g soln	1551134
		SD	72866
		CV	4.70
		µCi/g soln	0.699

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10	10		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6302 g
Mass of dilution B used in substrate prep	5.4302 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563872 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00791 µg/g soln.
µg/g soln.

a. µCi/g soln 0.699
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.563872 + 0.00791
= 0.571781 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.222 µCi/µg ASDN

776949 dpm/nmol

Assay Date	8/31/2005	Test Chemical ID	9	# Concentrations tested	8						
Technician ID		TD		Replicate #	3	Microsome type	placental	Microsome ID	6-041305		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.485	0.279	0.129	0.063	0.023	0.013	0.000			
	0.494	0.287	0.127	0.060	0.023	0.011	0.000			
	0.486	0.286	0.126	0.060	0.023	0.011	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.243	0.027	0.061
	0.247	0.025	0.060
			0.061

Standard concentration (µg/mL) LE in 3-20-06 BDL	Volume of stock used	Final volume of Std	mg Protein per µL		Used	mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200	0.0500		0.488	0.488	0.0482	m, b	0.101	-0.001
125	100	200	0.00013		200	0.0250		0.285	0.285	0.0275	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200	0.0100		0.128	0.128	0.0116	r^2, se_y	0.992	0.002
25	100	1000	0.00003		200	0.0050		0.061	0.061	0.0048	F, df	493	4
10	40	1000	0.00001		200	0.0020		0.023	0.023	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010		0.011	0.011	-0.0002			
				Blank	0.000	r^2=	0.992						
						m=	0.101						
						b=	-0.001						

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.243	0.243	0.023	200	1	1	0.000	0.000	0.117
QC 100	0.247	0.247	0.024	200	1	1	0.000		
QC 100									
QC 10	0.027	0.027	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.025	0.025	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.061	0.061	0.005	200	1	949	0.023	0.023	22.894
Microsomes	0.060	0.060	0.005	200	1	949	0.023		
Microsomes	0.061	0.061	0.005	200	1	949	0.023		

Assay Date	8/31/2005	Test Chemical ID	9	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.485	0.279	0.129	0.063	0.023	0.013	0.000			
	0.494	0.287	0.127	0.060	0.023	0.011	0.000			
	0.486	0.288	0.126	0.060	0.023	0.011	0.000			
Samples:	QC 100	QC 10	Microsomes							
	0.243	0.027	0.061							
	0.247	0.025	0.060							
			0.061							

Standard concentration (mg/mL) LE in 3-20-06 MBL	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.488	0.488	0.0425
125	100	200	0.00013	200	0.0250	0.285	0.285	0.0246
50	200	1000	0.00005	200	0.0100	0.128	0.128	0.0109
25	100	1000	0.00003	200	0.0050	0.061	0.061	0.0050
10	40	1000	0.00001	200	0.0020	0.023	0.023	0.0017
5	20	1000	0.00001	200	0.0010	0.011	0.011	0.0007
			Blank	0.000				

Variables	Regression results	
m, b	0.088	0.000
se_m, se_b	0.003	0.000
r^2, se_y	0.997	0.001
F, df	1085	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

r^2= 0.997
m= 0.088
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.243	0.243	0.021	200	1	1	0.000	0.000	0.106
QC 100	0.247	0.247	0.021	200	1	1	0.000		
QC 100									
QC 10	0.027	0.027	0.002	200	1	1	0.000	0.000	0.010
QC 10	0.025	0.025	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.061	0.061	0.005	200	1	949	0.024	0.024	23.873
Microsomes	0.060	0.060	0.005	200	1	949	0.024		
Microsomes	0.061	0.061	0.005	200	1	949	0.024		

Battelle Study No. G608316

Assay Date	8/31/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3572	7144.6	7183.6	14367.2	0.1	155113	9.26	14103	0.0182	1	0.013	15	0.0482
			0.5	2	3611	7222.6											
	2	2	0.5	1	3471	6942.4	6950.7	13901.4	0.1	155113	8.96	13637	0.0176	1	0.013	15	0.0466
			0.5	2	3480	6959											
	3	2	0.5	1	3344	6687.8	6710.9	13421.8	0.1	155113	8.65	13158	0.0169	1	0.013	15	0.0449
			0.5	2	3367	6734											
	4	2	0.5	1	3419	6838	6911	13822	0.1	155113	8.91	13558	0.0174	1	0.013	15	0.0463
			0.5	2	3492	6984											
Background control	1	2	0.5	1	73	145.78	139.05	278.1	0.1	155113	0.18	14	0.0000	1	0.013	15	0.0000
			0.5	2	66	132.32											
	2	2	0.5	1	75	150.74	144.66	289.32	0.1	155113	0.19	25	0.0000	1	0.013	15	0.0001
			0.5	2	69	138.58											
	3	2	0.5	1	68	136.08	128.26	256.52	0.1	155113	0.17	-6	0.0000	1	0.013	15	0.0000
			0.5	2	60	120.44											
	4	2	0.5	1	60	120.26	116.55	233.1	0.1	155113	0.15	-31	0.0000	1	0.013	15	-0.0001
			0.5	2	56	112.84											
Positive control	1	2	0.5	1	1755	3509.6	3509.1	7018.2	0.1	155113	4.52	6754	0.0087	1	0.013	15	0.0231
			0.5	2	1754	3508.6											
	2	2	0.5	1	1832	3664	3643.5	7287	0.1	155113	4.70	7023	0.0090	1	0.013	15	0.0240
			0.5	2	1812	3623											
	3	2	0.5	1	1741	3482.8	3471.2	6942.4	0.1	155113	4.48	6678	0.0086	1	0.013	15	0.0228
			0.5	2	1730	3459.6											
	4	2	0.5	1	1730	3460	3484	6968	0.1	155113	4.49	6704	0.0086	1	0.013	15	0.0229
			0.5	2	1754	3508											
Negative Control	1	2	0.5	1	3481	6961	7051.2	14102.4	0.1	155113	9.09	13838	0.0178	1	0.013	15	0.0473
			0.5	2	3571	7141.4											
	2	2	0.5	1	3693	7385.4	7299.9	14599.8	0.1	155113	9.41	14336	0.0185	1	0.013	15	0.0490
			0.5	2	3607	7214.4											
	3	2	0.5	1	3293	6586.2	6621.9	13243.8	0.1	155113	8.54	12980	0.0167	1	0.013	15	0.0443
			0.5	2	3329	6657.6											
	4	2	0.5	1	3231	6461.6	6458.8	12917.6	0.1	155113	8.33	12693	0.0163	1	0.013	15	0.0432
			0.5	2	3229	6458											
9	1-1	2	0.5	1	140	280.02	286.9	573.8	0.1	155113	0.37	310	0.0004	1	0.013	15	0.0011
			0.5	2	147	293.78											
	1-2	2	0.5	1	122	244.46	239.72	479.44	0.1	155113	0.31	215	0.0003	1	0.013	15	0.0007
			0.5	2	117	234.98											
	1-3	2	0.5	1	165	329.82	319.13	638.26	0.1	155113	0.41	374	0.0005	1	0.013	15	0.0013
			0.5	2	154	308.44											
	2-1	2	0.5	1	868	1735.8	1714.99	3429.98	0.1	155113	2.21	3166	0.0041	1	0.013	15	0.0108
			0.5	2	847	1694.18											
	2-2	2	0.5	1	843	1686.56	1687.34	3374.68	0.1	155113	2.18	3110	0.0040	1	0.013	15	0.0106
			0.5	2	844	1688.12											
	2-3	2	0.5	1	817	1633.6	1658.36	3316.72	0.1	155113	2.14	3052	0.0039	1	0.013	15	0.0104
			0.5	2	842	1683.12											
	3-1	2	0.5	1	1021	2041.46	2067.43	4134.86	0.1	155113	2.67	3871	0.0050	1	0.013	15	0.0132
			0.5	2	1047	2093.4											
	3-2	2	0.5	1	1073	2146.9	2154.62	4309.24	0.1	155113	2.78	4045	0.0052	1	0.013	15	0.0138
			0.5	2	1081	2162.74											
	3-3	2	0.5	1	1087	2174.98	2170.75	4341.5	0.1	155113	2.80	4077	0.0052	1	0.013	15	0.0139
			0.5	2	1083	2166.52											
	4-1	2	0.5	1	2867	5733.6	5767.9	11535.8	0.1	155113	7.44	11272	0.0145	1	0.013	15	0.0385
			0.5	2	2901	5802.2											
	4-2	2	0.5	1	2895	5790.8	5776.9	11553.8	0.1	155113	7.45	11290	0.0145	1	0.013	15	0.0385
			0.5	2	2882	5763											
	4-3	2	0.5	1	2811	5622.2	5662	11324	0.1	155113	7.30	11060	0.0142	1	0.013	15	0.0376
			0.5	2	2851	5701.8											
	5-1	2	0.5	1	3144	6288.4	6363.9	12727.8	0.1	155113	8.21	12464	0.0160	1	0.013	15	0.0426
			0.5	2	3220	6439.4											
	5-2	2	0.5	1	3049	6098.8	6182.6	12365.2	0.1	155113	7.97	12101	0.0156	1	0.013	15	0.0413
			0.5	2	3133	6266.4											
	5-3	2	0.5	1	3109	6217	6270.7	12541.4	0.1	155113	8.09	12277	0.0158	1	0.013	15	0.0419
			0.5	2	3162	6324.4											
	6-1	2	0.5	1	3171	6342	6487.8	12975.6	0.1	155113	8.37	12711	0.0164	1	0.013	15	0.0434
			0.5	2	3317	6633.6											
	6-2	2	0.5	1	3364	6728.4	6644.1	13288.2	0.1	155113	8.57	13024	0.0168	1	0.013	15	0.0445
			0.5	2	3280	6559.8											
	6-3	2	0.5	1	3493	6986.2	6996.4	13992.8	0.1	155113	9.02	13729	0.0177	1	0.013	15	0.0469
			0.5	2	3503	7006.6											
	7-1	2	0.5	1	3199	6397.4	6436.9	12873.8	0.1	155113	8.30	12610	0.0162	1	0.013	15	0.0431
			0.5	2	3238	6476.4											
	7-2	2	0.5	1	3539	7078.8	7080.1	14160.2	0.1	155113	9.13	13896	0.0179	1	0.013	15	0.0474
			0.5	2	3541	7081.4											
	7-3	2	0.5	1	3421	6841.8	6841	13682	0.1	155113	8.82	13418	0.0173	1	0.013	15	0.0458
			0.5	2	3420	6840.2											
	8-1	2	0.5	1	3236	6471.8	6429.6	12859.2	0.1	155113	8.29	12595	0.0162	1	0.013	15	0.0430
			0.5	2	3194	6387.4											
	8-2	2	0.5	1	3505	7009.8	6941.9	13883.8	0.1	155113	8.95	13620	0.0175	1	0.013	15	0.0465
			0.5	2	3437	6874											
	8-3	2	0.5	1	3484	6968	6796.1	13592.2	0.1	155113	8.76	13328	0.0172	1	0.013	15	0.0455
			0.5	2	3312	6624.2											

Assay Date	8/31/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0474	0.0011
Full activity	End	0.0456	0.0010
Full activity	Overall	0.0465	0.0013
Background	Beginning	0.0001	2.70907E-05
Background	End	-0.0001	5.65475E-05
Background	Overall	0.0000	8.48036E-05
Positive	Beginning	0.0235	0.0006
Positive	End	0.0228	0.0001
Positive	Overall	0.0232	0.0005
Negative	Beginning	0.0481	0.0012
Negative	End	0.0438	0.0008
Negative	Overall	0.0459	0.0026

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
9	1	1	1.00E-03	-3.00	0.0011
9	1	2	1.00E-03	-3.00	0.0007
9	1	3	1.00E-03	-3.00	0.0013
9	2	1	1.00E-04	-4.00	0.0108
9	2	2	1.00E-04	-4.00	0.0106
9	2	3	1.00E-04	-4.00	0.0104
9	3	1	5.00E-05	-4.30	0.0132
9	3	2	5.00E-05	-4.30	0.0138
9	3	3	5.00E-05	-4.30	0.0139
9	4	1	1.00E-05	-5.00	0.0385
9	4	2	1.00E-05	-5.00	0.0385
9	4	3	1.00E-05	-5.00	0.0378
9	5	1	1.00E-06	-6.00	0.0426
9	5	2	1.00E-06	-6.00	0.0413
9	5	3	1.00E-06	-6.00	0.0419
9	6	1	1.00E-07	-7.00	0.0434
9	6	2	1.00E-07	-7.00	0.0445
9	6	3	1.00E-07	-7.00	0.0469
9	7	1	1.00E-08	-8.00	0.0431
9	7	2	1.00E-08	-8.00	0.0474
9	7	3	1.00E-08	-8.00	0.0458
9	8	1	1.00E-09	-9.00	0.0430
9	8	2	1.00E-09	-9.00	0.0465
9	8	3	1.00E-09	-9.00	0.0455

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.27	1.58	2.75
2	-4.00	23.25	22.85	22.42
3	-4.30	28.43	29.71	29.95
4	-5.00	82.79	82.93	81.24
5	-6.00	91.55	88.89	90.18
6	-7.00	93.37	95.67	100.84
7	-8.00	92.62	102.07	98.56
8	-9.00	92.52	100.04	97.90

Assay Date	9/6/2005	Test Chemical ID	10	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0202	28559	1413812
2	0.0200	28554	1427700
3	0.0203	30715	1513054
4	0.0199	31567	1586281
5	0.0204	32586	1597353
		Average DPM/g soln	1507640
		SD	85814
		CV	5.69
		µCi/g soln	0.679

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.3	10		1030.00
Dilution A			100	10.30
Dilution B			10	1.03

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.5938 g
Mass of dilution B used in substrate prep	5.425 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.582433 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00769 µg/g soln.
µg/g soln.

a. µCi/g soln	0.679
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.582433 + 0.00769
= 0.590121 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.151 µCi/µg ASDN

731694 dpm/nmol

Assay Date	9/6/2005	Test Chemical ID	10	# Concentrations tested	8				
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305		

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.475	0.283	0.130	0.069	0.028	0.017	0.000	25	100	11343-77
	0.481	0.287	0.129	0.070	0.028	0.016	0.000			
	0.482	0.284	0.128	0.065	0.029	0.017	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.248	0.031	0.069
	0.249	0.032	0.069
			0.069

Standard concentration (mg/mL) LE 12-20-06 BdL	Volume of stock used	Final volume of Std	mg Protein per µL		Used	mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200	0.0500		0.479	0.479	0.0481	m, b	0.105	-0.002
125	100	200	0.00013		200	0.0250		0.285	0.285	0.0277	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200	0.0100		0.129	0.129	0.0114	r^2, se_y	0.991	0.002
25	100	1000	0.00003		200	0.0050		0.068	0.068	0.0050	F, df	449	4
10	40	1000	0.00001		200	0.0020		0.029	0.029	0.0010	SS_{reg}, SS_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010		0.017	0.017	-0.0003			
				Blank	0.000	r^2=	0.991						
						m=	0.105						
						b=	-0.002						

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.248	0.248	0.024	200	1	1	0.000	0.000	0.120
QC 100	0.249	0.249	0.024	200	1	1	0.000		
QC 100									
QC 10	0.031	0.031	0.001	200	1	1	0.000	0.000	0.006
QC 10	0.032	0.032	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.069	0.069	0.005	200	1	949	0.024	0.025	24.630
Microsomes	0.069	0.069	0.005	200	1	949	0.025		
Microsomes	0.069	0.069	0.005	200	1	949	0.025		

Assay Date	9/6/2005	Test Chemical ID	10	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	placental	Microsome ID	6-041305

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.475	0.283	0.130	0.069	0.028	0.017	0.000	25	100	11343-77
	0.481	0.287	0.129	0.070	0.028	0.016	0.000			
	0.482	0.284	0.128	0.065	0.029	0.017	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.248	0.031	0.069
	0.249	0.032	0.069
			0.069

Standard concentration (mg/mL) LE in 3-20-06 Book	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.479	0.479	0.0421
125	100	200	0.00013	200	0.0250	0.285	0.285	0.0247
50	200	1000	0.00005	200	0.0100	0.129	0.129	0.0107
25	100	1000	0.00003	200	0.0050	0.068	0.068	0.0052
10	40	1000	0.00001	200	0.0020	0.029	0.029	0.0017
5	20	1000	0.00001	200	0.0010	0.017	0.017	0.0007
			Blank	0.000				

r^2= 0.998
m= 0.090
b= -0.001

Variables	Regression results	
m, b	0.090	-0.001
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.001
F, df	1381	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.248	0.248	0.021	200	1	1	0.000	0.000	0.107
QC 100	0.249	0.249	0.021	200	1	1	0.000		
QC 100									
QC 10	0.031	0.031	0.002	200	1	1	0.000	0.000	0.010
QC 10	0.032	0.032	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.069	0.069	0.005	200	1	949	0.025	0.025	25.388
Microsomes	0.069	0.069	0.005	200	1	949	0.026		
Microsomes	0.069	0.069	0.005	200	1	949	0.025		

Assay Date	9/6/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3335	6669.6	6649.1	13298.2	0.1	150764	8.82	13004	0.0178	1	0.013	15	0.0443
			0.5	2	3314	6628.6											
	2	2	0.5	1	3652	7304	7348.9	14697.8	0.1	150764	9.75	14404	0.0197	1	0.013	15	0.0491
			0.5	2	3697	7393.8											
	3	2	0.5	1	3387	6774.8	6776.7	13553.4	0.1	150764	8.99	13259	0.0181	1	0.013	15	0.0452
			0.5	2	3389	6778.6											
	4	2	0.5	1	3420	6839.6	6901.6	13803.2	0.1	150764	9.16	13509	0.0185	1	0.013	15	0.0461
			0.5	2	3482	6963.6											
Background control	1	2	0.5	1	78	156.88	152.29	304.58	0.1	150764	0.20	11	0.0000	1	0.013	15	0.0000
			0.5	2	74	147.7											
	2	2	0.5	1	70	140.26	149.96	299.92	0.1	150764	0.20	6	0.0000	1	0.013	15	0.0000
			0.5	2	80	159.66											
	3	2	0.5	1	69	137.64	145.53	291.06	0.1	150764	0.19	-3	0.0000	1	0.013	15	0.0000
			0.5	2	77	153.42											
	4	2	0.5	1	72	144	140.17	280.34	0.1	150764	0.19	-14	0.0000	1	0.013	15	0.0000
			0.5	2	68	136.34											
Positive control	1	2	0.5	1	1770	3540.8	3523.2	7046.4	0.1	150764	4.67	6752	0.0092	1	0.013	15	0.0230
			0.5	2	1753	3505.6											
	2	2	0.5	1	1826	3651.4	3612.2	7224.4	0.1	150764	4.79	6930	0.0095	1	0.013	15	0.0236
			0.5	2	1787	3573											
	3	2	0.5	1	1732	3463.4	3472.4	6944.8	0.1	150764	4.61	6651	0.0091	1	0.013	15	0.0227
			0.5	2	1741	3481.4											
	4	2	0.5	1	1763	3525.2	3506.1	7012.2	0.1	150764	4.65	6718	0.0092	1	0.013	15	0.0229
			0.5	2	1744	3487											
Negative Control	1	2	0.5	1	3408	6816.6	6792.2	13584.4	0.1	150764	9.01	13290	0.0182	1	0.013	15	0.0453
			0.5	2	3384	6767.8											
	2	2	0.5	1	3182	6364.2	6468.9	12937.8	0.1	150764	8.58	12644	0.0173	1	0.013	15	0.0431
			0.5	2	3287	6573.6											
	3	2	0.5	1	3232	6464.2	6436.1	12872.2	0.1	150764	8.54	12578	0.0172	1	0.013	15	0.0429
			0.5	2	3204	6408											
	4	2	0.5	1	3271	6542.6	6628.5	13257	0.1	150764	8.79	12963	0.0177	1	0.013	15	0.0442
			0.5	2	3357	6714.4											
10	1-1	2	0.5	1	2823	5645.8	5644.5	11289	0.1	150764	7.49	10995	0.0150	1	0.013	15	0.0375
			0.5	2	2822	5643.2											
	1-2	2	0.5	1	2824	5647	5713.6	11427.2	0.1	150764	7.58	11133	0.0152	1	0.013	15	0.0380
			0.5	2	2890	5780.2											
	1-3	2	0.5	1	2808	5615.4	5623.5	11247	0.1	150764	7.46	10953	0.0150	1	0.013	15	0.0373
			0.5	2	2816	5631.6											
	2-1	2	0.5	1	3203	6406.6	6472	12944	0.1	150764	8.59	12650	0.0173	1	0.013	15	0.0431
			0.5	2	3269	6537.4											
	2-2	2	0.5	1	3316	6631.6	6606.9	13213.8	0.1	150764	8.76	12920	0.0177	1	0.013	15	0.0440
			0.5	2	3291	6582.2											
	2-3	2	0.5	1	3303	6605.8	6640	13280	0.1	150764	8.81	12986	0.0177	1	0.013	15	0.0443
			0.5	2	3337	6674.2											
	3-1	2	0.5	1	3540	7080.2	7060.4	14120.8	0.1	150764	9.37	13827	0.0189	1	0.013	15	0.0471
			0.5	2	3520	7040.6											
	3-2	2	0.5	1	3460	6919.4	7039.6	14079.2	0.1	150764	9.34	13785	0.0188	1	0.013	15	0.0470
			0.5	2	3580	7159.8											
	3-3	2	0.5	1	3527	7054.2	7093.9	14187.8	0.1	150764	9.41	13694	0.0190	1	0.013	15	0.0474
			0.5	2	3567	7133.6											
	4-1	2	0.5	1	3444	6888.6	6956.5	13913	0.1	150764	9.23	13619	0.0186	1	0.013	15	0.0464
			0.5	2	3512	7024.4											
	4-2	2	0.5	1	3422	6843	6892.7	13785.4	0.1	150764	9.14	13491	0.0184	1	0.013	15	0.0460
			0.5	2	3471	6942.4											
	4-3	2	0.5	1	3478	6956.4	7004.8	14009.6	0.1	150764	9.29	13716	0.0187	1	0.013	15	0.0468
			0.5	2	3527	7053.2											
	5-1	2	0.5	1	3283	6565.4	6686.7	13373.4	0.1	150764	8.87	13079	0.0179	1	0.013	15	0.0446
			0.5	2	3404	6808											
	5-2	2	0.5	1	3380	6760.6	6781.3	13562.6	0.1	150764	9.00	13269	0.0181	1	0.013	15	0.0452
			0.5	2	3401	6802											
	5-3	2	0.5	1	3417	6833.2	6800.6	13601.2	0.1	150764	9.02	13307	0.0182	1	0.013	15	0.0454
			0.5	2	3384	6768											
	6-1	2	0.5	1	3285	6570.8	6625.7	13251.4	0.1	150764	8.79	12957	0.0177	1	0.013	15	0.0442
			0.5	2	3340	6680.6											
	6-2	2	0.5	1	3423	6845.6	6898.4	13796.8	0.1	150764	9.15	13503	0.0185	1	0.013	15	0.0460
			0.5	2	3476	6951.2											
	6-3	2	0.5	1	3375	6749.8	6803.6	13607.2	0.1	150764	9.03	13313	0.0182	1	0.013	15	0.0454
			0.5	2	3429	6857.4											
	7-1	2	0.5	1	3323	6645	6649.3	13298.6	0.1	150764	8.82	13005	0.0178	1	0.013	15	0.0443
			0.5	2	3327	6653.6											
	7-2	2	0.5	1	3305	6610.6	6633.4	13266.8	0.1	150764	8.80	12973	0.0177	1	0.013	15	0.0442
			0.5	2	3328	6656.2											
	7-3	2	0.5	1	3245	6490.8	6581.5	13163	0.1	150764	8.73	12869	0.0176	1	0.013	15	0.0439
			0.5	2	3336	6672.2											
	8-1	2	0.5	1	3226	6451.2	6316.8	12633.6	0.1	150764	8.38	12340	0.0169	1	0.013	15	0.0421
			0.5	2	3091	6182.4											
	8-2	2	0.5	1	3257	6513.4	6580.7	13161.4	0.1	150764	8.73	12867	0.0176	1	0.013	15	0.0439
			0.5	2	3324	6648											
	8-3	2	0.5	1	3273	6545.4	6581	13162	0.1	150764	8.73	12868	0.0176	1	0.013	15	0.0439
			0.5	2	3308	6616.6											

Assay Date	9/6/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0467	0.0034
Full activity	End	0.0456	0.0006
Full activity	Overall	0.0462	0.0021
Background	Beginning	0.0000	1.12344E-05
Background	End	0.0000	2.58438E-05
Background	Overall	0.0000	3.6414E-05
Positive	Beginning	0.0233	0.0004
Positive	End	0.0228	0.0002
Positive	Overall	0.0231	0.0004
Negative	Beginning	0.0442	0.0016
Negative	End	0.0435	0.0009
Negative	Overall	0.0439	0.0011

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
10	1	1	1.00E-03	-3.00	0.0375
10	1	2	1.00E-03	-3.00	0.0380
10	1	3	1.00E-03	-3.00	0.0373
10	2	1	1.00E-04	-4.00	0.0431
10	2	2	1.00E-04	-4.00	0.0440
10	2	3	1.00E-04	-4.00	0.0443
10	3	1	1.00E-05	-5.00	0.0471
10	3	2	1.00E-05	-5.00	0.0470
10	3	3	1.00E-05	-5.00	0.0474
10	4	1	1.00E-06	-6.00	0.0464
10	4	2	1.00E-06	-6.00	0.0460
10	4	3	1.00E-06	-6.00	0.0468
10	5	1	1.00E-07	-7.00	0.0446
10	5	2	1.00E-07	-7.00	0.0452
10	5	3	1.00E-07	-7.00	0.0454
10	6	1	1.00E-08	-8.00	0.0442
10	6	2	1.00E-08	-8.00	0.0460
10	6	3	1.00E-08	-8.00	0.0454
10	7	1	1.00E-09	-9.00	0.0443
10	7	2	1.00E-09	-9.00	0.0442
10	7	3	1.00E-09	-9.00	0.0439
10	8	1	1.00E-10	-10.00	0.0421
10	8	2	1.00E-10	-10.00	0.0439
10	8	3	1.00E-10	-10.00	0.0439

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	81.18	82.20	80.87
2	-4.00	93.40	95.39	95.88
3	-5.00	102.09	101.78	102.58
4	-6.00	100.55	99.61	101.27
5	-7.00	96.57	97.97	98.25
6	-8.00	95.67	99.69	98.29
7	-9.00	96.02	95.78	95.02
8	-10.00	91.11	95.00	95.01

Assay Date	9/7/2005	Test Chemical ID	10	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	22200	1115578		
2	0.0202	23994	1187822		
3	0.0203	24939	1228522		
4	0.0201	26122	1299602		
5	0.0198	25883	1307222		
			Average DPM/g soln	1227749	
			SD	80087	
			CV	6.52	
			µCi/g soln	0.553	

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.6	10		1060.00
Dilution A			100	10.60
Dilution B			10	1.06

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6288 g
Mass of dilution B used in substrate prep	5.4093 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.59549 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00626 µg/g soln.
µg/g soln.

a. µCi/g soln	0.553
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.595490 + 0.00626
= 0.601751 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 0.919 µCi/µg ASDN

584340 dpm/nmol

Assay Date	9/7/2005	Test Chemical ID	10	# Concentrations tested	8						
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID	6-041305				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.480	0.282	0.123	0.061	0.024	0.011	0.000			
	0.476	0.281	0.124	0.064	0.030	0.011	0.000			
	0.471	0.279	0.119	0.060	0.023	0.009	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.239	0.024	0.059
	0.234	0.025	0.057
			0.056

Standard concentration (ng/mL) LE in 2-20-06 bgC	Volume of stock used	Final volume of Std	mg Protein per µL
250	200	200	0.00025
125	100	200	0.00013
50	200	1000	0.00005
25	100	1000	0.00003
10	40	1000	0.00001
5	20	1000	0.00001

	Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
	200	0.0500	0.476	0.476	0.0481
	200	0.0250	0.280	0.280	0.0278
	200	0.0100	0.122	0.122	0.0112
	200	0.0050	0.062	0.062	0.0050
	200	0.0020	0.026	0.026	0.0012
	200	0.0010	0.010	0.010	-0.0004
Blank	0.000				

Variables	Regression results	
m, b	0.104	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.992	0.002
F, df	474	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.992
m= 0.104
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.239	0.239	0.023	200	1	1	0.000	0.000	0.116
QC 100	0.234	0.234	0.023	200	1	1	0.000		
QC 100									
QC 10	0.024	0.024	0.001	200	1	1	0.000	0.000	0.006
QC 10	0.025	0.025	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.059	0.059	0.005	200	1	949	0.022	0.022	21.555
Microsomes	0.057	0.057	0.005	200	1	949	0.022		
Microsomes	0.056	0.056	0.004	200	1	949	0.021		

Assay Date	9/7/2005	Test Chemical ID	10	# Concentrations tested	8	
Technician ID	TD	Replicate #	2	Microsome type	placental	Microsome ID 6-041305

Standards:	250	125	50	25	10	5	0
	0.480	0.282	0.123	0.061	0.024	0.011	0.000
	0.476	0.281	0.124	0.064	0.030	0.011	0.000
	0.471	0.279	0.119	0.060	0.023	0.009	0.000

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25	100	11343-77

Samples:	QC 100	QC 10	Microsomes
	0.239	0.024	0.059
	0.234	0.025	0.057
			0.056

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
250	200	200	0.00025	200	0.0500	0.476	0.476	0.0422
125	100	200	0.00013	200	0.0250	0.280	0.280	0.0247
50	200	1000	0.00005	200	0.0100	0.122	0.122	0.0106
25	100	1000	0.00003	200	0.0050	0.062	0.062	0.0052
10	40	1000	0.00001	200	0.0020	0.026	0.026	0.0020
5	20	1000	0.00001	200	0.0010	0.010	0.010	0.0006

Variables	Regression results	
m, b	0.089	0.000
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.000
F, df	1977	3
ss_{reg}, ss_{resid}	0.000	0.000

Blank 0.000

r^2= 0.998
m= 0.089
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.239	0.239	0.021	200	1	1	0.000	0.000	0.104
QC 100	0.234	0.234	0.021	200	1	1	0.000		
QC 100									
QC 10	0.024	0.024	0.002	200	1	1	0.000	0.000	0.009
QC 10	0.025	0.025	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.059	0.059	0.005	200	1	949	0.023	0.023	22.739
Microsomes	0.057	0.057	0.005	200	1	949	0.023		
Microsomes	0.056	0.056	0.005	200	1	949	0.022		

Battelle Study No. G608316

B-159

Assay Date	9/7/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	2401	4802.4	4788.9	9577.8	0.1	122775	7.80	9209	0.0158	1	0.012	15	0.0439
			0.5	2	2388	4775.4											
	2	2	0.5	1	2406	4811	4763.5	9527	0.1	122775	7.76	9159	0.0157	1	0.012	15	0.0437
			0.5	2	2356	4716											
	3	2	0.5	1	2044	4087	4108.9	8217.8	0.1	122775	6.69	7849	0.0134	1	0.012	15	0.0374
			0.5	2	2065	4130.8											
	4	2	0.5	1	1947	3894.2	3916.4	7832.8	0.1	122775	6.38	7464	0.0128	1	0.012	15	0.0356
			0.5	2	1969	3938.6											
Background control	1	2	0.5	1	54	108.98	118.77	237.54	0.1	122775	0.19	-131	-0.0002	1	0.012	15	-0.0006
			0.5	2	64	128.56											
	2	2	0.5	1	128	255.78	211.51	423.02	0.1	122775	0.34	55	0.0001	1	0.012	15	0.0003
			0.5	2	84	167.24											
	3	2	0.5	1	82	164.92	167.24	334.48	0.1	122775	0.27	-34	-0.0001	1	0.012	15	-0.0002
			0.5	2	85	169.56											
	4	2	0.5	1	122	243.26	239.27	478.54	0.1	122775	0.39	110	0.0002	1	0.012	15	0.0005
			0.5	2	118	235.28											
Positive control	1	2	0.5	1	1269	2538.4	2524.8	5049.6	0.1	122775	4.11	4681	0.0080	1	0.012	15	0.0223
			0.5	2	1256	2511.2											
	2	2	0.5	1	1244	2487	2455	4910	0.1	122775	4.00	4542	0.0078	1	0.012	15	0.0216
			0.5	2	1212	2423											
	3	2	0.5	1	1072	2144.9	2159.85	4319.7	0.1	122775	3.52	3951	0.0068	1	0.012	15	0.0188
			0.5	2	1087	2174.8											
	4	2	0.5	1	1132	2264.8	2289.1	4578.2	0.1	122775	3.73	4210	0.0072	1	0.012	15	0.0201
			0.5	2	1157	2313.4											
Negative Control	1	2	0.5	1	2169	4337	4261.5	8523	0.1	122775	6.94	8155	0.0140	1	0.012	15	0.0389
			0.5	2	2093	4186											
	2	2	0.5	1	2031	4062.6	4077.9	8155.8	0.1	122775	6.64	7787	0.0133	1	0.012	15	0.0371
			0.5	2	2047	4093.2											
	3	2	0.5	1	1126	2252.6	2202.61	4405.22	0.1	122775	3.59	4037	0.0069	1	0.012	15	0.0192
			0.5	2	1076	2152.62											
	4	2	0.5	1	1997	3993.8	4054.3	8108.6	0.1	122775	6.60	7740	0.0132	1	0.012	15	0.0369
			0.5	2	2067	4114.8											
10	1-1	2	0.5	1	1744	3487.6	3518.2	7036.4	0.1	122775	5.73	6668	0.0114	1	0.012	15	0.0318
			0.5	2	1774	3548.8											
	1-2	2	0.5	1	1847	3693.4	3698.5	7397	0.1	122775	6.02	7029	0.0120	1	0.012	15	0.0335
			0.5	2	1852	3703.6											
	1-3	2	0.5	1	1860	3719.4	3729.9	7459.8	0.1	122775	6.08	7091	0.0121	1	0.012	15	0.0338
			0.5	2	1870	3740.4											
	2-1	2	0.5	1	1853	3706.8	3778.9	7557.8	0.1	122775	6.16	7189	0.0123	1	0.012	15	0.0343
			0.5	2	1926	3851											
	2-2	2	0.5	1	1817	3634	3610.8	7221.6	0.1	122775	5.88	6853	0.0117	1	0.012	15	0.0327
			0.5	2	1794	3587.6											
	2-3	2	0.5	1	1756	3511.2	3534.7	7069.4	0.1	122775	5.76	6701	0.0115	1	0.012	15	0.0319
			0.5	2	1779	3558.2											
	3-1	2	0.5	1	1705	3410.4	3403.1	6806.2	0.1	122775	5.54	6438	0.0110	1	0.012	15	0.0307
			0.5	2	1698	3395.8											
	3-2	2	0.5	1	1698	3396	3367	6734	0.1	122775	5.48	6366	0.0109	1	0.012	15	0.0303
			0.5	2	1669	3338											
	3-3	2	0.5	1	1816	3631.8	3623.4	7246.8	0.1	122775	5.90	6878	0.0118	1	0.012	15	0.0328
			0.5	2	1808	3615											
	4-1	2	0.5	1	1735	3469.4	3476.4	6952.8	0.1	122775	5.66	6584	0.0113	1	0.012	15	0.0314
			0.5	2	1742	3483.4											
	4-2	2	0.5	1	1913	3826.8	3830.2	7660.4	0.1	122775	6.24	7292	0.0125	1	0.012	15	0.0348
			0.5	2	1917	3833.6											
	4-3	2	0.5	1	1871	3742	3769.3	7538.6	0.1	122775	6.14	7170	0.0123	1	0.012	15	0.0342
			0.5	2	1898	3796.6											
	5-1	2	0.5	1	1852	3704.8	3676.3	7352.6	0.1	122775	5.99	6984	0.0120	1	0.012	15	0.0333
			0.5	2	1824	3647.8											
	5-2	2	0.5	1	1834	3668.6	3650	7300	0.1	122775	5.95	6932	0.0119	1	0.012	15	0.0330
			0.5	2	1816	3631.4											
	5-3	2	0.5	1	1899	3798.6	3775.7	7551.4	0.1	122775	6.15	7183	0.0123	1	0.012	15	0.0342
			0.5	2	1876	3752.8											
	6-1	2	0.5	1	2139	4277.2	4248.5	8497	0.1	122775	6.92	8129	0.0139	1	0.012	15	0.0387
			0.5	2	2110	4219.8											
	6-2	2	0.5	1	2023	4045.4	4039.9	8079.8	0.1	122775	6.58	7711	0.0132	1	0.012	15	0.0368
			0.5	2	2017	4034.4											
	6-3	2	0.5	1	2006	4011.8	4017.4	8034.8	0.1	122775	6.54	7666	0.0131	1	0.012	15	0.0365
			0.5	2	2012	4023											
	7-1	2	0.5	1	2017	4034.4	4000.9	8001.8	0.1	122775	6.52	7633	0.0131	1	0.012	15	0.0364
			0.5	2	1984	3967.4											
	7-2	2	0.5	1	2116	4231.2	4221.5	8443	0.1	122775	6.88	8075	0.0138	1	0.012	15	0.0385
			0.5	2	2106	4211.8											
	7-3	2	0.5	1	2113	4225	4243.4	8486.8	0.1	122775	6.91	8118	0.0139	1	0.012	15	0.0387
			0.5	2	2131	4261.8											
	8-1	2	0.5	1	2049	4098.2	4120	8240	0.1	122775	6.71	7872	0.0135	1	0.012	15	0.0375
			0.5	2	2071	4141.8											
	8-2	2	0.5	1	2109	4218.8	4226.9	8453.8	0.1	122775	6.89	8085	0.0138	1	0.012	15	0.0385
			0.5	2	2118	4235											
	8-3	2	0.5	1	2108	4216.2	4220.7	8441.4	0.1	122775	6.88	8073	0.0138	1	0.012	15	0.0385
			0.5	2	2113	4225.2											

Assay Date	9/7/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0438	0.0002
Full activity	End	0.0365	0.0013
Full activity	Overall	0.0401	0.0043
Background	Beginning	-0.0002	0.000625148
Background	End	0.0002	0.000485544
Background	Overall	0.0000	0.000502854
Positive	Beginning	0.0220	0.0005
Positive	End	0.0195	0.0009
Positive	Overall	0.0207	0.0016
Negative	Beginning	0.0380	0.0012
Negative	End	0.0281	0.0125
Negative	Overall	0.0330	0.0092

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
10	1	1	1.00E-03	-3.00	0.0318
10	1	2	1.00E-03	-3.00	0.0335
10	1	3	1.00E-03	-3.00	0.0338
10	2	1	9.00E-04	-3.05	0.0343
10	2	2	9.00E-04	-3.05	0.0327
10	2	3	9.00E-04	-3.05	0.0319
10	3	1	8.00E-04	-3.10	0.0307
10	3	2	8.00E-04	-3.10	0.0303
10	3	3	8.00E-04	-3.10	0.0328
10	4	1	7.50E-04	-3.12	0.0314
10	4	2	7.50E-04	-3.12	0.0348
10	4	3	7.50E-04	-3.12	0.0342
10	5	1	5.00E-04	-3.30	0.0333
10	5	2	5.00E-04	-3.30	0.0330
10	5	3	5.00E-04	-3.30	0.0342
10	6	1	1.00E-04	-4.00	0.0387
10	6	2	1.00E-04	-4.00	0.0368
10	6	3	1.00E-04	-4.00	0.0365
10	7	1	1.00E-05	-5.00	0.0364
10	7	2	1.00E-05	-5.00	0.0385
10	7	3	1.00E-05	-5.00	0.0387
10	8	1	1.00E-06	-6.00	0.0375
10	8	2	1.00E-06	-6.00	0.0385
10	8	3	1.00E-06	-6.00	0.0385

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	79.19	83.47	84.22
2	-3.05	85.38	81.39	79.58
3	-3.10	76.45	75.60	81.69
4	-3.12	78.20	86.60	85.15
5	-3.30	82.94	82.32	85.30
6	-4.00	96.53	91.58	91.05
7	-5.00	90.65	95.89	96.41
8	-6.00	93.48	96.02	95.87

Assay Date	9/8/2005	Test Chemical ID	10	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	21463	1078548		
2	0.0200	24677	1233850		
3	0.0198	25153	1270354		
4	0.0198	24935	1259343		
5	0.0198	26143	1320354		
			Average DPM/g soln		1232490
			SD		91617
			CV		7.43
			µCi/g soln		0.555

Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.3	10		1030.00
Dilution A			100	10.30
Dilution B			10	1.03

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	9.6008 g
Mass of dilution B used in substrate prep	5.4359 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.583178 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00628 µg/g soln.
µg/g soln.

a. µCi/g soln	0.555
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

µg ASDN/g soln.= µg cold ASDN/g soln. + µg [^{3}H]ASDN/g soln.

= 0.583178 + 0.00628
= 0.589463 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 0.942 µCi/µg ASDN

598825 dpm/nmol

Assay Date	9/8/2005	Test Chemical ID	10	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.492	0.293	0.133	0.067	0.028	0.014	0.000			
	0.492	0.287	0.132	0.066	0.027	0.012	0.000			
	0.493	0.292	0.135	0.068	0.028	0.014	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.246	0.027	0.068
	0.243	0.026	0.068
			0.066

Standard concentration (ng/mL) LE in 3-20-06 WSL	Volume of stock used	Final volume of Std	mg Protein per µL		Used		mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025		200		0.0500		0.492	0.492	0.0481	m, b	0.101	-0.002
125	100	200	0.00013		200		0.0250		0.291	0.291	0.0276	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200		0.0100		0.133	0.133	0.0117	r^2, se_y	0.991	0.002
25	100	1000	0.00003		200		0.0050		0.067	0.067	0.0050	F, df	435	4
10	40	1000	0.00001		200		0.0020		0.028	0.028	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.014	0.014	-0.0004			
				Blank	0.000									
						r^2=	0.991							
						m=	0.101							
						b=	-0.002							

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.246	0.246	0.023	200	1	1	0.000	0.000	0.115
QC 100	0.243	0.243	0.023	200	1	1	0.000		
QC 100									
QC 10	0.027	0.027	0.001	200	1	1	0.000	0.000	0.004
QC 10	0.026	0.026	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.068	0.068	0.005	200	1	949	0.024	0.024	23.748
Microsomes	0.068	0.068	0.005	200	1	949	0.024		
Microsomes	0.066	0.066	0.005	200	1	949	0.023		

Assay Date	9/8/2005	Test Chemical ID	10	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.492	0.293	0.133	0.067	0.028	0.014	0.000	25	100	11343-77
	0.492	0.287	0.132	0.066	0.027	0.012	0.000			
	0.493	0.292	0.135	0.068	0.028	0.014	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.246	0.027	0.068
	0.243	0.026	0.068
			0.066

Standard concentration (pg/mL) LE in 320-06 BZL	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
250	200	200	0.00025	200	0.0500	0.492	0.492	0.0421	m, b	0.087	-0.001
125	100	200	0.00013	200	0.0250	0.291	0.291	0.0246	se_m, se_b	0.003	0.000
50	200	1000	0.00005	200	0.0100	0.133	0.133	0.0109	r^2, se_y	0.997	0.001
25	100	1000	0.00003	200	0.0050	0.067	0.067	0.0052	F, df	865	3
10	40	1000	0.00001	200	0.0020	0.028	0.028	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.014	0.014	0.0005			
		Blank	0.000								

r^2= 0.997
m= 0.087
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
QC 100	0.246	0.246	0.021	200	1	1	0.000	0.000	0.103
QC 100	0.243	0.243	0.020	200	1	1	0.000		
QC 100									
QC 10	0.027	0.027	0.002	200	1	1	0.000	0.000	0.008
QC 10	0.026	0.026	0.002	200	1	1	0.000		
QC 10									
Microsomes	0.068	0.068	0.005	200	1	949	0.025	0.025	24.635
Microsomes	0.068	0.068	0.005	200	1	949	0.025		
Microsomes	0.066	0.066	0.005	200	1	949	0.024		

Assay Date	9/8/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type:	placental	Microsome ID	5-041305	Technician ID	TD	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	2428	4855.4	4800.6	9600.8	0.1	123249	7.79	9435	0.0158	1	0.013	15	0.0405
			0.5	2	2373	4745.4											
	2	2	0.5	1	2519	5037.4	5096.1	10192.2	0.1	123249	8.27	10027	0.0167	1	0.013	15	0.0430
			0.5	2	2577	5154.8											
	3	2	0.5	1	2414	4827	4640.7	9681.4	0.1	123249	7.86	9517	0.0159	1	0.013	15	0.0409
			0.5	2	2427	4854.4											
	4	2	0.5	1	2327	4654.8	4690.1	9380.2	0.1	123249	7.81	9215	0.0154	1	0.013	15	0.0396
			0.5	2	2363	4725.4											
Background control	1	2	0.5	1	29	58.16	63.49	126.98	0.1	123249	0.10	-38	-0.0001	1	0.013	15	-0.0002
			0.5	2	34	68.82											
	2	2	0.5	1	36	71.74	79.94	159.88	0.1	123249	0.13	-5	0.0000	1	0.013	15	0.0000
			0.5	2	44	88.14											
	3	2	0.5	1	49	98.76	102	204	0.1	123249	0.17	39	0.0001	1	0.013	15	0.0002
			0.5	2	53	105.24											
	4	2	0.5	1	44	86.94	84.15	168.3	0.1	123249	0.14	4	0.0000	1	0.013	15	0.0000
			0.5	2	40	79.36											
Positive control	1	2	0.5	1	1553	3108.6	3151.2	6302.4	0.1	123249	5.11	6139	0.0102	1	0.013	15	0.0263
			0.5	2	1598	3195.8											
	2	2	0.5	1	1530	3060.4	3087	6174	0.1	123249	5.01	6009	0.0100	1	0.013	15	0.0258
			0.5	2	1557	3113.6											
	3	2	0.5	1	1518	3036.2	3048.6	6097.2	0.1	123249	4.95	5932	0.0099	1	0.013	15	0.0255
			0.5	2	1531	3061											
	4	2	0.5	1	1402	2804.2	2798.7	5597.4	0.1	123249	4.54	5433	0.0091	1	0.013	15	0.0233
			0.5	2	1397	2793.2											
Negative Control	1	2	0.5	1	2515	5029.6	5087	10174	0.1	123249	8.25	10009	0.0167	1	0.013	15	0.0430
			0.5	2	2572	5144.4											
	2	2	0.5	1	2646	5292.8	5242.7	10485.4	0.1	123249	8.51	10321	0.0172	1	0.013	15	0.0443
			0.5	2	2596	5192.6											
	3	2	0.5	1	2396	4792.4	4815.1	9630.2	0.1	123249	7.81	9465	0.0158	1	0.013	15	0.0406
			0.5	2	2419	4837.8											
	4	2	0.5	1	2099	4197.4	4246.8	8497.6	0.1	123249	6.89	8333	0.0139	1	0.013	15	0.0358
			0.5	2	2150	4300.2											
10	1-1	2	0.5	1	2080	4159	4155	8310	0.1	123249	6.74	8145	0.0136	1	0.013	15	0.0350
			0.5	2	2076	4151											
	1-2	2	0.5	1	2056	4111.5	4126.5	8259	0.1	123249	6.70	8094	0.0135	1	0.013	15	0.0347
			0.5	2	2074	4147.4											
	1-3	2	0.5	1	2100	4199.8	4198.2	8396.4	0.1	123249	6.81	8232	0.0137	1	0.013	15	0.0353
			0.5	2	2098	4196.6											
	2-1	2	0.5	1	1977	3954	4054.1	8108.2	0.1	123249	6.58	7943	0.0133	1	0.013	15	0.0341
			0.5	2	2077	4154.2											
	2-2	2	0.5	1	2065	4130.2	4148.9	8297.8	0.1	123249	6.73	8133	0.0136	1	0.013	15	0.0349
			0.5	2	2084	4167.6											
	2-3	2	0.5	1	2111	4221.6	4238.7	8477.4	0.1	123249	6.88	8313	0.0139	1	0.013	15	0.0357
			0.5	2	2128	4255.8											
	3-1	2	0.5	1	2105	4210.6	4216.2	8432.4	0.1	123249	6.84	8268	0.0138	1	0.013	15	0.0355
			0.5	2	2111	4221.8											
	3-2	2	0.5	1	2072	4144.2	4155.3	8310.6	0.1	123249	6.74	8145	0.0136	1	0.013	15	0.0350
			0.5	2	2083	4166.4											
	3-3	2	0.5	1	2099	4196.6	4204.5	8409	0.1	123249	6.82	8244	0.0138	1	0.013	15	0.0354
			0.5	2	2105	4210.4											
	4-1	2	0.5	1	2049	4097.6	4112.1	8224.2	0.1	123249	6.67	8059	0.0135	1	0.013	15	0.0346
			0.5	2	2063	4126.6											
	4-2	2	0.5	1	2048	4096.4	4095.6	8191.2	0.1	123249	6.65	8026	0.0134	1	0.013	15	0.0345
			0.5	2	2047	4094.8											
	4-3	2	0.5	1	2135	4270.6	4229.1	8458.2	0.1	123249	6.86	8293	0.0138	1	0.013	15	0.0356
			0.5	2	2094	4187.6											
	5-1	2	0.5	1	2104	4208.6	4188	8376	0.1	123249	6.80	8211	0.0137	1	0.013	15	0.0353
			0.5	2	2084	4167.2											
	5-2	2	0.5	1	2107	4213	4190.5	8381.6	0.1	123249	6.78	8197	0.0137	1	0.013	15	0.0352
			0.5	2	2074	4148.6											
	5-3	2	0.5	1	2055	4110.2	4095.7	8191.4	0.1	123249	6.65	8027	0.0134	1	0.013	15	0.0345
			0.5	2	2041	4081.2											
	6-1	2	0.5	1	2287	4574.4	4563.4	9126.8	0.1	123249	7.41	8962	0.0150	1	0.013	15	0.0385
			0.5	2	2276	4552.4											
	6-2	2	0.5	1	2305	4609.8	4536.5	9073	0.1	123249	7.36	8906	0.0149	1	0.013	15	0.0382
			0.5	2	2232	4463.4											
	6-3	2	0.5	1	2263	4525.8	4510.4	9020.8	0.1	123249	7.32	8856	0.0148	1	0.013	15	0.0380
			0.5	2	2248	4495											
	7-1	2	0.5	1	2248	4496	4568.5	9137	0.1	123249	7.41	8972	0.0150	1	0.013	15	0.0385
			0.5	2	2321	4641											
	7-2	2	0.5	1	2184	4367	4366.1	8732.2	0.1	123249	7.09	8567	0.0143	1	0.013	15	0.0368
			0.5	2	2173	4345.2											
	7-3	2	0.5	1	2433	4866	4804.7	9609.4	0.1	123249	7.80	9445	0.0158	1	0.013	15	0.0405
			0.5	2	2372	4743.4											
	8-1	2	0.5	1	2281	4562.4	4592.5	9185	0.1	123249	7.45	9020	0.0151	1	0.013	15	0.0387
			0.5	2	2311	4622.6											
	8-2	2	0.5	1	2420	4839	4755.9	9511.8	0.1	123249	7.72	9347	0.0156	1	0.013	15	0.0401
			0.5	2	2336	4672.8											
	8-3	2	0.5	1	2349	4698.6	4712.3	9424.6	0.1	123249	7.65	9260	0.0155	1	0.013	15	0.0398
			0.5	2	2363	4726											

Assay Date	9/8/2005	Test Chemical ID	10	# Concentrations tested	Microsome type	placental	Microsome ID 6-041305	Technician ID TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0418	0.0018
Full activity	End	0.0402	0.0009
Full activity	Overall	0.0410	0.0015
Background	Beginning	-0.0001	9.98756E-05
Background	End	0.0001	0.000108376
Background	Overall	0.0000	0.00013584
Positive	Beginning	0.0261	0.0004
Positive	End	0.0244	0.0015
Positive	Overall	0.0252	0.0013
Negative	Beginning	0.0436	0.0009
Negative	End	0.0382	0.0034
Negative	Overall	0.0409	0.0038

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
10	1	1	1.00E-03	-3.00	0.0350
10	1	2	1.00E-03	-3.00	0.0347
10	1	3	1.00E-03	-3.00	0.0353
10	2	1	9.00E-04	-3.05	0.0341
10	2	2	9.00E-04	-3.05	0.0349
10	2	3	9.00E-04	-3.05	0.0357
10	3	1	8.00E-04	-3.10	0.0355
10	3	2	8.00E-04	-3.10	0.0350
10	3	3	8.00E-04	-3.10	0.0354
10	4	1	7.50E-04	-3.12	0.0346
10	4	2	7.50E-04	-3.12	0.0345
10	4	3	7.50E-04	-3.12	0.0356
10	5	1	5.00E-04	-3.30	0.0353
10	5	2	5.00E-04	-3.30	0.0352
10	5	3	5.00E-04	-3.30	0.0345
10	6	1	1.00E-04	-4.00	0.0385
10	6	2	1.00E-04	-4.00	0.0382
10	6	3	1.00E-04	-4.00	0.0380
10	7	1	1.00E-05	-5.00	0.0385
10	7	2	1.00E-05	-5.00	0.0368
10	7	3	1.00E-05	-5.00	0.0405
10	8	1	1.00E-06	-6.00	0.0387
10	8	2	1.00E-06	-6.00	0.0401
10	8	3	1.00E-06	-6.00	0.0398

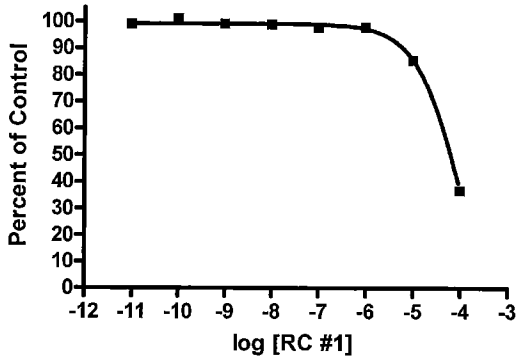
Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	85.30	84.77	86.21
2	-3.05	83.19	85.17	87.05
3	-3.10	86.58	85.31	86.34
4	-3.12	84.40	84.06	86.85
5	-3.30	85.99	85.84	84.06
6	-4.00	93.85	93.29	92.74
7	-5.00	93.96	89.72	98.91
8	-6.00	94.46	97.89	96.97

APPENDIX C

Prism Output. C-1

Placental microsomes Assay 7-20-05

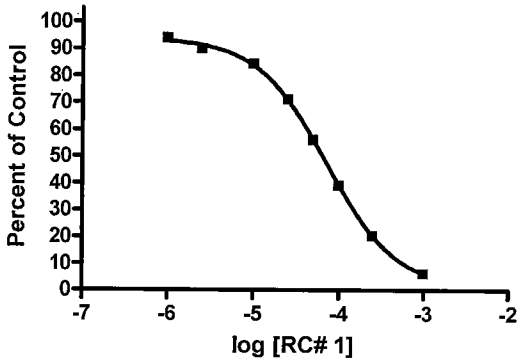
Replicate 1 (Aminoglutethimide)



-4.00	36.01	36.81	36.46
-5.00	85.78	85.88	84.07
-6.00	97.89	98.33	96.29
-7.00	97.78	97.67	97.05
-8.00	98.38	100.01	97.30
-9.00	98.68	98.37	99.65
-10.00	99.02	101.95	101.75
-11.00	98.20	101.10	96.83

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-29.54
TOP	99.04
LOGEC50	-3.975
HILLSLOPE	-0.8987
EC50	0.0001060
Std. Error	
BOTTOM	86.82
TOP	0.3968
LOGEC50	0.6421
HILLSLOPE	0.2421
95% Confidence Intervals	
BOTTOM	-210.6 to 151.6
TOP	98.21 to 99.87
LOGEC50	-5.314 to -2.635
HILLSLOPE	-1.404 to -0.3937
EC50	4.851e-006 to 0.002315
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9959
Absolute Sum of Squares	41.39
Sy.x	1.439
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

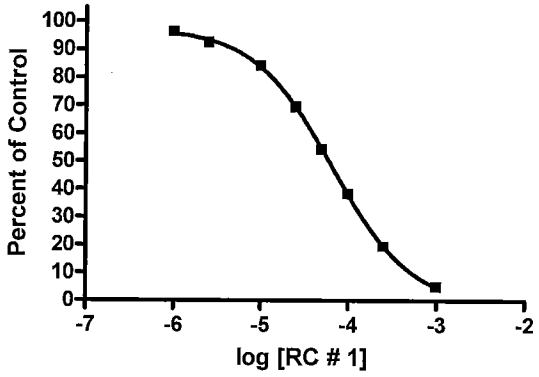
Replicate 2 (Aminoglutethimide)



-3.00	6.05	6.25	6.32
-3.60	20.22	20.74	19.91
-4.00	38.48	39.25	39.20
-4.30	55.19	56.76	55.97
-4.60	70.96	71.04	71.00
-5.00	82.66	84.80	85.26
-5.60	90.57	91.08	87.94
-6.00	95.57	90.29	95.77

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	1.114
TOP	93.81
LOGEC50	-4.148
HILLSLOPE	-1.073
EC50	7.114e-005
Std. Error	
BOTTOM	1.386
TOP	0.7374
LOGEC50	0.01712
HILLSLOPE	0.04396
95% Confidence Intervals	
BOTTOM	-1.778 to 4.006
TOP	92.27 to 95.35
LOGEC50	-4.184 to -4.112
HILLSLOPE	-1.164 to -0.9810
EC50	6.553e-005 to 7.724e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9982
Absolute Sum of Squares	41.11
Sy.x	1.434
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Replicate 3 (Aminoglutethimide)

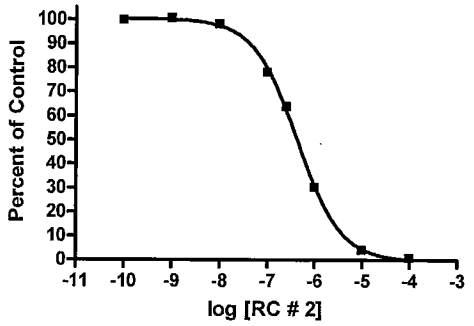


-3.00	5.20	4.89	4.52
-3.60	19.48	19.18	19.20
-4.00	38.50	38.15	37.97
-4.30	54.71	53.35	54.02
-4.60	70.16	69.23	68.75
-5.00	85.86	83.42	82.88
-5.60	93.87	91.84	91.63
-6.00	94.25	97.63	96.82

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-1.797
TOP	97.33
LOGEC50	-4.181
HILLSLOPE	-0.9702
EC50	6.592e-005
Std. Error	
BOTTOM	1.116
TOP	0.6089
LOGEC50	0.01319
HILLSLOPE	0.02912
95% Confidence Intervals	
BOTTOM	-4.124 to 0.5309
TOP	96.06 to 98.60
LOGEC50	-4.208 to -4.153
HILLSLOPE	-1.031 to -0.9095
EC50	6.188e-005 to 7.024e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9991
Absolute Sum of Squares	21.32
Sy.x	1.032
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental Microsomes (08-11-2005)

Replicate 1 (Ketoconazole)

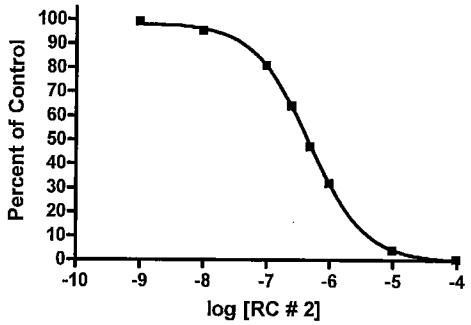


-4.00	0.99	0.51	0.41
-5.00	4.18	4.04	4.02
-6.00	29.99	30.99	29.90
-6.60	65.23	62.69	63.33
-7.00	79.09	79.07	75.89
-8.00	100.54	98.52	94.56
-9.00	101.23	100.10	100.34
-10.00	100.73	99.89	98.37

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.4299
TOP	100.3
LOGEC50	-6.371
HILLSLOPE	-0.9384
EC50	4.258e-007
Std. Error	
BOTTOM	0.8579
TOP	0.6107
LOGEC50	0.01735
HILLSLOPE	0.03367
95% Confidence Intervals	
BOTTOM	-2.219 to 1.360
TOP	99.01 to 101.6
LOGEC50	-6.407 to -6.335
HILLSLOPE	-1.009 to -0.8682
EC50	3.917e-007 to 4.628e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9986
Absolute Sum of Squares	54.34
Sy.x	1.648
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-12-2005)

Replicate 2 (Ketoconazole)

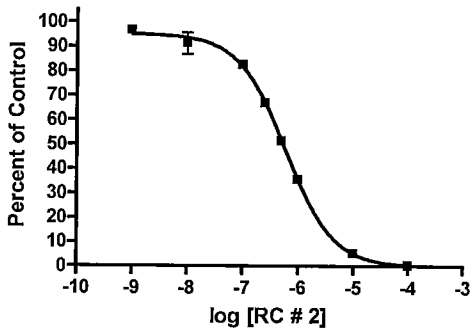


-4.00	0.64	-0.16	-0.15
-5.00	4.26	3.74	4.28
-6.00	32.27	31.66	31.46
-6.30	47.76	46.32	47.07
-6.60	64.02	64.88	63.11
-7.00	81.67	82.06	78.97
-8.00	95.02	95.29	94.80
-9.00	99.66	98.69	98.40

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.5072
TOP	98.31
LOGEC50	-6.325
HILLSLOPE	-0.9801
EC50	4.733e-007
Std. Error	
BOTTOM	0.4716
TOP	0.4283
LOGEC50	0.008478
HILLSLOPE	0.02055
95% Confidence Intervals	
BOTTOM	-1.491 to 0.4767
TOP	97.42 to 99.21
LOGEC50	-6.343 to -6.307
HILLSLOPE	-1.023 to -0.9373
EC50	4.544e-007 to 4.930e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9995
Absolute Sum of Squares	17.07
Sy.x	0.9239
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-15-2005)

Replicate 3 (Ketoconazole)

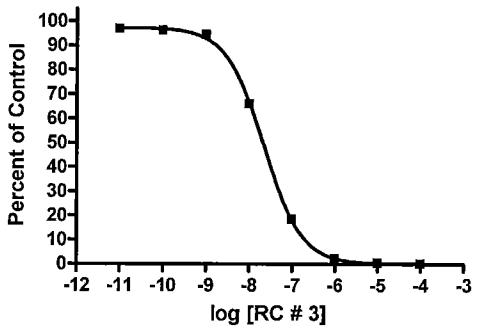


-4.00	0.29	0.36	0.36
-5.00	5.15	5.42	5.21
-6.00	34.86	36.07	35.59
-6.30	50.71	51.54	51.34
-6.60	68.86	63.11	68.67
-7.00	82.27	83.24	82.02
-8.00	95.59	95.54	82.61
-9.00	98.20	97.18	94.28

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.09188
TOP	95.03
LOGEC50	-6.225
HILLSLOPE	-1.009
EC50	5.961e-007
Std. Error	
BOTTOM	1.526
TOP	1.307
LOGEC50	0.02796
HILLSLOPE	0.07104
95% Confidence Intervals	
BOTTOM	-3.276 to 3.092
TOP	92.31 to 97.76
LOGEC50	-6.283 to -6.166
HILLSLOPE	-1.158 to -0.8612
EC50	5.212e-007 to 6.818e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9942
Absolute Sum of Squares	170.9
Sy.x	2.923
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes 08-02-05

Replicate 1 (Prochloraz)

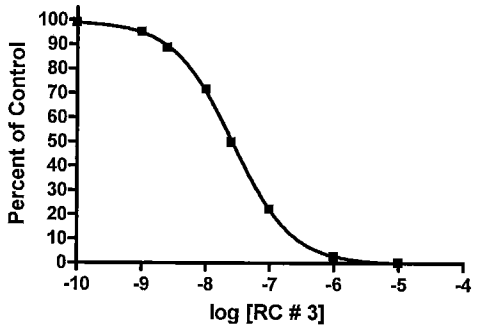


-4.00	0.29	0.00	-0.08
-5.00	0.49	0.18	0.15
-6.00	2.43	2.18	2.27
-7.00	18.41	18.91	17.34
-8.00	65.10	66.92	65.66
-9.00	92.77	93.70	96.38
-10.00	95.86	95.04	97.70
-11.00	96.59	96.48	97.06

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.1382
TOP	97.27
LOGEC50	-7.662
HILLSLOPE	-0.9853
EC50	2.176e-008
Std. Error	
BOTTOM	0.4018
TOP	0.4193
LOGEC50	0.01321
HILLSLOPE	0.02416
95% Confidence Intervals	
BOTTOM	-0.6999 to 0.9763
TOP	96.40 to 98.14
LOGEC50	-7.690 to -7.635
HILLSLOPE	-1.036 to -0.9349
EC50	2.042e-008 to 2.319e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9995
Absolute Sum of Squares	23.89
Sy.x	1.093
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes 08-03-05

Replicate 2 (Prochloraz)

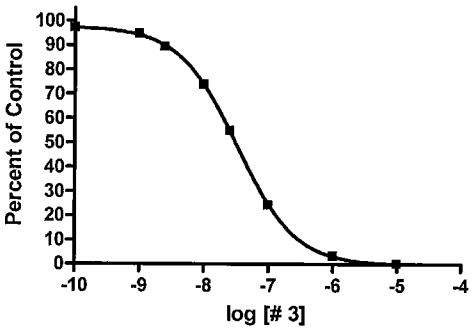


-5.00	0.15	-0.06	0.12
-6.00	2.60	2.42	2.58
-7.00	22.18	22.46	21.58
-7.60	47.61	50.00	51.41
-8.00	71.75	71.24	71.97
-8.60	89.05	89.33	87.07
-9.00	95.87	95.30	94.35
-10.00	99.21	99.06	98.22

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.4169
TOP	99.28
LOGEC50	-7.580
HILLSLOPE	-0.9386
EC50	2.629e-008
Std. Error	
BOTTOM	0.5029
TOP	0.5226
LOGEC50	0.01087
HILLSLOPE	0.02175
95% Confidence Intervals	
BOTTOM	-1.466 to 0.6322
TOP	98.19 to 100.4
LOGEC50	-7.603 to -7.558
HILLSLOPE	-0.9840 to -0.8932
EC50	2.495e-008 to 2.770e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9994
Absolute Sum of Squares	21.45
Sy.x	1.036
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes 08-04-05

Replicate 3 (Prochloraz)

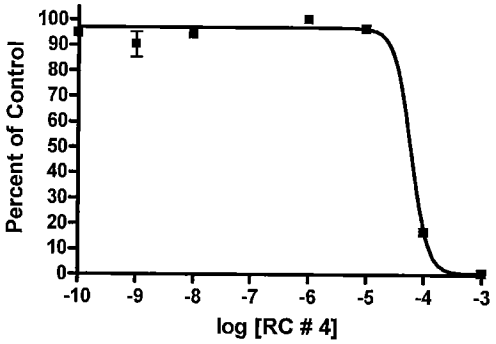


-5.00	-0.05	0.08	0.22
-6.00	3.24	3.15	3.23
-7.00	24.60	23.97	24.19
-7.60	56.57	54.05	54.20
-8.00	75.66	71.97	73.67
-8.60	87.05	89.33	91.55
-9.00	93.51	96.35	94.03
-10.00	100.16	94.50	96.66

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.3238
TOP	97.48
LOGEC50	-7.487
HILLSLOPE	-0.9641
EC50	3.260e-008
Std. Error	
BOTTOM	0.7289
TOP	0.6999
LOGEC50	0.01558
HILLSLOPE	0.03239
95% Confidence Intervals	
BOTTOM	-1.844 to 1.197
TOP	96.02 to 98.94
LOGEC50	-7.519 to -7.454
HILLSLOPE	-1.032 to -0.8966
EC50	3.025e-008 to 3.513e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9987
Absolute Sum of Squares	43.88
Sy.x	1.481
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (09-27-2005)

Replicate 1 (4-Nonylphenol)

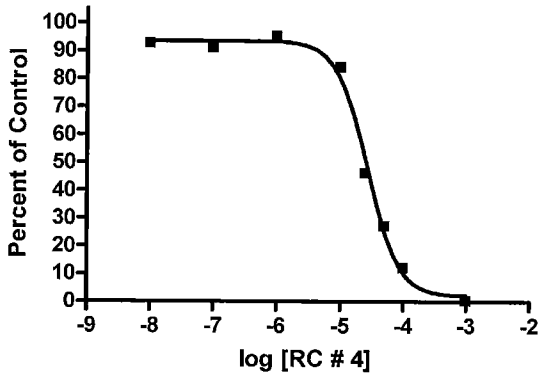


-3.00	3.81	-1.58	-0.50
-4.00	17.68	15.47	17.09
-5.00	96.33	96.37	96.64
-6.00	101.51	100.03	98.72
-7.00	107.22	102.08	106.41
-8.00	93.18	93.10	96.17
-9.00	80.56	96.55	93.56
-10.00	94.81	92.81	96.56

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.5591
TOP	96.88
LOGEC50	-4.228
HILLSLOPE	-3.042
EC50	5.911e-005
Std. Error	
BOTTOM	3.187
TOP	1.421
LOGEC50	0.2662
HILLSLOPE	3.520
95% Confidence Intervals	
BOTTOM	-6.088 to 7.206
TOP	93.92 to 99.85
LOGEC50	-4.784 to -3.673
HILLSLOPE	-10.39 to 4.302
EC50	1.646e-005 to 0.0002123
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9832
Absolute Sum of Squares	605.4
Sy.x	5.502
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (09-28-2005)

Replicate 2 (4-Nonylphenol)

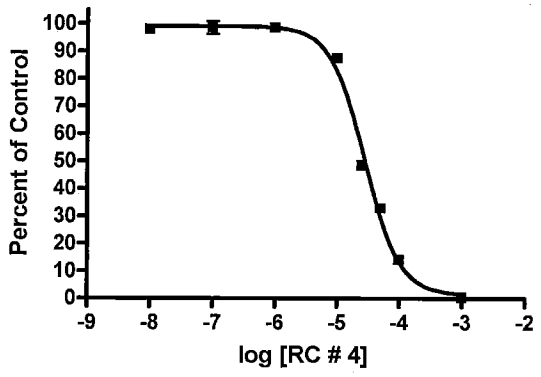


-3.00	1.51	0.01	-0.77
-4.00	11.99	11.12	13.10
-4.30	26.66	27.96	26.71
-4.60	45.81	46.13	46.38
-5.00	83.98	85.93	82.09
-6.00	94.08	95.59	95.94
-7.00	88.00	93.52	91.24
-8.00	90.81	91.22	95.46

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	2.043
TOP	93.58
LOGEC50	-4.571
HILLSLOPE	-1.766
EC50	2.684e-005
Std. Error	
BOTTOM	1.802
TOP	1.114
LOGEC50	0.02105
HILLSLOPE	0.1297
95% Confidence Intervals	
BOTTOM	-1.715 to 5.801
TOP	91.26 to 95.90
LOGEC50	-4.615 to -4.527
HILLSLOPE	-2.037 to -1.496
EC50	2.425e-005 to 2.969e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9932
Absolute Sum of Squares	223.0
Sy.x	3.339
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (09-29-2005)

Replicate 3 (4-Nonylphenol)

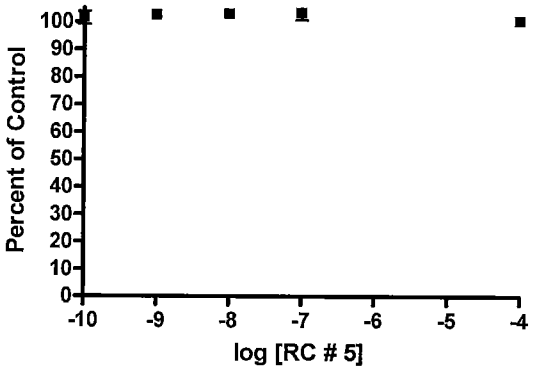


-3.00	0.29	0.31	0.41
-4.00	12.94	13.90	15.36
-4.30	32.58	32.19	33.63
-4.60	46.98	48.25	50.00
-5.00	87.59	85.67	88.61
-6.00	99.41	99.13	96.45
-7.00	95.54	96.66	102.95
-8.00	98.49	98.38	96.40

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	1.341
TOP	98.95
LOGEC50	-4.553
HILLSLOPE	-1.558
EC50	2.798e-005
Std. Error	
BOTTOM	2.095
TOP	1.239
LOGEC50	0.02412
HILLSLOPE	0.1167
95% Confidence Intervals	
BOTTOM	-3.028 to 5.710
TOP	96.37 to 101.5
LOGEC50	-4.603 to -4.503
HILLSLOPE	-1.802 to -1.315
EC50	2.492e-005 to 3.141e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9923
Absolute Sum of Squares	269.5
Sy.x	3.671
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (09-30-2005)

Replicate 1 (Dibenz[a,h]anthracene)

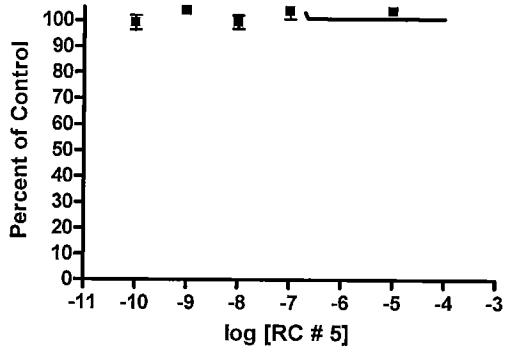


-4.00	99.72	98.94	102.33
-5.00	105.92	104.93	105.15
-6.00	107.50	107.77	110.72
-6.60	107.45	104.55	104.15
-7.00	106.33	98.22	104.73
-8.00	102.85	100.76	104.85
-9.00	105.47	101.82	100.07
-10.00	97.59	105.47	101.18

Sigmoidal dose-response (variable slope)	Does not converge.
Best-fit values	
BOTTOM	
TOP	
LOGEC50	
HILLSLOPE	
EC50	
Std. Error	
BOTTOM	
TOP	
LOGEC50	
HILLSLOPE	
95% Confidence Intervals	
BOTTOM	
TOP	
LOGEC50	
HILLSLOPE	
EC50	
Goodness of Fit	
Degrees of Freedom	
R^2	
Absolute Sum of Squares	
Sy.x	
Data	
Number of X values	
Number of Y replicates	
Total number of values	
Number of missing values	

Placental microsomes (10-03-2005)

Replicate 2 (Dibenz[a,h]anthracene)

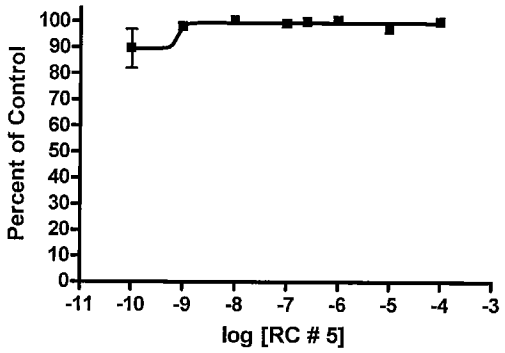


-4.00	107.58	102.70	108.55
-5.00	100.69	106.43	103.51
-6.00	102.22	107.97	106.00
-6.60	106.80	108.95	106.64
-7.00	103.33	98.57	109.09
-8.00	100.44	94.40	103.21
-9.00	107.45	103.19	100.77
-10.00	93.48	101.72	102.08

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	105.1
TOP	100.8
LOGEC50	-6.696
HILLSLOPE	38.53
EC50	2.014e-007
Std. Error	
BOTTOM	1.991
TOP	2.048
LOGEC50	1.167e+009
HILLSLOPE	4.684e+011
95% Confidence Intervals	
BOTTOM	100.9 to 109.3
TOP	96.49 to 105.0
LOGEC50	-2434000000 to 2.434e+009
HILLSLOPE	-977100000000 to 9.771e+011
EC50	
Goodness of Fit	
Degrees of Freedom	20
R^2	-0.8236
Absolute Sum of Squares	755.0
Sy.x	6.144
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (10-04-2005)

Replicate 3 (Dibenz[a,h]anthracene)

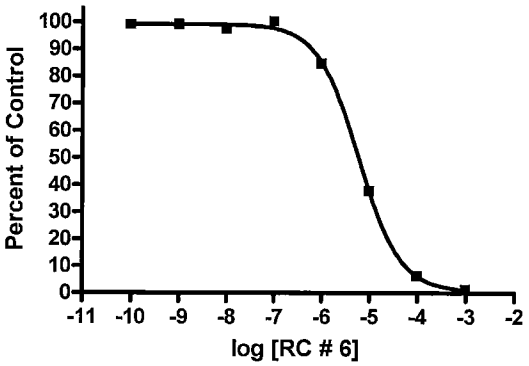


-4.00	100.89	97.59	100.59
-5.00	95.47	96.49	98.99
-6.00	101.72	102.46	96.64
-6.60	96.79	100.40	101.81
-7.00	97.86	99.46	99.95
-8.00	99.34	99.68	101.84
-9.00	97.75	99.67	96.20
-10.00	97.75	96.30	74.54

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	89.53
TOP	99.33
LOGEC50	-9.107
HILLSLOPE	7.107
EC50	7.824e-010
Std. Error	
BOTTOM	94.84
TOP	1.183
LOGEC50	136415
HILLSLOPE	9.056e+006
95% Confidence Intervals	
BOTTOM	-108.3 to 287.4
TOP	96.86 to 101.8
LOGEC50	-284600 to 284553
HILLSLOPE	-18890000 to 1.889e+007
EC50	
Goodness of Fit	
Degrees of Freedom	20
R^2	0.3705
Absolute Sum of Squares	419.8
Sy.x	4.581
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-05-2005)

Replicate 1 (Fenarimol)

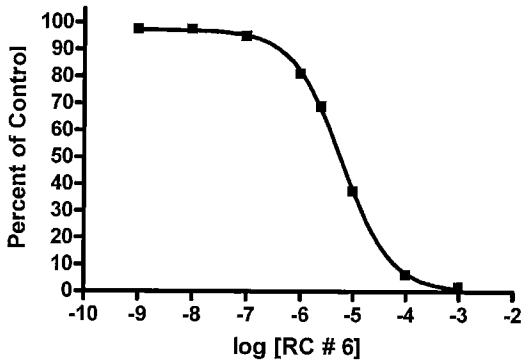


-3.00	1.26	1.06	1.32
-4.00	6.42	6.05	5.88
-5.00	33.80	39.37	39.61
-6.00	83.57	84.50	85.60
-7.00	97.86	102.56	99.85
-8.00	97.69	95.75	98.98
-9.00	97.46	99.51	100.18
-10.00	96.98	100.08	99.68

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.6679
TOP	99.20
LOGEC50	-5.224
HILLSLOPE	-1.005
EC50	5.966e-006
Std. Error	
BOTTOM	1.031
TOP	0.6040
LOGEC50	0.02338
HILLSLOPE	0.04852
95% Confidence Intervals	
BOTTOM	-1.484 to 2.819
TOP	97.94 to 100.5
LOGEC50	-5.273 to -5.176
HILLSLOPE	-1.106 to -0.9034
EC50	5.332e-006 to 6.675e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9981
Absolute Sum of Squares	77.32
Sy.x	1.966
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-08-2005)

Replicate 2 (Fenarimol)

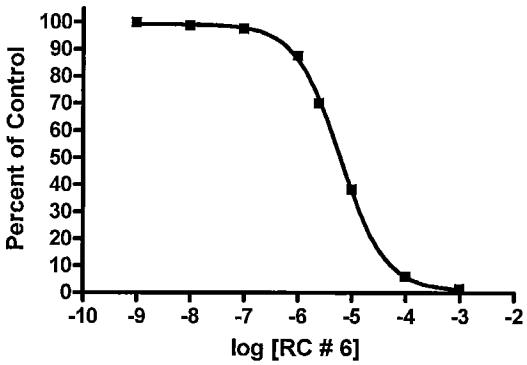


-3.00	1.45	1.71	1.62
-4.00	6.40	6.13	5.85
-5.00	36.62	38.25	37.42
-5.60	68.35	67.04	71.03
-6.00	79.10	82.58	80.91
-7.00	97.38	93.82	93.36
-8.00	95.44	100.80	96.30
-9.00	96.69	98.33	97.47

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.2286
TOP	97.27
LOGEC50	-5.218
HILLSLOPE	-0.9361
EC50	6.056e-006
Std. Error	
BOTTOM	0.9234
TOP	0.6043
LOGEC50	0.01935
HILLSLOPE	0.03584
95% Confidence Intervals	
BOTTOM	-1.698 to 2.155
TOP	96.01 to 98.53
LOGEC50	-5.258 to -5.177
HILLSLOPE	-1.011 to -0.8614
EC50	5.518e-006 to 6.646e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9984
Absolute Sum of Squares	55.49
Sy.x	1.666
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-09-2005)

Replicate 3 (Fenarimol)

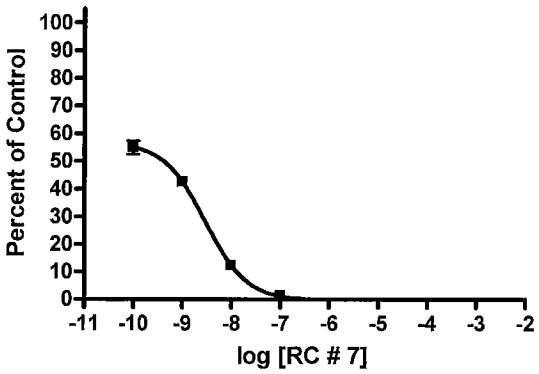


-3.00	1.30	1.27	1.17
-4.00	5.77	5.66	5.90
-5.00	38.04	38.22	37.86
-5.60	70.27	73.37	66.50
-6.00	86.32	87.94	88.02
-7.00	96.73	98.05	97.21
-8.00	101.56	95.65	98.25
-9.00	98.23	101.29	99.36

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.7629
TOP	99.13
LOGEC50	-5.214
HILLSLOPE	-1.039
EC50	6.113e-006
Std. Error	
BOTTOM	0.8948
TOP	0.6117
LOGEC50	0.01790
HILLSLOPE	0.04006
95% Confidence Intervals	
BOTTOM	-1.104 to 2.629
TOP	97.85 to 100.4
LOGEC50	-5.251 to -5.176
HILLSLOPE	-1.123 to -0.9559
EC50	5.610e-006 to 6.662e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9983
Absolute Sum of Squares	60.53
Sy.x	1.740
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-17-2005)

Replicate 1 (Econazole)

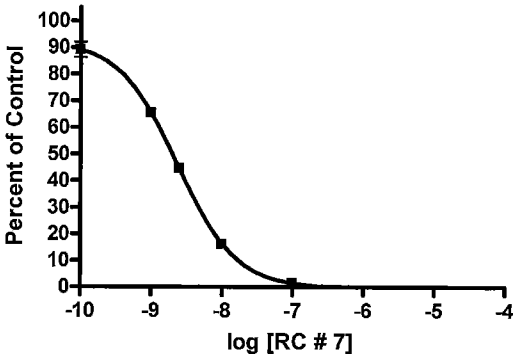


-3.00	-0.37	-0.40	-0.26
-4.00	-0.38	-0.66	-0.59
-5.00	0.10	-0.75	-0.37
-6.00	-0.40	0.07	-0.31
-7.00	1.29	1.25	1.25
-8.00	13.18	11.64	11.99
-9.00	40.68	43.03	44.08
-10.00	57.01	57.86	50.24

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.3735
TOP	56.78
LOGEC50	-8.531
HILLSLOPE	-1.025
EC50	2.942e-009
Std. Error	
BOTTOM	0.4024
TOP	1.155
LOGEC50	0.03646
HILLSLOPE	0.06242
95% Confidence Intervals	
BOTTOM	-1.213 to 0.4659
TOP	54.37 to 59.19
LOGEC50	-8.607 to -8.455
HILLSLOPE	-1.155 to -0.8950
EC50	2.470e-009 to 3.506e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9959
Absolute Sum of Squares	42.94
Sy.x	1.465
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-18-2005)

Replicate 2 (Econazole)

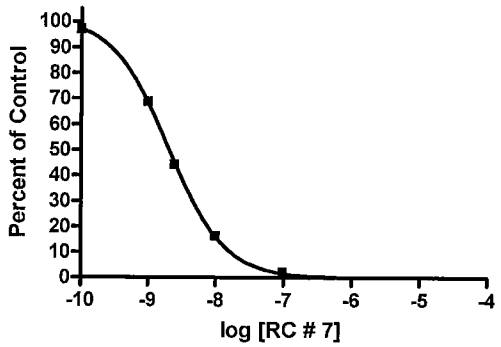


-4.00	-0.39	0.09	-0.39
-5.00	-0.37	0.34	-0.22
-6.00	-0.33	-0.26	-0.48
-7.00	1.48	1.91	1.47
-8.00	16.07	16.44	16.28
-8.60	45.70	44.67	43.61
-9.00	63.51	65.02	67.62
-10.00	83.75	90.14	93.56

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.2682
TOP	92.38
LOGEC50	-8.628
HILLSLOPE	-1.048
EC50	2.352e-009
Std. Error	
BOTTOM	0.5394
TOP	1.388
LOGEC50	0.02032
HILLSLOPE	0.04851
95% Confidence Intervals	
BOTTOM	-1.393 to 0.8569
TOP	89.48 to 95.27
LOGEC50	-8.671 to -8.586
HILLSLOPE	-1.149 to -0.9468
EC50	2.134e-009 to 2.594e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9976
Absolute Sum of Squares	61.63
Sy.x	1.755
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-19-2005)

Replicate 3 (Econazole)

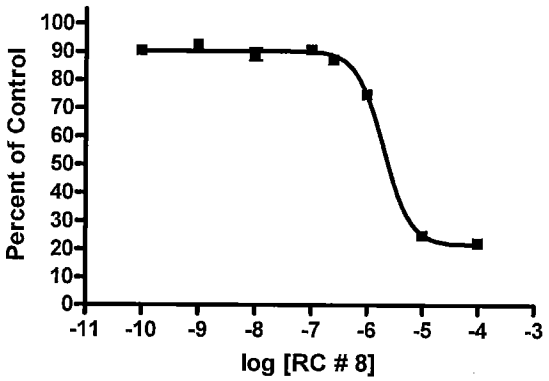


-4.00	-0.11	-0.44	-0.38
-5.00	-0.36	-0.56	0.16
-6.00	-0.08	-0.32	-0.14
-7.00	2.28	2.15	1.79
-8.00	16.94	15.84	16.01
-8.60	45.08	41.79	45.58
-9.00	69.42	68.39	68.29
-10.00	94.06	100.48	96.44

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.1287
TOP	101.4
LOGEC50	-8.698
HILLSLOPE	-1.038
EC50	2.005e-009
Std. Error	
BOTTOM	0.4020
TOP	1.104
LOGEC50	0.01447
HILLSLOPE	0.03437
95% Confidence Intervals	
BOTTOM	-0.9673 to 0.7099
TOP	99.13 to 103.7
LOGEC50	-8.728 to -8.668
HILLSLOPE	-1.110 to -0.9667
EC50	1.870e-009 to 2.149e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9988
Absolute Sum of Squares	34.47
Sy.x	1.313
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-23-2005)

Replicate 1 (Chrysin)

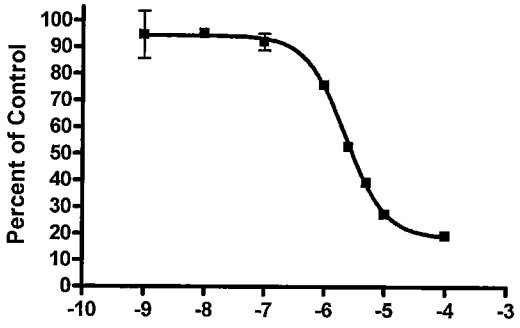


-4.00	20.54	20.28	25.14
-5.00	25.00	25.28	23.80
-6.00	71.56	76.56	76.28
-6.60	86.28	86.93	87.77
-7.00	92.20	90.66	88.60
-8.00	85.40	92.99	88.69
-9.00	88.71	93.99	94.51
-10.00	93.26	90.36	87.09

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	21.55
TOP	90.29
LOGEC50	-5.706
HILLSLOPE	-1.785
EC50	1.967e-006
Std. Error	
BOTTOM	1.520
TOP	0.7205
LOGEC50	0.05148
HILLSLOPE	0.2565
95% Confidence Intervals	
BOTTOM	18.38 to 24.72
TOP	88.79 to 91.79
LOGEC50	-5.813 to -5.599
HILLSLOPE	-2.320 to -1.250
EC50	1.536e-006 to 2.519e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9929
Absolute Sum of Squares	136.2
Sy.x	2.609
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-24-2005)

Replicate 2 (Chrysin)

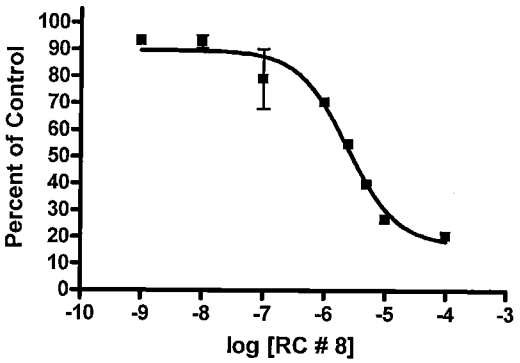


-4.00	19.50	17.12	20.78
-5.00	27.72	26.71	27.42
-5.30	37.61	39.81	40.15
-5.60	51.06	52.96	53.79
-6.00	74.76	74.52	78.20
-7.00	98.23	88.77	88.73
-8.00	94.82	96.98	93.28
-9.00	76.98	102.13	104.57

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	18.57
TOP	94.35
LOGEC50	-5.647
HILLSLOPE	-1.325
EC50	2.253e-006
Std. Error	
BOTTOM	3.176
TOP	1.876
LOGEC50	0.05260
HILLSLOPE	0.1951
95% Confidence Intervals	
BOTTOM	11.95 to 25.20
TOP	90.43 to 98.26
LOGEC50	-5.757 to -5.538
HILLSLOPE	-1.732 to -0.9180
EC50	1.750e-006 to 2.900e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9730
Absolute Sum of Squares	573.4
Sy.x	5.354
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-25-2005)

Replicate 3 (Chrysin)

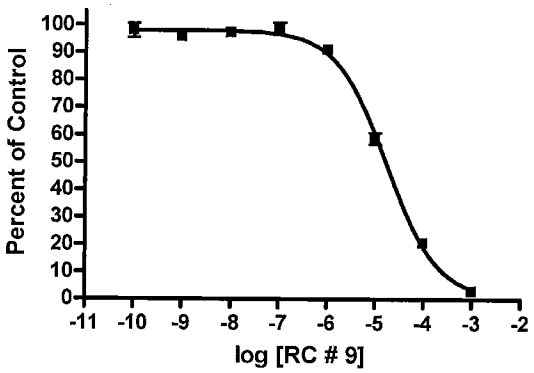


-4.00	19.83	20.79	20.76
-5.00	27.89	26.81	25.56
-5.30	39.98	39.89	39.68
-5.60	53.85	54.80	55.92
-6.00	72.26	68.66	70.28
-7.00	90.20	56.70	90.53
-8.00	94.70	88.10	95.79
-9.00	93.84	94.74	91.43

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	17.37
TOP	89.76
LOGEC50	-5.620
HILLSLOPE	-1.075
EC50	2.400e-006
Std. Error	
BOTTOM	5.169
TOP	2.807
LOGEC50	0.09618
HILLSLOPE	0.2503
95% Confidence Intervals	
BOTTOM	6.583 to 28.15
TOP	83.90 to 95.61
LOGEC50	-5.820 to -5.419
HILLSLOPE	-1.597 to -0.5525
EC50	1.512e-006 to 3.809e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9365
Absolute Sum of Squares	1150
Sy.x	7.582
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-29-2005)

Replicate 1 (Dicofol)

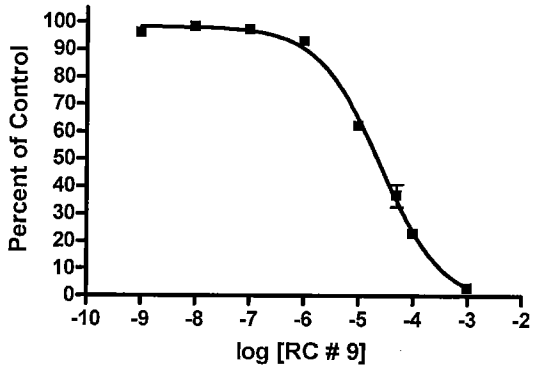


-3.00	2.56	2.63	3.68
-4.00	20.00	20.16	21.39
-5.00	61.78	59.69	54.70
-6.00	89.09	94.22	89.73
-7.00	95.14	98.50	102.65
-8.00	97.40	95.93	98.11
-9.00	94.89	96.20	96.28
-10.00	100.76	100.48	92.89

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.3831
TOP	98.01
LOGEC50	-4.759
HILLSLOPE	-0.8235
EC50	1.741e-005
Std. Error	
BOTTOM	2.418
TOP	0.8680
LOGEC50	0.04907
HILLSLOPE	0.06481
95% Confidence Intervals	
BOTTOM	-4.661 to 5.427
TOP	96.20 to 99.82
LOGEC50	-4.862 to -4.657
HILLSLOPE	-0.9587 to -0.6883
EC50	1.375e-005 to 2.204e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9948
Absolute Sum of Squares	165.8
Sy.x	2.879
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-30-2005)

Replicate 2 (Dicofol)

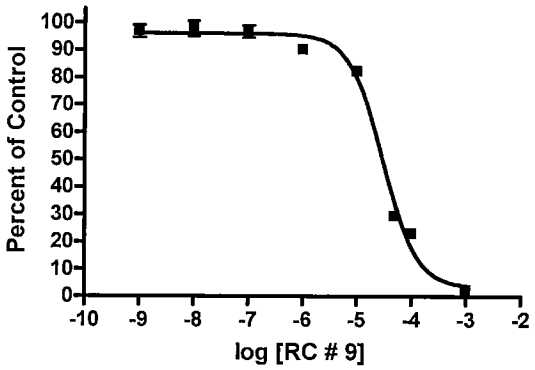


-3.00	3.05	2.58	2.75
-4.00	26.03	21.58	20.71
-4.30	34.86	44.55	30.66
-5.00	62.19	60.34	63.59
-6.00	92.56	92.16	94.31
-7.00	96.36	97.80	97.37
-8.00	98.88	97.23	98.81
-9.00	94.73	96.80	96.84

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-2.536
TOP	98.27
LOGEC50	-4.606
HILLSLOPE	-0.7637
EC50	2.475e-005
Std. Error	
BOTTOM	3.025
TOP	1.129
LOGEC50	0.05031
HILLSLOPE	0.06113
95% Confidence Intervals	
BOTTOM	-8.845 to 3.773
TOP	95.91 to 100.6
LOGEC50	-4.711 to -4.501
HILLSLOPE	-0.8912 to -0.6362
EC50	1.944e-005 to 3.152e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9935
Absolute Sum of Squares	202.5
Sy.x	3.182
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (08-31-2005)

Replicate 3 (Dicofol)

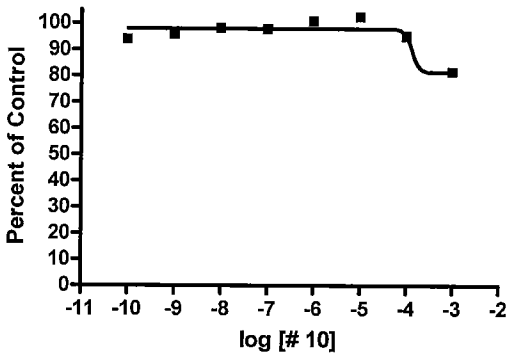


-3.00	2.27	1.58	2.75
-4.00	23.25	22.85	22.42
-4.30	28.43	29.71	29.95
-5.00	82.79	82.93	81.24
-6.00	91.55	88.89	90.18
-7.00	93.37	95.67	100.84
-8.00	92.62	102.07	98.56
-9.00	92.52	100.04	97.90

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	3.244
TOP	95.91
LOGEC50	-4.519
HILLSLOPE	-1.408
EC50	3.027e-005
Std. Error	
BOTTOM	2.584
TOP	1.263
LOGEC50	0.03954
HILLSLOPE	0.1327
95% Confidence Intervals	
BOTTOM	-2.147 to 8.635
TOP	93.27 to 98.54
LOGEC50	-4.601 to -4.436
HILLSLOPE	-1.685 to -1.131
EC50	2.504e-005 to 3.661e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9890
Absolute Sum of Squares	363.9
Sy.x	4.265
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (09-06-2005)

Replicate 1 (Atrazine)

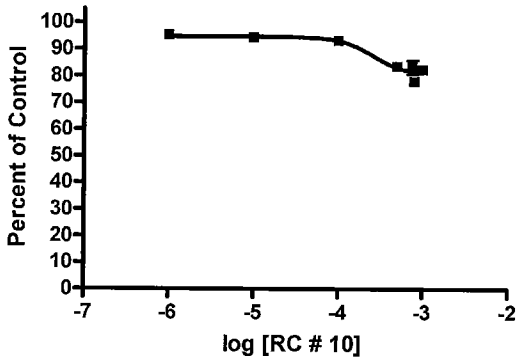


-3.00	81.18	82.20	80.87
-4.00	93.40	95.39	95.88
-5.00	102.09	101.78	102.58
-6.00	100.55	99.61	101.27
-7.00	96.57	97.97	98.25
-8.00	95.67	99.69	98.29
-9.00	96.02	95.78	95.02
-10.00	91.11	95.00	95.01

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	81.42
TOP	97.90
LOGEC50	-3.882
HILLSLOPE	-5.527
EC50	0.0001311
Std. Error	
BOTTOM	35.80
TOP	0.7520
LOGEC50	1013
HILLSLOPE	44463
95% Confidence Intervals	
BOTTOM	6.738 to 156.1
TOP	96.33 to 99.47
LOGEC50	-2117 to 2109
HILLSLOPE	-92750 to 92743
EC50	
Goodness of Fit	
Degrees of Freedom	20
R^2	0.8049
Absolute Sum of Squares	169.7
Sy.x	2.912
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (09-07-2005)

Replicate 2 (Atrazine)

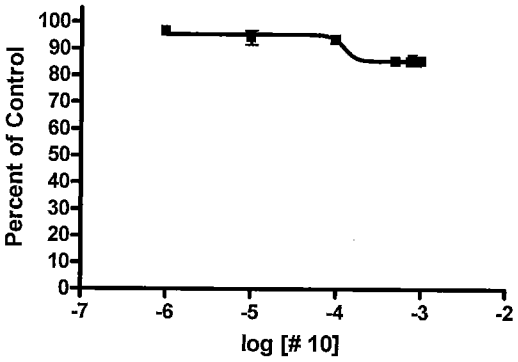


-3.00	79.19	83.47	84.22
-3.05	85.38	81.39	79.58
-3.10	76.45	75.60	81.69
-3.12	78.20	86.60	85.15
-3.30	82.94	82.32	85.30
-4.00	96.53	91.58	91.05
-5.00	90.65	95.89	96.41
-6.00	93.48	96.02	95.87

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	80.63
TOP	94.70
LOGEC50	-3.597
HILLSLOPE	-2.243
EC50	0.0002527
Std. Error	
BOTTOM	2.467
TOP	1.292
LOGEC50	0.2231
HILLSLOPE	1.395
95% Confidence Intervals	
BOTTOM	75.48 to 85.77
TOP	92.00 to 97.39
LOGEC50	-4.063 to -3.132
HILLSLOPE	-5.153 to 0.6672
EC50	8.656e-005 to 0.0007378
Goodness of Fit	
Degrees of Freedom	20
R^2	0.8150
Absolute Sum of Squares	197.2
Sy.x	3.140
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Placental microsomes (09-08-2005)

Replicate 3 (Atrazine)



-3.00	85.30	84.77	86.21
-3.05	83.19	85.17	87.05
-3.10	86.58	85.31	86.34
-3.12	84.40	84.06	86.85
-3.30	85.99	85.84	84.06
-4.00	93.85	93.29	92.74
-5.00	93.96	89.72	98.91
-6.00	94.46	97.89	96.97

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	85.41
TOP	95.32
LOGEC50	-3.903
HILLSLOPE	-6.088
EC50	0.0001250
Std. Error	
BOTTOM	0.6026
TOP	0.7907
LOGEC50	5.055
HILLSLOPE	300.5
95% Confidence Intervals	
BOTTOM	84.15 to 86.66
TOP	93.67 to 96.97
LOGEC50	-14.45 to 6.642
HILLSLOPE	-632.9 to 620.7
EC50	3.565e-015 to 4.383e+006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.8667
Absolute Sum of Squares	75.02
Sy.x	1.937
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

APPENDIX D

Statistician's Report D-1

DRAFT REPORT

On

PLACENTAL AROMATASE VALIDATION STUDY
INTRALABORATORY STATISTICAL ANALYSIS OF BATTELLE DATA

CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-16, TASK 7

May 17, 2006

Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
ENDOCRINE DISRUPTOR SCREENING PROGRAM
WASHINGTON, D.C.

Prepared by

BATTELLE
505 King Avenue
Columbus, Ohio 43201

Placental Aromatase Validation Study

Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories

Intralaboratory Statistical Analysis of Battelle Data

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-16, TASK 7

Ying-Liang Chou, Author	Date
Paul I. Feder, Reviewer	Date

Offsite Quality Assurance Statement

Study Number:

This study was inspected by the Quality Assurance Unit and reports were submitted to the Study Director and Management as follows:

Phase Inspected	Battelle Management Inspection Date	Date Reported to Battelle Task Leader/ Management
Audit study file	5/11/2006	5/11/2006
Audit draft report	5/11/2006	5/11/2006

Quality Assurance Unit **Date**

Hillary Flory 5-17-06

This report discusses the methods and results of the intralaboratory statistical analysis on the data collected at Battelle with the placental aromatase assay for WA 4-16 Task 7: Conduct Studies with Microsomes Prepared in Participating Laboratories.

Summary and Conclusions

There were ten test substances analyzed in this task, including the four chemicals in Task 5: aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, dibenz[a,h]anthracene, fenarimol, econazole, chrysin, dicofol, and atrazine (coded: Chemicals I, II, III, IV, V, VI, VII, VIII, IX, and X, respectively). Battelle analyzed five chemicals with microsomal preparation made at Battelle (aminoglutethimide, ketoconazole, dibenz[a,h]anthracene, dicofol, atrazine) and five chemicals with microsomal preparation made at In Vitro Technologies (IVT) (prochloraz, 4-nonylphenol, fenarimol, econazole, chrysin) and provided summary results from the concentration response curve fits from each chemical for statistical analysis. Three replicates were carried out for each chemical. For each chemical the statistical analyses were based on the data from all the replicates.

Two types of data were obtained: inhibition curve fit data and control activity data. Statistical analyses were carried out separately for these two types of data. The response variable was the "percent of control". Percent of control is defined as the ratio of the background adjusted aromatase activity in the tube under consideration to the average background adjusted aromatase activity among the four full enzyme activity control tubes within the replicate, times 100. Control activity data consists of four types of controls: full enzyme activity control, background activity control, positive control (4-OH ASDN), and negative control (lindane). Statistical analyses were performed separately for these four types of controls.

For the inhibition curve fit data separate analyses were carried out for each of the ten test substances. For each test substance concentration response curves were fitted within each replicate to describe the relation between test substance concentrations and extent of inhibition. The concentration response curves were summarized by four parameters: the baseline response (bottom), the maximum response (top), the concentration that provokes a response halfway between baseline and maximum (IC_{50}), and the slope. Results were compared across replicates within test substance. For the control activity data, statistical analyses were carried out for the controls combined across test substances. Full enzyme activity, background activity, positive, and negative control tube responses were compared between the beginning and the end of each replicate to identify differences within replicates, differences among replicates within test substances, and differences among test substances.

The following results were observed:

1. Test substances V (dibenz[a,h]anthracene) and X (atrazine) are non-inhibitors. The majority of concentration responses were above 80% of control.

2. For Chemical VIII (chrysin) the low threshold of responses was in the range of 17% and 25%. Bottom parameter estimates ranged from 17.370 to 21.550 for three replicates.
3. Among the four parameters, the top parameter had the most replicate-to-replicate variation. Other three parameters all had zero, close to zero, or minimal replicate-to-replicate variations.
4. Except for Chemical IV Replicate 1, the within-replicate variations were all close to zero for $\log_{10}IC_{50}$ and slope.
5. Except for background controls, the other three control types the differences between the beginning and the end portions, when averaged across replicates, were significant. The end portion was significantly lower than the beginning portion. This implies a reduction in aromatase activity between the beginning and the end of a replicate.
6. For all the control types, chemical main effect and chemical by portion interaction effect were not significant and were not included in the tables.
7. Source of microsomes did not have significant effect on the activities of four controls.
8. For Chemical VII the variation in replicate 1 for the full enzyme activity controls, the positive controls, and the negative controls was substantially larger and out of line with than that for any replicate for all the chemicals (Figures 11b, 13b, 14b).
9. For positive controls the majority of variation was from replicate-to-replicate within chemicals. This is seen in Figures 13a and 13b.

Introduction and Background

In Task 7 of the Placental Aromatase Validation Study, each laboratory carried out multiple independent replicates of the placental aromatase assay with ten test substances according to a common test protocol: five chemicals with microsomal preparation made at Battelle and five chemicals with microsomal preparation made at In Vitro Technologies (IVT). For each test substance concentration response, curves were fitted to the results from each replicate. Graphical displays and analysis of variance summary comparisons of the concentration response curves and the full enzyme activity control, background activity control, positive, and negative controls were prepared for each inhibitor compound.

This report discusses the methods and results of the statistical analyses of the data collected at Battelle for the ten test substances: aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, dibenz[a,h]anthracene, fenarimol, econazole, chrysin, dicofol, and atrazine, which were coded: Chemicals I, II, III, IV, V, VI, VII, VIII, IX, and X, respectively. Four chemicals: aminoglutethimide, ketoconazole, econazole, and chrysin were previously analyzed and discussed in Task 5.

Data Used in the Analyses

Aromatase activity levels were determined for the full enzyme activity control, background activity control, positive control, and negative control, and for eight graded concentrations of each of the ten test substances. The specific concentration levels varied for each of the test substances.

Three replicates were carried out for each chemical. Within each replicate of each test substance three repetitions were run at each of the eight concentrations. In addition, two repeat tubes of the full enzyme activity, background activity, positive, and negative controls were run prior to the inhibition concentration runs and two repeat tubes of each the four control types were run following the inhibition concentration runs.

Statistical analyses were carried out on the "percent of control" responses. Percent of control is defined as the ratio of the background adjusted aromatase activity in the tube under consideration to the background adjusted average aromatase activity among the four full enzyme activity control tubes within the replicate, times 100. The average percent of control among the four full enzyme activity control tubes is necessarily 100 percent within each replicate. The average percent of control among the four background activity control tubes is necessarily 0% within each replicate.

Nominally for an inhibitor the percent of control activity values vary between approximately 0% near the high inhibition concentrations and approximately 100% near the low inhibition concentrations, but this may vary with the inhibitor. Chemical VIII (chrysin) departs from this behavior with respect to the bottom threshold. Chemicals V (dibenz[a,h]anthracene) and X (atrazine) are apparent non-inhibitors.

The percent of control activity responses at varying inhibitor concentrations within each replicate are displayed in Tables A-1a through A-1j for Chemicals I through X, respectively. The full enzyme activity, background activity, positive, and negative controls background adjusted aromatase activity and the percent of control data within each replicate are displayed in Tables A-2a through A-2j for Chemicals I through X, respectively.

Several potentially outlying responses were identified in the data, in that they differed from surrounding data values more than typically:

- Chemical II – Replicate 3 (assay date 8-15-05), level 7, Percent of Control value 82.61
- Chemical IV – Replicate 1 (assay date 9-27-05), level 7, Percent of Control value 80.56
- Chemical VIII – Replicate 2 (assay date 8-24-05), level 6, Percent of Control value 98.23
- Chemical VIII – Replicate 2 (assay date 8-24-05), level 8, Percent of Control value 76.98
- Chemical VIII – Replicate 3 (assay date 8-25-05), level 6, Percent of Control value 56.70

- Chemical VIII – Replicate 3 (assay date 8-25-05), level 7, Percent of Control value 88.10

There were no indications for any of these values of departures from protocol test procedures or of clerical errors. It was thus decided to include all these values in the statistical analyses.

Objectives

The primary objectives of the statistical analysis are:

1. Fit concentration curves within each replicate to describe the trend in the percent of control activity across varying inhibitor concentrations for each of the ten test substances.
2. Estimate baseline response, maximum response, the IC_{50} concentration, slope, and associated standard errors within each replicate of each test substance.
3. Combine results across replicates within test substances to determine the average baseline response, average maximum response, average IC_{50} concentration, average slope, and associated standard errors among replicates.
4. Determine whether there are differences between the full enzyme activity, background activity, positive, and negative controls obtained at the beginning and those obtained at the end of each replicate within each test substance.
5. Assess the consistency of test conditions within replicates, across replicates within test substances, and across test substances based on the full enzyme activity, background activity, positive, and negative control values.
6. Assess whether there are any differences in control responses between the microsomes that were made at Battelle and those that were made at In Vitro Technologies.

Statistical Analysis Methods

Concentration Response Trend Curves

For each test substance and replicate within test substance a concentration response curve was fitted to the percent of control activity values at the three repetitions at each of the eight graded test substance inhibitor concentrations.

For purposes of response curve fitting, concentration was expressed on the log scale. In agreement with past convention, common logarithms (i.e. base 10) were used. Let X denote the logarithm of the concentration of inhibitor compound (e.g. if concentration = 10^{-5} then X = -5). Let

Y ≡ (background corrected) percent of control in the inhibitor tube

X ≡ logarithm (base 10) of the concentration
DAVG ≡ average (not corrected for background) DPMs across the repeat tubes with the same inhibitor concentration
T ≡ top of plateau (maximum response)
B ≡ bottom of plateau (baseline response)
β ≡ slope of the concentration response curve (β is negative)
$\mu \equiv \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to the percent of control halfway between baseline and maximum responses)

The following four parameter concentration response curve was fitted to relate percent of control activity to logarithm of concentration within each replicate

$$Y = \mathbf{B} + (\mathbf{T}-\mathbf{B})/[1 + 10^{(\mu - X)\beta}] + \epsilon$$

where ϵ is the variation among repetitions, distributed with mean 0 and variance proportional to DAVG (based on Poisson distribution theory for radiation counts) and also approximately proportional to the response Y.

The response curve was fitted by non-weighted least squares nonlinear regression analysis. Model fits were carried out using PRISM software (Version 4).

The slope "β" is an alternative and equivalent notation to what is referred to in amendment 2 to the protocol as the hillslope "H". The random variation term "ϵ" is not explicitly included in the concentration response curve equation stated in Amendment 2 to the protocol but is tacitly assumed to be included in the model specified there.

For each replicate, the estimated baseline response (**B**) and its associated standard error, the estimated maximum response (**T**) and its associated standard error, the estimated $\log_{10}IC_{50}$ (μ) and its associated standard error, the IC_{50} and its associated geometric standard error, the slope (β) and its associated standard error, and the "Status" of each response curve are reported. The "Status" of each response curve is indicated as "C", complete, if the concentration response curve inhibition ranges from essentially 0 percent to at least 80 percent of control. It is indicated as "I", incomplete, if the concentration response curve inhibition ranges from essentially 100% inhibition to less than 50% inhibition. It is also indicated as "I", incomplete, if the concentration response curve decreases to between 80% and 50% of control. If the concentration response curve inhibition does not go below 80% of control, the test substance is a non-inhibitor, parameter estimates are not calculated.

For each of the test substances a one-way random effects analysis of variance model with heterogeneous variances among the replicates was fitted to the parameter estimates top (**T**), bottom (**B**), $\log_{10}IC_{50}$ (μ), and slope (β), from the concentration response curve fits within each replicates. The random effect was replicate within test substance. The within replicate variances were estimated as the squares of the standard errors for each replicate. The analysis of variance fits provide estimated averages effects (mean) across the replicates within test substances and their associated standard errors.

These standard errors include both within replicate and between replicate components of variation. Degrees of freedom associated with the mean effects were calculated based on Satterthwaite's approximation.

The estimated IC_{50} for the test substance was estimated as 10 to the power mean $\log_{10}IC_{50}$. The geometric standard error associated with the estimated IC_{50} was calculated as 10 to the power standard error associated with mean $\log_{10}IC_{50}$.

Top (**T**), bottom (**B**), slope (β), and $\log_{10}IC_{50}$ (μ) were each compared across replicates within test substances based on the one-way random effects analysis of variance model fits. For each of **T**, **B**, β, and μ, plots were prepared that display the parameters within each replicate with associated 95% confidence intervals based on the within replicate standard error and the average across replicates with associated 95% confidence interval incorporating replicate-to-replicate variation.

Concentration response curves were fitted to the averages of the three repetitions within each replicate. Estimates and associated standard errors (or geometric standard error) for top (**T**), bottom (**B**), $\log_{10}IC_{50}$ (μ), IC_{50}, and slope (β) were displayed. The averages of the three repetitions for each replicate were plotted in the same plot with plotting symbols distinguishing among replicates. The concentration response curves for each replicate, fitted to the average data, were superimposed on the same plot to compare the percent of control activity values across replicates.

On a separate plot the average percent of control values for each replicate were plotted versus logarithm of inhibitor concentrations. The average concentration response curve across replicates was superimposed on the same plot. The average response curve was calculated as

$$Y_{avg} = \mathbf{B}_{avg} + (\mathbf{T}_{avg} - \mathbf{B}_{avg})/[1 + 10^{\beta_{avg}(\mu_{avg} - X)}]$$

where $\mathbf{B}_{avg}$, $\mathbf{T}_{avg}$, β_{avg}, and μ_{avg} were estimated across the three replicates, based on the random effects one-way analysis of variance model discussed above.

All concentration response curves were fitted to the data using the non-linear regression analysis features in the PRISM statistical analysis package, Version 4. Supplemental statistical analyses and displays such as summary tables, graphical displays, analysis of variance, and multiple comparisons were carried out using PRISM and the SAS statistical analysis system- Version 9.

Analysis of Full Enzyme Activity, Background Activity, Positive, and Negative Controls Across Test substances and Replicates Within Test substances

Within each test substance and replicate within test substance, quadruplicate repetitions were made of the full enzyme activity, background activity, positive, and negative control responses. Half the repetitions were carried out at the beginning of the replicate and half at the end. If the test conditions were consistent throughout the

replicate, the control tube responses at the beginning should be equivalent to those at the end.

The control response analyses were carried out with the control data combined across 30 replicates within the ten test substances. The control responses were expressed as percent of control. The full enzyme activity, background activity, positive, and negative controls percent of control responses were plotted across replicates, with plotting symbol distinguishing between beginning and end, and with reference line at 0% (background activity controls), at 100% (full enzyme activity controls), or at 100% (negative controls). These plots indicate the extent of consistency across replicates with respect to average value and variability, and provide comparisons of beginning versus end of each replicate. Two separate plots (with the same vertical scale) were generated for chemicals with microsomal preparation made in the two laboratories. Therefore, Chemicals I, II, V, IX, and X were included in one plot (microsomal preparation made at Battelle); Chemicals III, IV, VI, VII, and VIII were included in the other plot (microsomal preparation made at IVT). Additional plots were prepared displaying the difference of the average of the first two percent of control values (i.e. those based on the "beginning" tubes) and the average of the last two percent of control values (i.e. those based on the "end" tubes) across replicates (end minus beginning). Each plot has a reference line at 0. Also, two separate plots (with the same vertical scale) were generated for chemicals with microsomal preparation made in the two laboratories.

Mixed effects analysis of variance models were fitted to the full enzyme activity, background activity, positive, and negative controls percent of control responses. The fixed effect factors in the analysis of variance were source of microsomes, test substance, portion (beginning or end), and portion by test substance interaction. The random effects were replicate nested within test substance and portion by replicate within test substance interaction. The residual error variation was based on the variation among repetitions within replicate and portion. The response was percent of control. For the background activity and full enzyme activity controls the average of the repetitions within a replicate are constrained to be 0 and 100 respectively, which implies that the variation associated with the replication effect is necessarily constrained to be 0.

Round off

Some derived numbers in the results tables may differ from those in the computer printouts or from those obtained using hand calculations by several units in the least significant digit due to round off in intermediate numbers and in intermediate calculations.

Statistical Analysis Results

Concentration Response Trend Curves

Concentration response curves were fitted separately to the repeat tubes data

within each replicate and to the averages of the repetitions within each replicate. Percent of control, the response variable for the statistical analyses, is displayed in Tables A-1a through A-1j for Chemicals I through X, respectively. The estimated parameters of the fitted concentration response curves are displayed in Tables 1-1, 2-1, 3-1, 4-1, 6-1, 7-1, 8-1, and 9-1 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively. The concentration response curves fitted to the averages of the three repetitions within each replicate for Chemicals I, II, III, IV, VI, VII, VIII, and IX are displayed in Figures 1-1, 2-1, 3-1, 4-1, 6-1, 7-1, 8-1, and 9-1.

The parameters of the average concentration response curves, based on random effects analysis of variance model fits with replicate as a random effect are displayed in Tables 1-1, 2-1, 3-1, 4-1, 6-1, 7-1, 8-1, and 9-1 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively. ("Mean" under "Replicate" column). The parameters within each replicate are also displayed. The average concentration response curves, along with the averages of three repetitions within each replicate are plotted together in Figures 1-2, 2-2, 3-2, 4-2, 6-2, 7-2, 8-2, and 9-2 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively.

The parameter estimates for each replicate and the average parameter estimates across replicates and their associated 95% confidence intervals are displayed in Tables 1-2, 2-2, 3-2, 4-2, 6-2, 7-2, 8-2, and 9-2 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively. The corresponding graphs for bottom parameter are presented in Figures 1-3, 2-3, 3-3, 4-3, 6-3, 7-3, 8-3, and 9-3; for top parameter, 1-4, 2-4, 3-4, 4-4, 6-4, 7-4, 8-4, and 9-4; for $\log_{10}IC_{50}$, Figures 1-5, 2-5, 3-5, 4-5, 6-5, 7-5, 8-5, and 9-5; and for slope, Figures 1-6, 2-6, 3-6, 4-6, 6-6, 7-6, 8-6, and 9-6.

The results of analyses of variance for these estimates are presented in Tables 1-3, 2-3, 3-3, 4-3, 6-3, 7-3, 8-3, and 9-3 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively. For each replicate the squares of the standard errors associated with each parameter are given. These estimates include only within replicate variation. Across replicates, the replicate-to-replicate variation and the square of the standard error of the overall average are displayed. These estimates include both within replicate variation and replicate-to-replicate variation.

All responses were above 80% or close to 80% of control for Chemical V (dibenz[a,h]anthracene) and Chemical X (atrazine). Both chemicals were determined to be non-inhibitors. Their parameters were not estimable. Response curve analysis was not performed for these two chemicals. Averages percent of control of the three repetitions within each replicate are displayed in Figures 5-1 and 10-1 for Chemicals V and X, respectively. Figures 5-2, 5-3, and 5-4 display the percent of control values of the three repetitions for each of the replicates 1, 2, and 3, respectively for Chemical V. Figures 10-2, 10-3, 10-4 display the percent of control values of the three repetitions for each of the replicates 1, 2, and 3, respectively for Chemical X. Percent of control values are shown in Tables A-1e for Chemical V and A-1j for Chemical X.

Chemical I (aminoglutethimide): Response Curve Analysis

Figures 1-1 and 1-2 show that all three replicates are in line with the fitted response curve. However, data for replicate 1 did not go below 30%, resulting in large standard errors for bottom, $\log_{10}IC_{50}$, and slope estimates, as seen in Figures 1-3, 1-5, and 1-6 and Table 1-1. The relatively low top plateau in replicate 2 inflates the estimate of the replicate-to-replicate variation and results in relatively large standard error of the overall mean for the top estimate, as seen in Figures 1-4 and Tables 1-1 and 1-3. The parameter estimates and their 95% confidence intervals are displayed in Table 1-2 and plotted in Figures 1-3 to 1-6.

Table 1-3 shows the estimated variance components for the parameter estimates. For bottom, $\log_{10}IC_{50}$, and slope parameters, the within-replicate variation for replicate 1 was orders of magnitude higher than the within-replicate variations for replicates 2 and 3 and the replicate-to-replicate variation. For the top parameter, the replicate-to-replicate variation was more than ten times the individual replicate within-replicate variances due to the relatively low top plateau for replicate 2. For $\log_{10}IC_{50}$ and slope, the within-replicate variations for replicates 2 and 3 and the replicate-to-replicate variation were close to zero.

Chemical II (ketoconazole): Response Curve Analysis

Figures 2-1 and 2-2 show that all three replicates are in line with the fitted response curve. Table A1-b and Table 2-3 show that an outlying value in replicate 3 ($\log_{10}$ concentration = -8, 82.61% of control) increased the within replicate variability of the bottom, top, $\log_{10}IC_{50}$, and the slope estimates as compared to the other two replicates. This also resulted in larger standard errors for the parameter estimates of replicate 3, as shown in Tables 2-1, and Figures 2-3 to 2-6.

Table 2-3 also shows that the replicate-to-replicate variation accounted for the majority of the overall variation for top and $\log_{10}IC_{50}$. For bottom and slope, the replicate-to-replicate variations were essentially zero.

Chemical III (prochloraz): Response Curve Analysis

Figures 3-1 and 3-2 show that all three replicates are in line with the fitted response curve. The bottom estimate was close to zero, as shown in Table 3-1 and Figure 3-3. Table 3-1 and Figure 3-4 show that for all three replicates, top estimate was close to, but not reaching 100%. As shown in Table 3-2 and in Figure 3-5, parameter estimates for $\log_{10}IC_{50}$ are consistent across three replicates. Table 3-2 and Figure 3-6 show that the slope estimates were close to -1 for all three replicates.

Table 3-3 shows that the replicate-to-replicate variation was essentially zero for bottom parameter. For the bottom parameter, the majority of the overall variation was from replicate 3, whose within replicate variation was three times and two times higher than the within replicate variations from replicates 1 and 2, respectively. For top parameter, the replicate-to-replicate variation was about six times the individual replicate

within-replicate variance for replicate 1, more than three times the individual replicate within-replicate variance for replicate 2, and more than two times the individual replicate within-replicate variance for replicate 3. For $\log_{10}IC_{50}$ and slope, the individual replicate within-replicate variances and the replicate-to-replicate variation were all close to zero.

Chemical IV (4-Nonylphenol): Response Curve Analysis

Figures 4-1 and 4-2 show that the fitted response curves in replicates 2 and 3 were in line, but the response curve in replicate 1 differed. Replicate 1 did not have enough responses between $\log_{10}$ concentrations at -4 and -5, which resulted in a big jump of percent of control values between $\log_{10}$ concentrations at -4 and -5. Replicates 2 and 3 remedied this by inserting responses at $\log_{10}$ concentrations at -4.3 and -4.6, in the central portions of the response curves. As shown in Table 4-1 and Figures 4-5 and 4-6, $\log_{10}IC_{50}$ and slope parameters had large standard errors in replicate 1. For the top parameter, as shown in Table 4-1 and Figure 4-4, it was relatively consistent across three replicates, since the big jump of percent of control values did not occur near the top. However variation in top among the replicates resulted in the relatively large standard error of the overall mean for top, as seen in Table 4-1, Table 4-3, and Figure 4-4 for the top parameter.

Table 4-3 shows that for bottom parameter, the replicate-to-replicate variation was zero and the individual replicate within-replicate variances for three replicates were within the same order of magnitude. For top parameter, the replicate-to-replicate variation was more than three times the individual replicate within-replicate variances for replicates 1, 2, and 3. For $\log_{10}IC_{50}$, the replicate-to-replicate variation and the individual replicate within-replicate variances for replicates 2 and 3 were zero or close to zero. The majority variation for $\log_{10}IC_{50}$ was from replicate 1's within-replicate variance. For slope, replicate 1's within-replicate variance was extremely large and the replicate-to-replicate variation and the individual replicate within-replicate variances for replicates 2 and 3 were all close to zero.

Chemical V (dibenz[a,h]anthracene): Response Curve Analysis

This is a non-inhibitor chemical. All responses were above 90% of control for replicates 1 and 2. Except for one response which was below 80% of control (75%), all other responses were above 90% of control for replicate 3. Response curve analysis was not performed for this chemical since its parameters were not estimable. Average percent of control of the three repetitions within each replicate are displayed in Figure 5-1. The percent of control values of the three repetitions for each of the three replicates are plotted in Figures 5-2, 5-3, and 5-4 and the values are shown in Tables A-1e.

Chemical VI (fenarimol): Response Curve Analysis

Figures 6-1 and 6-2 indicate that all three replicates are in line. Table 6-1 and Figure 6-3 show that the bottom parameter estimates were between 0% and 1% for all three replicates. Table 6-1 and Figure 6-4 show that the top parameter estimates were

close to 100 for all three replicates, with replicates 1 and 3 higher than the average and replicate 2 lower than the average. As shown in Table 6-1 and in Figure 6-5, parameter estimates for $\log_{10}IC_{50}$ were consistent across three replicates. For slope, the estimates were close to -1 for all three replicates, as shown in Table 6-1 and Figure 6-6.

Table 6-3 shows that for bottom parameter, the replicate-to-replicate variation was zero and the individual replicate within-replicate variances for three replicates were close to each other. For top parameter, the replicate-to-replicate variation was more than two times the individual replicate within-replicate variances for replicates 1, 2, and 3. For $\log_{10}IC_{50}$ and slope, the replicate-to-replicate variations and the individual replicate within-replicate variances for replicates 1, 2 and 3 were zero or close to zero, with the replicate to replicate variation for slope about the same size as the within replicate variation.

Chemical VII (econazole): Response Curve Analysis

Figure 7-1 and Table A-1g show that all the replicate 1 responses were below 60%. Replicates 2 and 3's fitted response curves were more in line with each other. For bottom parameter, the estimates were pretty consistent across three replicates, as seen in Figure 7-3 and Table 7-2. Econazole is a very strong inhibitor. There are no top plateaus in Figure 7-1 and very long bottom plateaus (total inhibition) for concentrations greater than 10^{-7}. Table 7-1 and Figure 7-4 show a large standard error for the overall mean for top parameter. This is due to replicate 1's relatively low percent of control values at low $\log_{10}$ concentrations (-10, -9, and -8). There is large variability among replicates. Note that for replicate 1 the "IC50" concentration in fact corresponds to approximately 28% inhibition, since the top plateau is at about 56%. The slope estimates were all close to the average for three replicates, as shown in Figure 7-6 and Table 7-1.

Table 7-3 shows that the replicate-to-replicate variation was zero for the bottom parameter and that the highest variation was from the individual replicate within-replicate variance for replicate 2. The replicate-to-replicate variation accounted for nearly all the variation for the top parameter. For top parameter, the replicate-to-replicate variation was hundreds times the individual replicate within-replicate variances. For slope, the replicate-to-replicate variation was consistent with the individual replicate within-replicate variances for replicates 1, 2 and 3, and was close to zero. For $\log_{10}IC_{50}$ the replicate to replicate variation was large relative to the within replicate variation.

Chemical VIII (chrysin): Response Curve Analysis

Figures 8-1, 8-2, and Table A-1h show that the low threshold of responses was in the range of 17% to 22%. The response curves fitted to three replicates were in line with each other, as seen in Figures 8-1 and 8-2. The bottom parameter was generally consistent among replicates. Figure 8-4 shows for the top parameter, the estimates were close to the average for three replicates. All three replicates' $\log_{10}IC_{50}$ estimates were close to the average, as seen in Figure 8-5 and Table 8-1. The slope estimate showed some variation among replicates. This is seen in Figure 8-6 and Table 8-1.

Table 8-3 shows that outlying values in replicate 2 (log_{10} concentration = -7, 98.23%; log_{10} concentration = -9, 76.98%) and in replicate 3 (log_{10} concentration = -7, 56.70%; log_{10} concentration = -8, 88.10%) increased the within replicate variability of the bottom and top estimates as compared to replicate 1. For bottom parameter, the replicate-to-replicate variation was zero; the individual replicate within-replicate variance for replicate 3 was three times the individual replicate within-replicate variance for replicate 2 and ten times the individual replicate within-replicate variance for replicate 1. For the top parameter, the replicate-to-replicate variation was comparable to or smaller than the within-replicate variances for replicates 2 and 3. For $log_{10}IC_{50}$, the replicate-to-replicate variation was zero and the individual replicate within-replicate variances for replicates 1, 2 and 3 were close to zero. For slope the replicate to replicate variation was comparable to the within replicate variation.

Chemical IX (dicofol): Response Curve Analysis

Figure 9-1 shows that while replicates 1 and 2's fitted response curves were in line with each other, replicate 3's fitted response curve's slope was steeper. This is due to higher percent of control responses at log_{10} concentration = -5 in replicate 3, as shown in Figure 9-2 and Table A-1i. It also increases the bottom and $log_{10}IC_{50}$ estimates and decreases the slope estimate for replicate 3, thereby increasing the standard errors of the overall means, as seen in Figures 9-3, 9-5, and 9-6 and Table 9-1. Table 9-1 and Figure 9-4 show that the top estimates were consistent and close to the average across three replicates.

Table 9-3 shows that the replicate-to-replicate variations for the top and bottom parameters are zero. The replicate-to-replicate variation was larger than the within-replicate variances for $log_{10}IC_{50}$. For slope, the replicate-to-replicate variation was seven time the individual replicate within-replicate variance for replicate 3 and an order of magnitude greater than the within replicate variances for replicate 1 and 2.

Chemical X (atrazine): Response Curve Analysis

This is a non-inhibitor chemical. All responses were above 80% of control for replicates 1 and 3. All responses were above 75% of control for replicate 2. Response curve analysis was not performed for this chemical since its parameters were not estimable. Averages percent of control of the three repetitions within each replicate are displayed in Figure 10-1. The percent of control values of the three repetitions for each of the three replicates are plotted in Figures 10-2, 10-3, and 10-4 and the values are shown in Tables A-1j.

Analysis of Full Enzyme Activity, Background Activity, Positive, and Negative Controls Across Test substances and Replicates Within Test Substances

The control data were combined across the 30 replicates among the ten test

substances. The control responses were expressed as percent of control. The full enzyme activity, background activity, positive, and negative control responses for each replicate are displayed in Tables A-2a through A-2j for Chemicals I through X, respectively. The percent of control data are plotted by replicate in Figures 11a, 11b, 12a, 12b, 13a, 13b, 14a, and 14b with plotting symbol distinguishing between beginning and end of the replicate. Figures 15a, 15b, 16a, 16b, 17a, 17b, 18a, and 18b show the differences between the averages at the beginning and at the end within each replicate (end minus beginning). In the horizontal axis of these figures, "I-1" stands for replicate 1 of Chemical I, "V-2" stands for replicate 2 of Chemical V, and so on. The chemicals in these figures were grouped by the laboratory that prepared the microsomes. Figures 11a, 12a, 13a, 14a, 15a, 16a, 17a, and 18a are for chemicals whose microsomal preparations were made at Battelle; Figures 11b, 12b, 13b, 14b, 15b, 16b, 17b, and 18b are for chemicals whose microsomal preparations were made at IVT. For the same figure number, "a" plots and "b" plots have the same vertical scale.

Mixed effects analysis of variance models were fitted to the full enzyme activity, background activity, positive, and negative controls percent of control data. The fixed effect factors in the analysis of variance were test substance, portion (beginning or end), and portion by test substance interaction. The random effects were replicate nested within test substance and portion by replicate within test substance interaction. The residual error variation corresponds to variation among repetitions within test substance, replicate, and portion. For the background and full enzyme activity control responses the component of variation due to replicate is constrained to be 0 by the definitions of these control responses. The analysis results for the four types of controls are displayed in Table 11. The left panel of the table displays the results of the tests for the differences between the responses collected at the beginning and at the end of a replicate. The right panel displays the estimated variance components across replicate within test substances and across portion by replicate interaction within test substances. The chemicals were grouped by the laboratory that prepared the microsomes.

The source of microsomes main effect, chemical main effect, and chemical by portion interaction effect are not significant for any of the four controls and are not included in Table 11.

Figures 11a, 11b, 14a, and 14b show that the control responses were nearly always lower at the end of a replicate than at the beginning for the full enzyme activity and negative controls. This is reflected in the curves in Figures 15a, 15b, 18a, and 18b always falling below zero and in the significant portion effects for the full enzyme activity and negative control activity in Table 11 ($p=0.0014$; 0.0053). Positive control activity also shows a significant portion effect ($p=0.0263$), with the end lower than the beginning.

Figure 11a show that the average levels and the variability about the average levels were consistent across all the replicates for the full enzyme activity for test substances whose microsomes were made at Battelle. The test conditions appear to have been consistent among the test substances and the replicates within test substances for the

full enzyme activity for test substances whose microsomes were made at Battelle. Figure 11b (for test substances whose microsomes were made at IVT) shows that except for the first replicate for test substance VII, the average levels and the variability about the average levels were also consistent across all the replicates for the full enzyme activity. The first replicate for test substance VII had much higher full enzyme activity at the beginning and much lower full enzyme activity at the end than other replicates. This also results in high replicate by portion interaction effect variance, as shown in Table 11. Figures 11a and 11b show that test substances prepared at IVT had greater variability between the beginning and the end for the full enzyme activity of the replicates than test substances prepared at Battelle.

Figures 12a and 16a (for test substances whose microsomes were made at Battelle) show that Chemical I had very little variability between the beginning and the end; and the differences between the beginning and the end were essentially zero for all three replicates for background activity. Replicates 2 and 3 for test substance V and replicate 2 for test substance X, at both the beginning and the end, had great variability for the background control activity. Figures 12b and 16b (for test substances whose microsomes were made at IVT) show that Chemical VI had very little variability between the beginning and the end for all three replicates for background activity. The three replicates for test substance IV show greater variability for the background control activity than the other replicates. The variances for replicate effect and replicate by portion interaction effect were all zero, except for Chemical V's replicate by portion interaction, whose variance was also small.

Figure 13a shows that positive controls had upward trend with chemical number/replicate number for chemicals whose microsomal preparations were made at Battelle. The differences between the beginning and the end of the replicates were small for the positive control activity, as seen in Figure 17a. In Figure 13b, the majority positive controls were between 40% and 50% for chemicals whose microsomal preparations were made at IVT. Replicates 2 and 3 for test substance III and replicate 1 for test substance IV, both at the beginning and the end, had positive controls over 50%. Replicate 1 for test substance VII had positive controls at the beginning portion over 60% and was under 40% at the end portion. The big difference between the beginning and the end for the positive controls for replicate 1 for test substance VII is shown in Figure 17b. This big difference also results in large positive controls variability in replicate by portion interaction effect for Chemical VII, as seen in Table 11. Table 11 also shows that relatively large replicate-to-replicate variations in Chemicals X and III.

For the negative controls, Figure 14a shows that except for one activity value, all other activity values were between 85% and 105% for chemicals whose microsomal preparations were made at Battelle. The outlying negative control was at the end portion for replicate 2 for test substance X. It was below 50%. The relatively big difference between the beginning and the end for replicate 2 for test substance X is seen in Figure 18a. This also results in large variability in replicate effect and in replicate by portion interaction effect for Chemical X, as shown in Table 11 for negative controls. For chemicals whose microsomal preparations were made at IVT, Figure 14b shows that the

majority negative controls were between 80% and 110%. Outlying negative controls were found in replicate 1 for test substance VII. They were over 130% at the beginning and under 62% at the end. The big difference between the beginning and the end for the negative controls for replicate 1 for test substance VII is shown in Figure 18b. This big difference also results in large negative controls variability in replicate by portion interaction effect for Chemical VII, as seen in Table 11.

In general, except for a few replicates in some test substances (for example, replicate 1 for test substance VII), the test system appears to be consistent across all the replicates within all the chemicals. However there was consistent decrease in control activity between the beginning and the end of nearly all the test replicates, which was significant for the full enzyme activity, the negative, and the positive control responses. This can result in potential bias or potential loss of precision due to decrease of aromatase activity within replicates. If the inhibition concentration tubes are tested in order of inhibitor concentration, the decrease in aromatase activity across the replicate can accentuate the apparent inhibition if the test substance concentrations are varied from low to high or can lessen the apparent inhibition if the test substance concentrations are varied from high to low. If the 24 tubes (8 inhibitor concentrations × 3 repetitions per concentration) were tested in randomized order then the decrease in aromatase activity within the replicate would increase the residual variation about the fitted concentration response curves for each replicate but would avoid resulting in biased response curves.

Table 1-1. Reference Chemical I: Aminoglutethimide.
Microsomes Prepared by Battelle.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-29.540 (86.820)	99.040 (0.397)	-3.975 (0.642)	1.060E-04 (4.386)	-0.899 (0.242)	C
2	1.114 (1.386)	93.810 (0.737)	-4.148 (0.017)	7.114E-05 (1.040)	-1.073 (0.044)	C
3	-1.797 (1.116)	97.330 (0.609)	-4.181 (0.013)	6.592E-05 (1.031)	-0.970 (0.029)	C
Mean [c]	-0.466 (1.449)	96.774 (1.532)	-4.166 (0.016)	6.821E-05 (1.038)	-1.011 (0.048)	C
Average Values [b]						
1	-30.110 (125.400)	99.040 (0.567)	-3.971 (0.923)	1.070E-04 (8.368)	-0.897 (0.345)	C
2	1.116 (1.553)	93.810 (0.826)	-4.148 (0.019)	7.114E-05 (1.045)	-1.073 (0.049)	C
3	-1.800 (1.161)	97.320 (0.633)	-4.181 (0.014)	6.595E-05 (1.032)	-0.970 (0.030)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 1-2. Reference Chemical I: Aminoglutethimide.
Microsomes Prepared by Battelle.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay

Parameter	Estimate (95% CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	-29.540 (210.643,151.563)	1.114 (-1.777,4.005)	-1.797 (-4.125,0.531)	-0.466 (-3.489,2.557)
Top	99.040 (98.212,99.868)	93.810 (92.272,95.348)	97.330 (96.060,98.600)	96.774 (90.124,103.424)
$Log_{10}IC_{50}$	-3.975 (-5.314,-2.636)	-4.148 (-4.184,-4.112)	-4.181 (-4.209,-4.153)	-4.166 (-4.200,-4.132)
Slope	-0.899 (-1.404,-0.394)	-1.073 (-1.165,-0.981)	-0.970 (-1.031,-0.909)	-1.011 (-1.113,-0.910)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.

b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 1-3. Reference Chemical I: Aminoglutethimide.
Microsomes Prepared by Battelle
Variances Associated with Estimated Parameters of Concentration Response Curves. Percent of Control Activity.
Placental Aromatase Assay

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	7537.71240 /df=20	1.92100 /df=20	1.24546 /df=20	2.64671 /df=2 (p=0.329)	2.10089 /df=20.058
Top	0.15745 /df=20	0.54376 /df=20	0.37076 /df=20	6.68344 /df=2 (p=0.172)	2.34573 /df=1.981
$Log_{10}IC_{50}$	0.41229 /df=20	0.00029 /df=20	0.00017 /df=20	0.00031 /df=2 (p=0.343)	0.00027 /df=20.130
Slope	0.05861 /df=20	0.00193 /df=20	0.00085 /df=20	0.00337 /df=2 (p=0.295)	0.00227 /df=14.718

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \sum(S_r^2 + S_i^2))^2/(var(S_r^2)+(2/K^2)* \sum (Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 2-1. Reference Chemical II: Ketoconazole.
Microsomes Prepared by Battelle.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-0.430 (0.858)	100.300 (0.611)	-6.371 (0.017)	4.258E-07 (1.041)	-0.938 (0.034)	C
2	-0.507 (0.472)	98.310 (0.428)	-6.325 (0.008)	4.733E-07 (1.020)	-0.980 (0.021)	C
3	-0.092 (1.526)	95.030 (1.307)	-6.225 (0.028)	5.961E-07 (1.066)	-1.009 (0.071)	C
Mean [c]	-0.462 (0.399)	98.069 (1.442)	-6.310 (0.041)	4.901E-07 (1.100)	-0.971 (0.017)	C
Average Values [b]						
1	-0.436 (1.192)	100.300 (0.849)	-6.371 (0.024)	4.259E-07 (1.057)	-0.938 (0.047)	C
2	-3.573 (12.750)	100.000 (10.590)	-6.286 (0.236)	5.172E-07 (1.722)	-0.748 (0.358)	C
3	-3.294 (12.370)	96.010 (9.237)	-6.159 (0.236)	6.933E-07 (1.721)	-0.784 (0.364)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 2-2. Reference Chemical II: Ketoconazole.
Microsomes Prepared by Battelle.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay

Parameter	Estimate (95% CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	-0.430 (-2.219,1.360)	-0.507 (-1.491,0.477)	-0.092 (-3.275,3.091)	-0.462 (-1.271,0.347)
Top	100.300 (99.026,101.574)	98.310 (97.417,99.203)	95.030 (92.304,97.756)	98.069 (91.092,105.046)
$Log_{10}IC_{50}$	-6.371 (-6.407,-6.335)	-6.325 (-6.343,-6.307)	-6.225 (-6.283,-6.167)	-6.310 (-6.503,-6.117)
Slope	-0.938 (-1.009,-0.868)	-0.980 (-1.023,-0.937)	-1.009 (-1.157,-0.861)	-0.971 (-1.006,-0.936)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.

b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 2-3. Reference Chemical II: Ketoconazole.
Microsomes Prepared by Battelle.
Variances Associated with Estimated Parameters of Concentration Response Curves. Percent of Control Activity.
Placental Aromatase Assay

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	0.73599 /df=20	0.22241 /df=20	2.32868 /df=20	0.00000 /df=2 (p=NA)	0.15912 /df=35.933
Top	0.37295 /df=20	0.18344 /df=20	1.70825 /df=20	5.54756 /df=2 (p=0.203)	2.07808 /df=1.785
$Log_{10}IC_{50}$	0.00030 /df=20	0.00007 /df=20	0.00078 /df=20	0.00477 /df=2 (p=0.187)	0.00171 /df=1.849
Slope	0.00113 /df=20	0.00042 /df=20	0.00505 /df=20	0.00000 /df=2 (p=NA)	0.00029 /df=32.373

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \sum(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \sum (Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 3-1. Reference Chemical III: Prochloraz.
Microsomes Prepared by IVT.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	0.138 (0.402)	97.270 (0.419)	-7.662 (0.013)	2.176E-08 (1.031)	-0.985 (0.024)	C
2	-0.418 (0.503)	99.280 (0.523)	-7.580 (0.011)	2.629E-08 (1.025)	-0.939 (0.022)	C
3	-0.324 (0.729)	97.480 (0.700)	-7.487 (0.016)	3.260E-08 (1.037)	-0.964 (0.032)	C
Mean [c]	-0.117 (0.288)	98.015 (0.656)	-7.577 (0.050)	2.651E-08 (1.123)	-0.961 (0.016)	C
Average Values [b]						
1	0.136 (0.576)	97.270 (0.602)	-7.662 (0.019)	2.176E-08 (1.045)	-0.985 (0.035)	C
2	-0.418 (0.710)	99.280 (0.738)	-7.580 (0.015)	2.629E-08 (1.036)	-0.939 (0.031)	C
3	-0.317 (0.331)	97.480 (0.318)	-7.487 (0.007)	3.259E-08 (1.016)	-0.964 (0.015)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 3-2. Reference Chemical III: Prochloraz.
Microsomes Prepared by IVT.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay

Parameter	Estimate (95% CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	0.138 (-0.700,0.976)	-0.418 (-1.467,0.631)	-0.324 (-1.844,1.197)	-0.117 (-0.697,0.463)
Top	97.270 (96.395,98.145)	99.280 (98.190,100.370)	97.480 (96.020,98.940)	98.015 (95.376,100.654)
$Log_{10}IC_{50}$	-7.662 (-7.690,-7.634)	-7.580 (-7.603,-7.557)	-7.487 (-7.519,-7.455)	-7.577 (-7.795,-7.358)
Slope	-0.985 (-1.036,-0.935)	-0.939 (-0.984,-0.893)	-0.964 (-1.032,-0.897)	-0.961 (-1.013,-0.909)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.

b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 3-3. Reference Chemical III: Prochloraz.
Microsomes Prepared by IVT.
Variances Associated with Estimated Parameters of Concentration Response Curves. Percent of Control Activity.
Placental Aromatase Assay

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	0.16144 /df=20	0.25291 /df=20	0.53130 /df=20	0.00000 /df=2 (p=NA)	0.08312 /df=48.039
Top	0.17581 /df=20	0.27311 /df=20	0.48986 /df=20	0.98939 /df=2 (p=0.215)	0.42986 /df=2.151
$Log_{10}IC_{50}$	0.00017 /df=20	0.00012 /df=20	0.00024 /df=20	0.00742 /df=2 (p=0.165)	0.00253 /df=1.983
Slope	0.00058 /df=20	0.00047 /df=20	0.00105 /df=20	0.00014 /df=2 (p=0.421)	0.00026 /df=2.900

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \sum(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \sum(Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 4-1. Reference Chemical IV: 4-Nonylphenol.
Microsomes Prepared by IVT.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	0.559 (3.187)	96.880 (1.421)	-4.228 (0.266)	5.911E-05 (1.846)	-3.042 (3.520)	C
2	2.043 (1.802)	93.580 (1.114)	-4.571 (0.021)	2.684E-05 (1.050)	-1.766 (0.130)	C
3	1.341 (2.095)	98.950 (1.239)	-4.553 (0.024)	2.798E-05 (1.057)	-1.558 (0.117)	C
Mean [c]	1.560 (1.256)	96.423 (1.608)	-4.562 (0.016)	2.741E-05 (1.037)	-1.655 (0.104)	C
Average Values [b]						
1	0.563 (5.862)	96.880 (2.614)	-4.228 (0.493)	5.915E-05 (3.112)	-3.046 (6.540)	C
2	2.044 (3.608)	93.580 (2.230)	-4.571 (0.042)	2.684E-05 (1.102)	-1.766 (0.260)	C
3	1.345 (4.196)	98.950 (2.482)	-4.553 (0.048)	2.798E-05 (1.118)	-1.558 (0.234)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 4-2. Reference Chemical IV: 4-Nonylphenol.
Microsomes Prepared by IVT.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay

Parameter	Estimate (95% CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	0.559 (-6.089,7.207)	2.043 (-1.716,5.802)	1.341 (-3.029,5.711)	1.560 (-0.965,4.086)
Top	96.880 (93.916,99.844)	93.580 (91.256,95.904)	98.950 (96.365,101.535)	96.423 (89.851,102.996)
$Log_{10}IC_{50}$	-4.228 (-4.783,-3.673)	-4.571 (-4.615,-4.527)	-4.553 (-4.603,-4.503)	-4.562 (-4.595,-4.529)
Slope	-3.042 (-10.385,4.301)	-1.766 (-2.037,-1.495)	-1.558 (-1.801,-1.315)	-1.655 (-1.872,-1.439)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

**Table 4-3. Reference Chemical IV: 4-Nonylphenol.
Microsomes Prepared by IVT.
Variances Associated with Estimated Parameters of Concentration Response Curves. Percent of Control Activity.
Placental Aromatase Assay**

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	10.15697 /df=20	3.24720 /df=20	4.38903 /df=20	0.00000 /df=2 (p=NA)	1.57666 /df=47.619
Top	2.01924 /df=20	1.24100 /df=20	1.53512 /df=20	6.17469 /df=2 (p=0.207)	2.58668 /df=2.115
$Log_{10}IC_{50}$	0.07086 /df=20	0.00044 /df=20	0.00058 /df=20	0.00000 /df=2 (p=NA)	0.00025 /df=20.581
Slope	12.39040 /df=20	0.01682 /df=20	0.01362 /df=20	0.00640 /df=2 (p=0.417)	0.01074 /df=20.150

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \sum(S_r^2 + S_i^2))^2/(var(S_r^2)+(2/K^2)* \sum(Si^4/df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 5-1. Reference Chemical V: Dibenz[a, h]anthracene. Microsomes Prepared by Battelle.

Only one data point was below 80% (but above 74%) of control value for this chemical. This is a non-inhibition chemical. Parameters were not estimable.
See Figures 5-1 to 5-4 for scatter data plots and Table A-1e for data values.

Table 6-1. Reference Chemical VI: Fenarimol.
Microsomes Prepared by IVT.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	0.668 (1.031)	99.200 (0.604)	-5.224 (0.023)	5.966E-06 (1.055)	-1.005 (0.049)	C
2	0.229 (0.923)	97.270 (0.604)	-5.218 (0.019)	6.056E-06 (1.046)	-0.936 (0.036)	C
3	0.763 (0.895)	99.130 (0.612)	-5.214 (0.018)	6.113E-06 (1.042)	-1.039 (0.040)	C
Mean [c]	0.550 (0.545)	98.532 (0.633)	-5.218 (0.011)	6.056E-06 (1.027)	-0.990 (0.033)	C
Average Values [b]						
1	0.660 (1.373)	99.200 (0.804)	-5.224 (0.031)	5.968E-06 (1.074)	-1.004 (0.065)	C
2	0.234 (0.964)	97.270 (0.631)	-5.218 (0.020)	6.055E-06 (1.048)	-0.936 (0.037)	C
3	0.767 (0.879)	99.130 (0.601)	-5.214 (0.018)	6.113E-06 (1.041)	-1.040 (0.039)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 6-2. Reference Chemical VI: Fenarimol.
Microsomes Prepared by IVT.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay

Parameter	Estimate (95% CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	0.668 (-1.483,2.819)	0.229 (-1.698,2.155)	0.763 (-1.104,2.629)	0.550 (-0.541,1.641)
Top	99.200 (97.940,100.460)	97.270 (96.009,98.531)	99.130 (97.854,100.406)	98.532 (95.808,101.256)
$Log_{10}IC_{50}$	-5.224 (-5.273,-5.175)	-5.218 (-5.258,-5.178)	-5.214 (-5.251,-5.177)	-5.218 (-5.241,-5.195)
Slope	-1.005 (-1.106,-0.904)	-0.936 (-1.011,-0.861)	-1.039 (-1.123,-0.955)	-0.990 (-1.113,-0.868)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.

b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 6-3. Reference Chemical VI: Fenarimol.
Microsomes Prepared by IVT.
Variances Associated with Estimated Parameters of Concentration Response Curves. Percent of Control Activity.
Placental Aromatase Assay

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	1.06296 /df=20	0.85267 /df=20	0.80067 /df=20	0.00000 /df=2 (p=NA)	0.29740 /df=59.073
Top	0.36482 /df=20	0.36518 /df=20	0.37418 /df=20	0.83340 /df=2 (p=0.244)	0.40048 /df=1.999
$Log_{10}IC_{50}$	0.00055 /df=20	0.00037 /df=20	0.00032 /df=20	0.00000/ df=2 (p=NA)	0.00013 /df=56.908
Slope	0.00235 /df=20	0.00128 /df=20	0.00160 /df=20	0.00158 /df=2 (p=0.301)	0.00109 /df=2.378

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \sum(S_r^2 + S_i^2))^2/(var(S_r^2)+(2/K^2)* \sum(Si^4/df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 7-1. Reference Chemical VII: Econazole.
Microsomes Prepared by IVT.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-0.374 (0.402)	56.780 (1.155)	-8.531 (0.036)	2.942E-09 (1.088)	-1.025 (0.062)	I
2	-0.268 (0.539)	92.380 (1.388)	-8.628 (0.020)	2.352E-09 (1.048)	-1.048 (0.049)	C
3	-0.129 (0.402)	101.400 (1.104)	-8.698 (0.014)	2.005E-09 (1.034)	-1.038 (0.034)	C
Mean [c]	-0.255 (0.252)	83.518 (13.625)	-8.624 (0.047)	2.379E-09 (1.115)	-1.039 (0.026)	C
Average Values [b]						
1	-0.372 (0.054)	56.770 (0.154)	-8.531 (0.005)	2.944E-09 (1.011)	-1.026 (0.008)	I
2	-0.268 (0.116)	92.380 (0.299)	-8.629 (0.004)	2.352E-09 (1.010)	-1.048 (0.010)	C
3	-0.128 (0.262)	101.400 (0.720)	-8.698 (0.009)	2.005E-09 (1.022)	-1.039 (0.022)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 7-2. Reference Chemical VII: Econazole.
Microsomes Prepared by IVT.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay

Parameter	Estimate (95% CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	-0.374 (-1.213,0.466)	-0.268 (-1.393,0.857)	-0.129 (-0.967,0.710)	-0.255 (-0.759,0.249)
Top	56.780 (54.371,59.189)	92.380 (89.485,95.275)	101.400 (99.097,103.703)	83.518 (24.926,142.110)
$Log_{10}IC_{50}$	-8.531 (-8.607,-8.455)	-8.628 (-8.670,-8.586)	-8.698 (-8.728,-8.668)	-8.624 (-8.835,-8.412)
Slope	-1.025 (-1.155,-0.895)	-1.048 (-1.149,-0.947)	-1.038 (-1.110,-0.966)	-1.039 (-1.090,-0.987)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.

b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

**Table 7-3. Reference Chemical VII: Econazole.
Microsomes Prepared by IVT.
Variances Associated with Estimated Parameters of Concentration Response Curves. Percent of Control Activity.
Placental Aromatase Assay**

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	0.16193 /df=20	0.29095 /df=20	0.16160 /df=20	0.00000 /df=2 (p=NA)	0.06329 /df=55.127
Top	1.33403 /df=20	1.92654 /df=20	1.21882 /df=20	555.41526 /df=2 (p=0.159)	185.63607 /df=2.001
$Log_{10}IC_{50}$	0.00133 /df=20	0.00041 /df=20	0.00021 /df=20	0.00605 /df=2 (p=0.188)	0.00222 /df=1.915
Slope	0.00390 /df=20	0.00235 /df=20	0.00118 /df=20	0.00000 /df=2 (p=NA)	0.00065 /df=49.938

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \sum(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \sum(Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 8-1. Reference Chemical VIII: Chrysin.
Microsomes Prepared by IVT.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	21.550 (1.520)	90.290 (0.721)	-5.706 (0.051)	1.967E-06 (1.126)	-1.785 (0.257)	I
2	18.570 (3.176)	94.350 (1.876)	-5.647 (0.053)	2.253E-06 (1.129)	-1.325 (0.195)	I
3	17.370 (5.169)	89.760 (2.807)	-5.620 (0.096)	2.400E-06 (1.248)	-1.075 (0.250)	I
Mean [c]	20.756 (1.325)	91.382 (1.395)	-5.670 (0.034)	2.139E-06 (1.082)	-1.386 (0.191)	I
Average Values [b]						
1	21.540 (1.538)	90.290 (0.729)	-5.706 (0.052)	1.969E-06 (1.127)	-1.784 (0.259)	I
2	18.570 (1.202)	94.350 (0.710)	-5.647 (0.020)	2.253E-06 (1.047)	-1.325 (0.074)	I
3	17.370 (6.293)	89.750 (3.420)	-5.620 (0.117)	2.400E-06 (1.309)	-1.076 (0.306)	I

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 8-2. Reference Chemical VIII: Chrysin.
Microsomes Prepared by IVT.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay

Parameter	Estimate (95% CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	21.550 (18.379,24.721)	18.570 (11.945,25.195)	17.370 (6.588,28.152)	20.756 (18.072,23.441)
Top	90.290 (88.787,91.793)	94.350 (90.437,98.263)	89.760 (83.905,95.615)	91.382 (86.775,95.990)
$Log_{10}IC_{50}$	-5.706 (-5.813,-5.599)	-5.647 (-5.757,-5.537)	-5.620 (-5.821,-5.419)	-5.670 (-5.739,-5.601)
Slope	-1.785 (-2.320,-1.250)	-1.325 (-1.732,-0.918)	-1.075 (-1.597,-0.553)	-1.386 (-2.371,-0.402)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.

b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

**Table 8-3. Reference Chemical VIII: Chrysin.
Microsomes Prepared by IVT.
Variances Associated with Estimated Parameters of Concentration Response Curves. Percent of Control Activity.
Placental Aromatase Assay**

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	2.31040 /df=20	10.08698 /df=20	26.71856 /df=20	0.00000 /df=2 (p=NA)	1.75626 /df=37.275
Top	0.51912 /df=20	3.51938 /df=20	7.87925 /df=20	3.14497 /df=2 (p=0.298)	1.94674 /df=2.818
$Log_{10}IC_{50}$	0.00265 /df=20	0.00277 /df=20	0.00925 /df=20	0.00000 /df=2 (p=NA)	0.00118 /df=42.919
Slope	0.06579 /df=20	0.03806 /df=20	0.06265 /df=20	0.05583 /df=2 (p=0.322)	0.03661 /df=1.693

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \sum(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \sum(Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 9-1. Reference Chemical IX: Dicofol.
Microsomes Prepared by Battelle.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	0.383 (2.418)	98.010 (0.868)	-4.759 (0.049)	1.741E-05 (1.120)	-0.824 (0.065)	C
2	-2.536 (3.025)	98.270 (1.129)	-4.606 (0.050)	2.475E-05 (1.123)	-0.764 (0.061)	C
3	3.244 (2.584)	95.910 (1.263)	-4.519 (0.040)	3.027E-05 (1.095)	-1.408 (0.133)	C
Mean [c]	0.638 (1.525)	97.604 (0.604)	-4.626 (0.071)	2.366E-05 (1.177)	-0.983 (0.198)	C
Average Values [b]						
1	0.384 (2.965)	98.020 (1.064)	-4.759 (0.060)	1.741E-05 (1.149)	-0.823 (0.079)	C
2	-2.539 (4.006)	98.270 (1.494)	-4.606 (0.067)	2.475E-05 (1.166)	-0.764 (0.081)	C
3	3.245 (4.802)	95.910 (2.346)	-4.519 (0.073)	3.027E-05 (1.184)	-1.408 (0.247)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 9-2. Reference Chemical IX: Dicofol.
Microsomes Prepared by Battelle.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay

Parameter	Estimate (95% CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	0.383 (-4.661,5.427)	-2.536 (-8.846,3.774)	3.244 (-2.146,8.634)	0.638 (-2.415,3.690)
Top	98.010 (96.199,99.821)	98.270 (95.915,100.625)	95.910 (93.275,98.545)	97.604 (96.393,98.815)
$Log_{10}IC_{50}$	-4.759 (-4.861,-4.657)	-4.606 (-4.711,-4.501)	-4.519 (-4.601,-4.437)	-4.626 (-4.926,-4.326)
Slope	-0.824 (-0.959,-0.688)	-0.764 (-0.891,-0.636)	-1.408 (-1.685,-1.131)	-0.983 (-1.902,-0.064)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.

b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 9-3. Reference Chemical IX: Dicofol.
Microsomes Prepared by Battelle.
Variances Associated with Estimated Parameters of Concentration Response Curves. Percent of Control Activity.
Placental Aromatase Assay

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	5.84672 /df=20	9.15063 /df=20	6.67706 /df=20	0.00000 /df=2 (p=NA)	2.32512 /df=57.819
Top	0.75342 /df=20	1.27464 /df=20	1.59517 /df=20	0.00000 /df=2 (p=NA)	0.36514 /df=55.428
$Log_{10}IC_{50}$	0.00241 /df=20	0.00253 /df=20	0.00156 /df=20	0.01280 /df=2 (p=0.194)	0.00498 /df=2.028
Slope	0.00420 /df=20	0.00374 /df=20	0.01761 /df=20	0.10895 /df=2 (p=0.186)	0.03904 /df=1.851

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \sum(S_r^2 + S_i^2))^2/(var(S_r^2) + (2/K^2)* \sum(Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 10-1. Reference Chemical X: Atrazine.
Microsomes Prepared by Battelle.

Only a few data points were below 80% (but above 75%) of control value for this chemical. This is a non-inhibition chemical. Parameters were not estimable.
See Figures 10-1 to 10-4 for scatter data plots and Table A-1j for data values.

Table 11. **All Chemicals All Replicates: Variance Components of the Percent of Control Values for Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control. Position Effects and Variation Across Replicates of Portion Effects Within Replicates.**

Parameter	Difference Between Beginning and End Portions (End-Beginning)		Variance Components											
				Chemical										Residual (Repetition)
				Battelle Microsomes					IVT Microsomes					
	Estimate (%) (Std. Error)	p-Value / Degree of Freedom		I	II	V	IX	X	III	IV	VI	VII	VIII	
Full Enzyme Activity Control	-10.444 (1.837)	0.0014 /df=5.81	Replicate	0	0	0	0	0	0	0	0	0	0	15.87
			Replicate * Portion	0	0	0	0	11.05	3.67	0	0.65	411.64	0	
Background Activity Control	0.062 (0.090)	0.4945 /df=61.80	Replicate	0	0	0	0	0	0	0	0	0	0	0.22
			Replicate * Portion	0	0	0.13	0	0	0	0	0	0	0	
Positive Control	-2.233 (0.765)	0.0263 /df=6.07	Replicate	0	13.06	6.56	1.56	37.81	26.73	16.48	0.13	0	2.38	2.33
			Replicate * Portion	0	0	0	0	2.16	4.08	0	0.39	69.47	0	
Negative Control	-8.294 (2.088)	0.0053 /df=7.04	Replicate	5.28	0	0	0	48.13	0	1.82	0	0	17.41	25.74
			Replicate * Portion	0	0	0	0	54.88	0	0	0	470.29	0	

Note: The source of microsomes effect, the chemical main effect and the chemical by portion interaction fixed effect was not significant for all four types of control activity data. These effects were not summarized in this table.

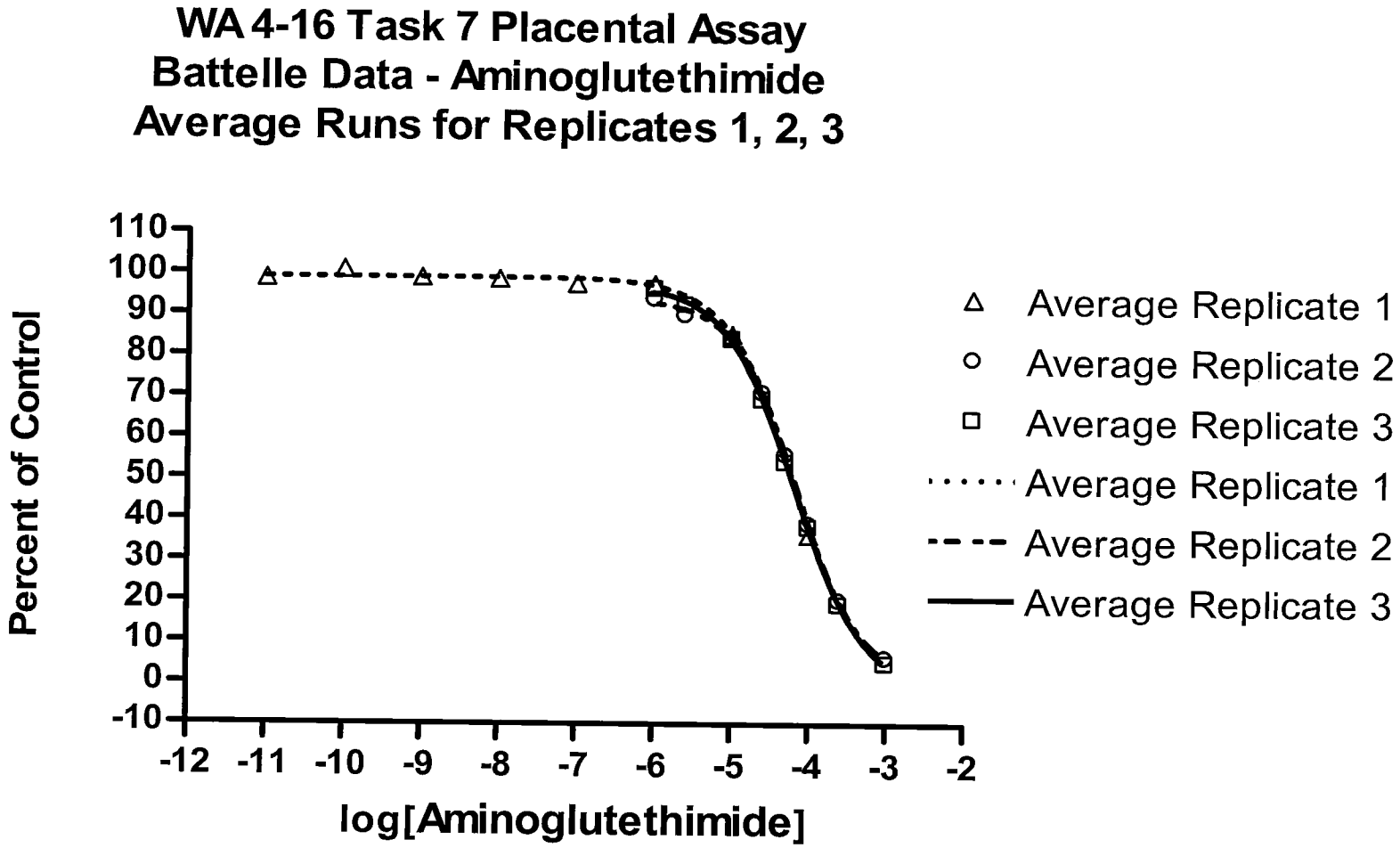


Figure 1-1. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by Battelle. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

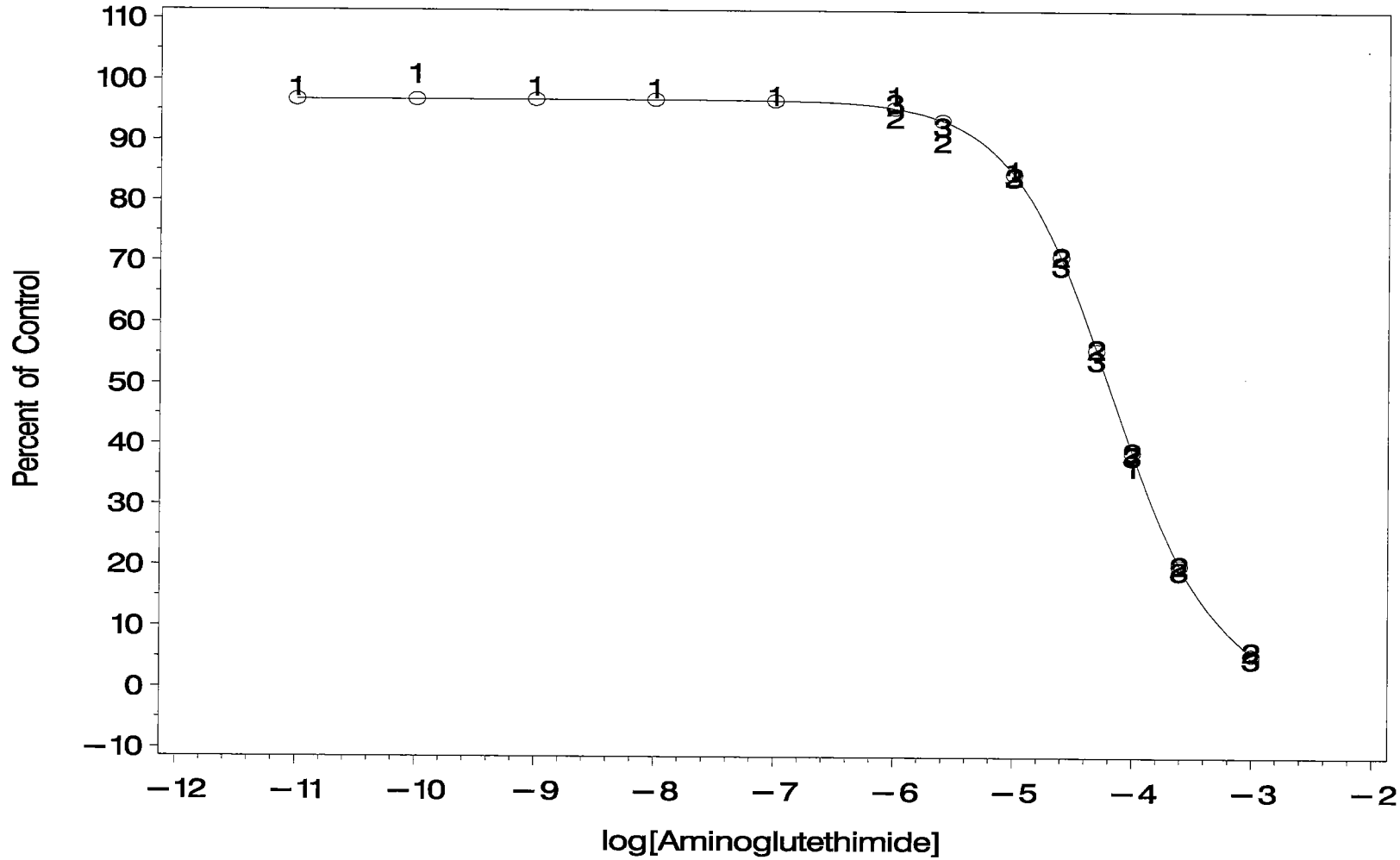


Figure 1-2. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by Battelle. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

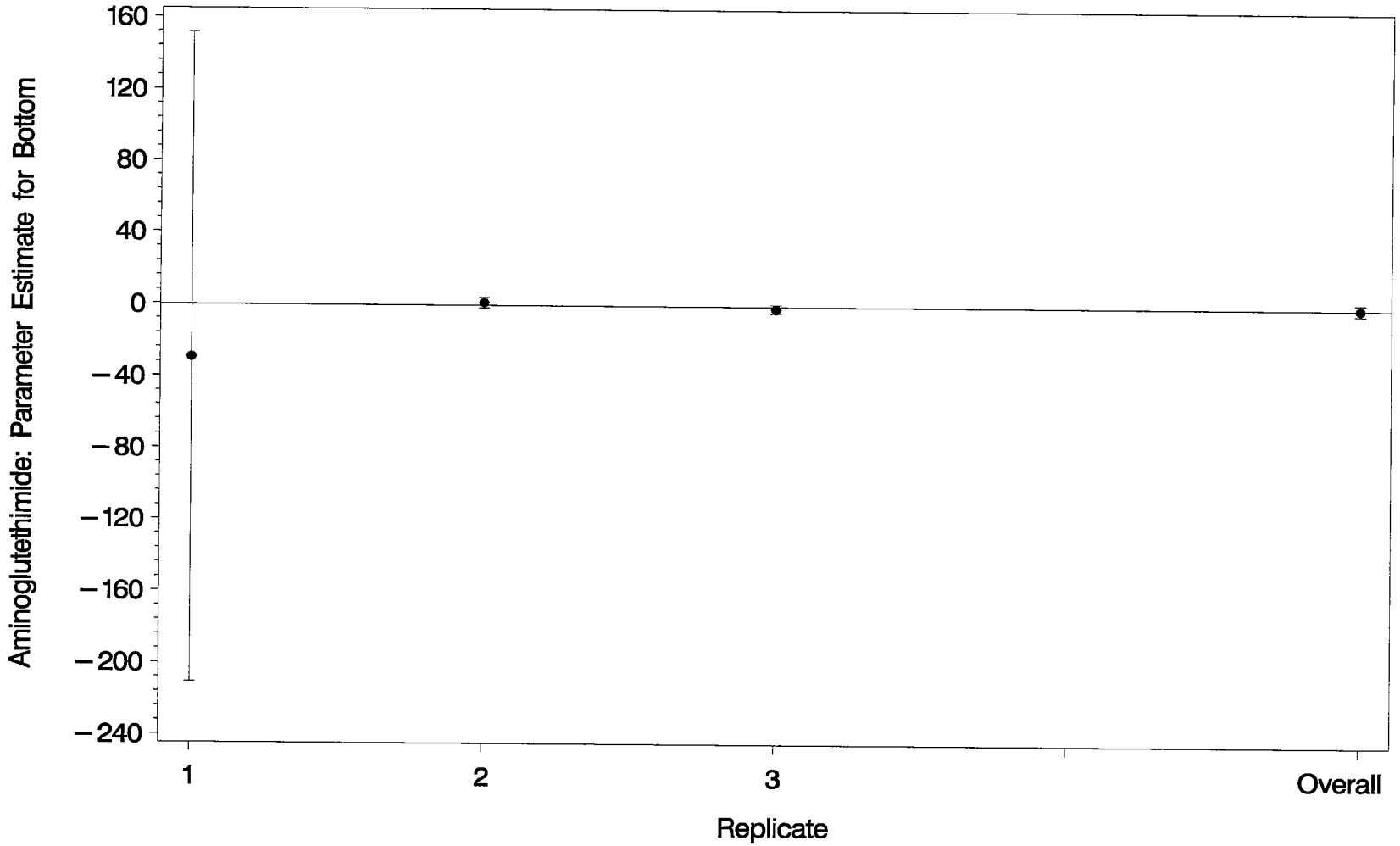


Figure 1-3. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by Battelle. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

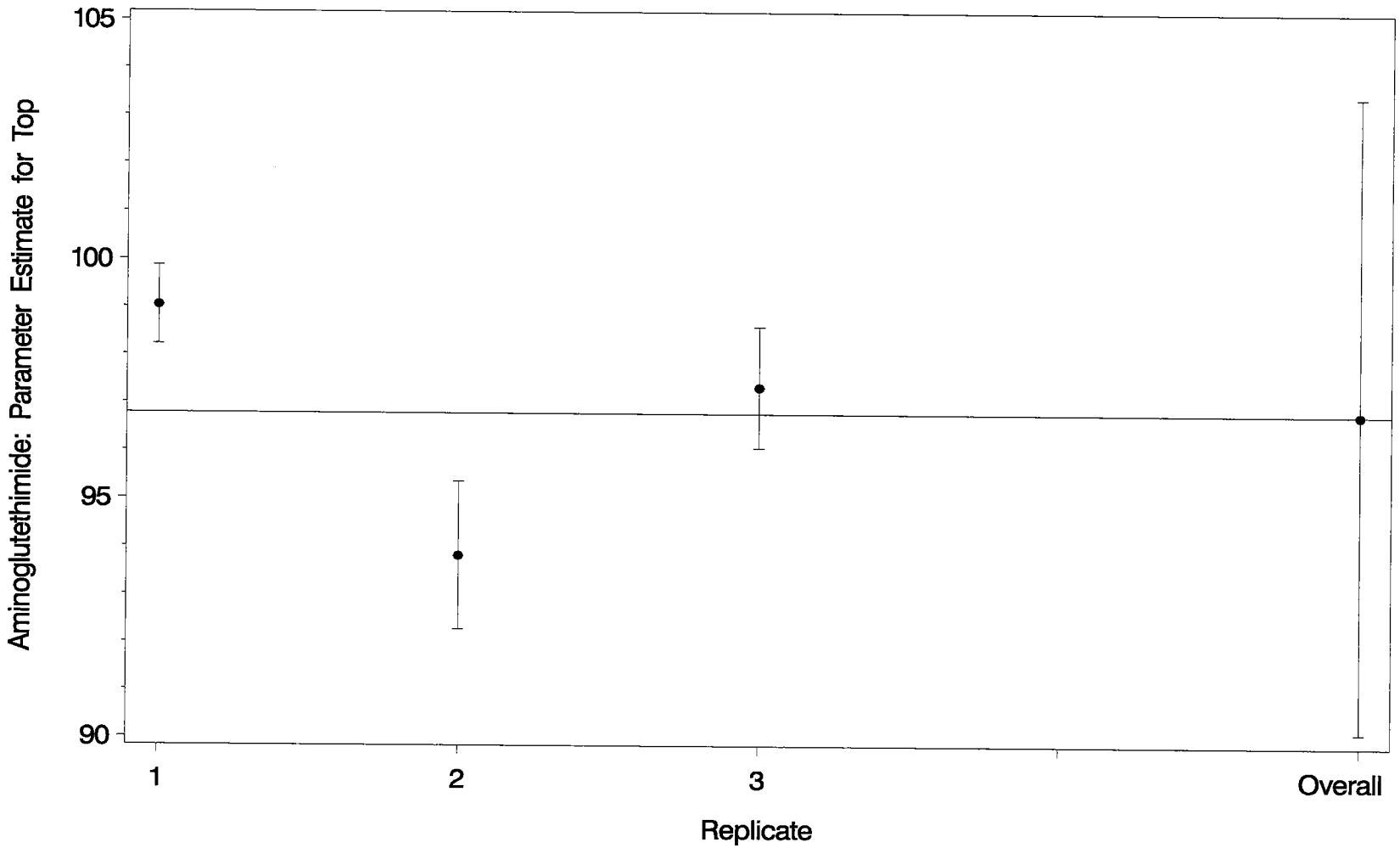


Figure 1-4. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by Battelle. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

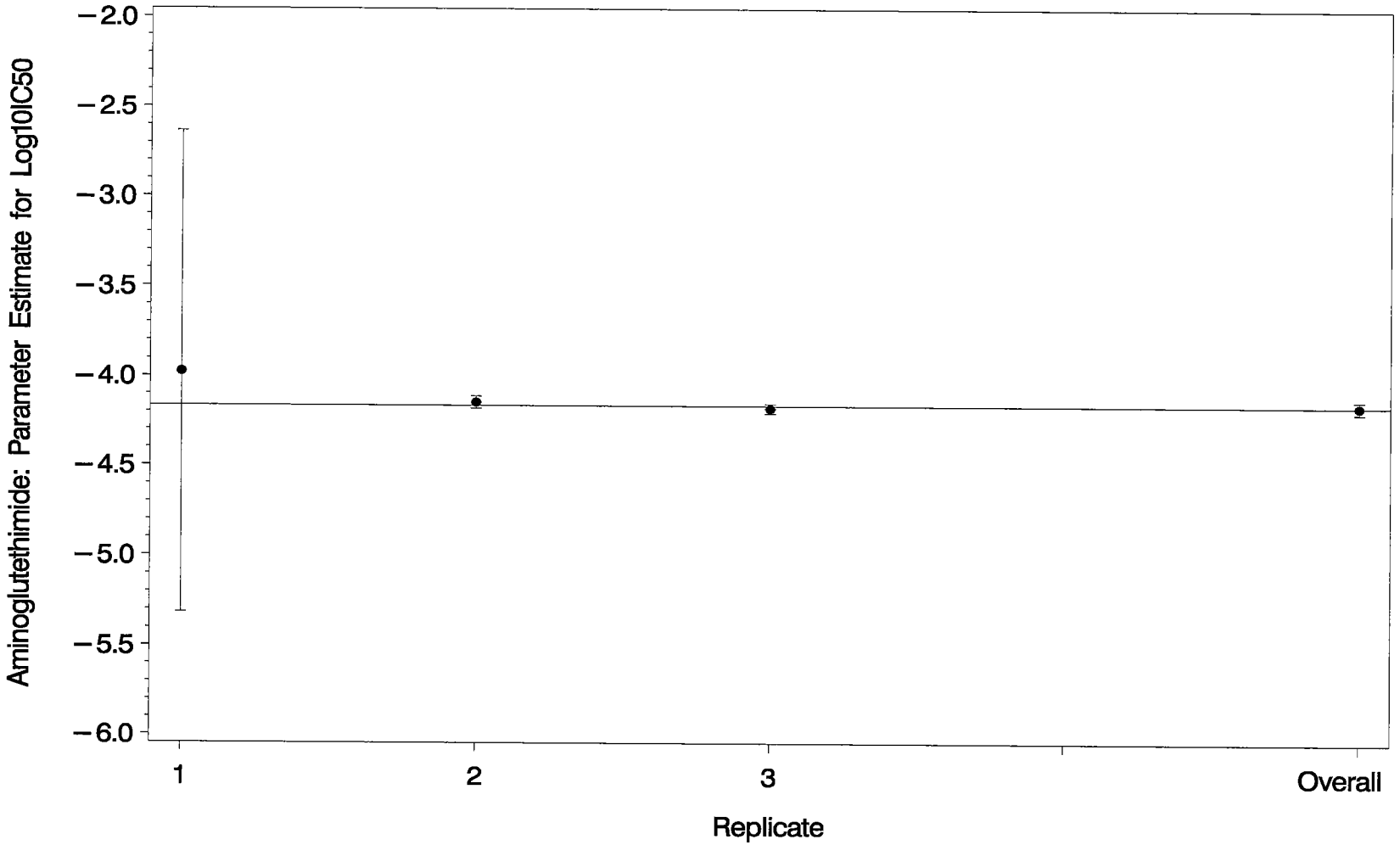


Figure 1-5. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by Battelle. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

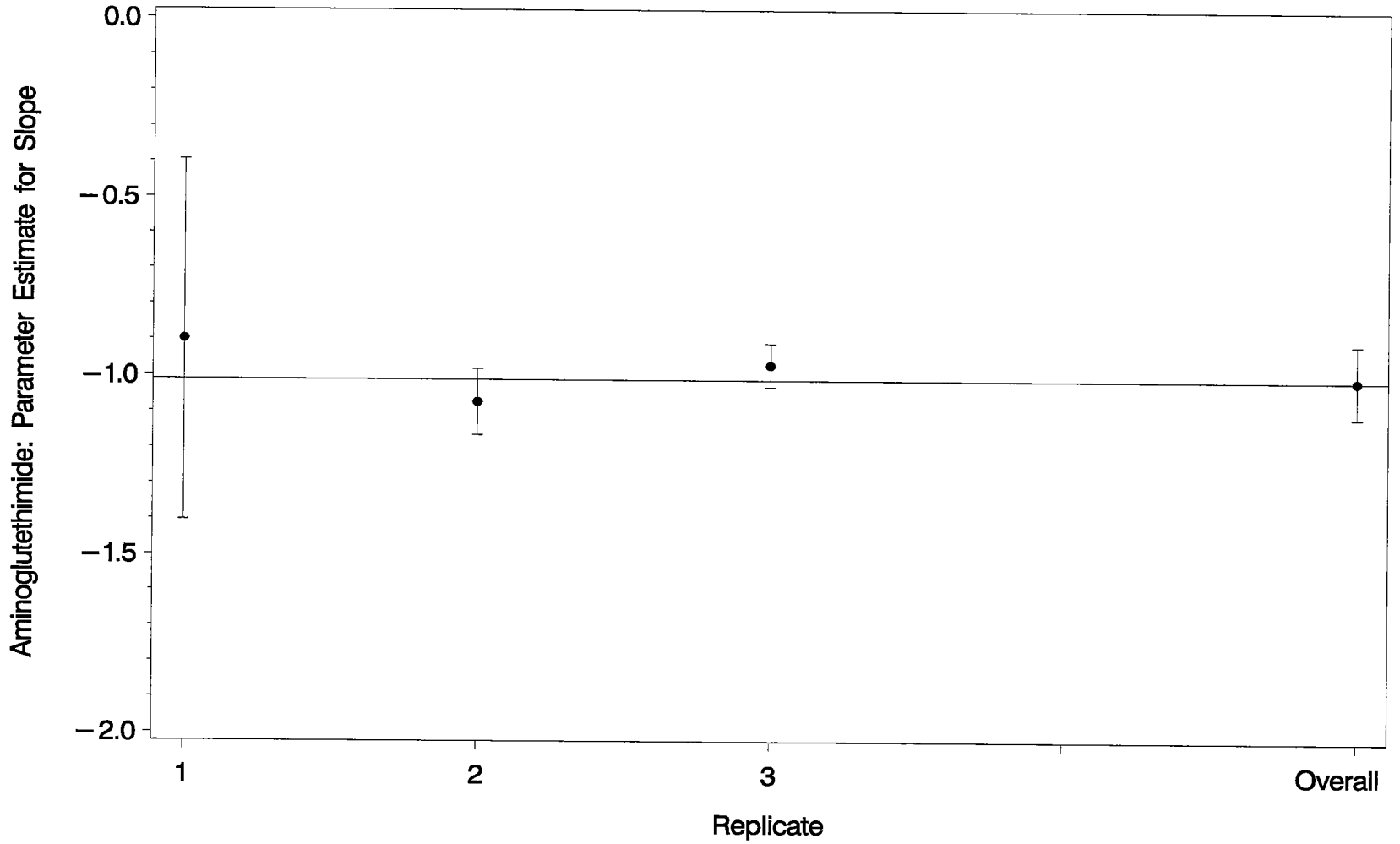


Figure 1-6. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by Battelle. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

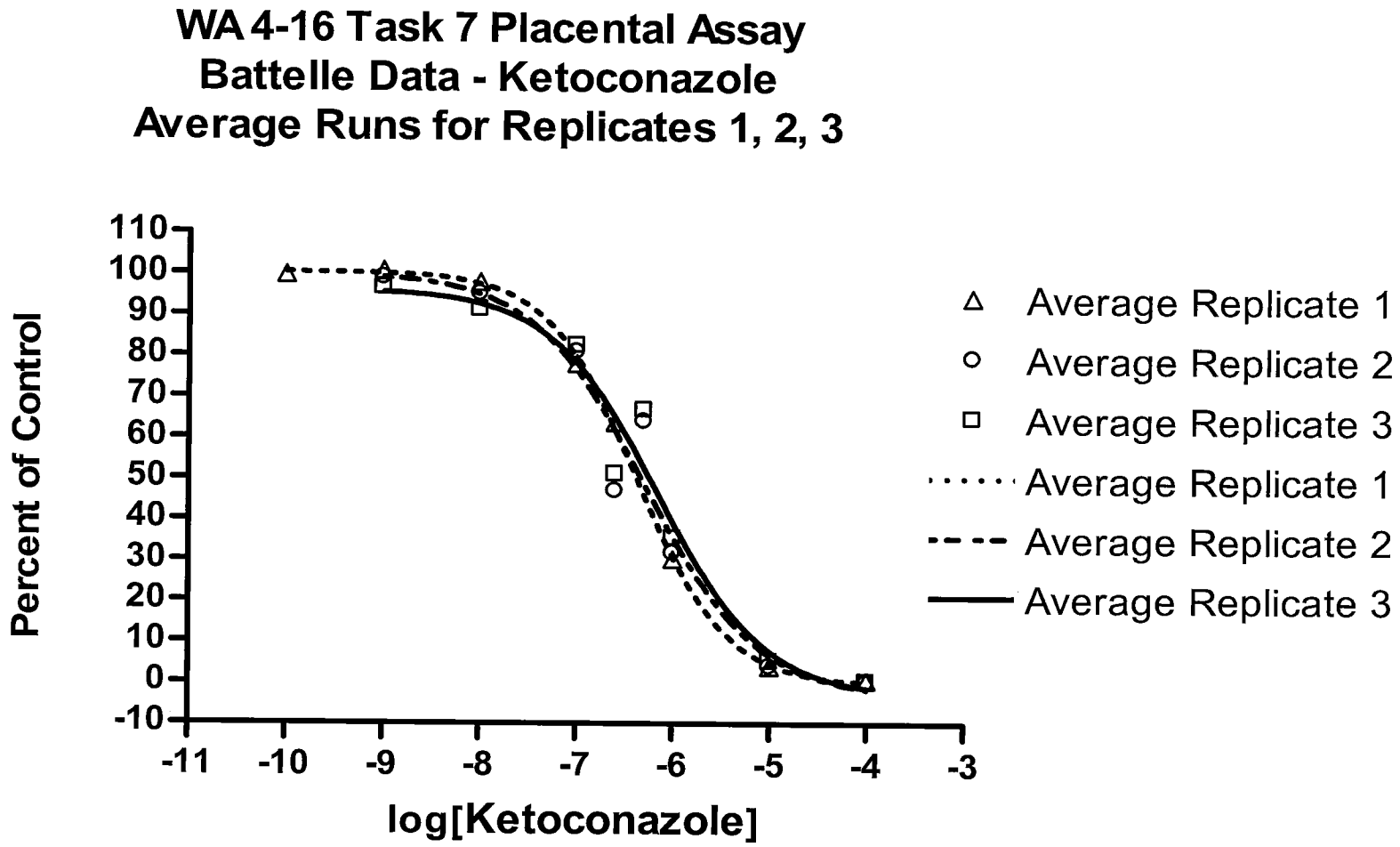


Figure 2-1. **Reference Chemical II: Ketoconazole. Microsomes Prepared by Battelle. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

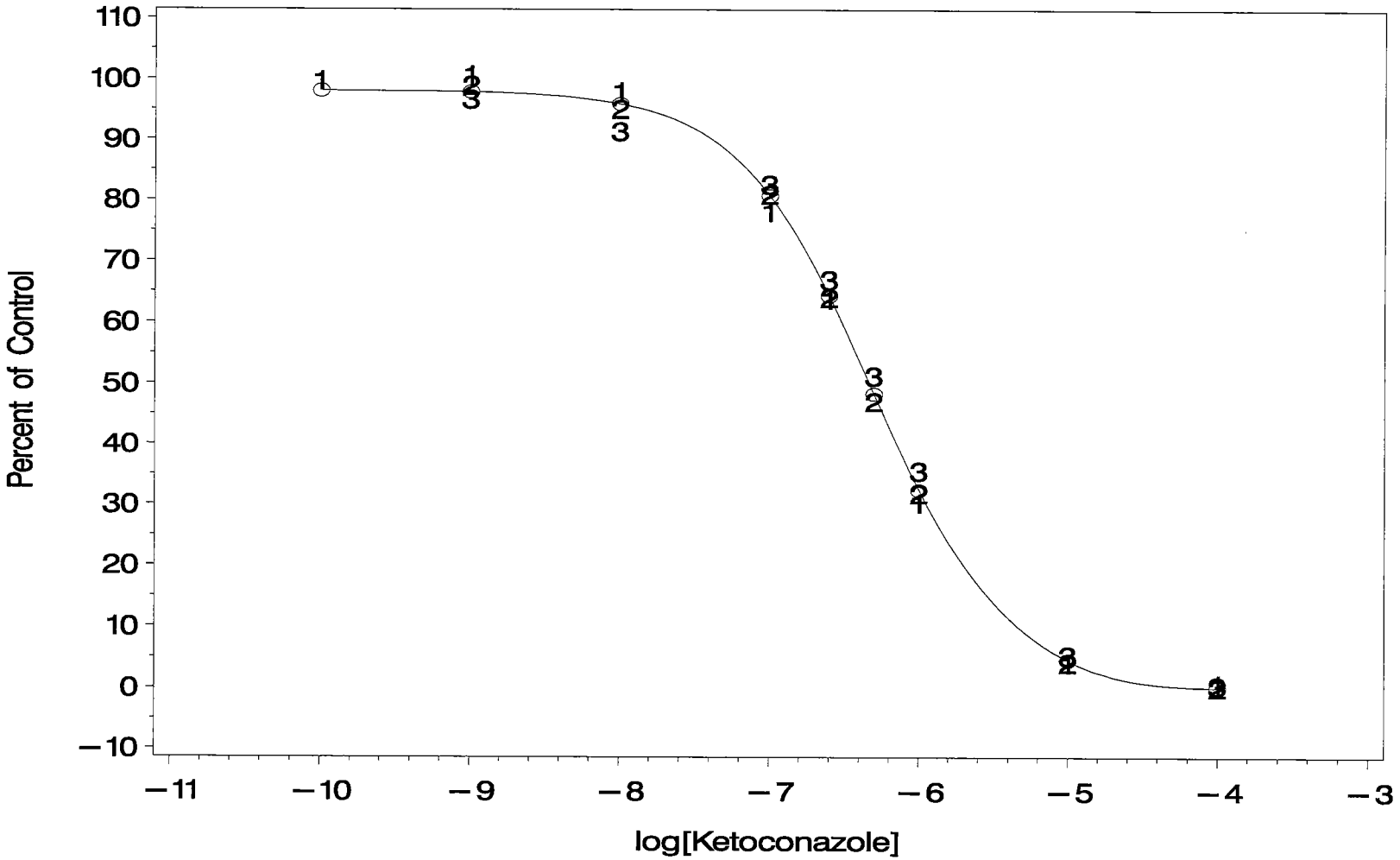


Figure 2-2. **Reference Chemical II: Ketoconazole. Microsomes Prepared by Battelle. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

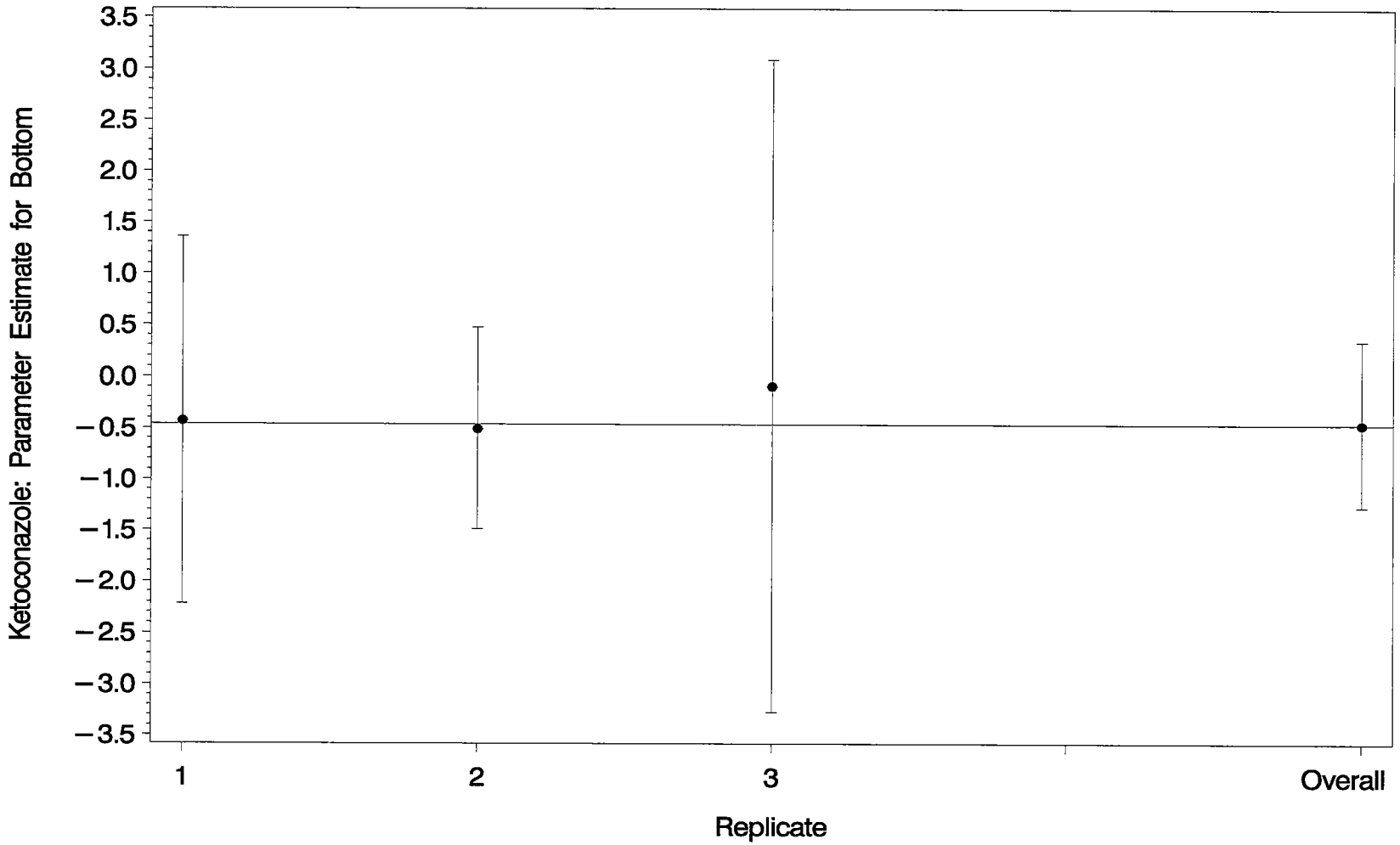


Figure 2-3. Reference Chemical II: Ketoconazole. Microsomes Prepared by Battelle. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

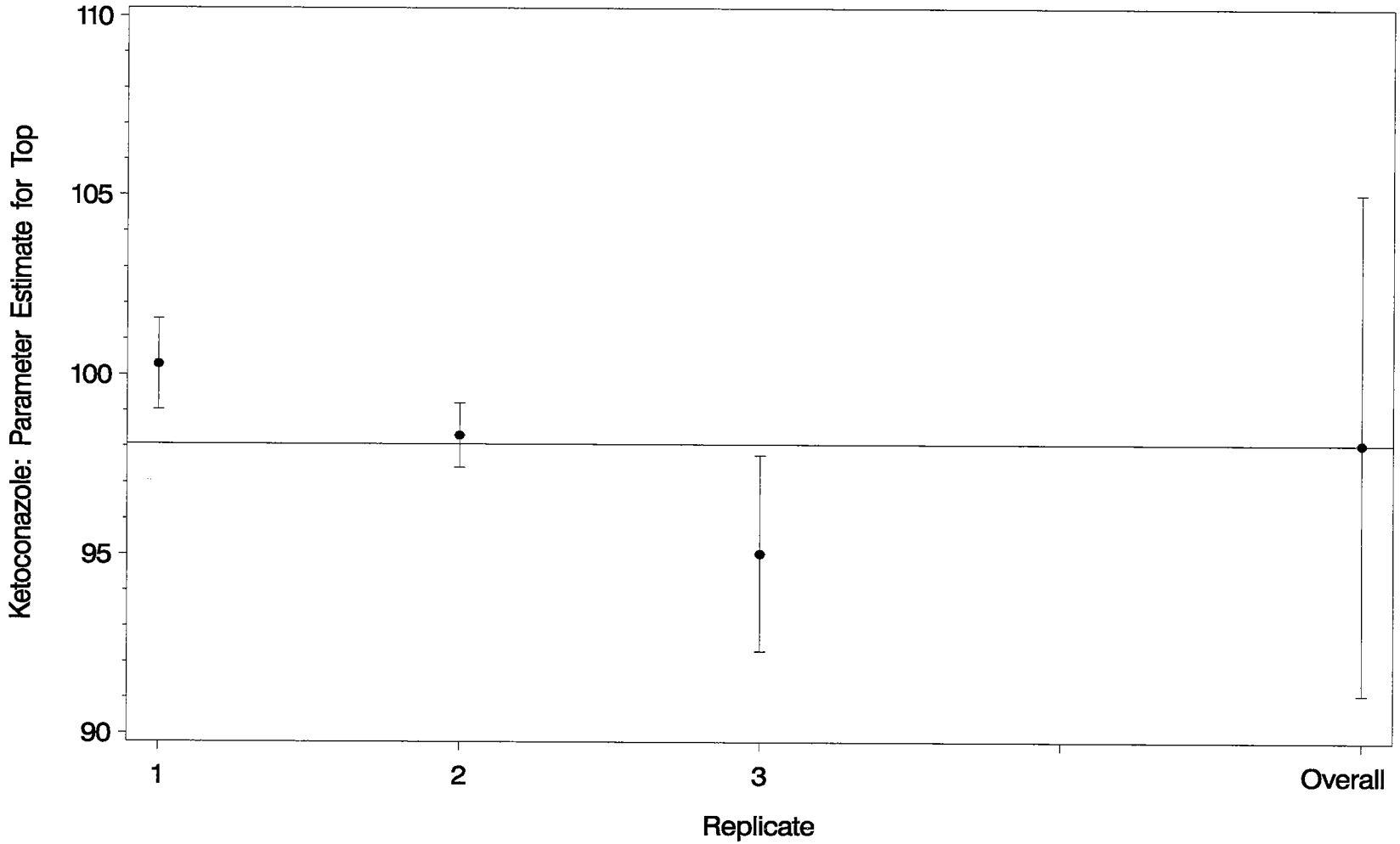


Figure 2-4. **Reference Chemical II: Ketoconazole. Microsomes Prepared by Battelle. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

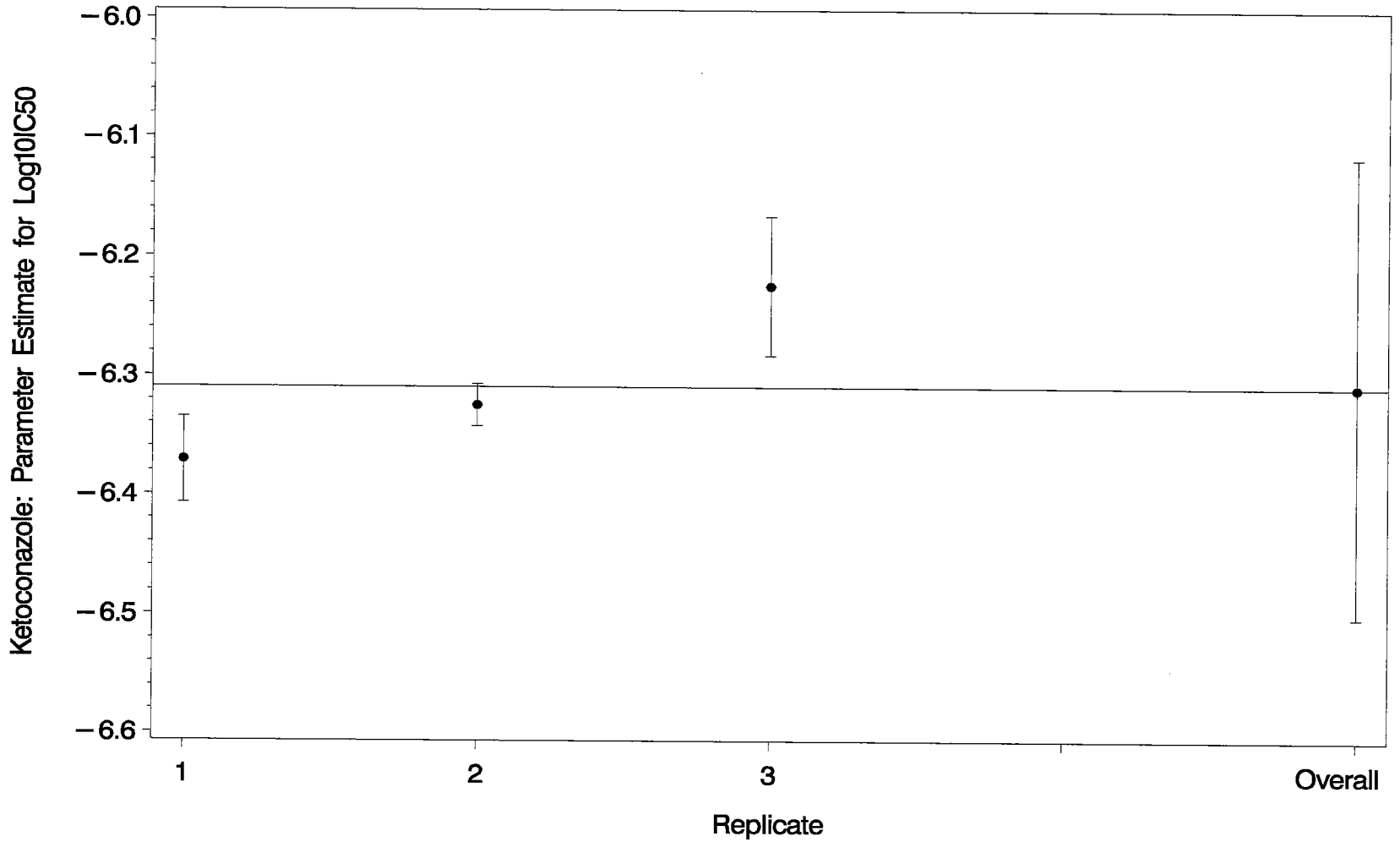


Figure 2-5. **Reference Chemical II: Ketoconazole. Microsomes Prepared by Battelle. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

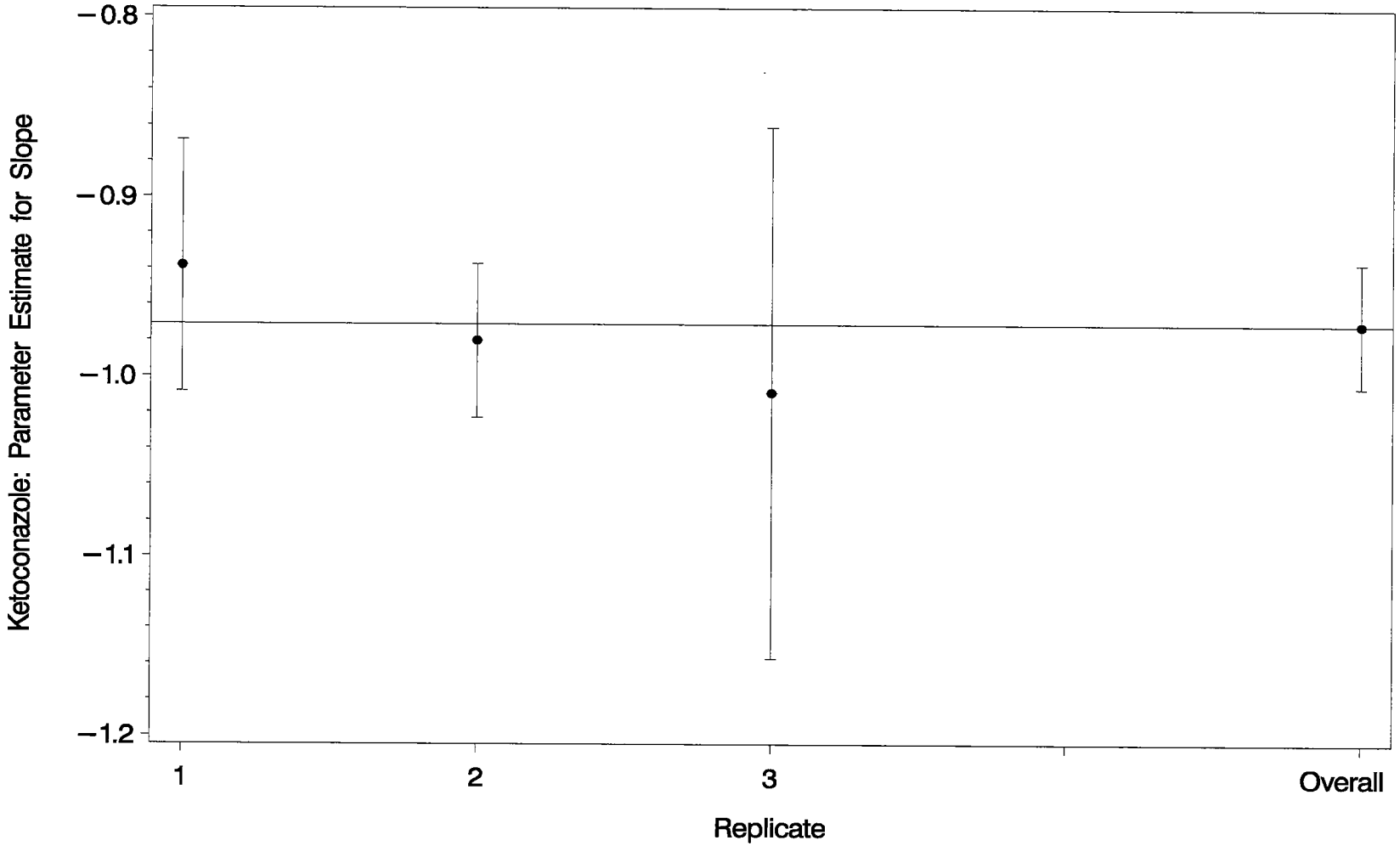


Figure 2-6. **Reference Chemical II: Ketoconazole. Microsomes Prepared by Battelle. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates**

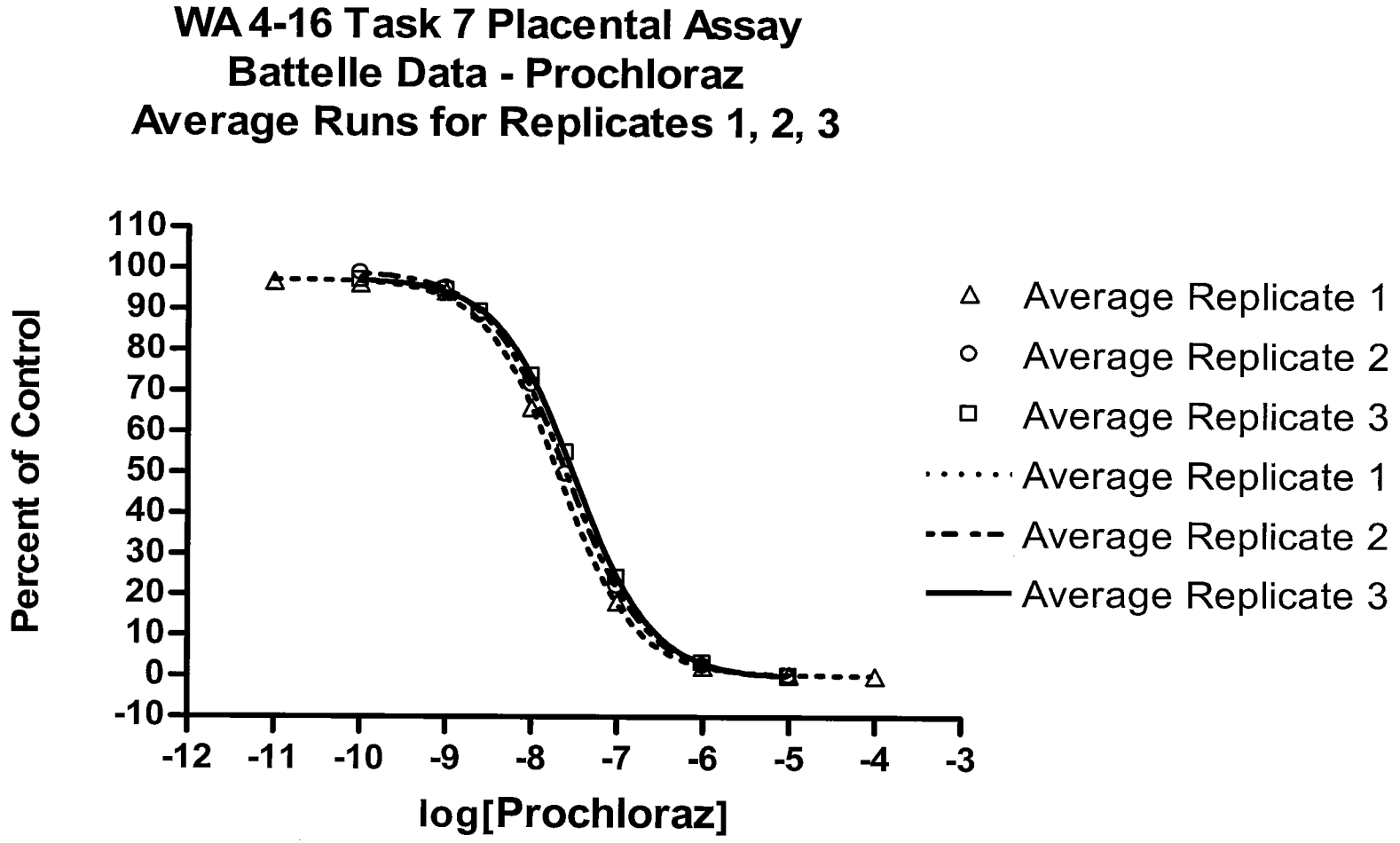


Figure 3-1. **Reference Chemical III: Prochloraz. Microsomes Prepared by IVT. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

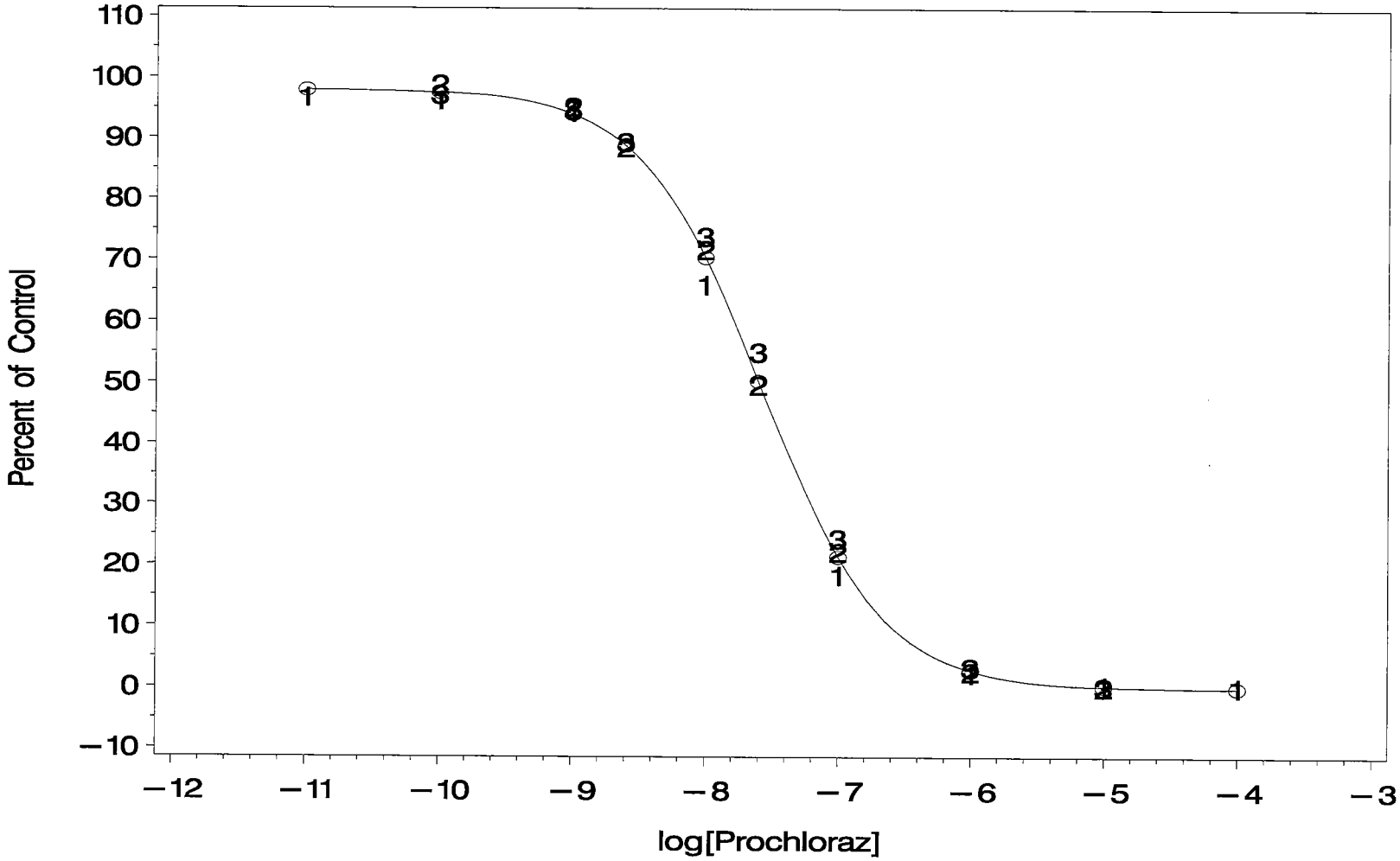


Figure 3-2. **Reference Chemical III: Prochloraz. Microsomes Prepared by IVT. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

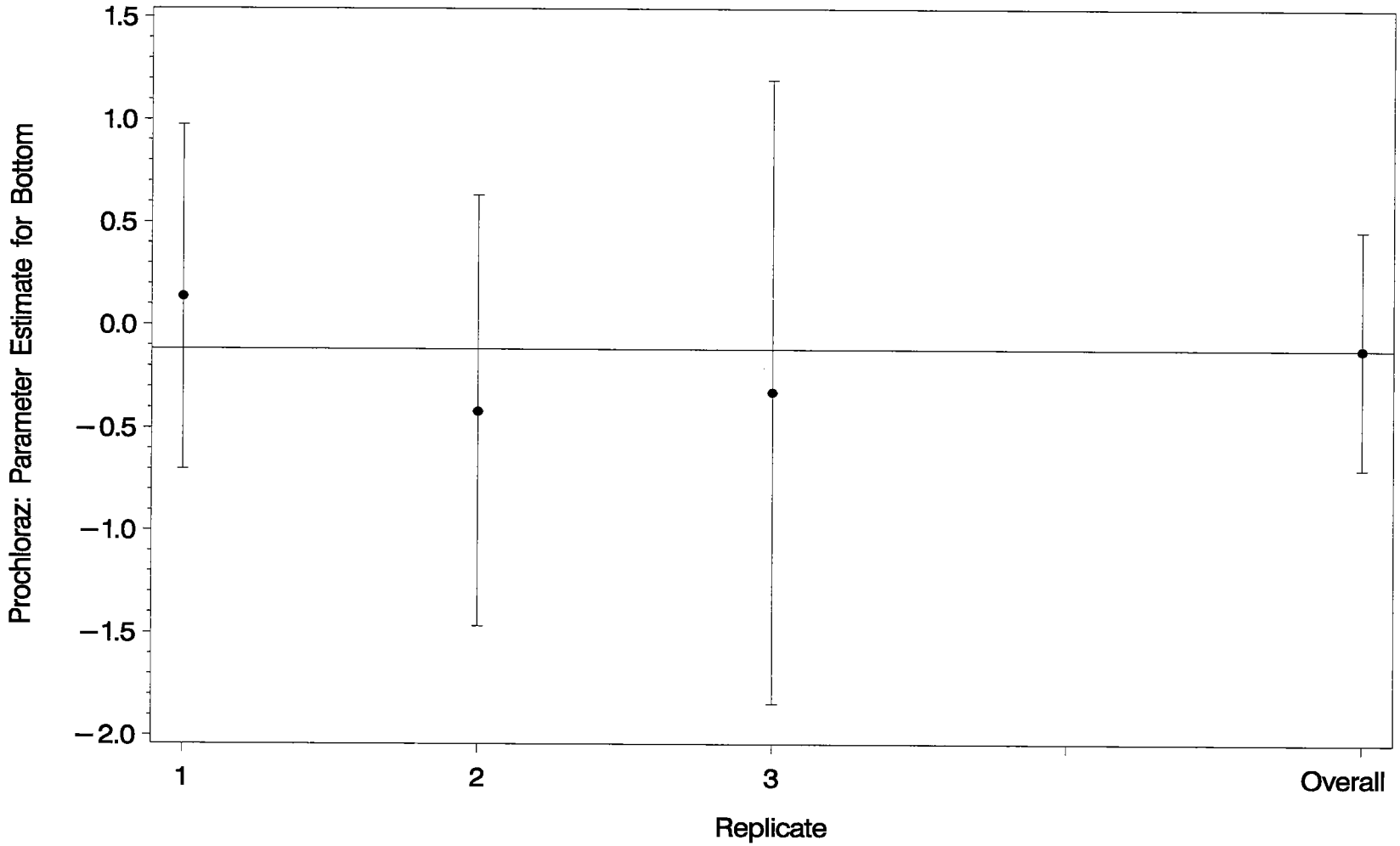


Figure 3-3. Reference Chemical III: Prochloraz. Microsomes Prepared by IVT. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

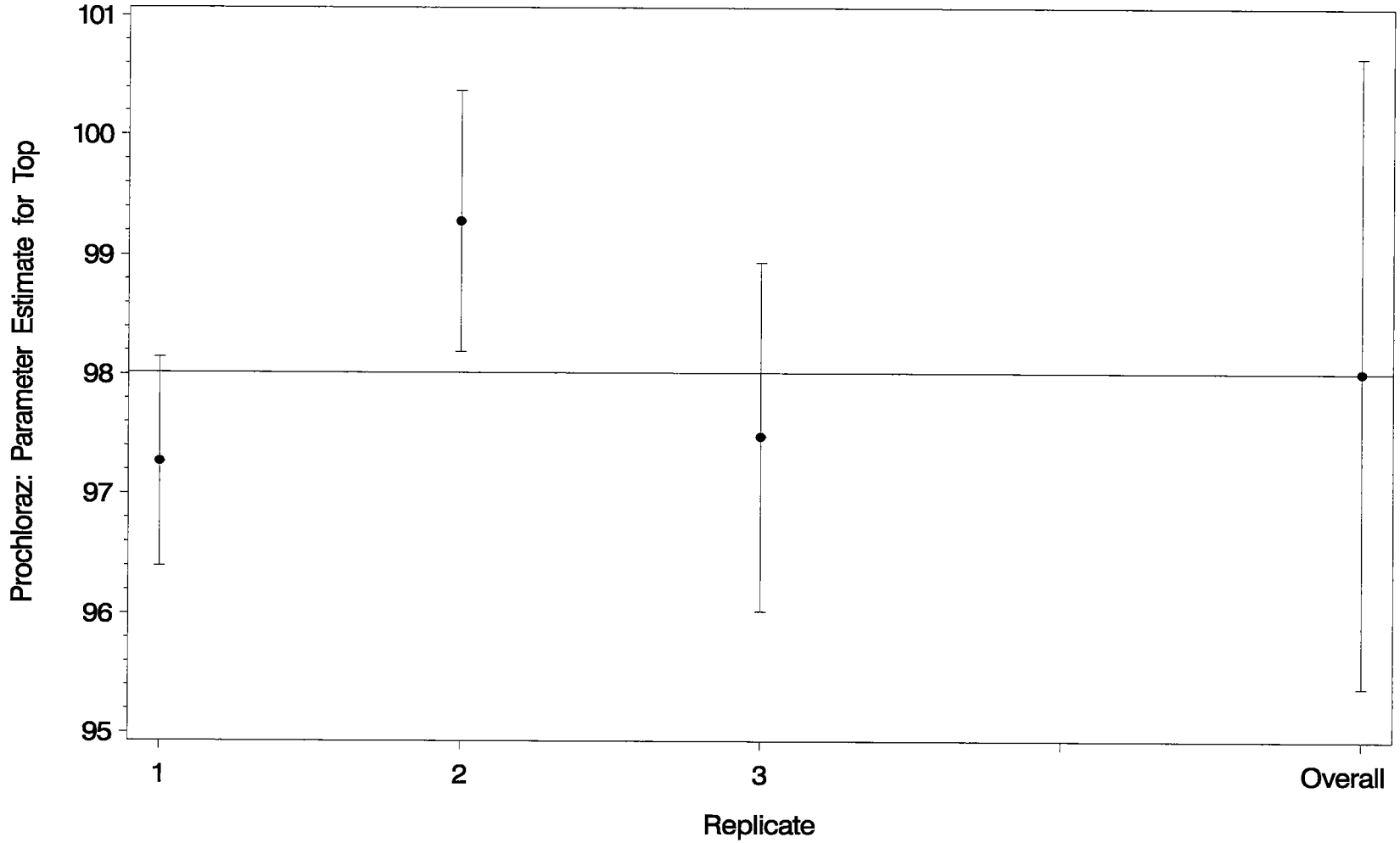


Figure 3-4. **Reference Chemical III: Prochloraz. Microsomes Prepared by IVT. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

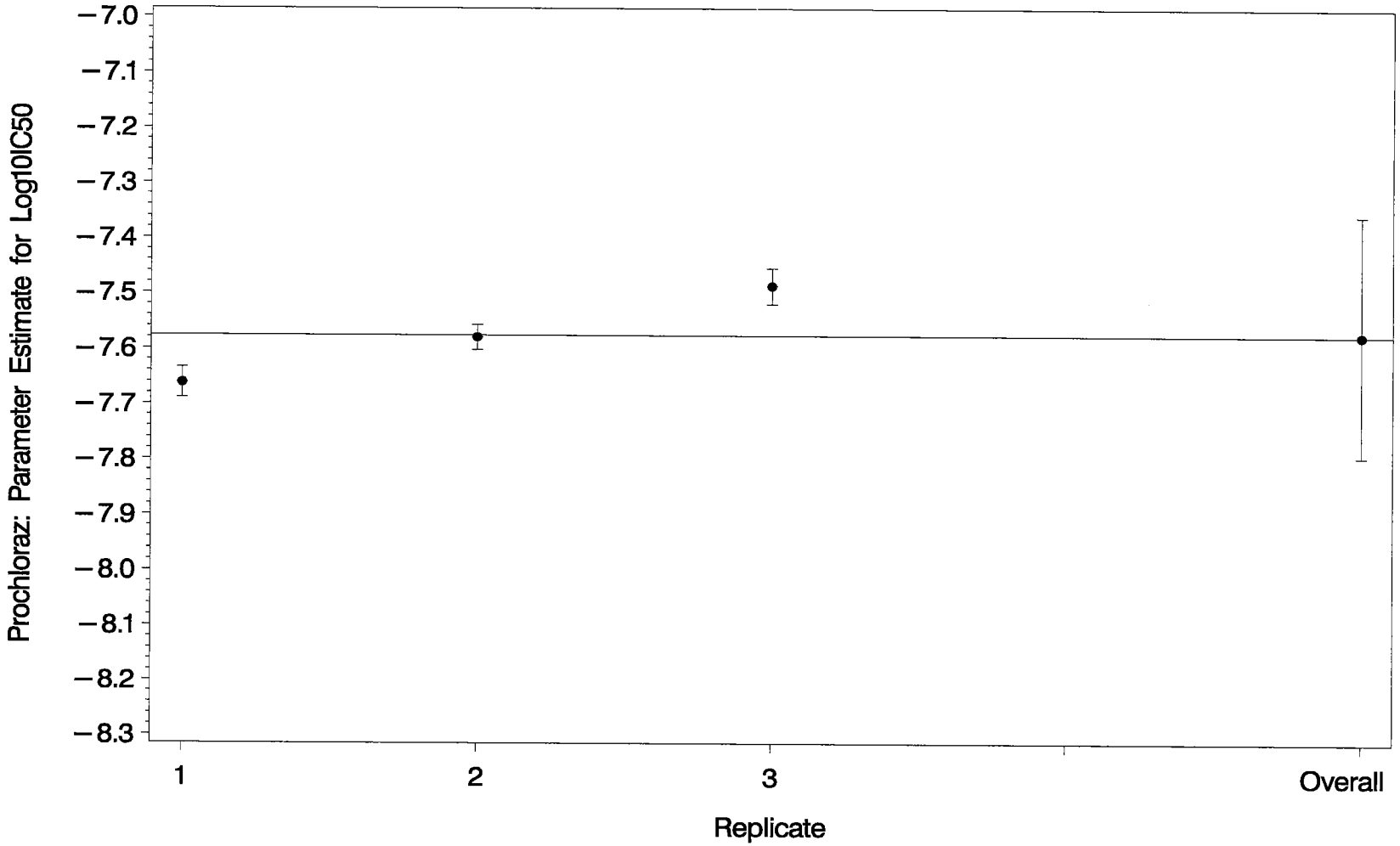


Figure 3-5. Reference Chemical III: Prochloraz. Microsomes Prepared by IVT. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

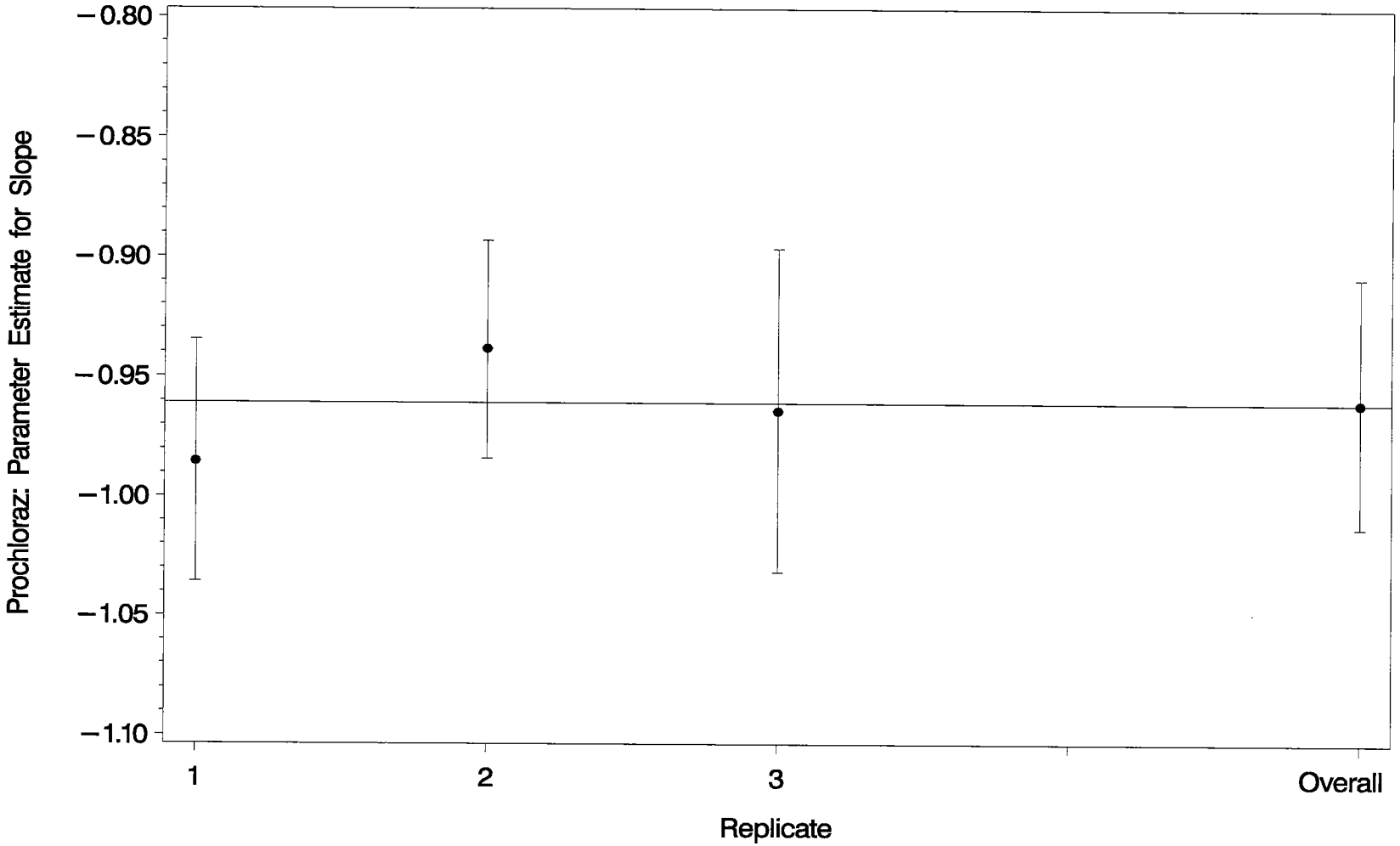


Figure 3-6. **Reference Chemical III: Prochloraz. Microsomes Prepared by IVT. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

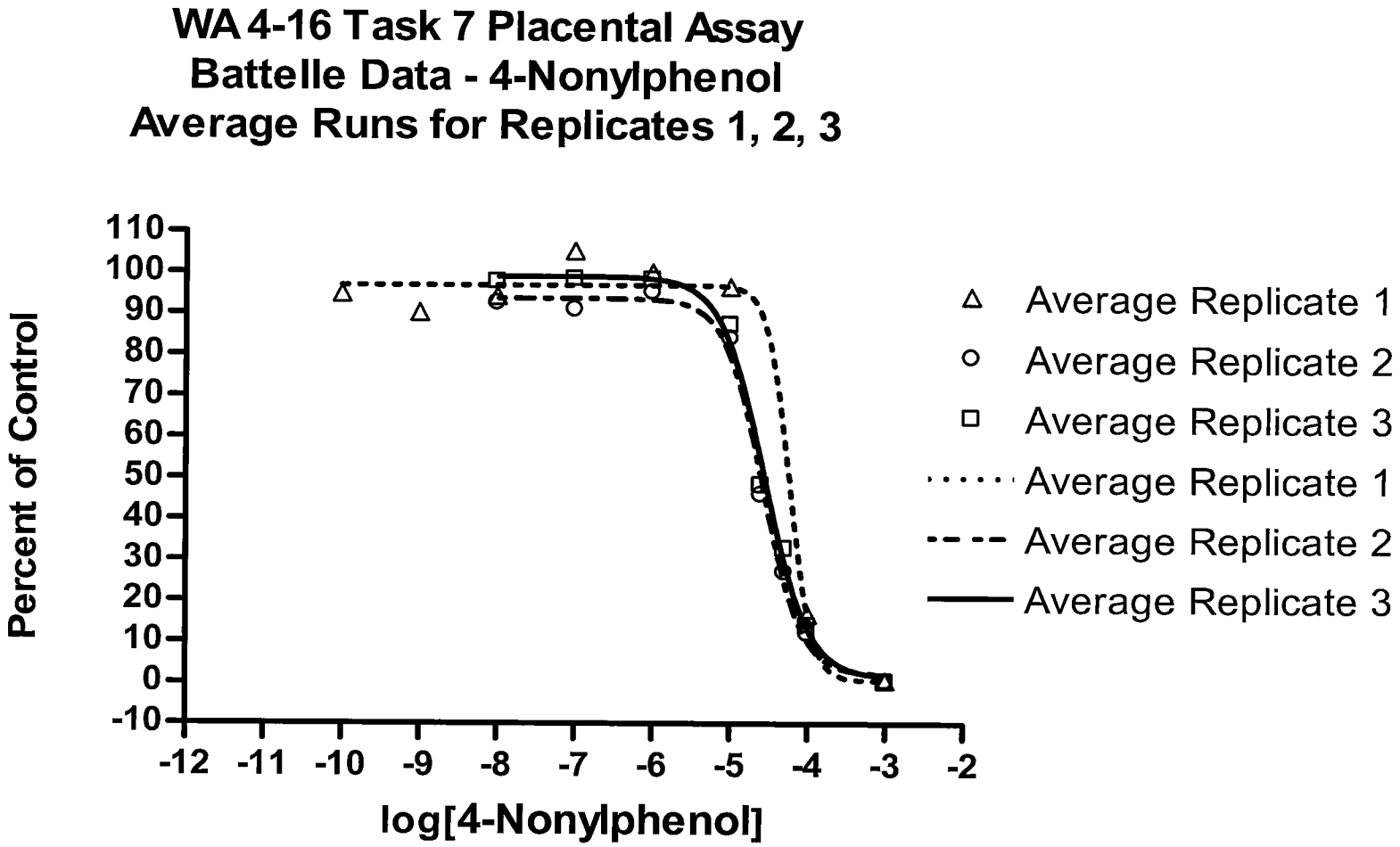


Figure 4-1. **Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

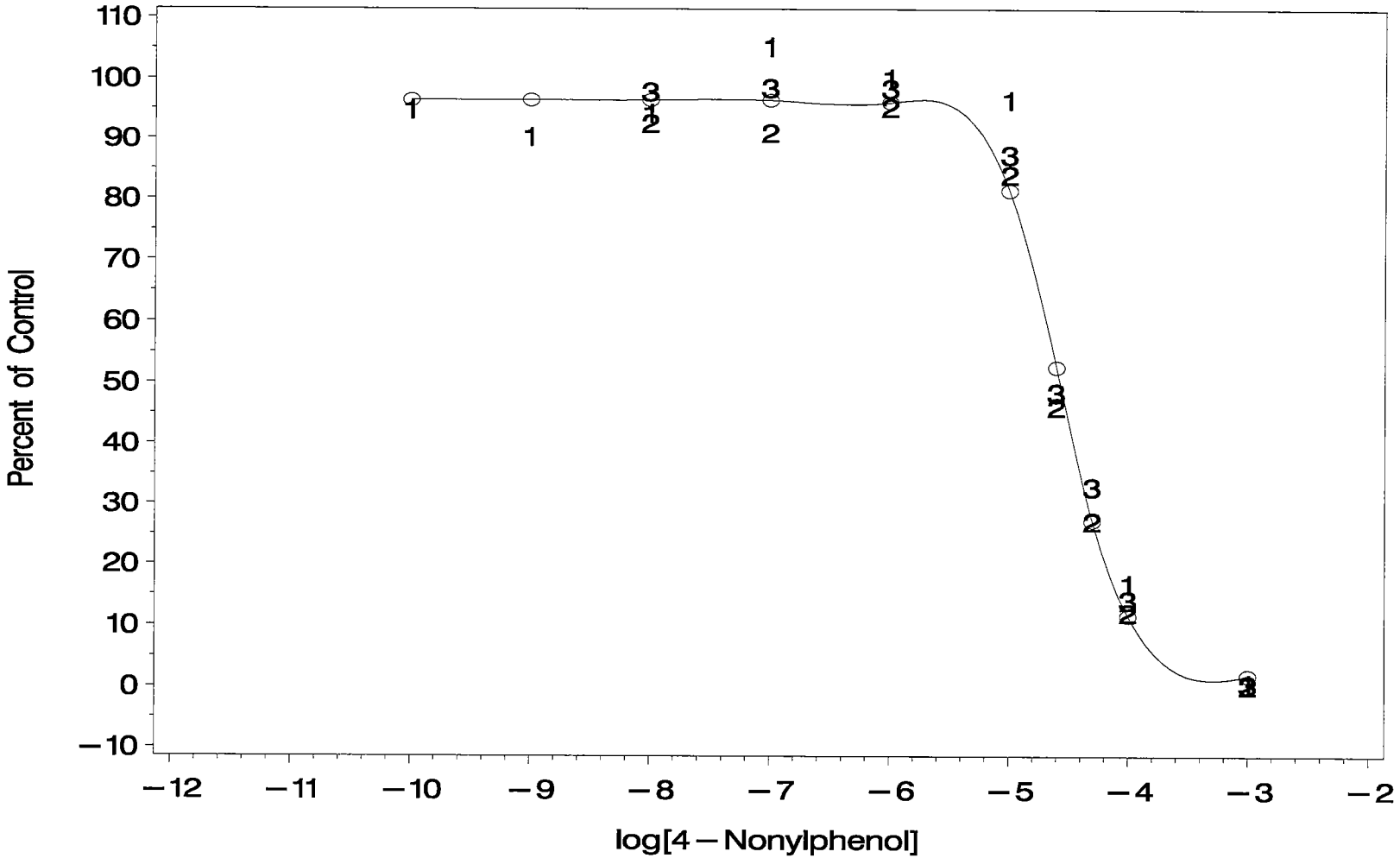


Figure 4-2. **Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

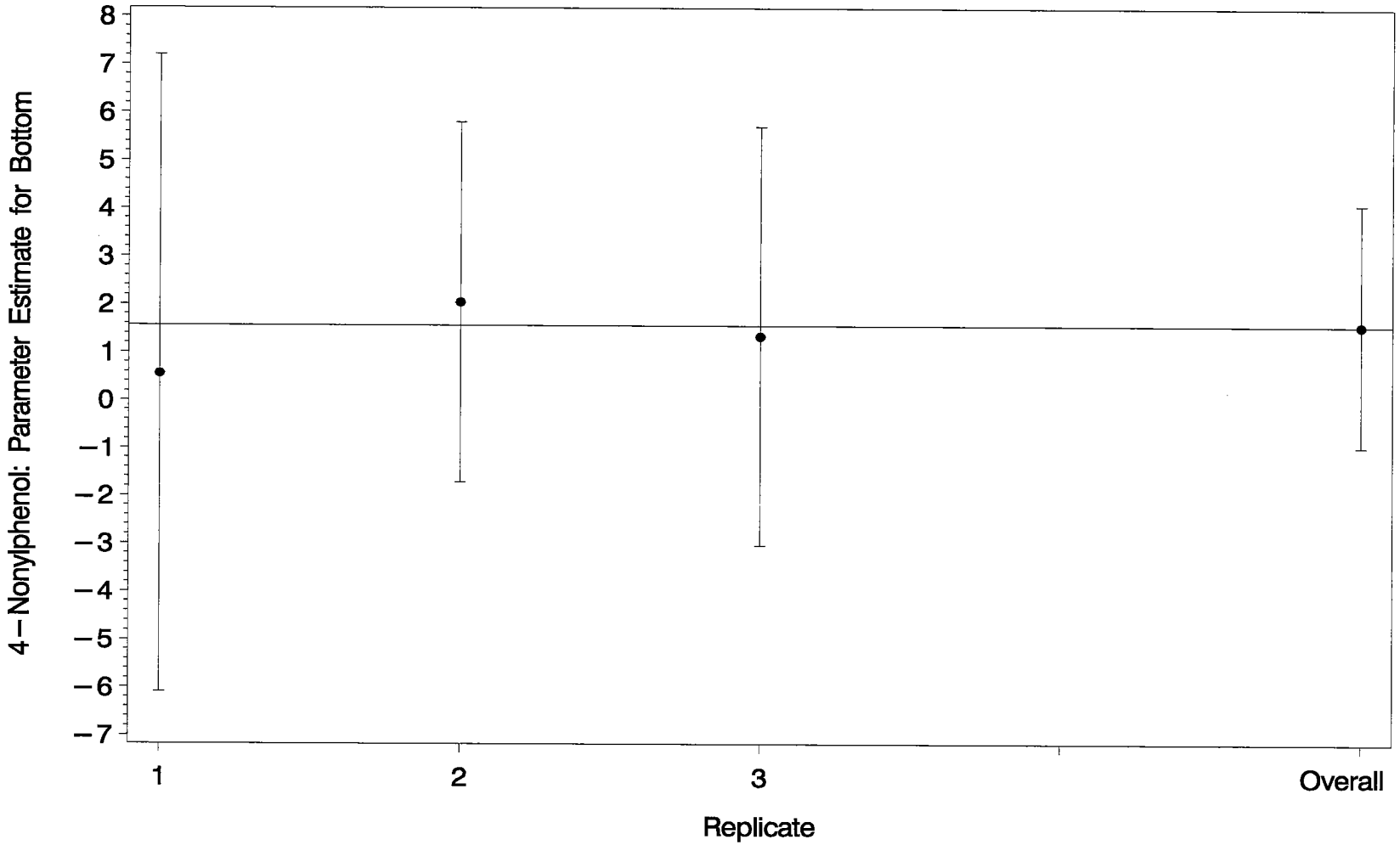


Figure 4-3. **Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

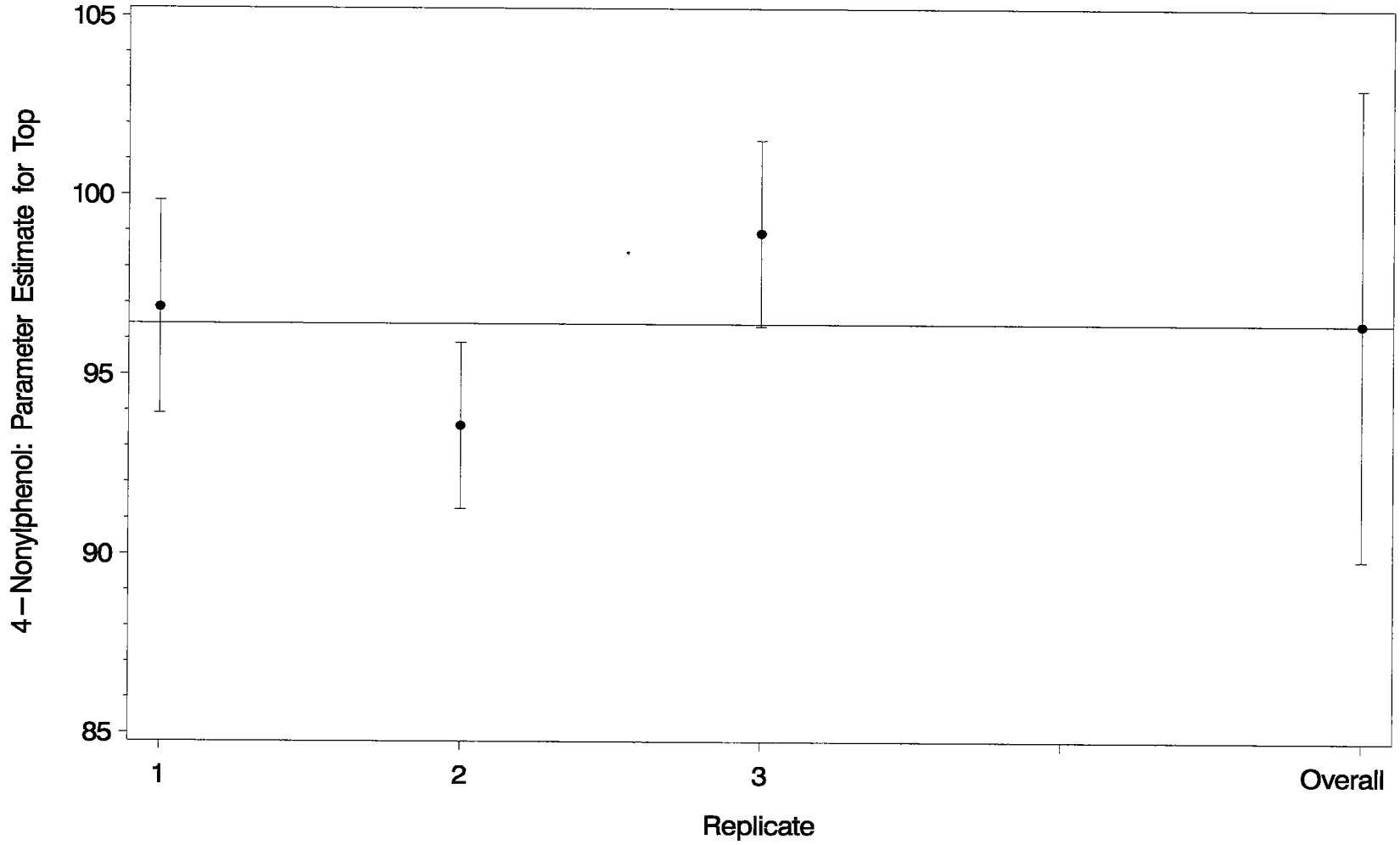


Figure 4-4. **Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

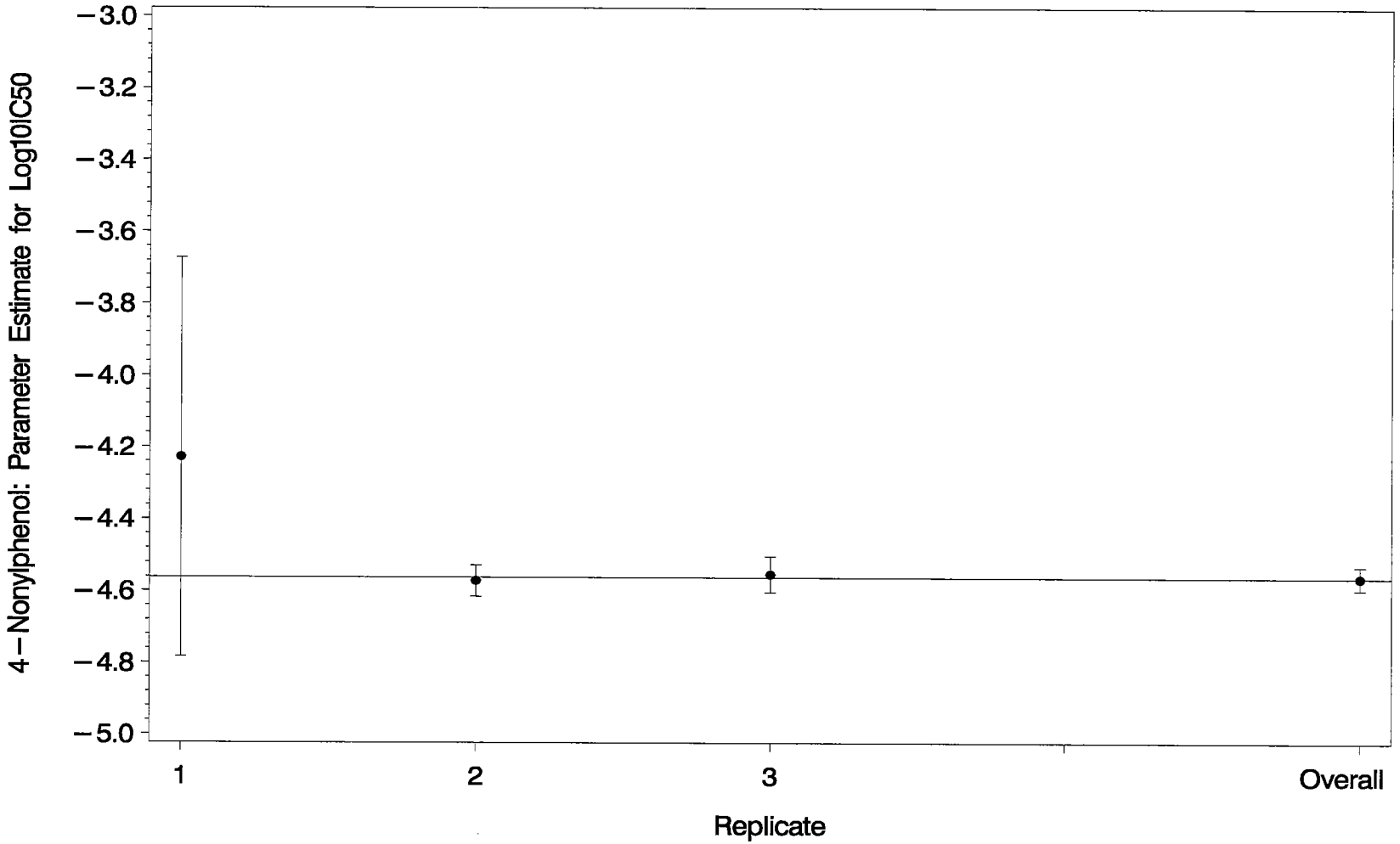


Figure 4-5. Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

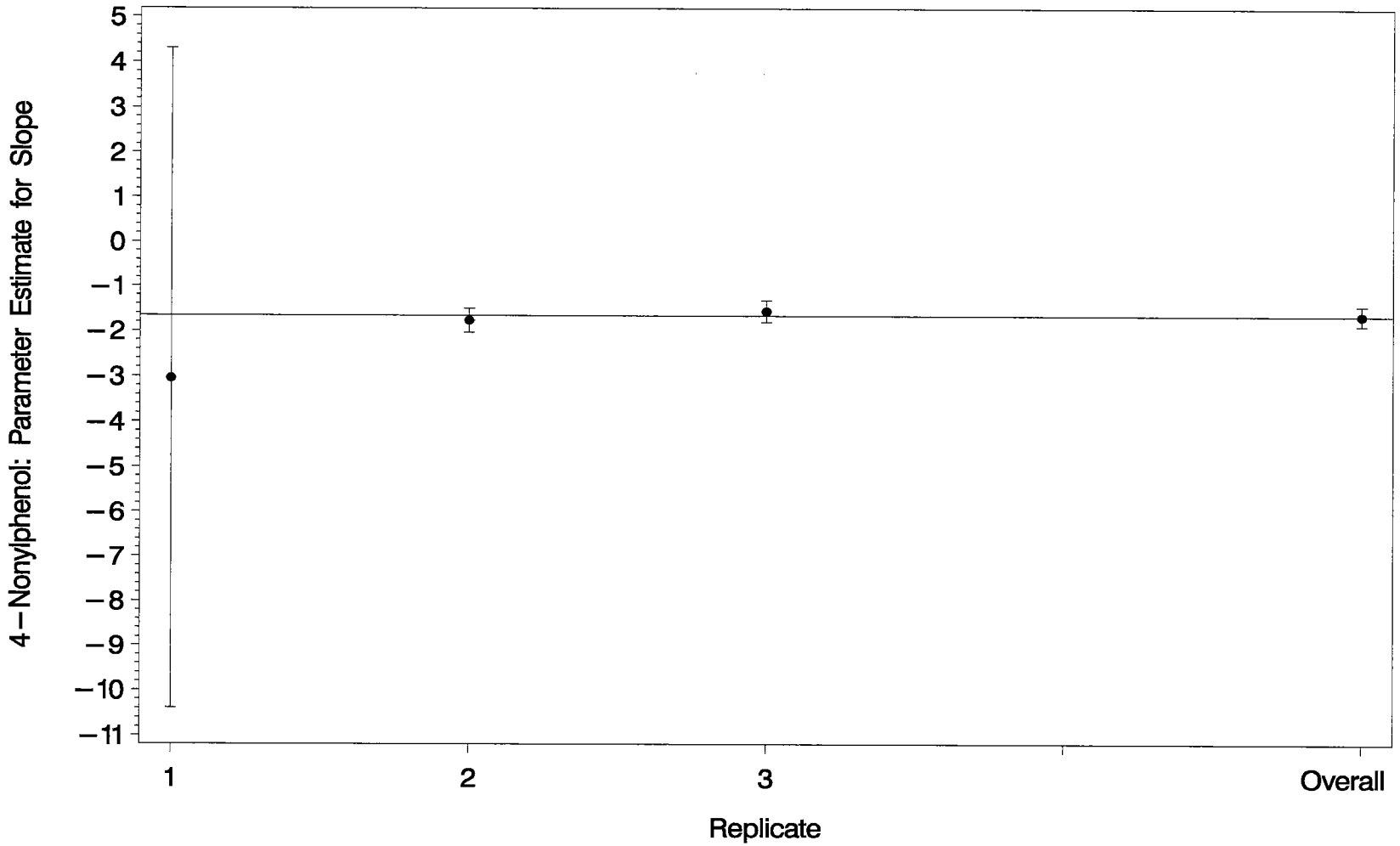


Figure 4-6. Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

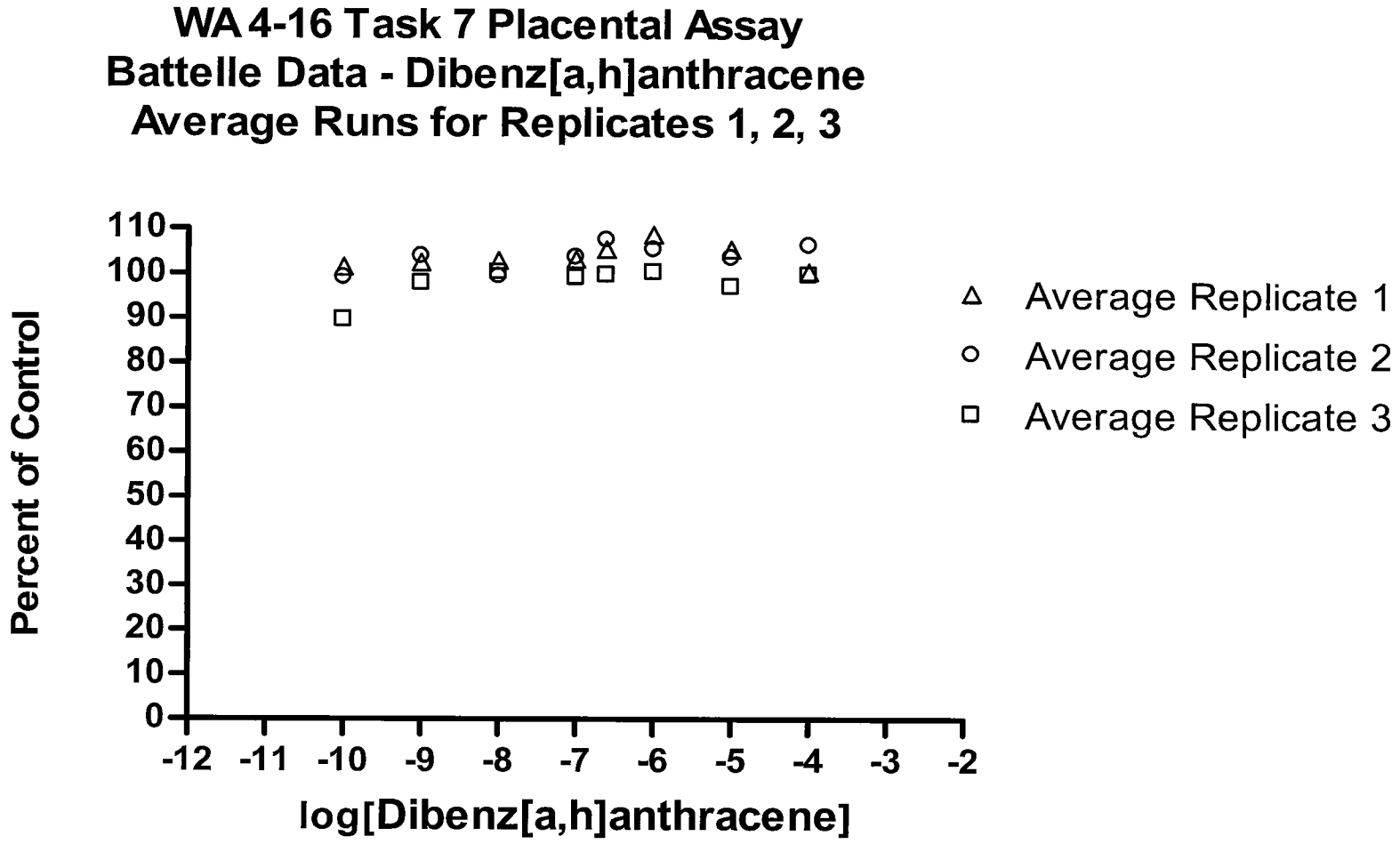


Figure 5-1. **Reference Chemical V: Dibenz[a, h]anthracene. Microsomes Prepared by Battelle. Scatter Plot for Averages of Repetitions within Concentration for Each Replicate. Placental Aromatase Assay.**

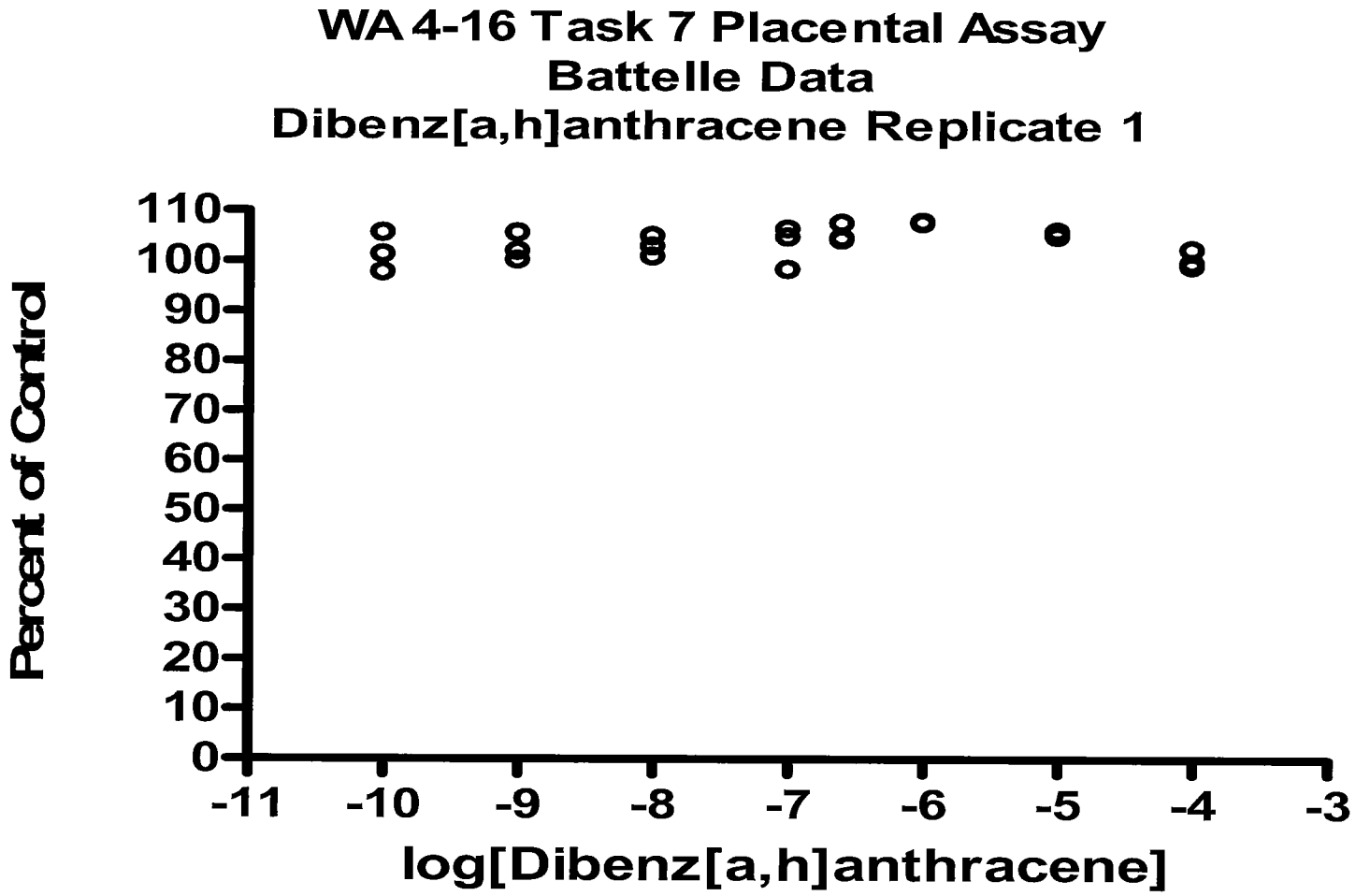


Figure 5-2. **Reference Chemical V: Dibenz[a, h]anthracene. Microsomes Prepared by Battelle. Scatter Plot for Replicate 1 Data. Placental Aromatase Assay.**

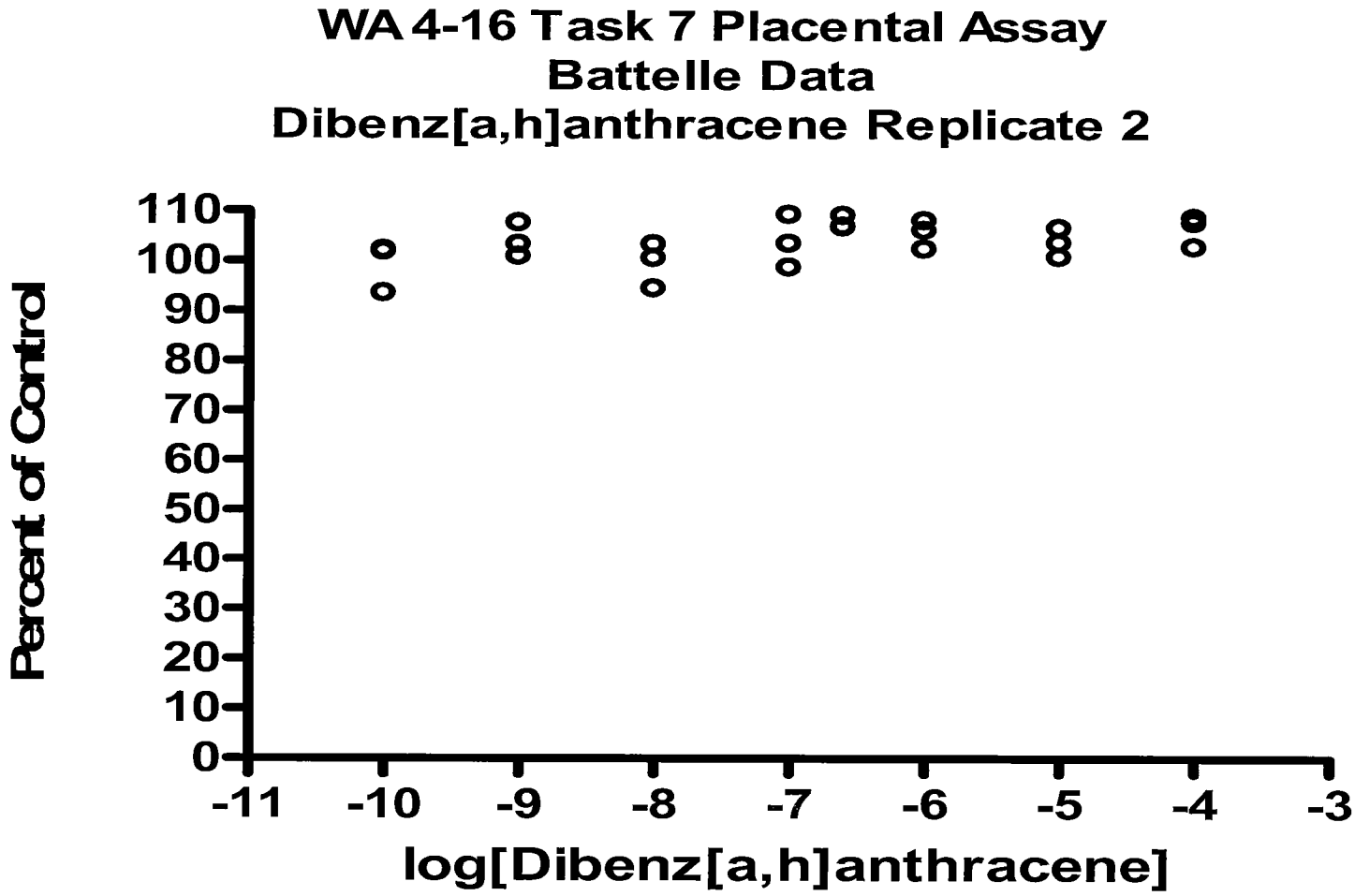


Figure 5-3. **Reference Chemical V: Dibenz[a, h]anthracene. Microsomes Prepared by Battelle. Scatter Plot for Replicate 2 Data. Placental Aromatase Assay.**

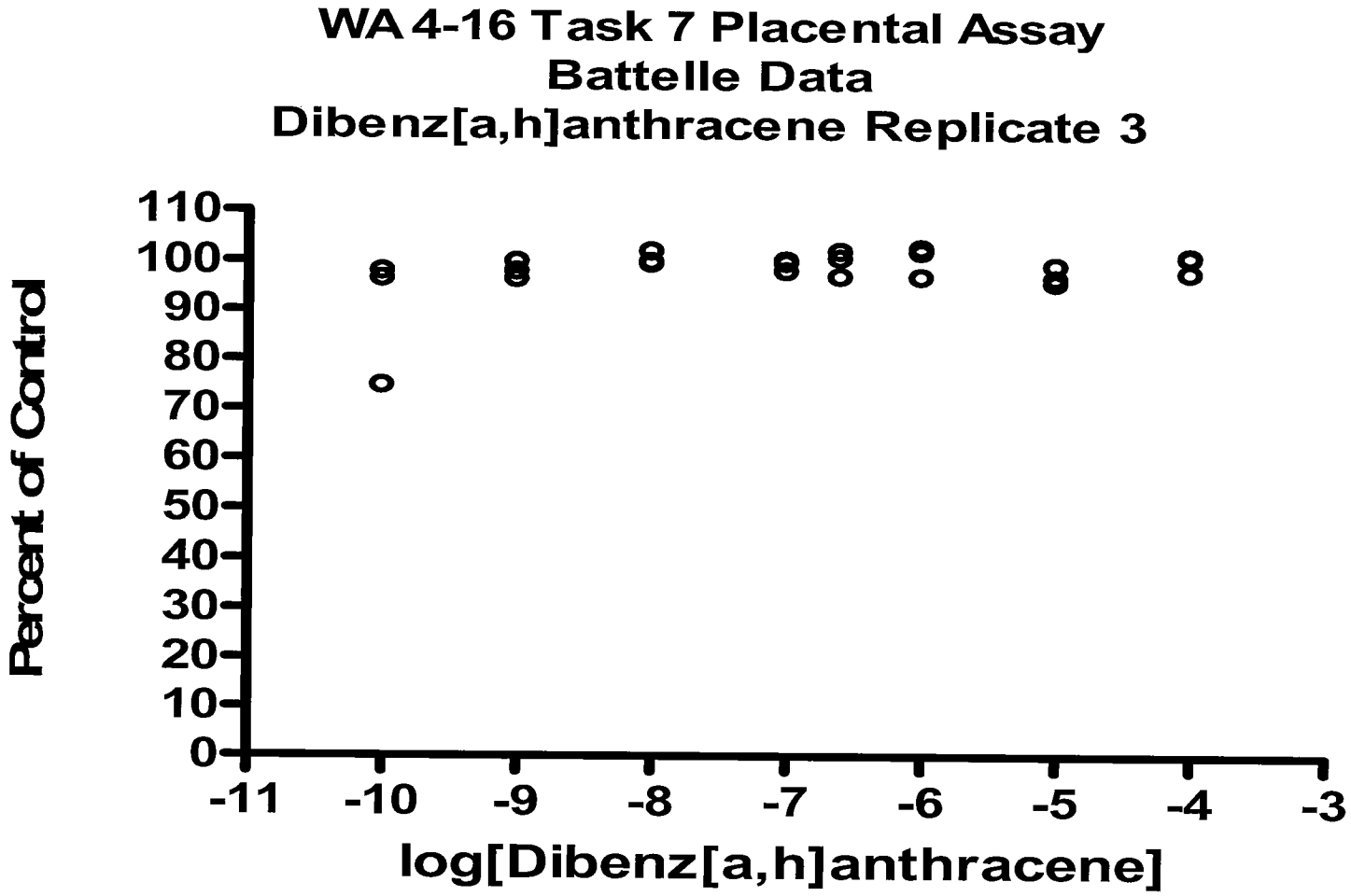


Figure 5-4. **Reference Chemical V: Dibenz[a, h]anthracene. Microsomes Prepared by Battelle. Scatter Plot for Replicate 3 Data. Placental Aromatase Assay.**

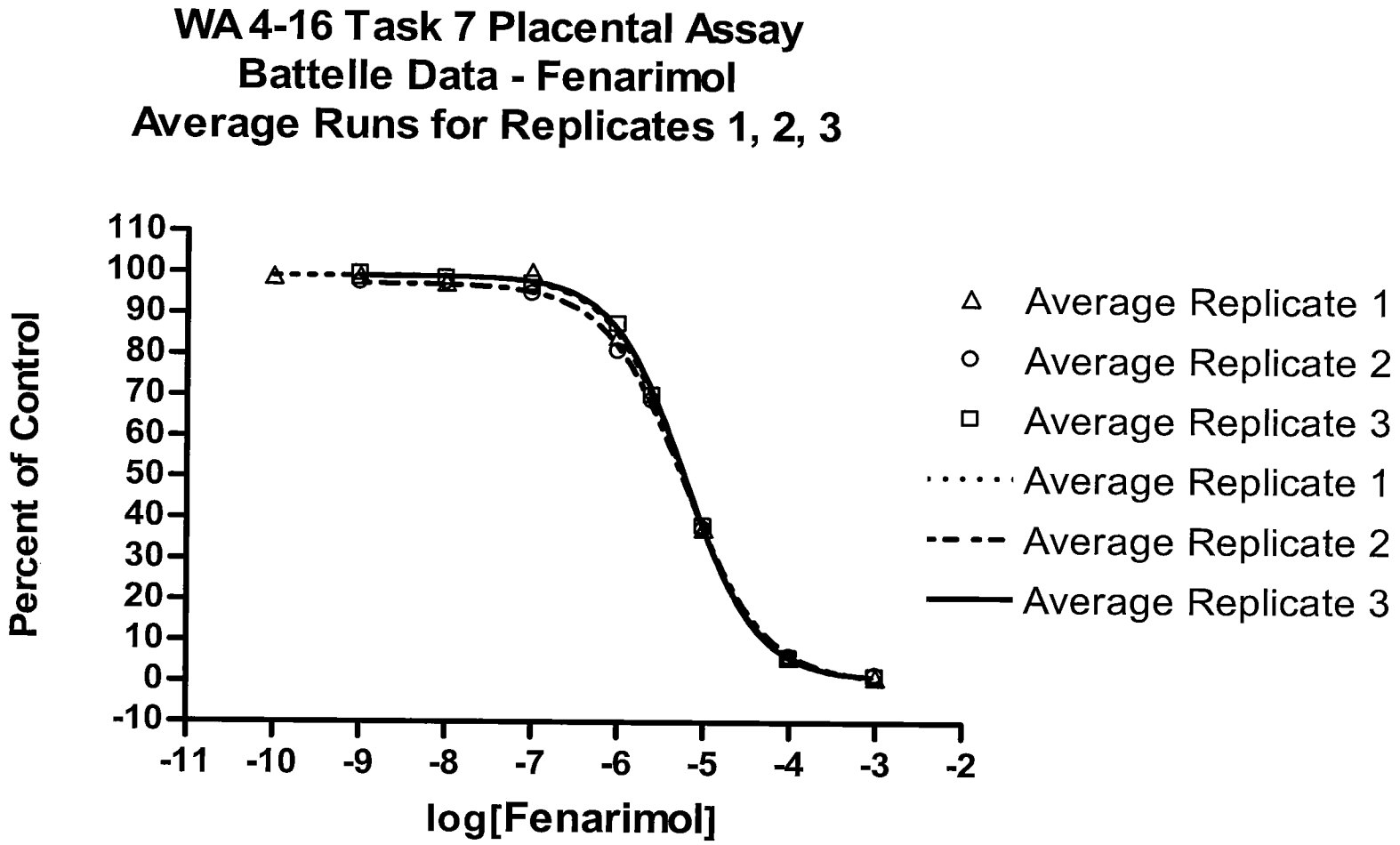


Figure 6-1. **Reference Chemical VI: Fenarimol. Microsomes Prepared by IVT. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

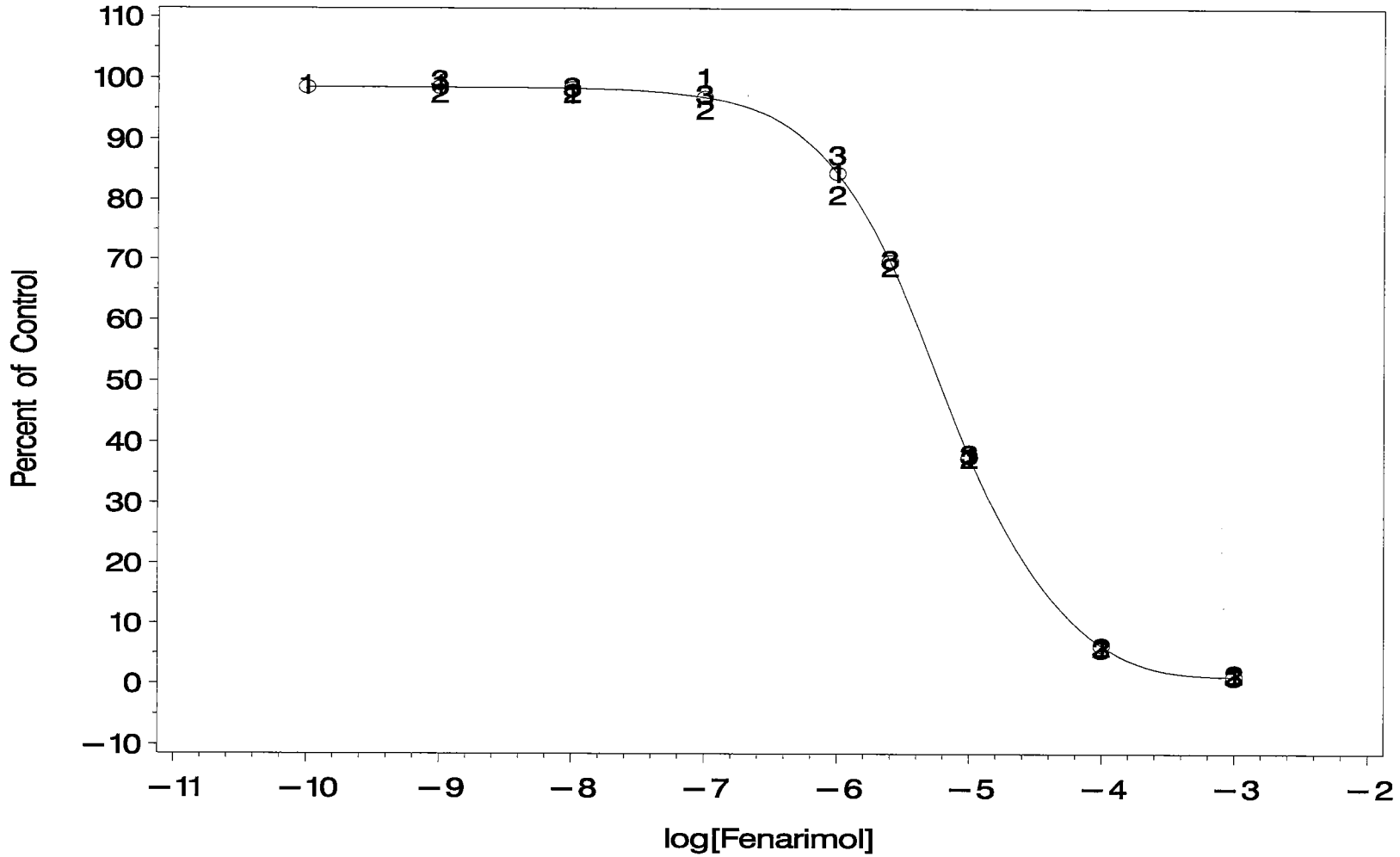


Figure 6-2. Reference Chemical VI: Fenarimol. Microsomes Prepared by IVT. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

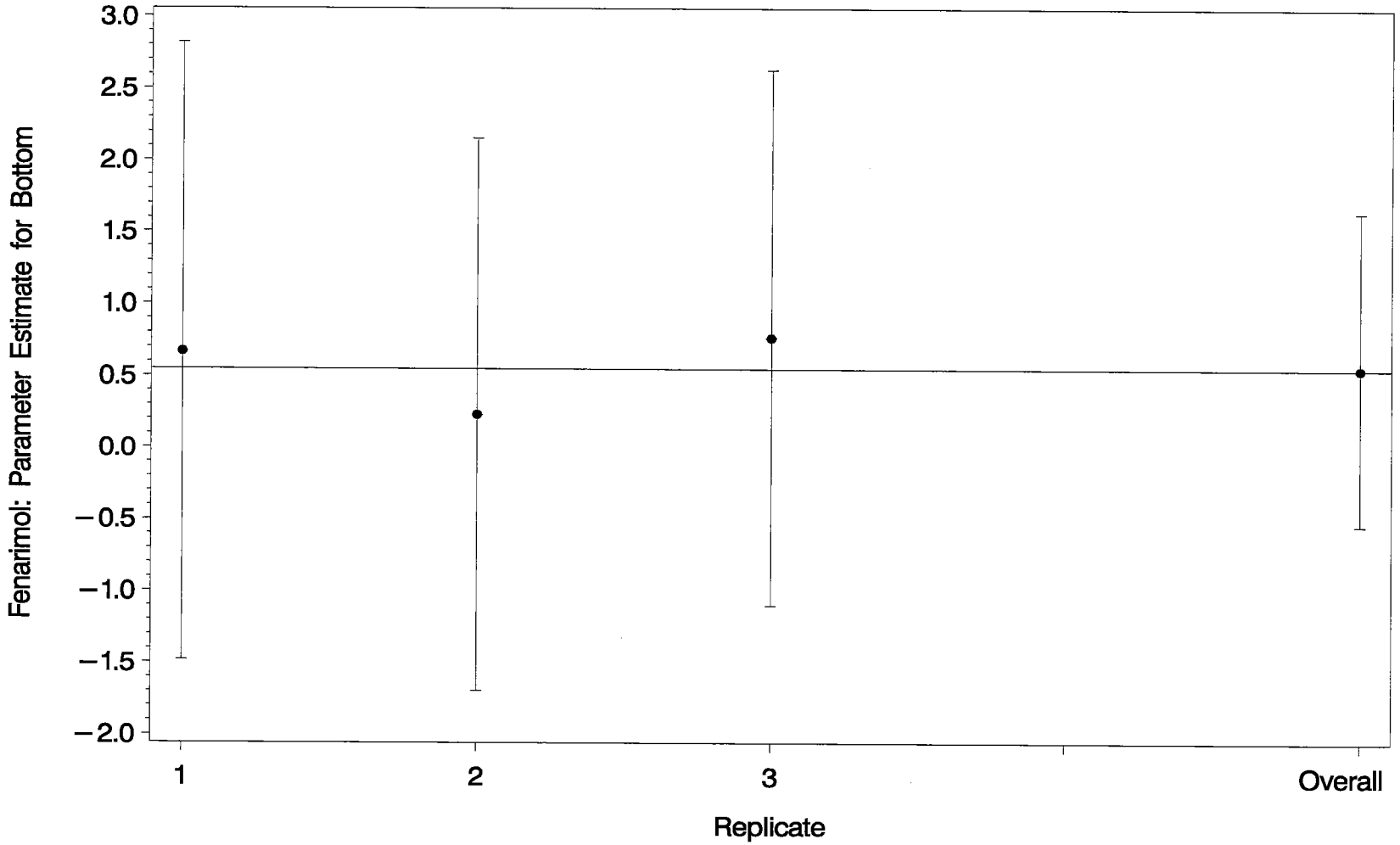


Figure 6-3. Reference Chemical VI: Fenarimol. Microsomes Prepared by IVT. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

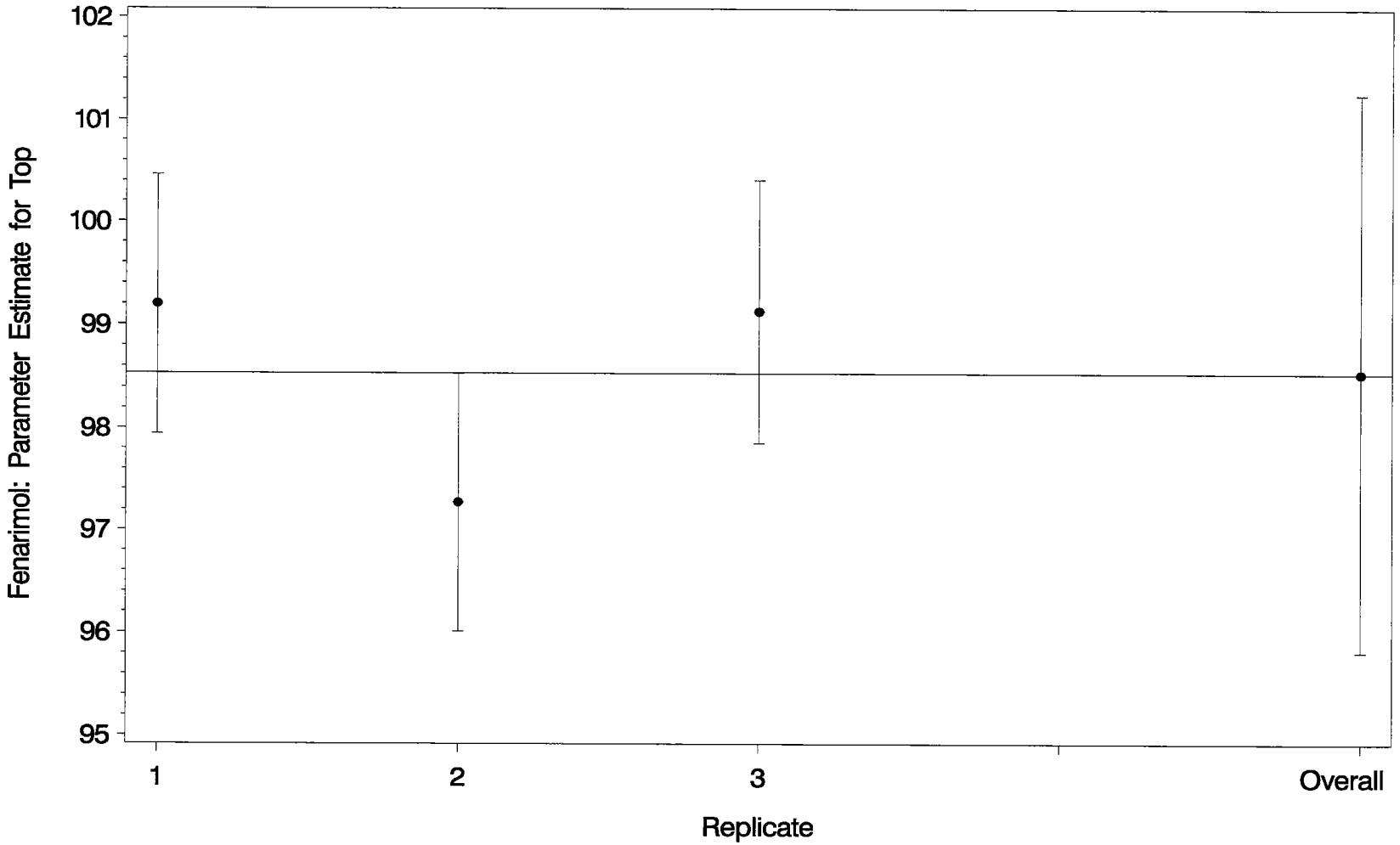


Figure 6-4. Reference Chemical VI: Fenarimol. Microsomes Prepared by IVT. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

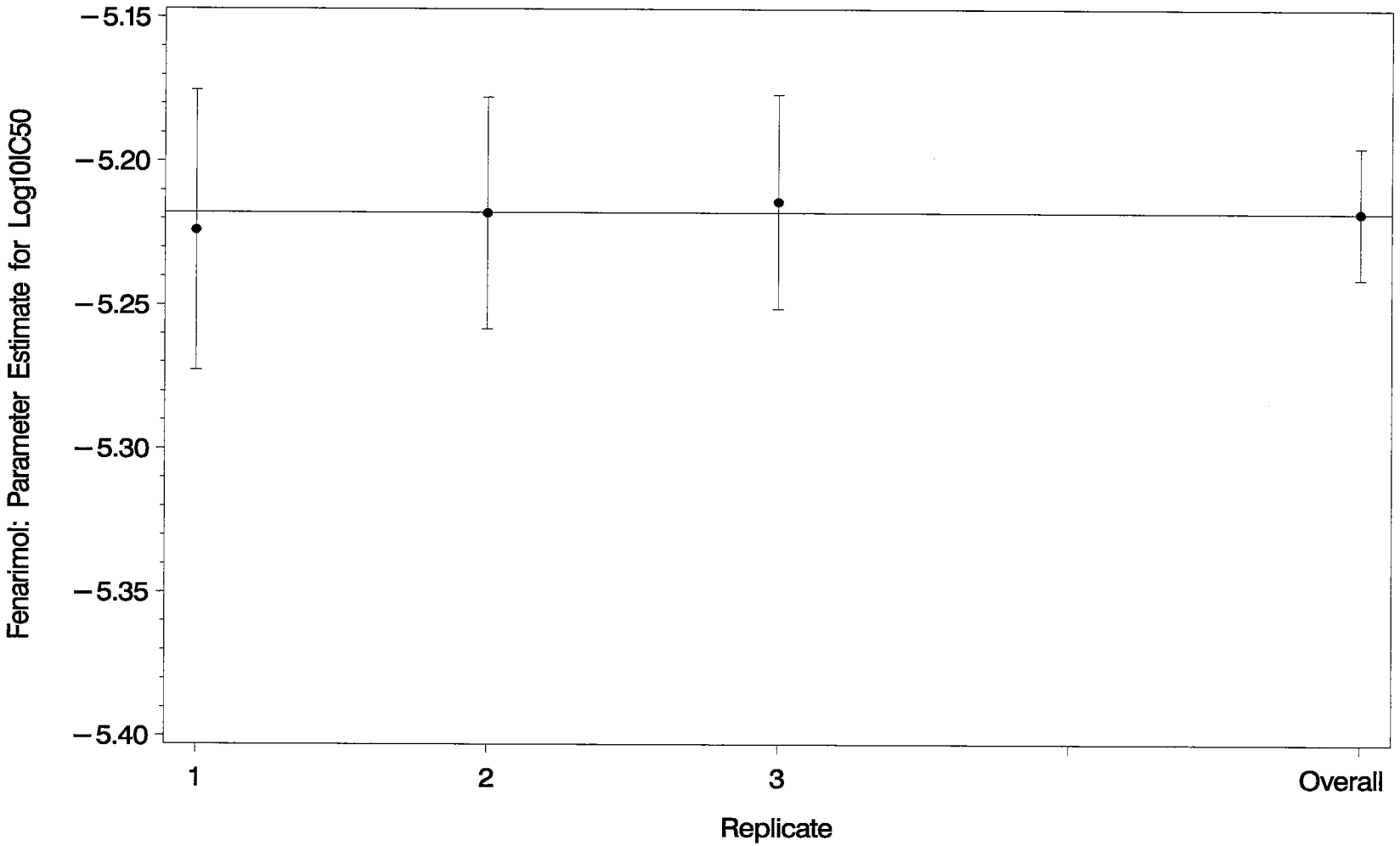


Figure 6-5. Reference Chemical VI: Fenarimol. Microsomes Prepared by IVT. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

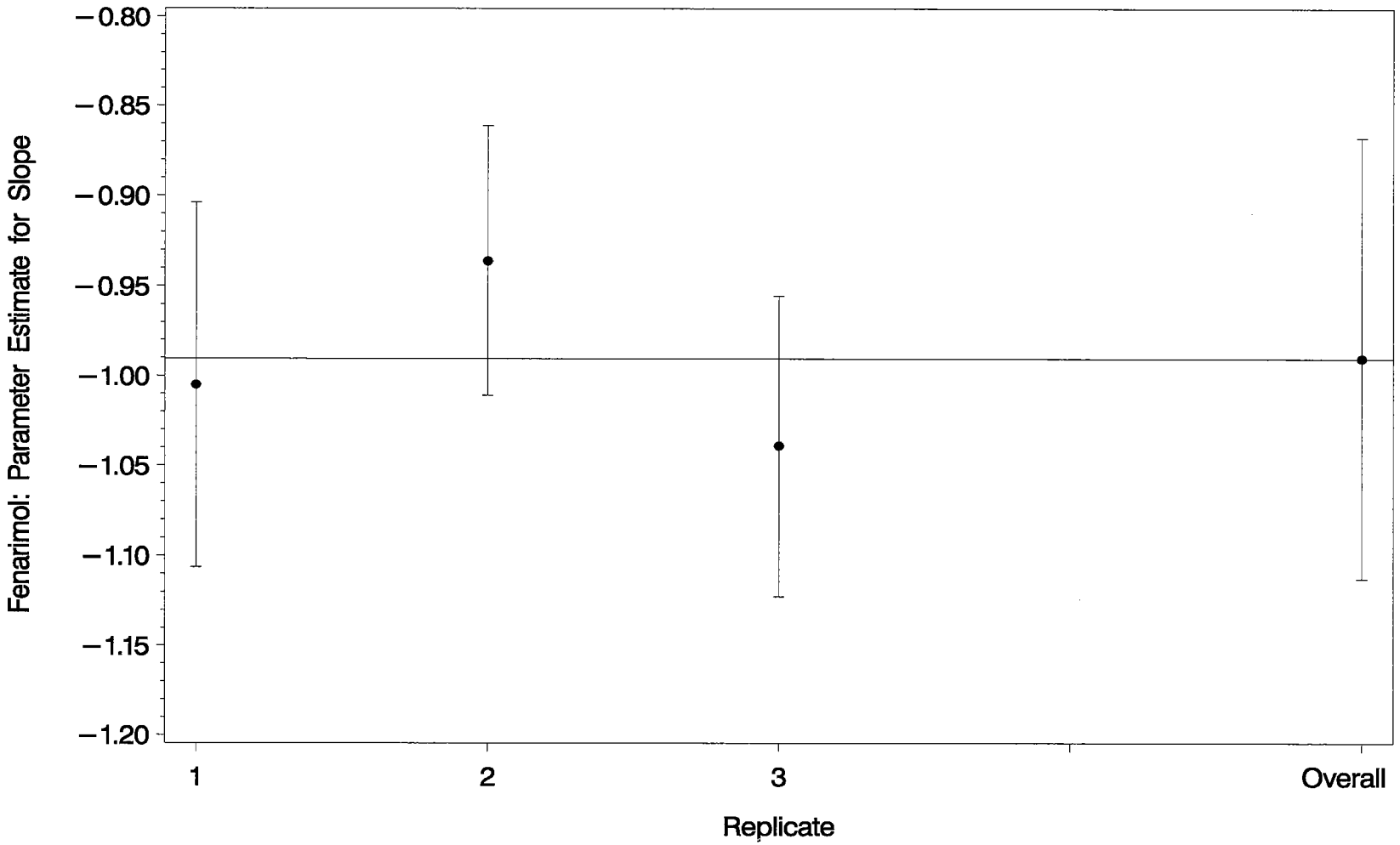


Figure 6-6. Reference Chemical VI: Fenarimol. Microsomes Prepared by IVT. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

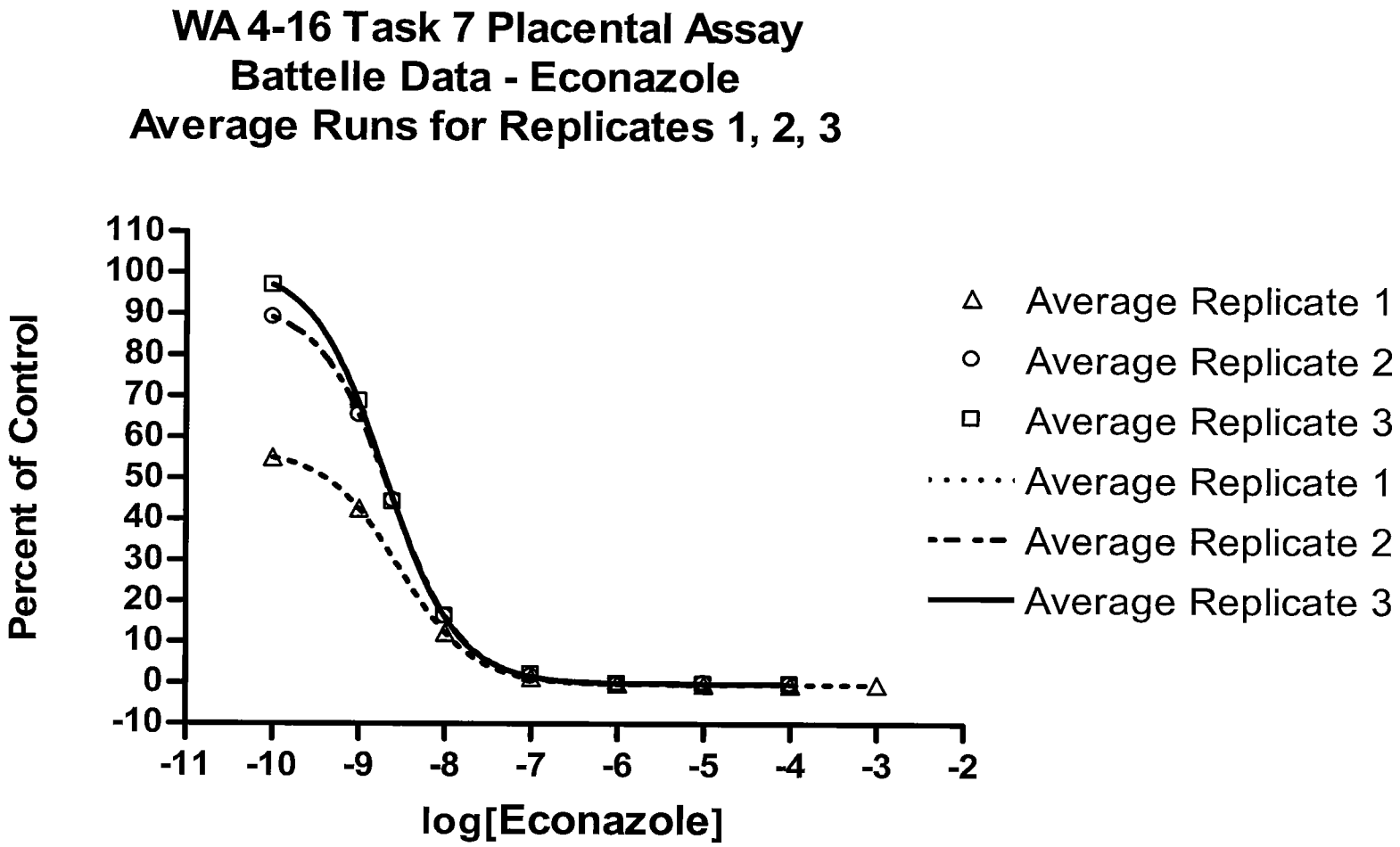


Figure 7-1. **Reference Chemical VII: Econazole. Microsomes Prepared by IVT. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

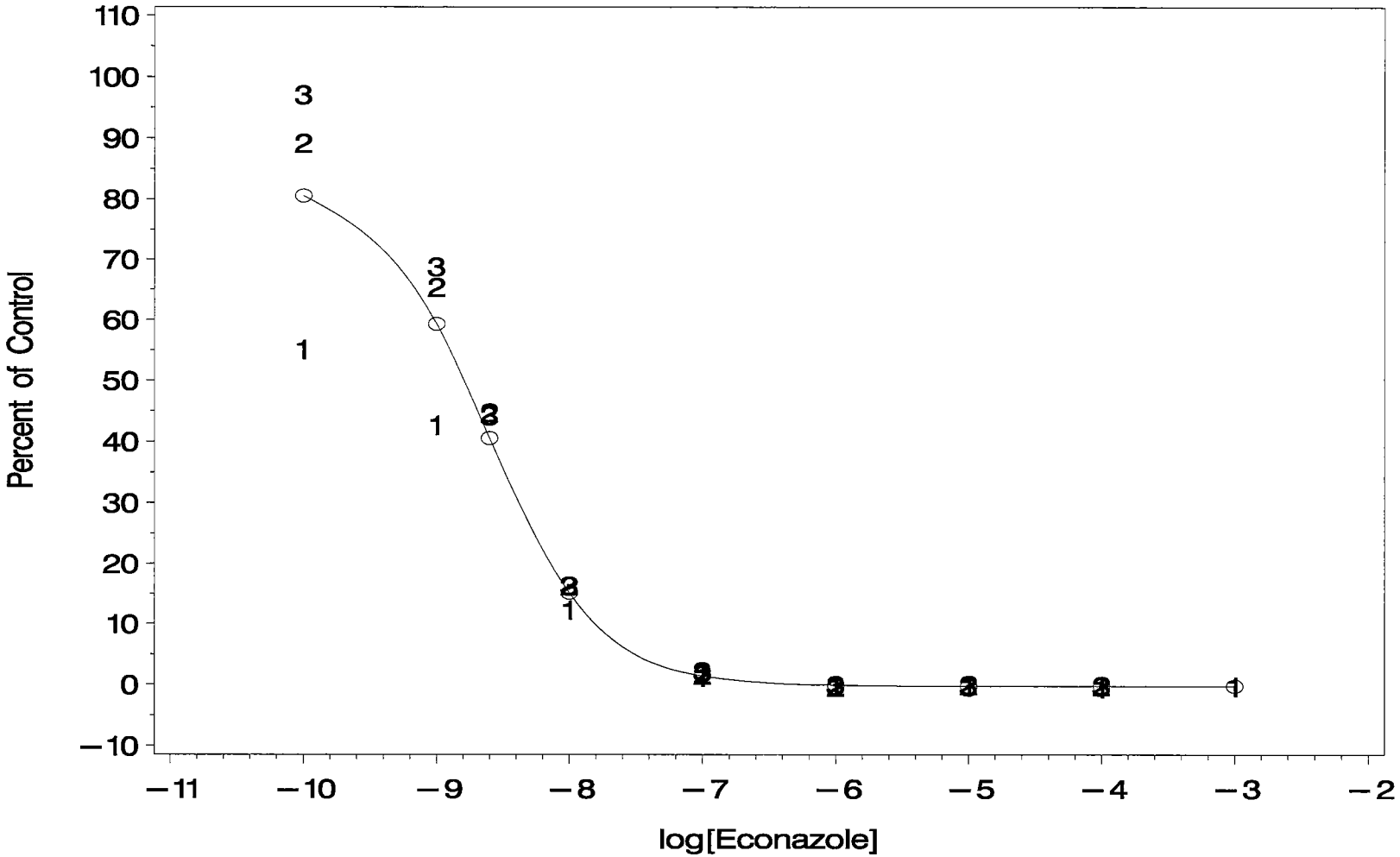


Figure 7-2. Reference Chemical VII: Econazole. Microsomes Prepared by IVT. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

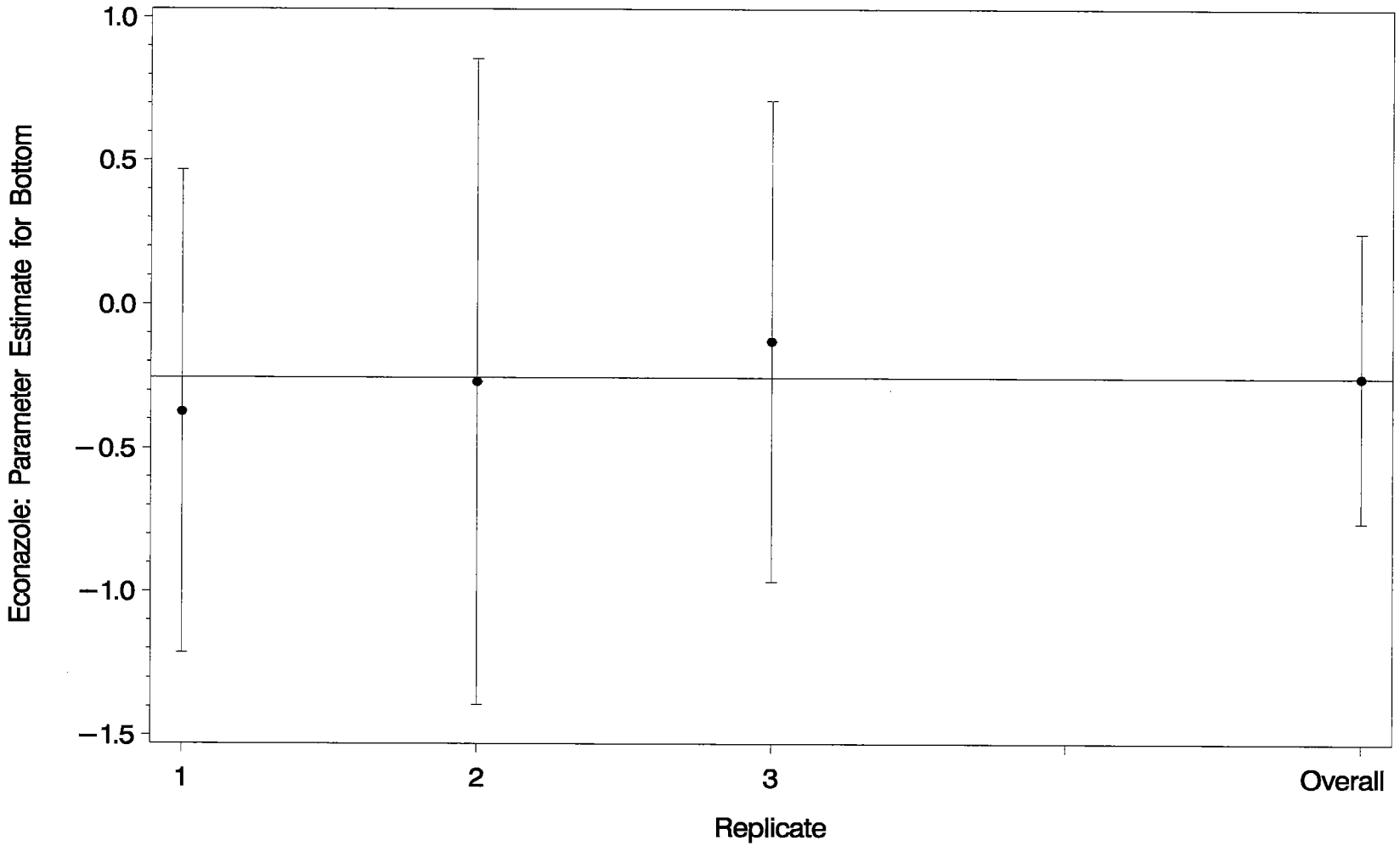


Figure 7-3. Reference Chemical VII: Econazole. Microsomes Prepared by IVT. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

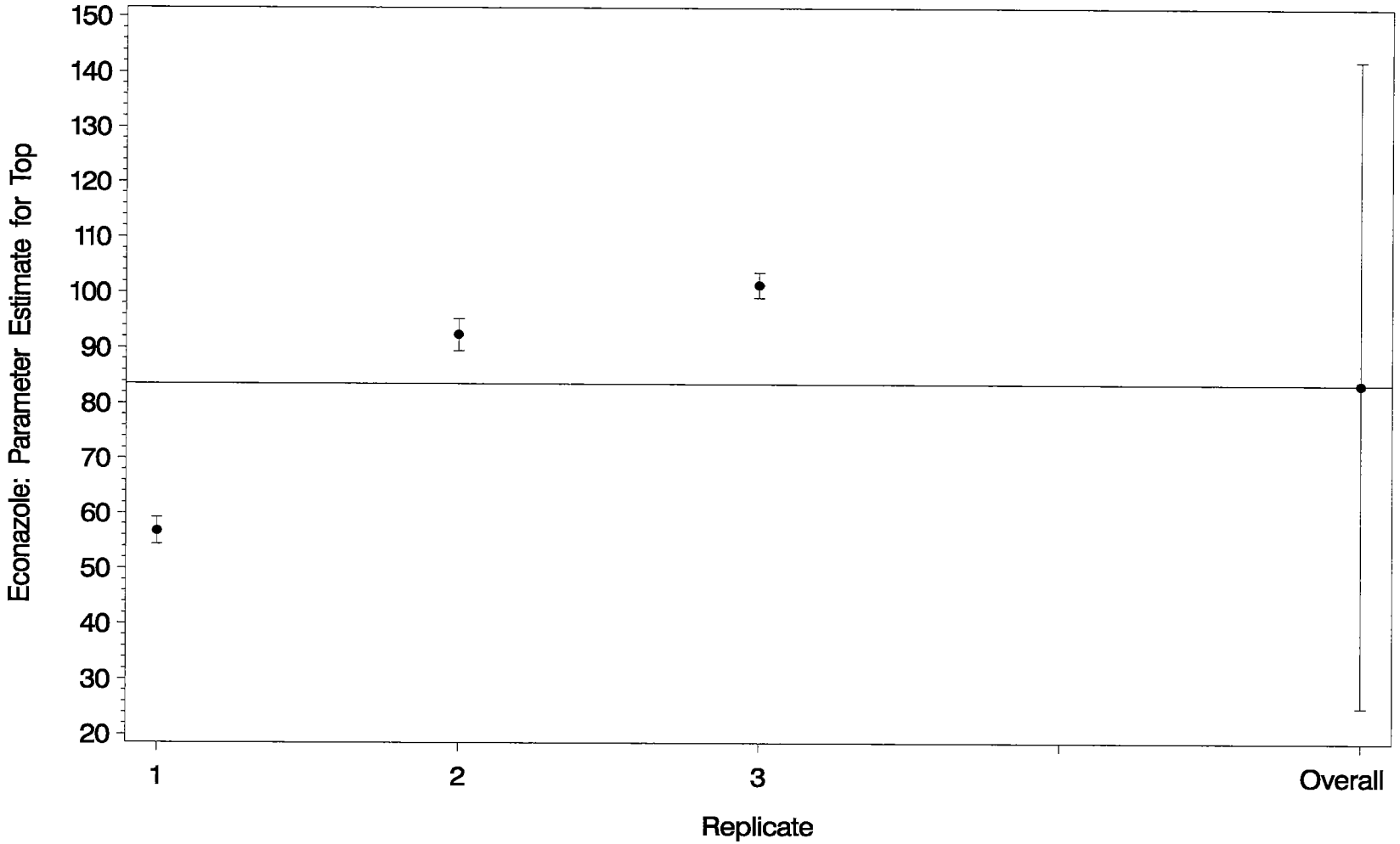


Figure 7-4. Reference Chemical VII: Econazole. Microsomes Prepared by IVT. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

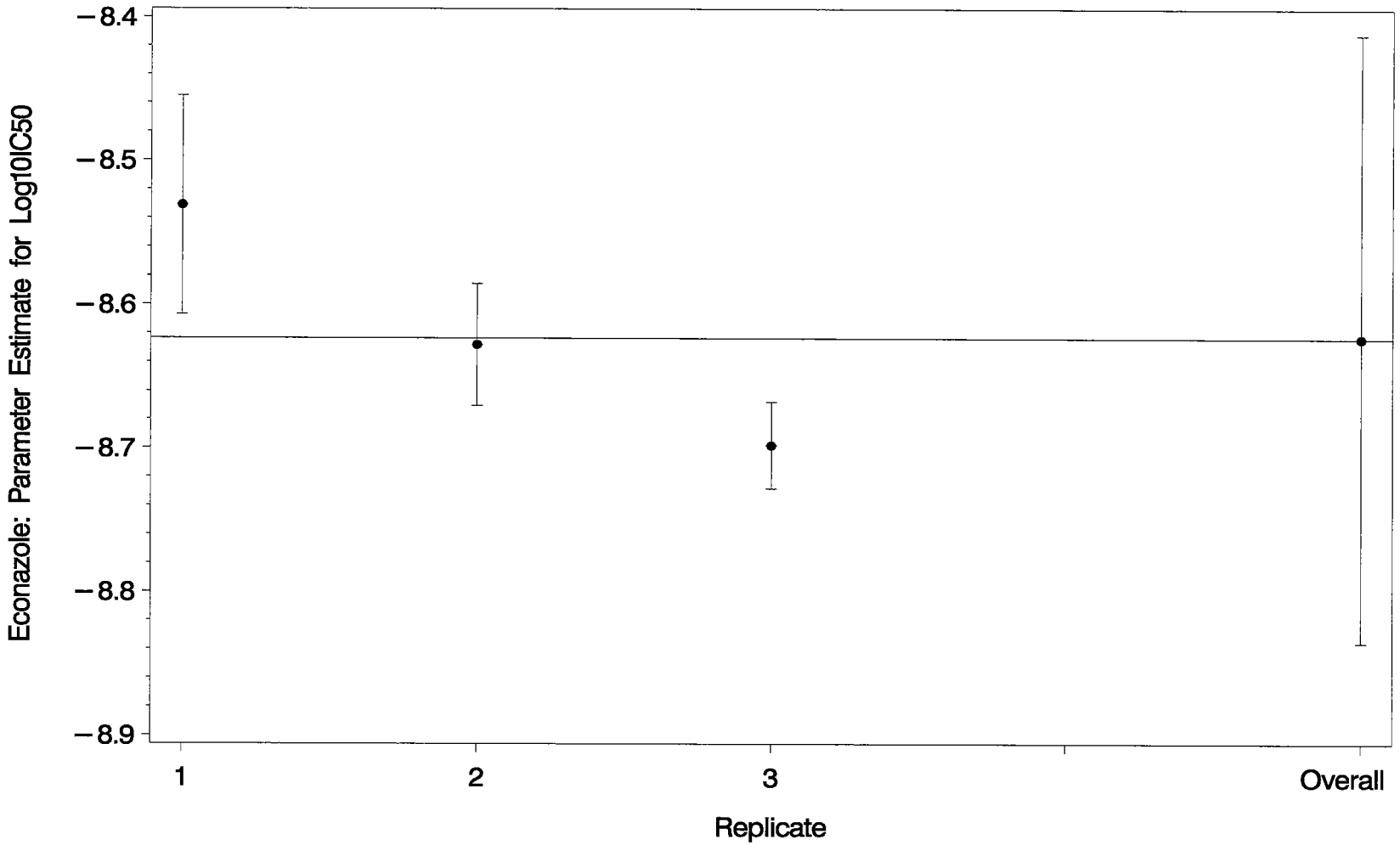


Figure 7-5. Reference Chemical VII: Econazole. Microsomes Prepared by IVT. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

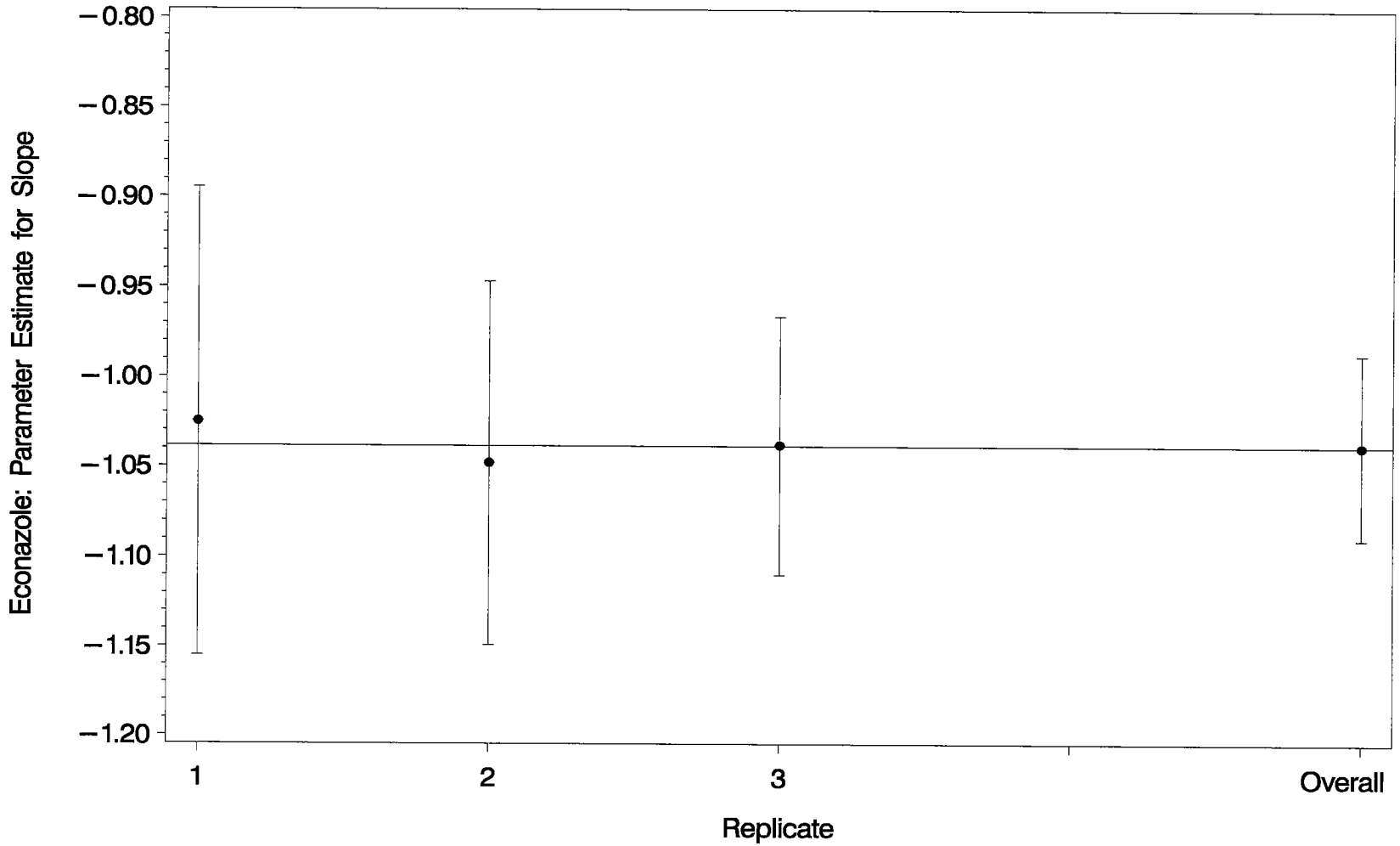


Figure 7-6. Reference Chemical VII: Econazole. Microsomes Prepared by IVT. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

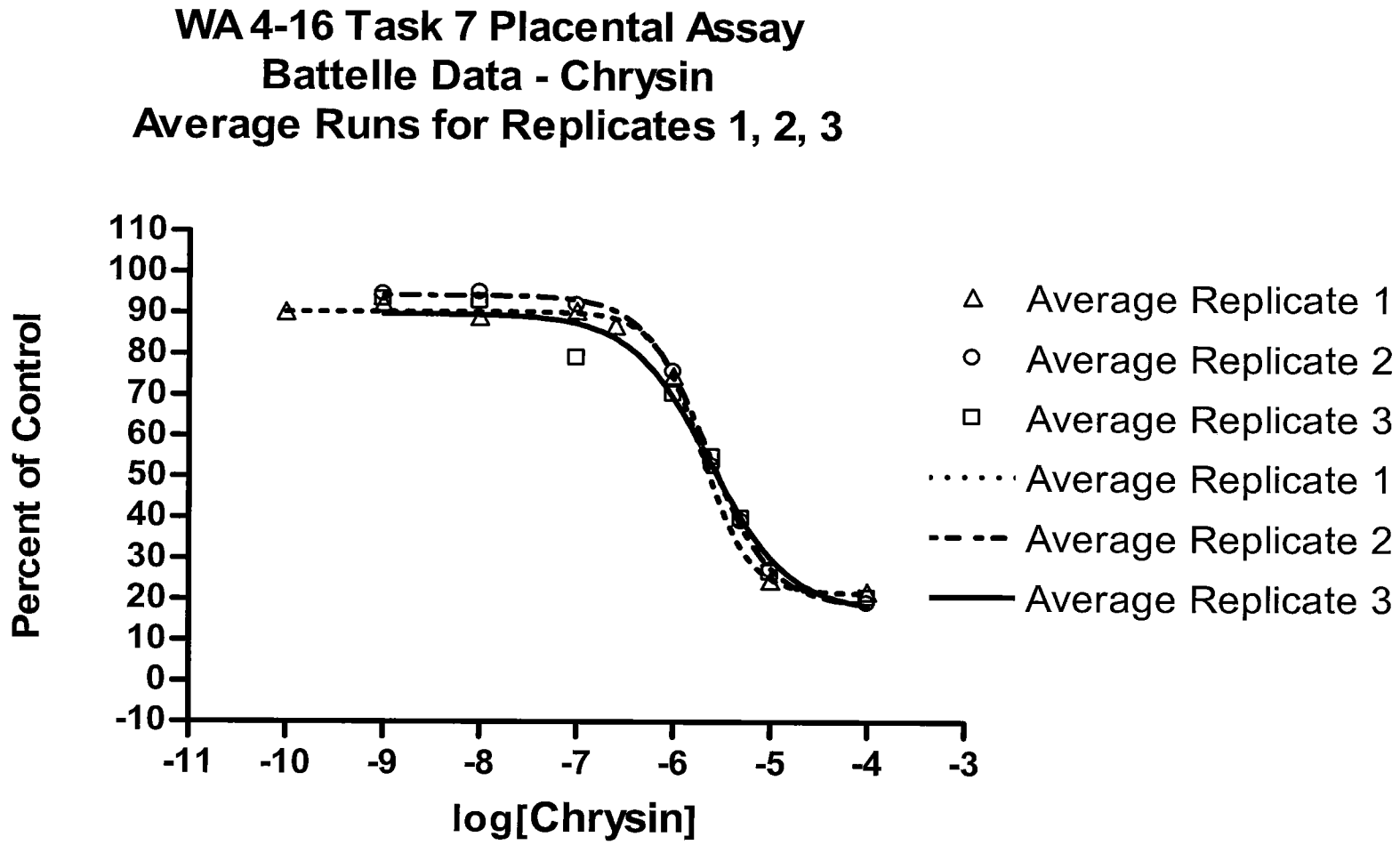


Figure 8-1. **Reference Chemical VIII: Chrysin. Microsomes Prepared by IVT. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

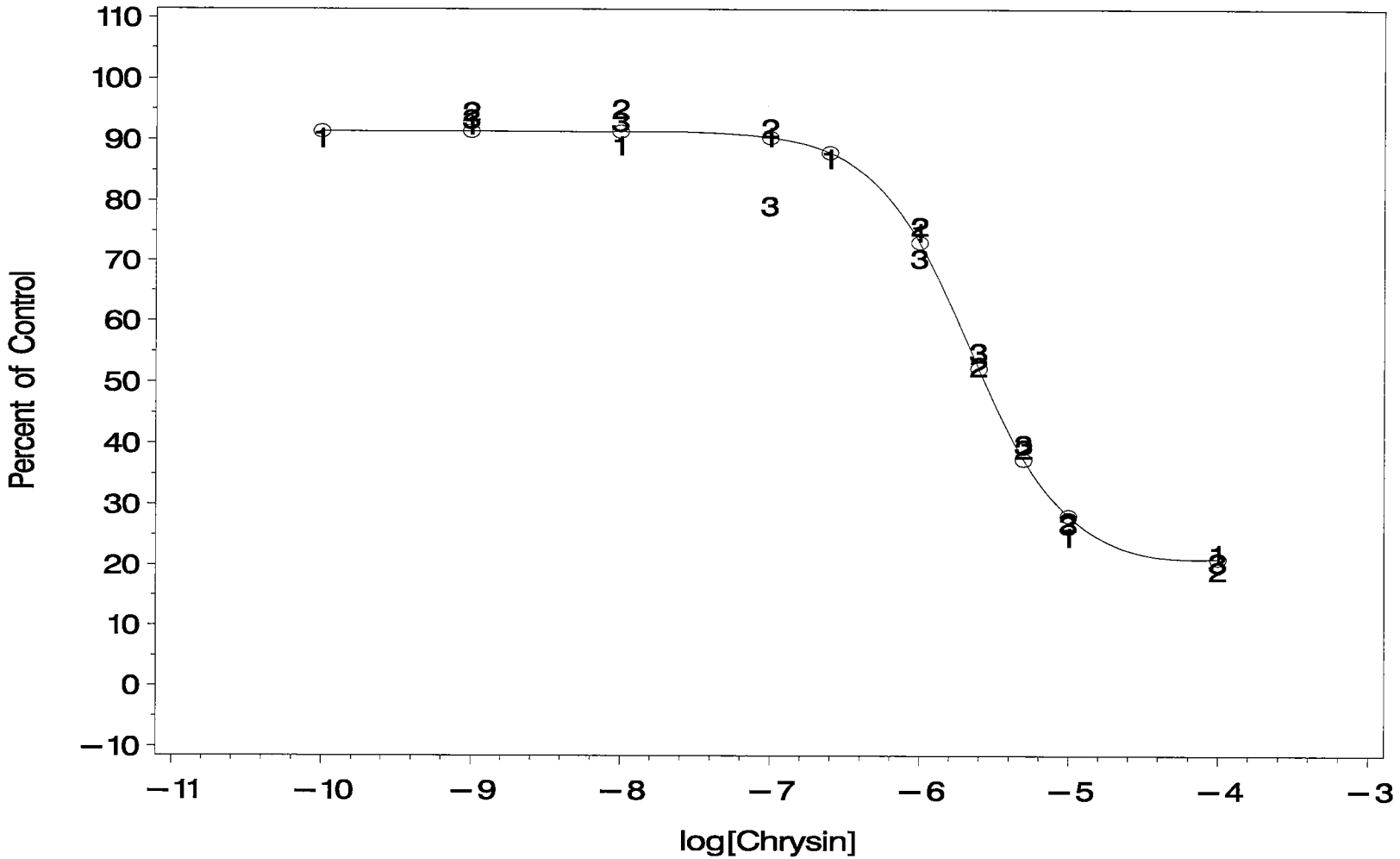


Figure 8-2. Reference Chemical VIII: Chrysin. Microsomes Prepared by IVT. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

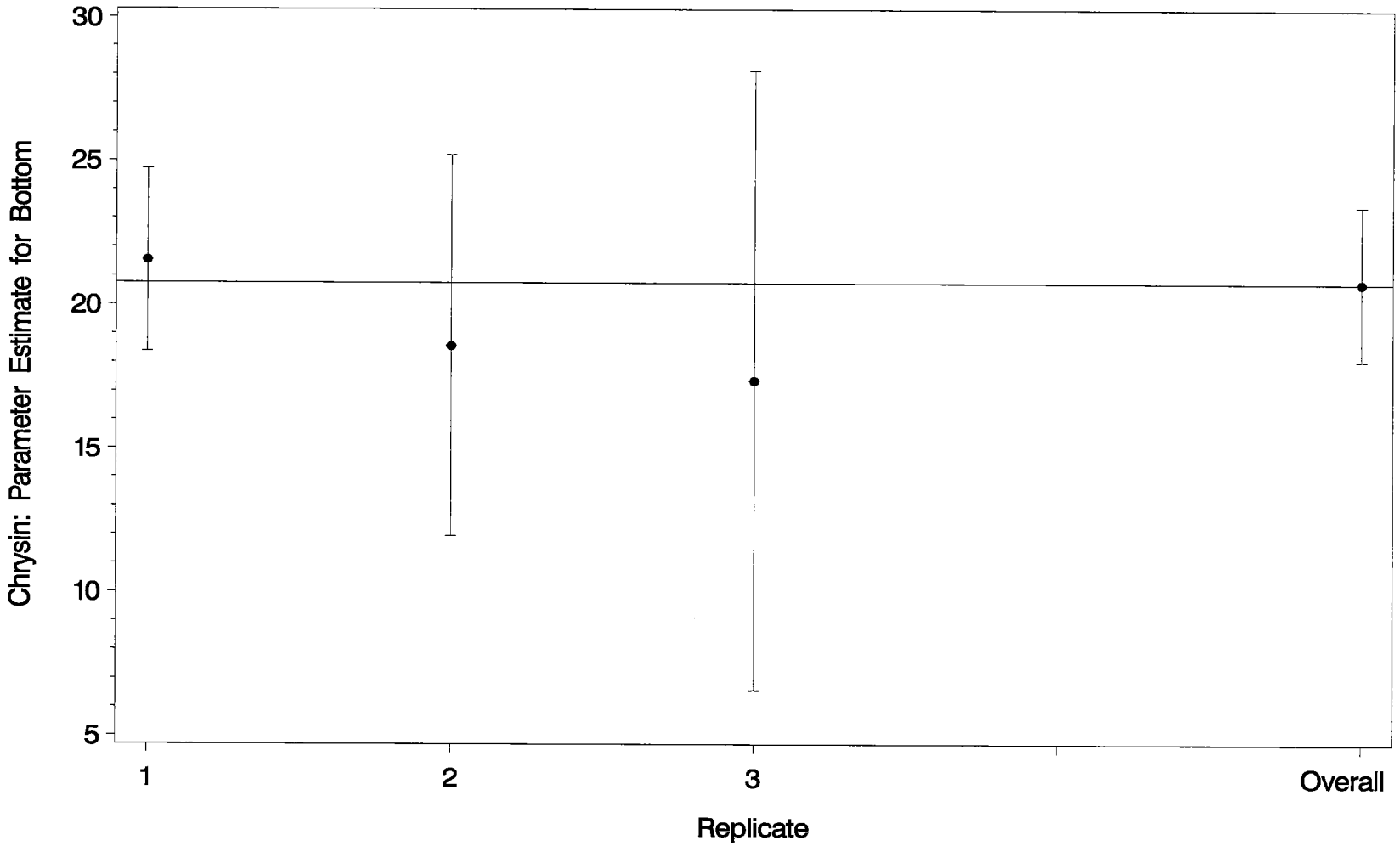


Figure 8-3. Reference Chemical VIII: Chrysin. Microsomes Prepared by IVT. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

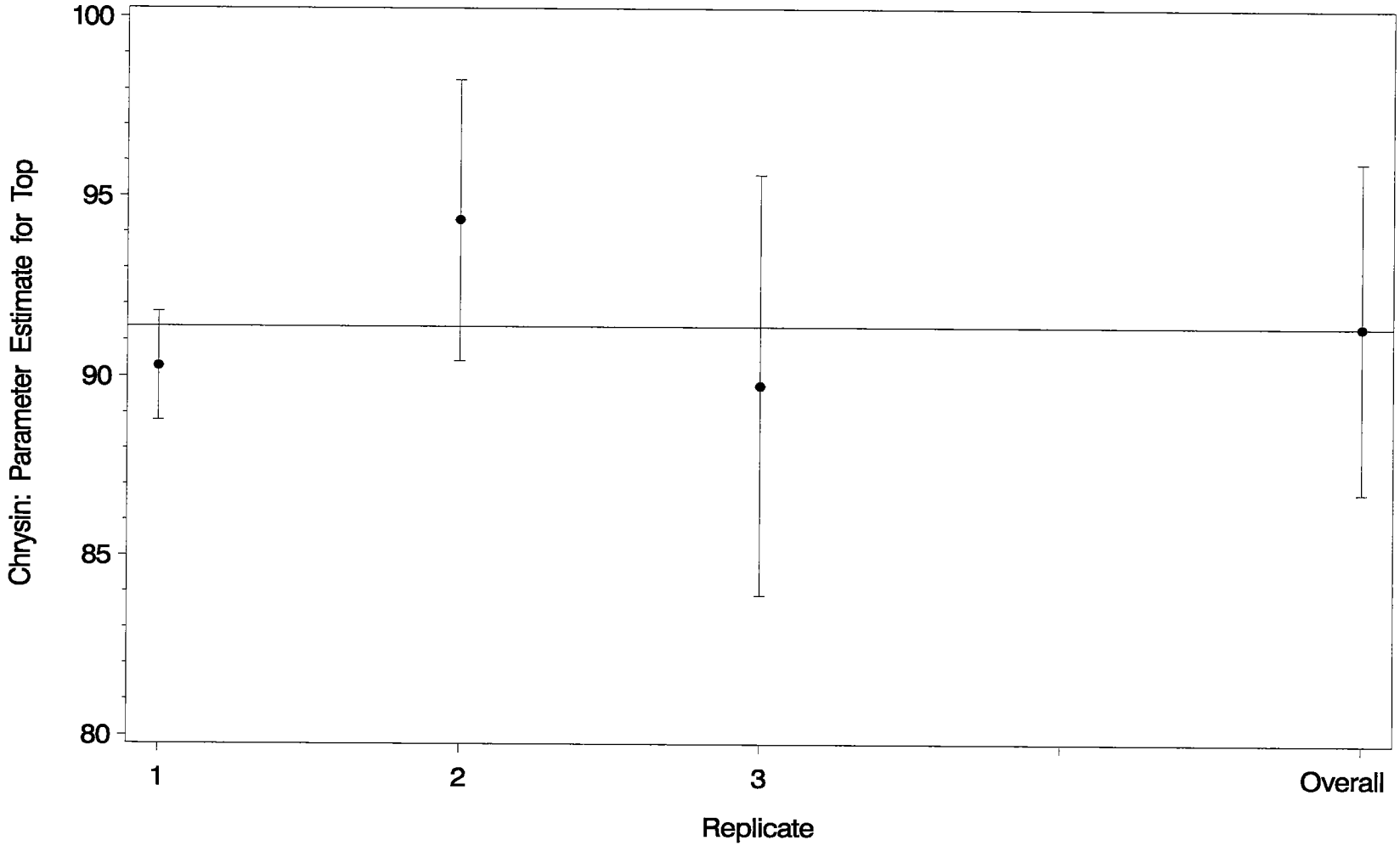


Figure 8-4. **Reference Chemical VIII: Chrysin. Microsomes Prepared by IVT. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

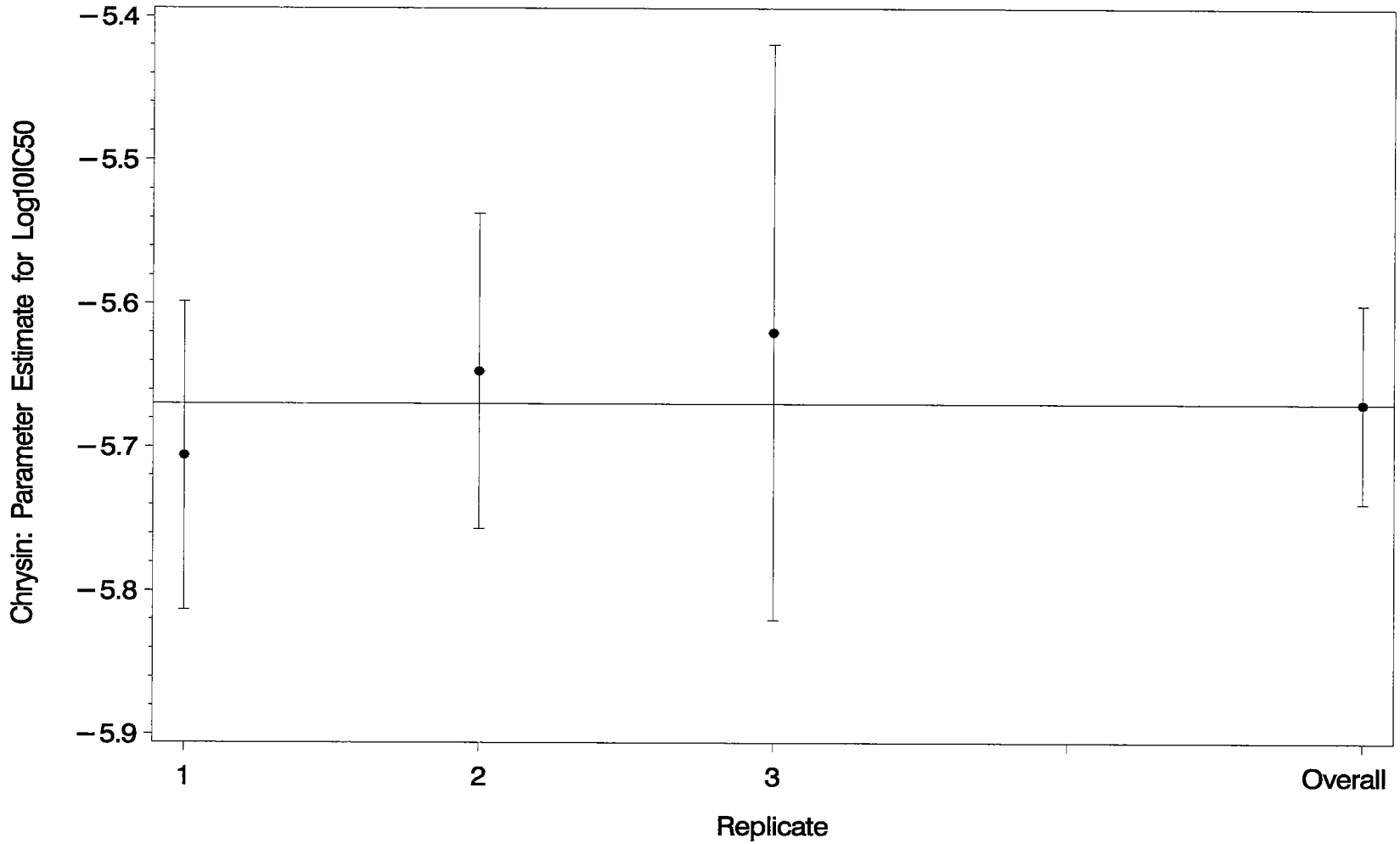


Figure 8-5. Reference Chemical VIII: Chrysin. Microsomes Prepared by IVT. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

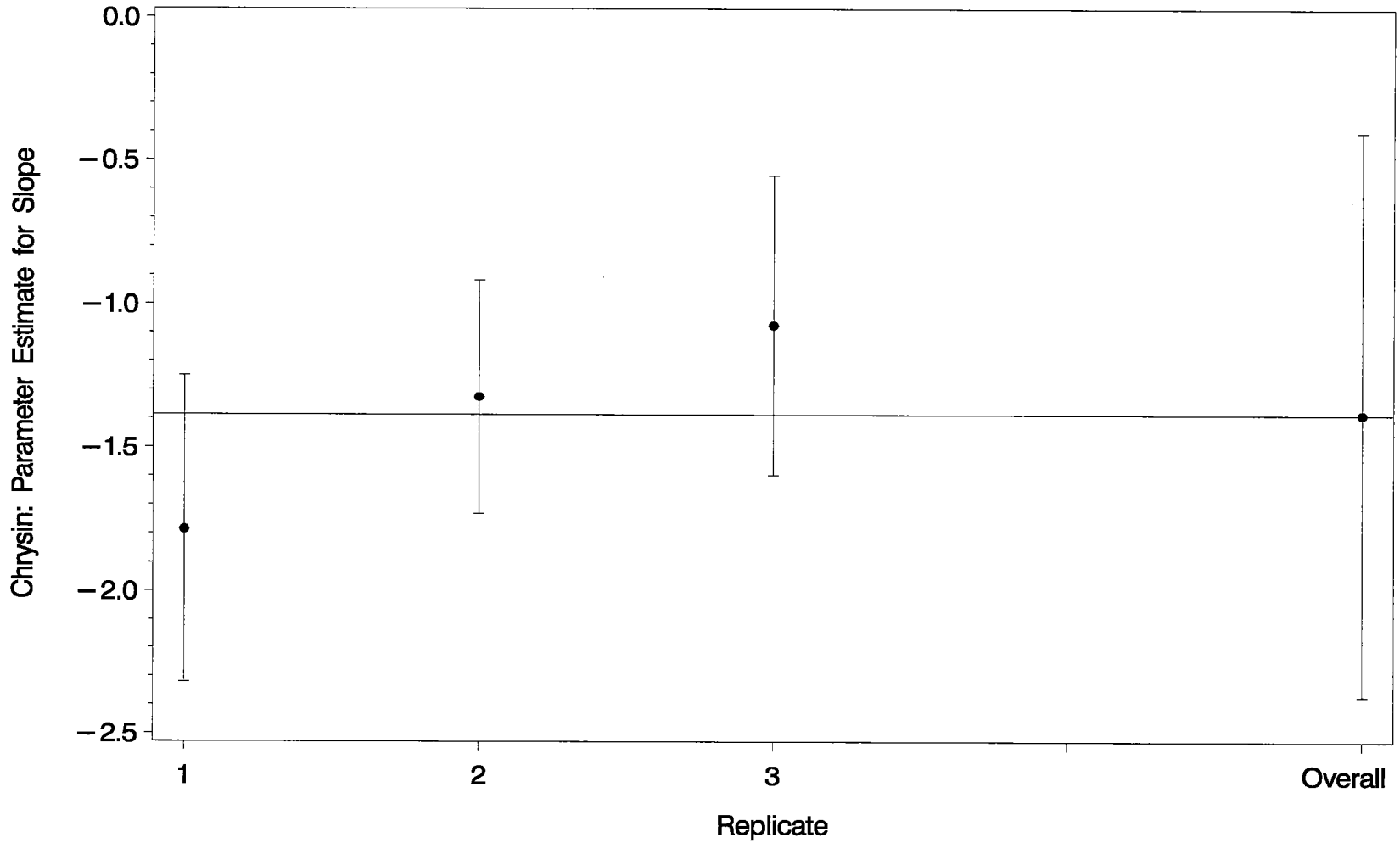


Figure 8-6. Reference Chemical VIII: Chrysin. Microsomes Prepared by IVT. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

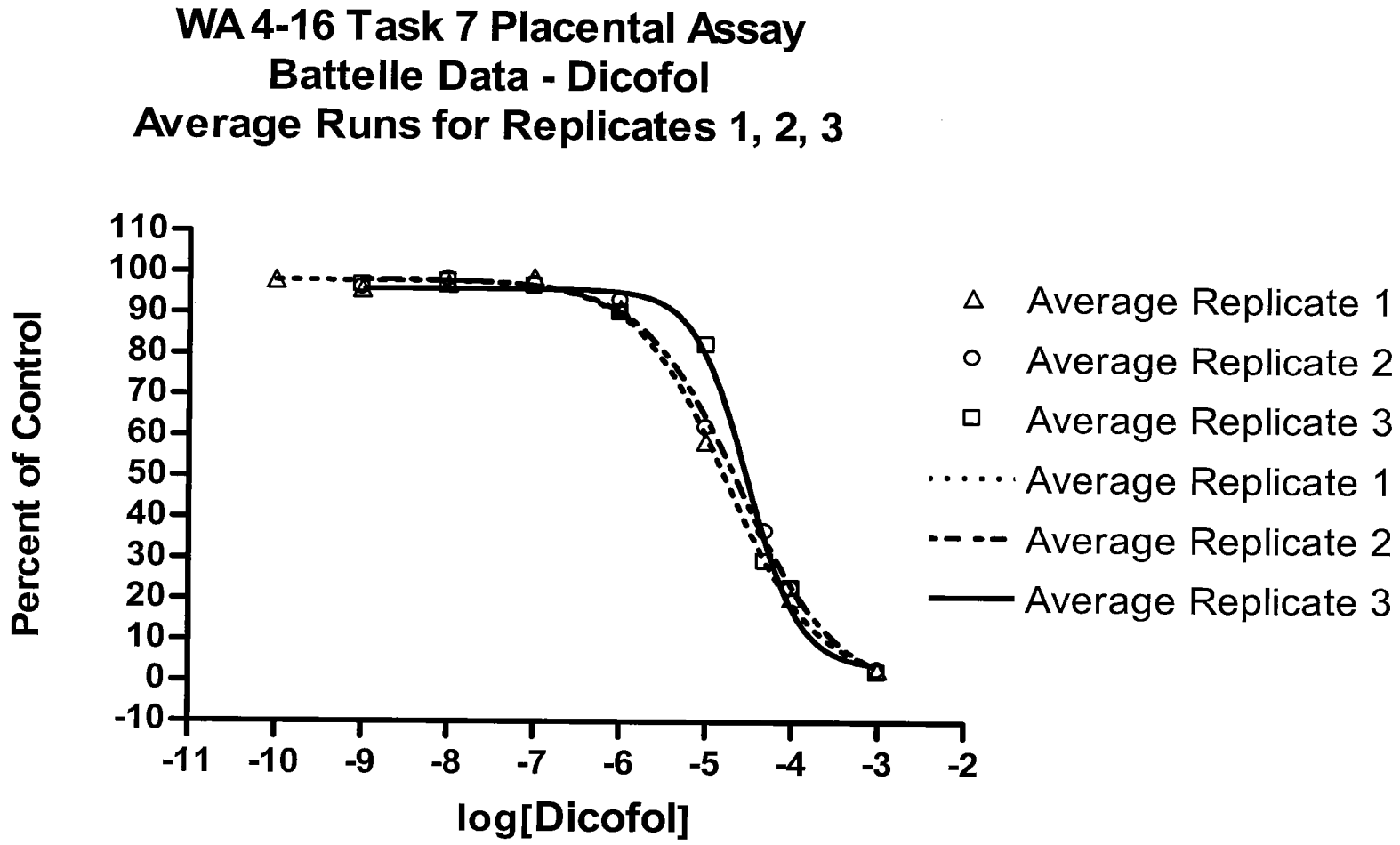


Figure 9-1. **Reference Chemical IX: Dicofol. Microsomes Prepared by Battelle. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

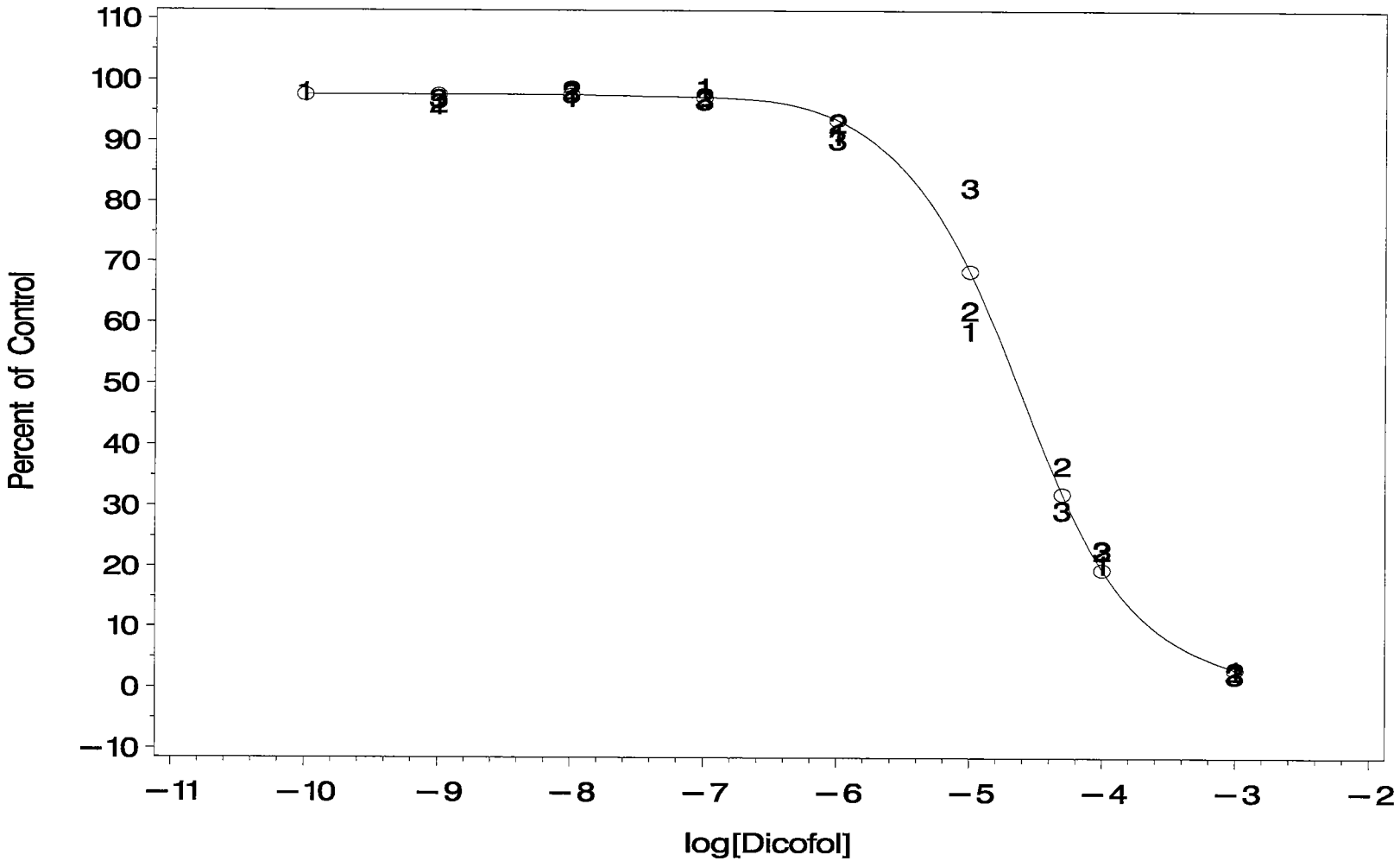


Figure 9-2. Reference Chemical IX: Dicofol. Microsomes Prepared by Battelle. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

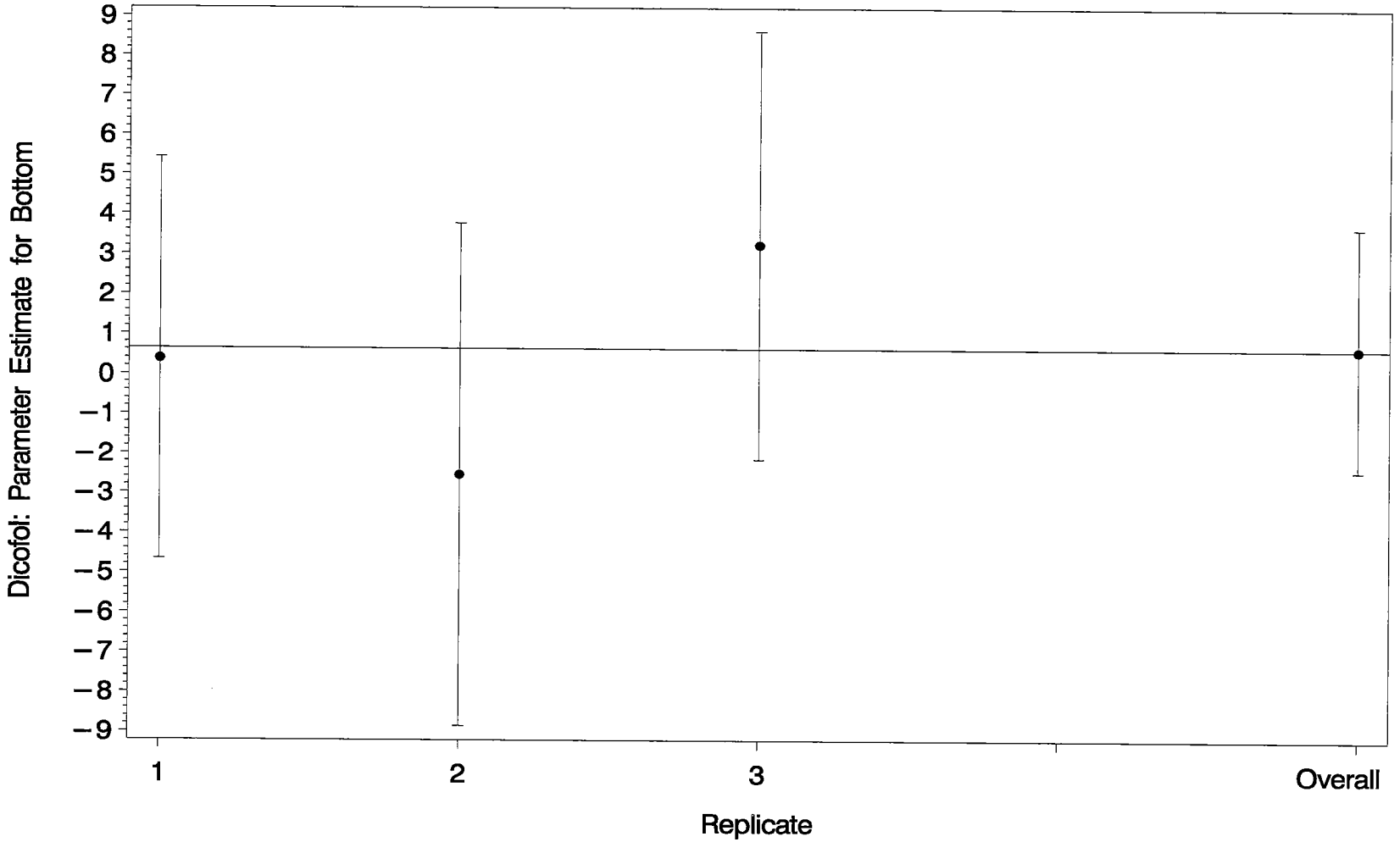


Figure 9-3. **Reference Chemical IX: Dicofol. Microsomes Prepared by Battelle. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

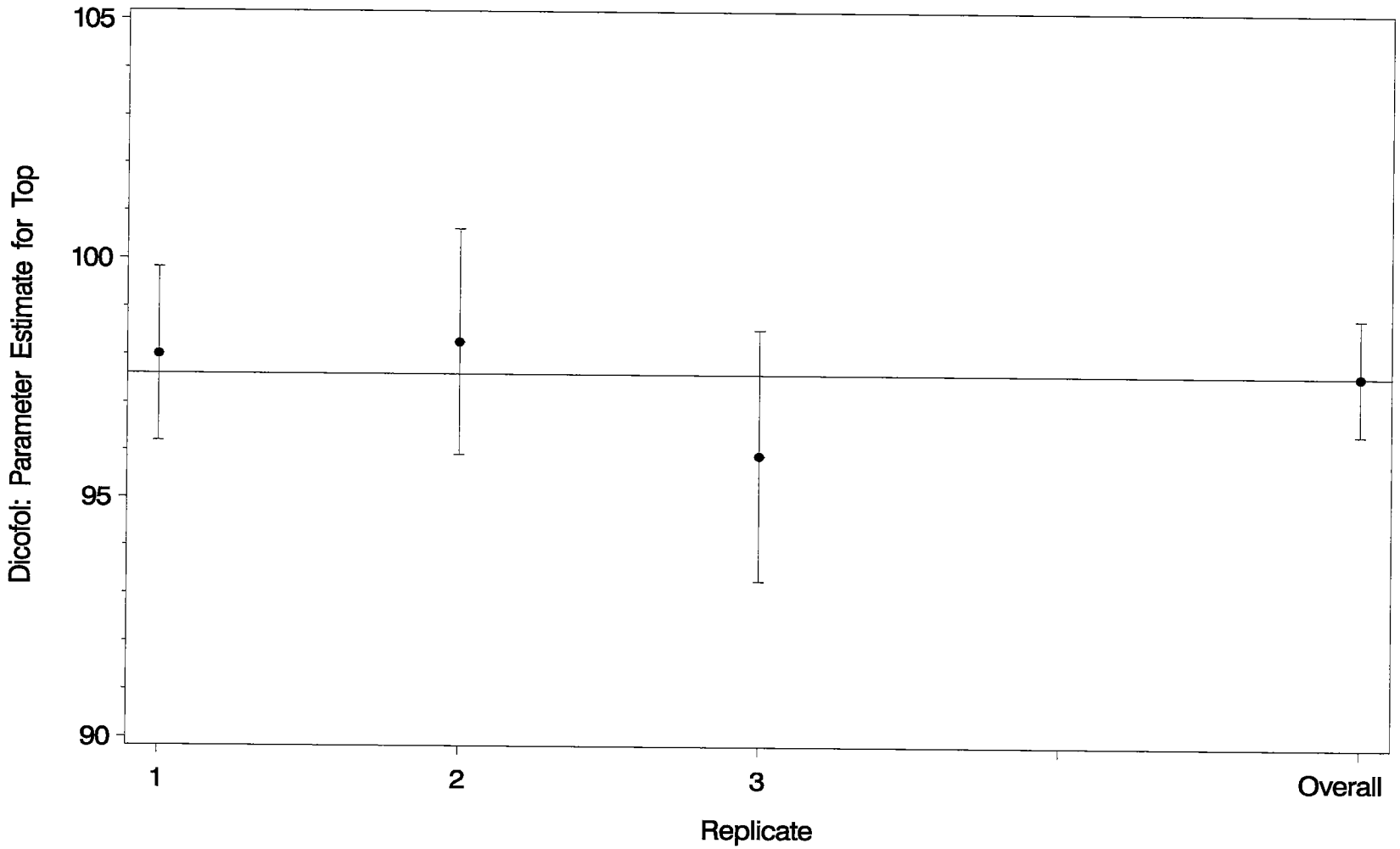


Figure 9-4. **Reference Chemical IX: Dicofol. Microsomes Prepared by Battelle. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

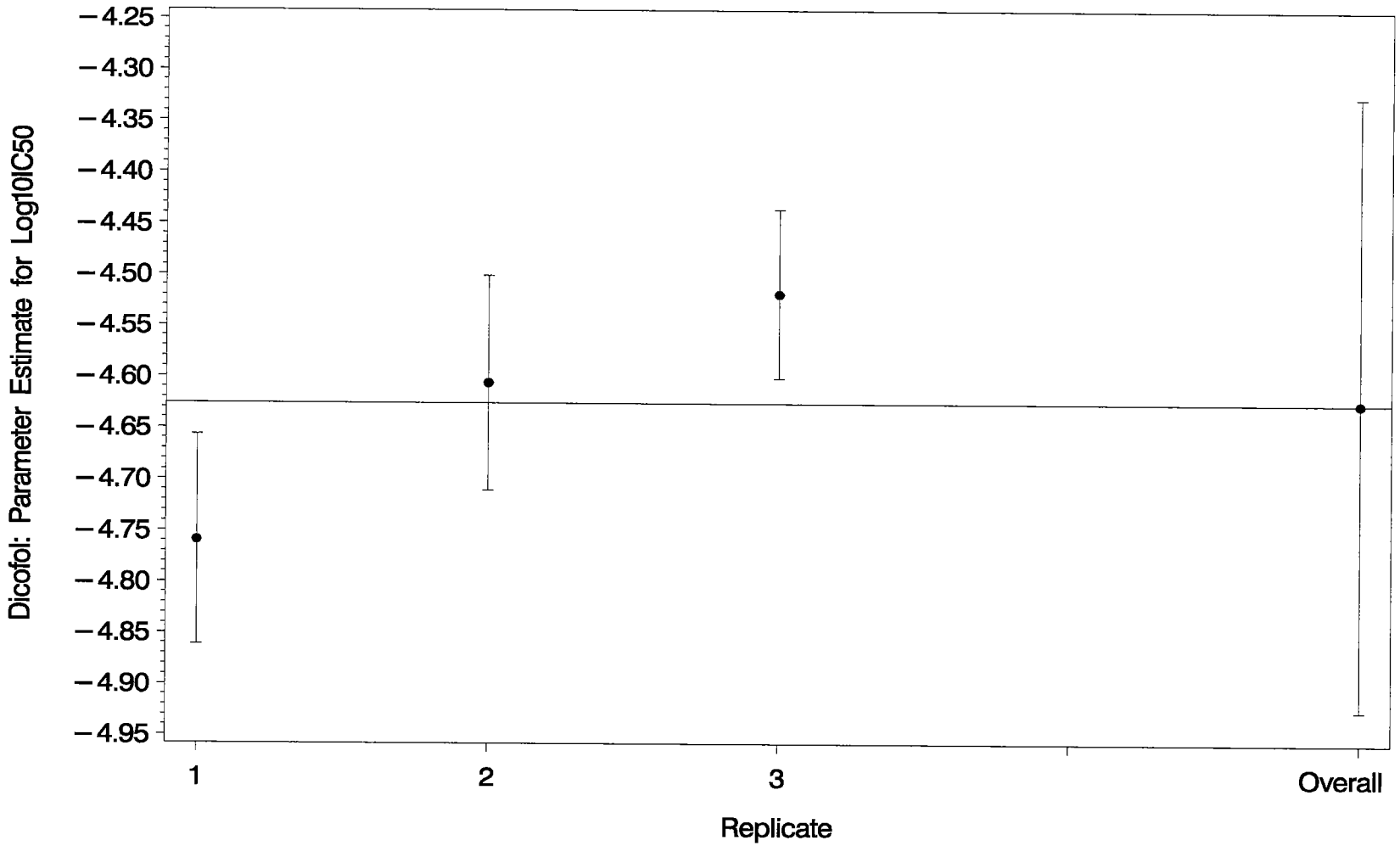


Figure 9-5. Reference Chemical IX: Dicofol. Microsomes Prepared by Battelle. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

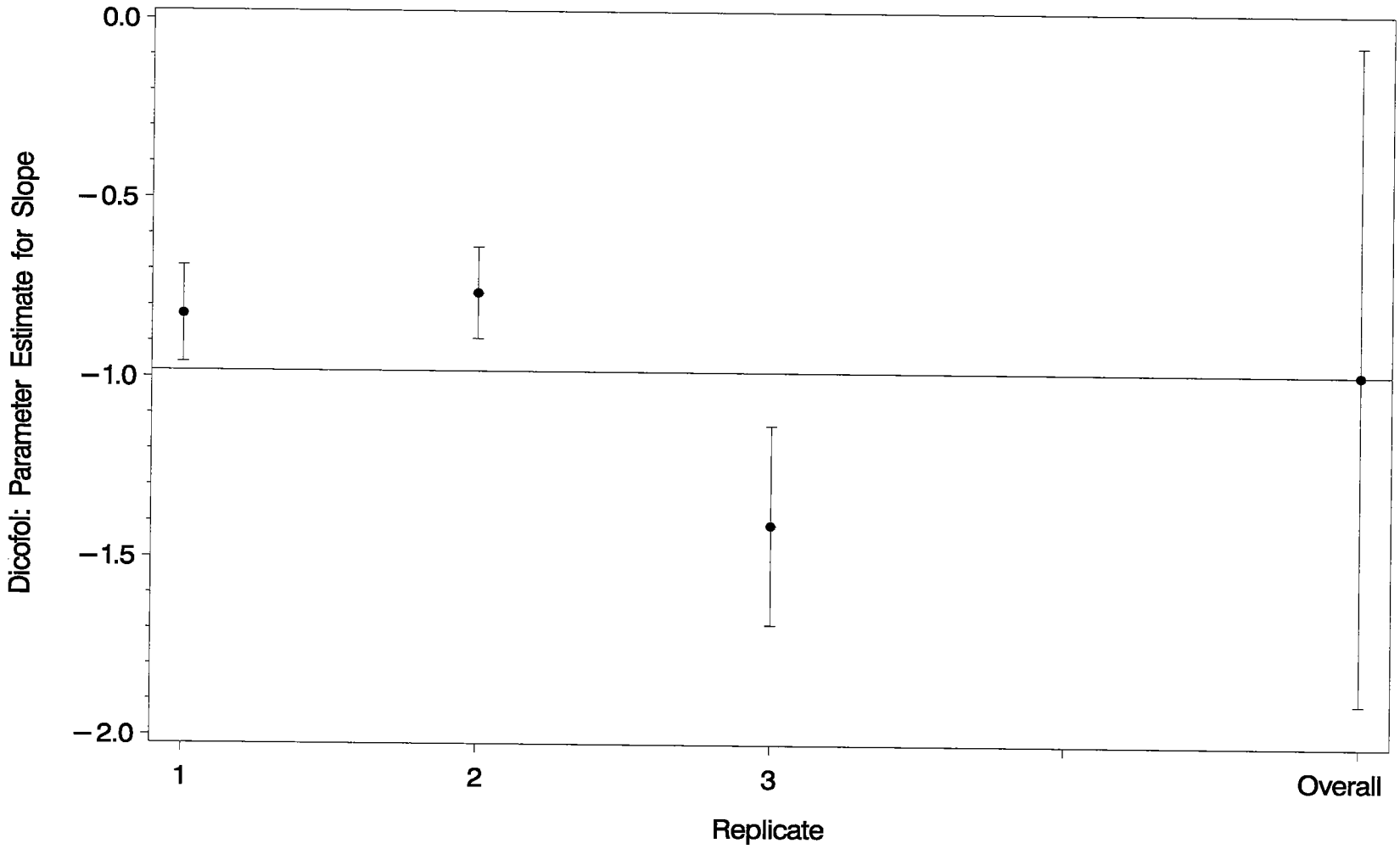


Figure 9-6. **Reference Chemical IX: Dicofol. Microsomes Prepared by Battelle. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

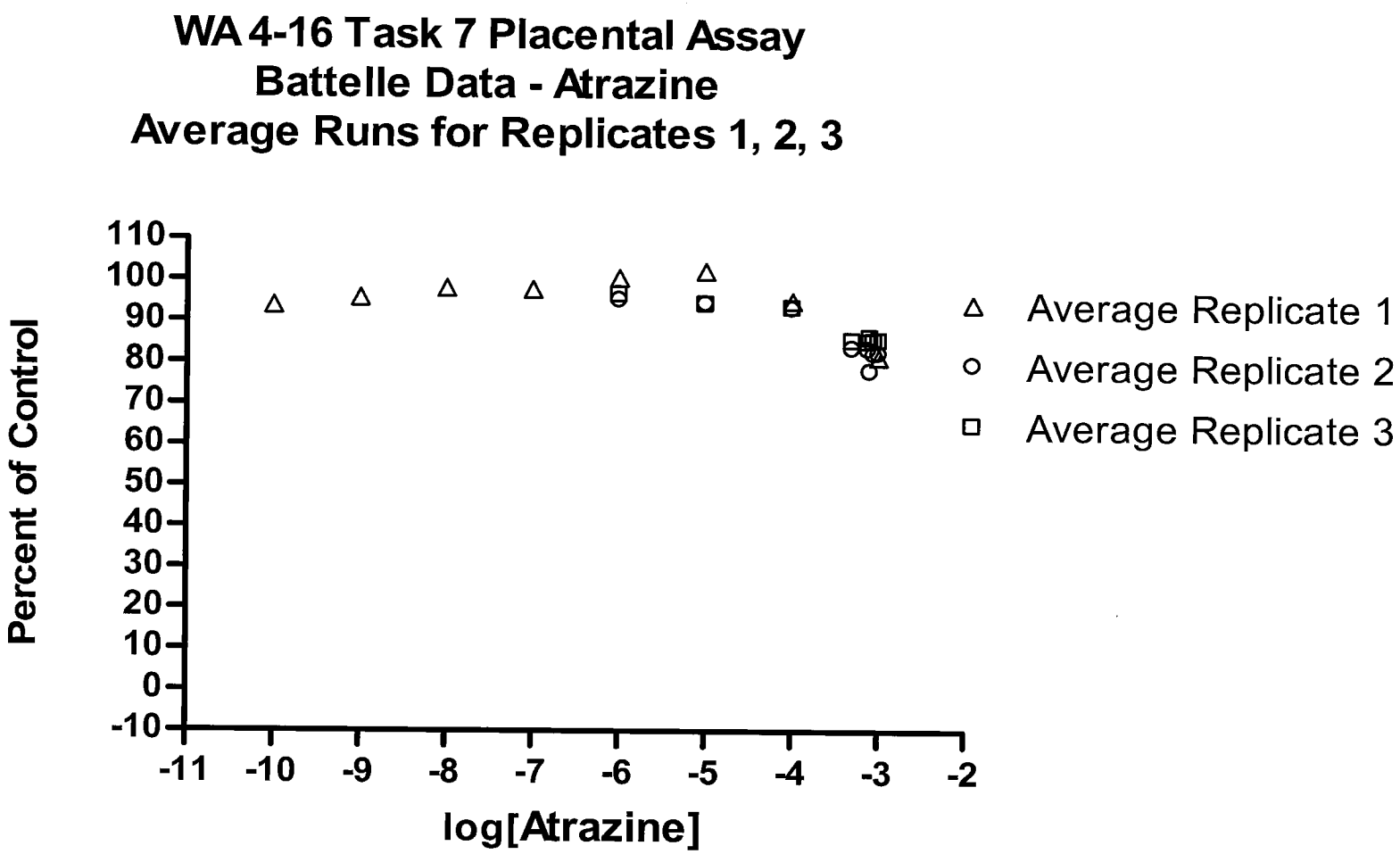


Figure 10-1. Reference Chemical X: Atrazine. Microsomes Prepared by Battelle. Scatter Plot for Averages of Repetitions within Concentration for Each Replicate.

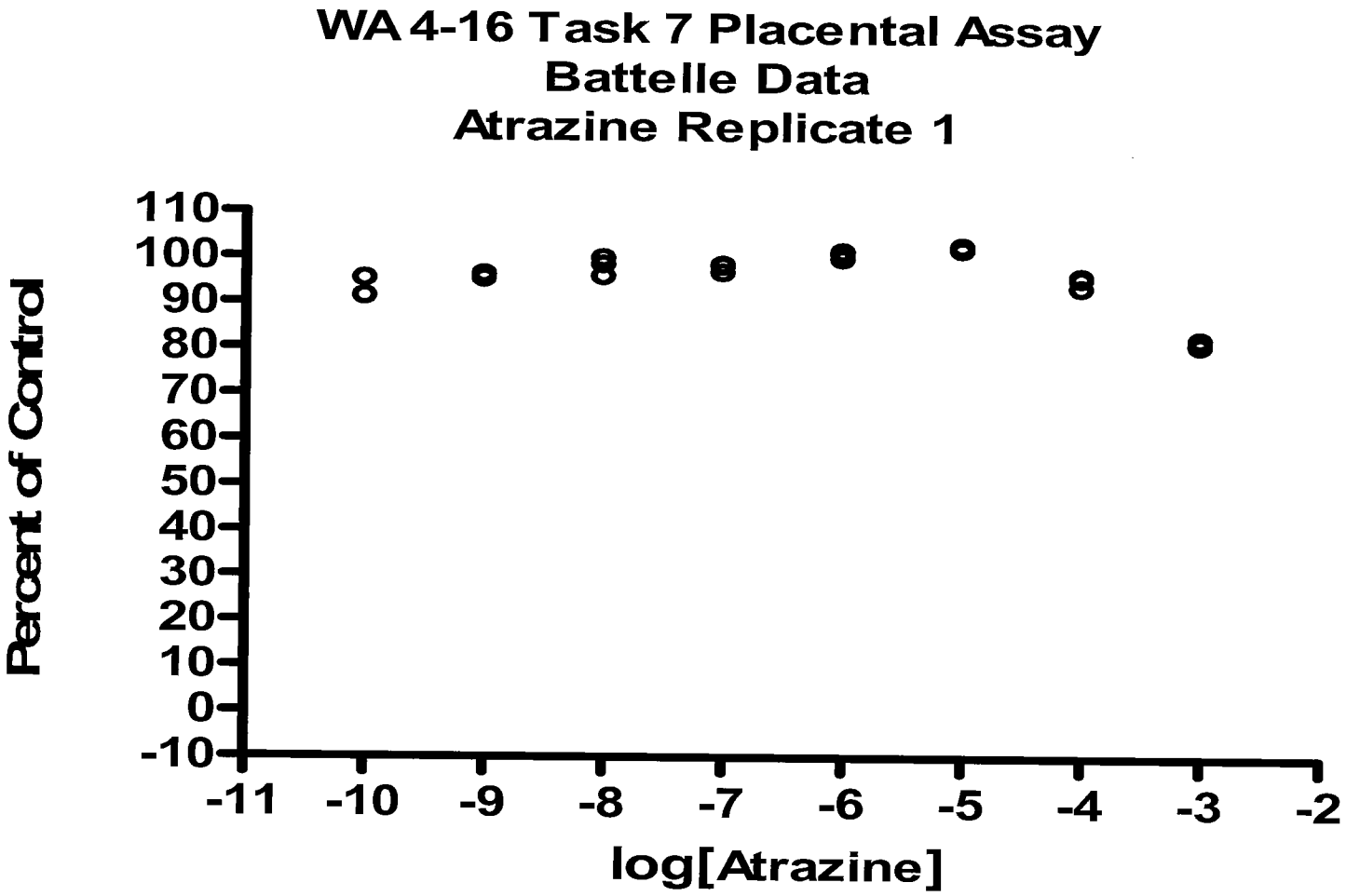


Figure 10-2. Reference Chemical X: Atrazine. Microsomes Prepared by Battelle. Scatter Plot for Replicate 1 Data.

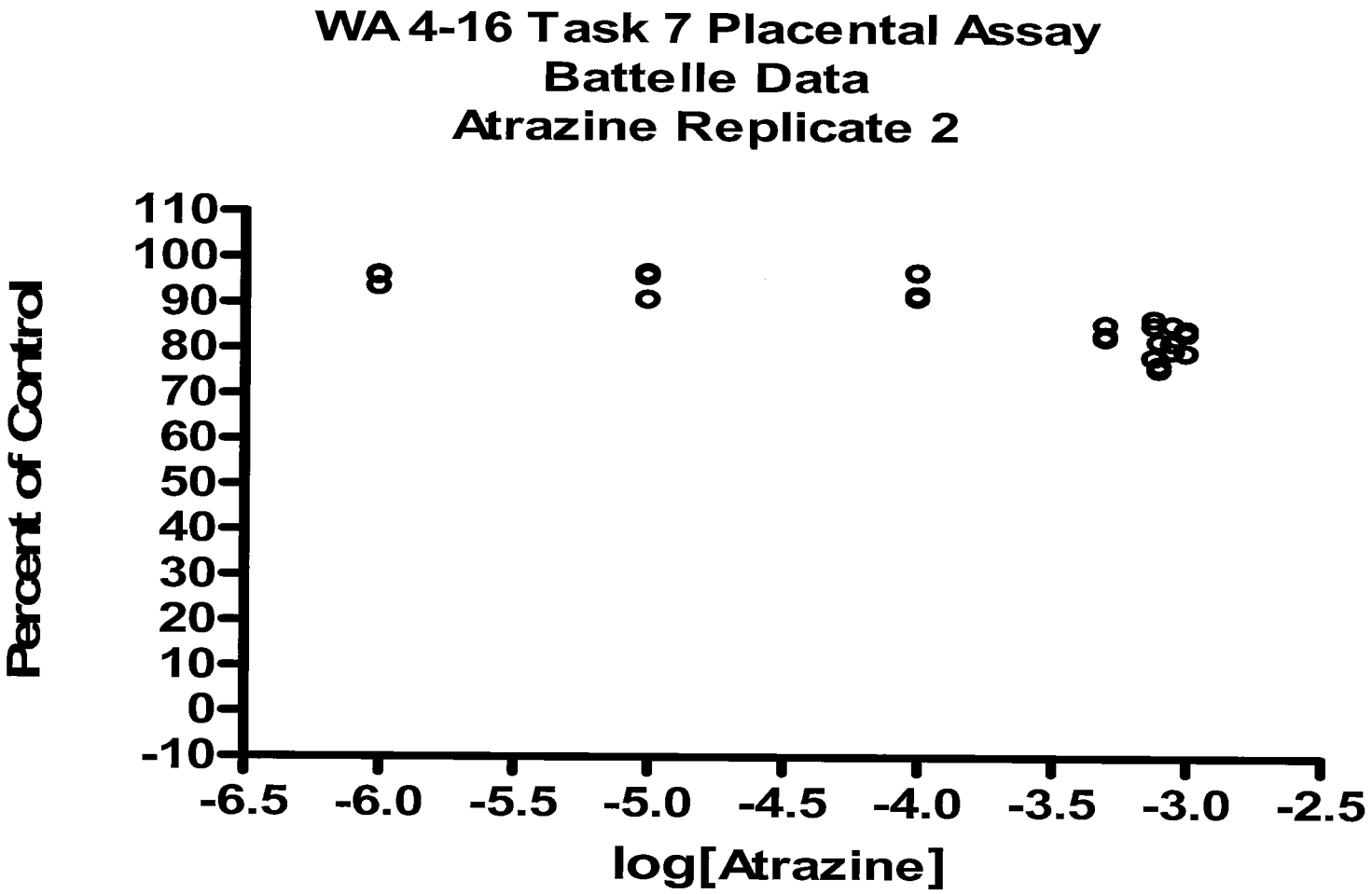


Figure 10-3. Reference Chemical X: Atrazine. Microsomes Prepared by Battelle. Scatter Plot for Replicate 2 Data.

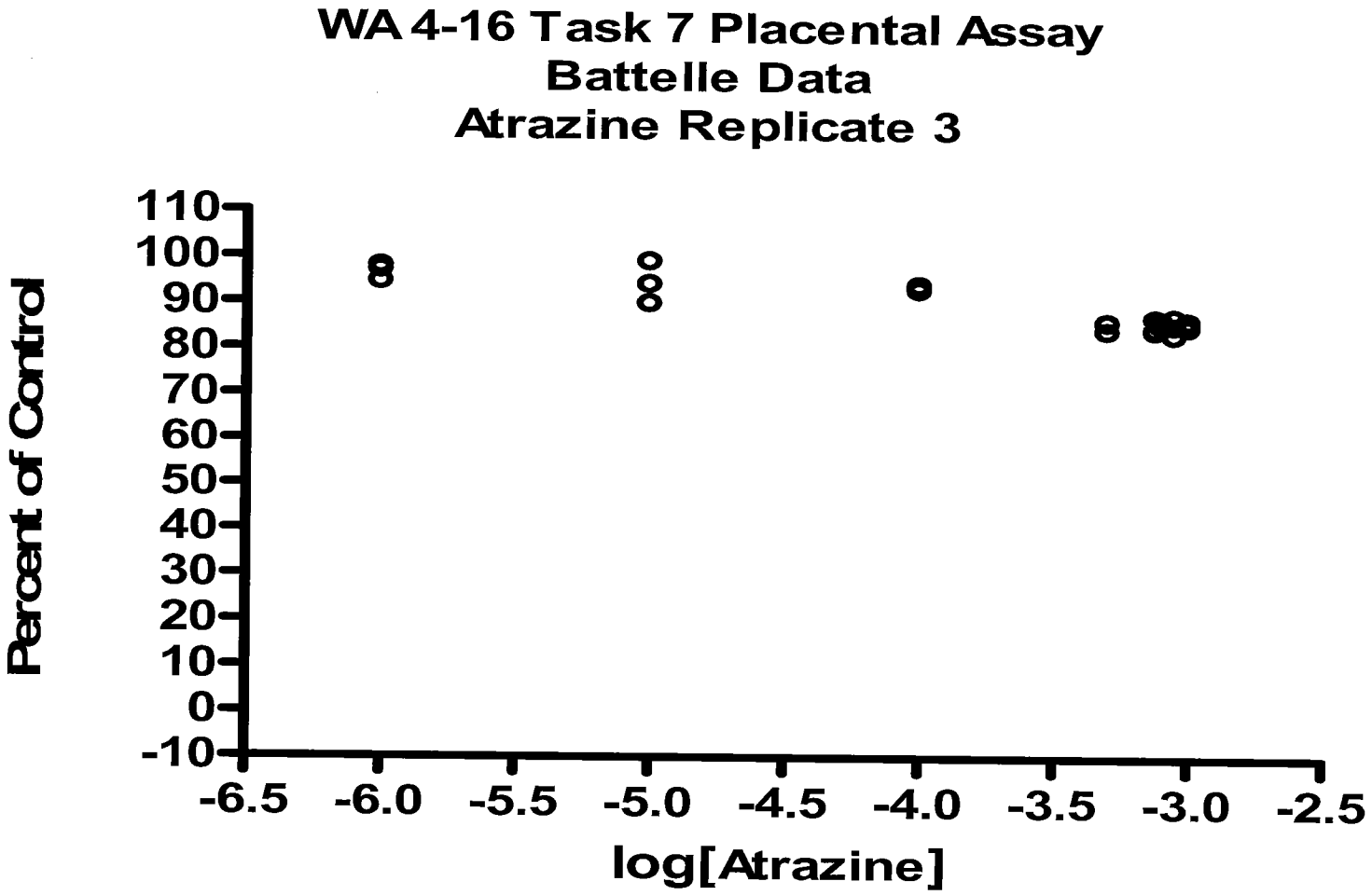


Figure 10-4. Reference Chemical X: Atrazine. Microsomes Prepared by Battelle. Scatter Plot for Replicate 3 Data.

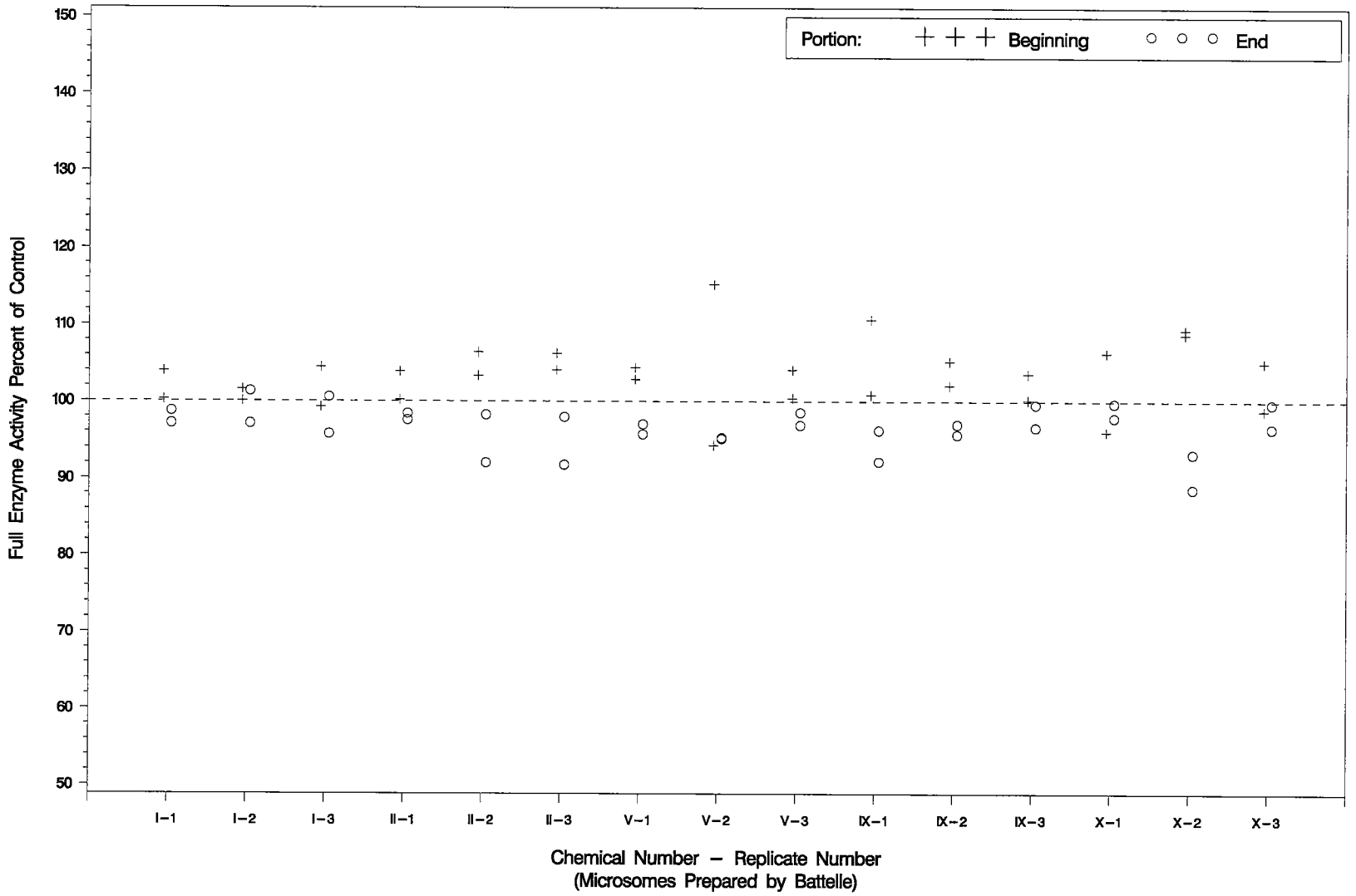


Figure 11a. **Full Enzyme Activity Control Data. Microsomes Prepared by Battelle. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 100%.**

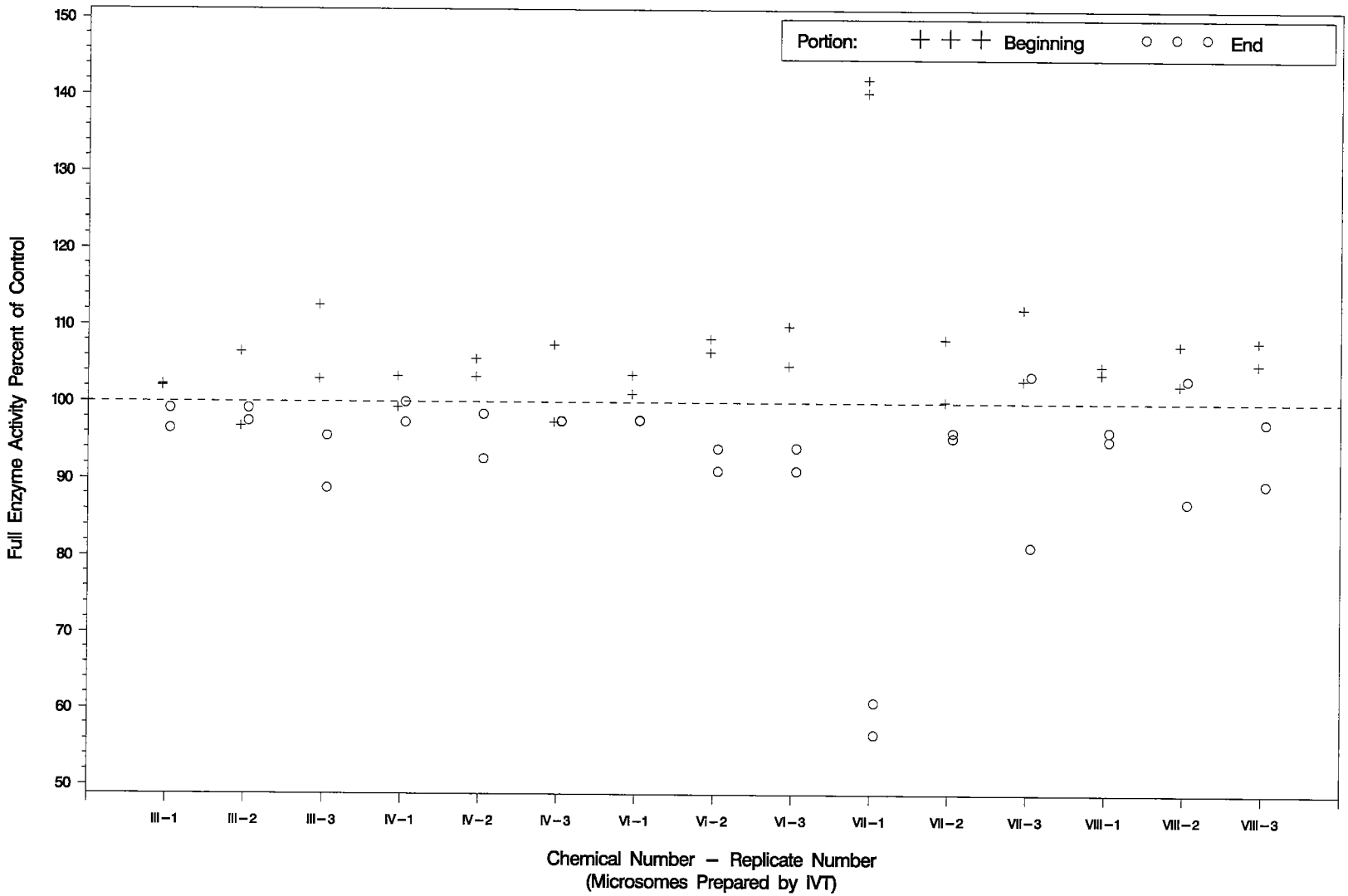


Figure 11b. **Full Enzyme Activity Control Data. Microsomes Prepared by IVT. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 100%.**

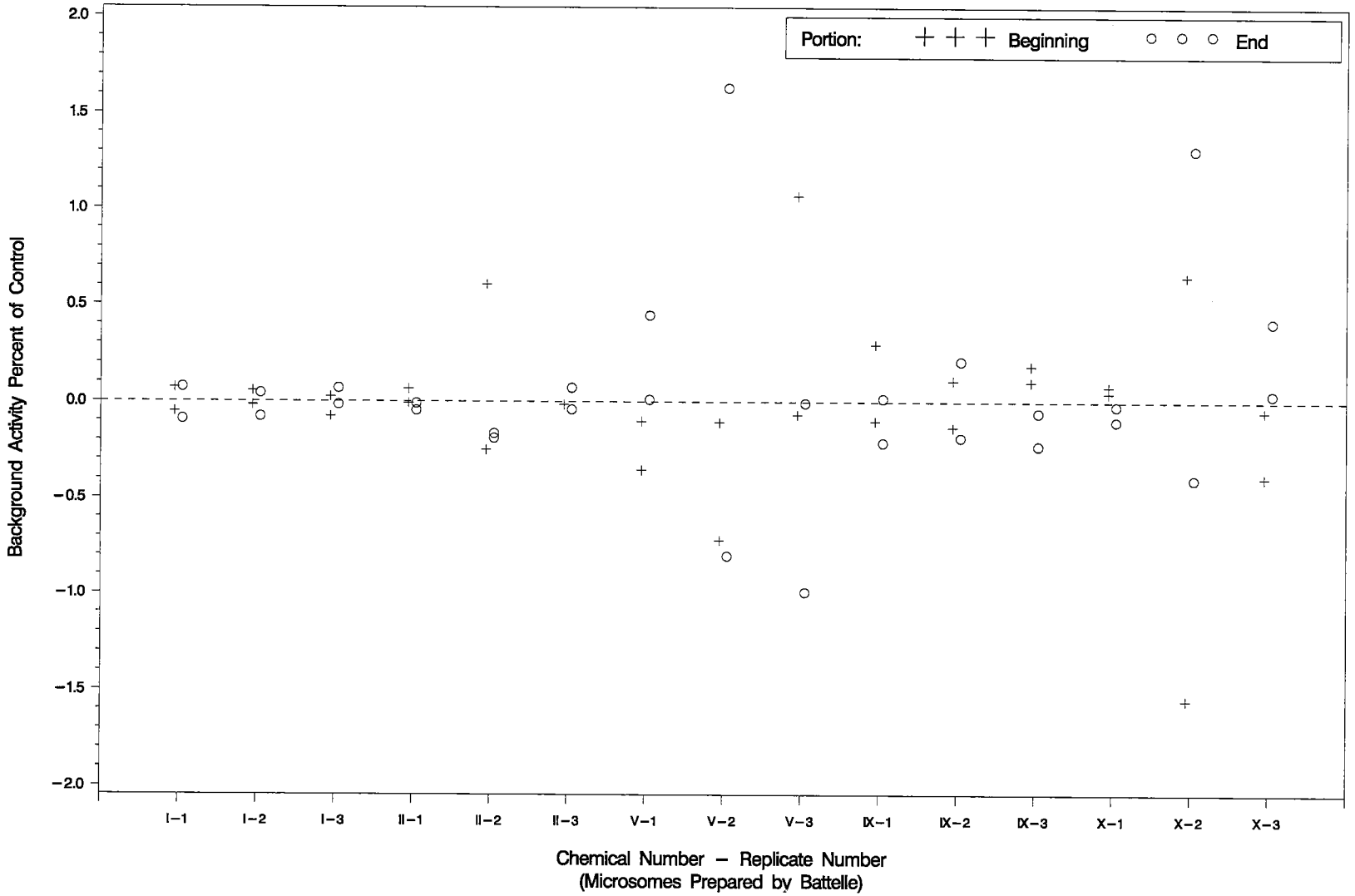


Figure 12a. **Background Activity Control Data. Microsomes Prepared by Battelle. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 0%.**

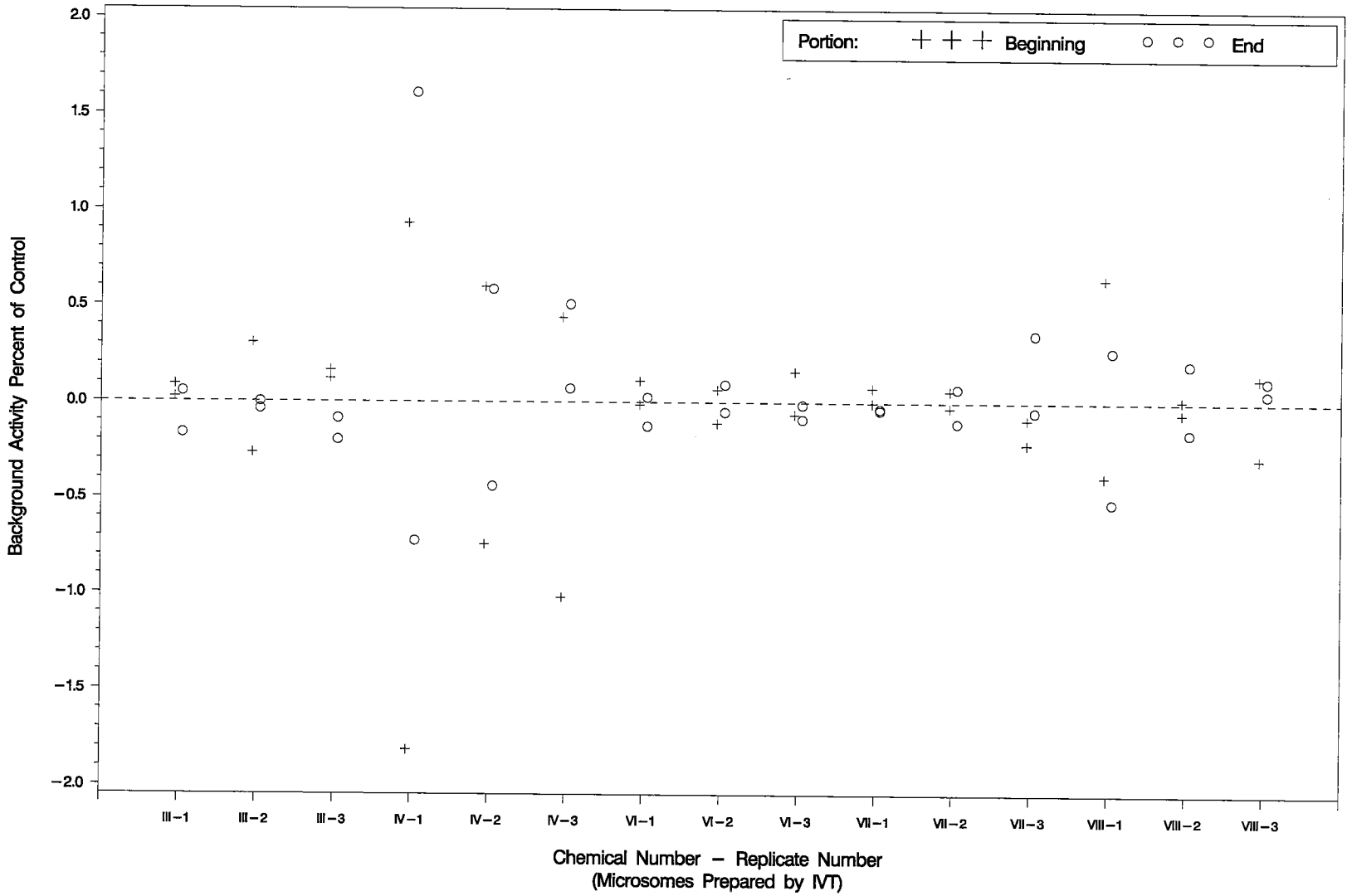


Figure 12b. **Background Activity Control Data. Microsomes Prepared by IVT. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 0%.**

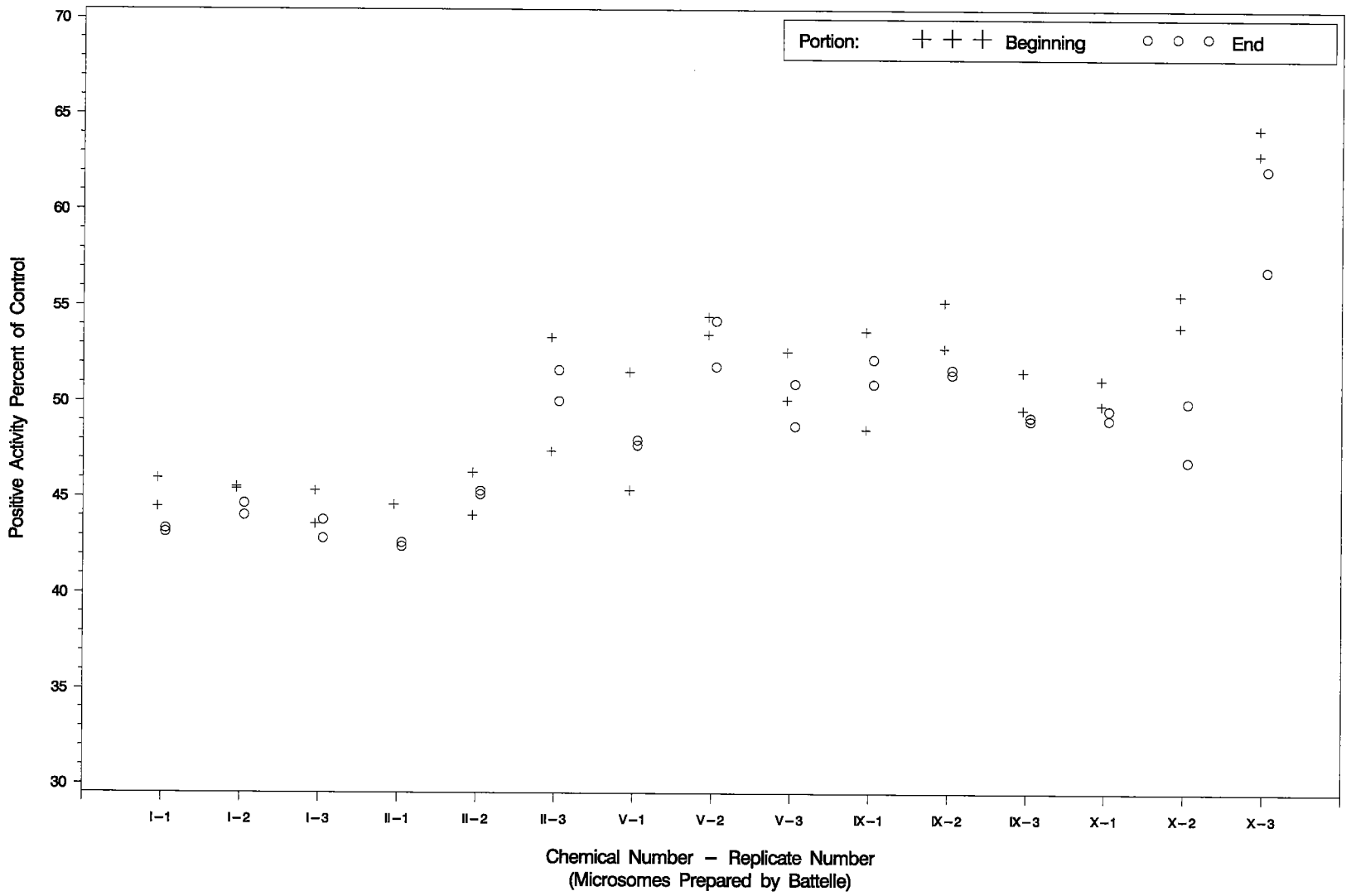


Figure 13a. **Positive Control Data. Microsomes Prepared by Battelle. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay.**

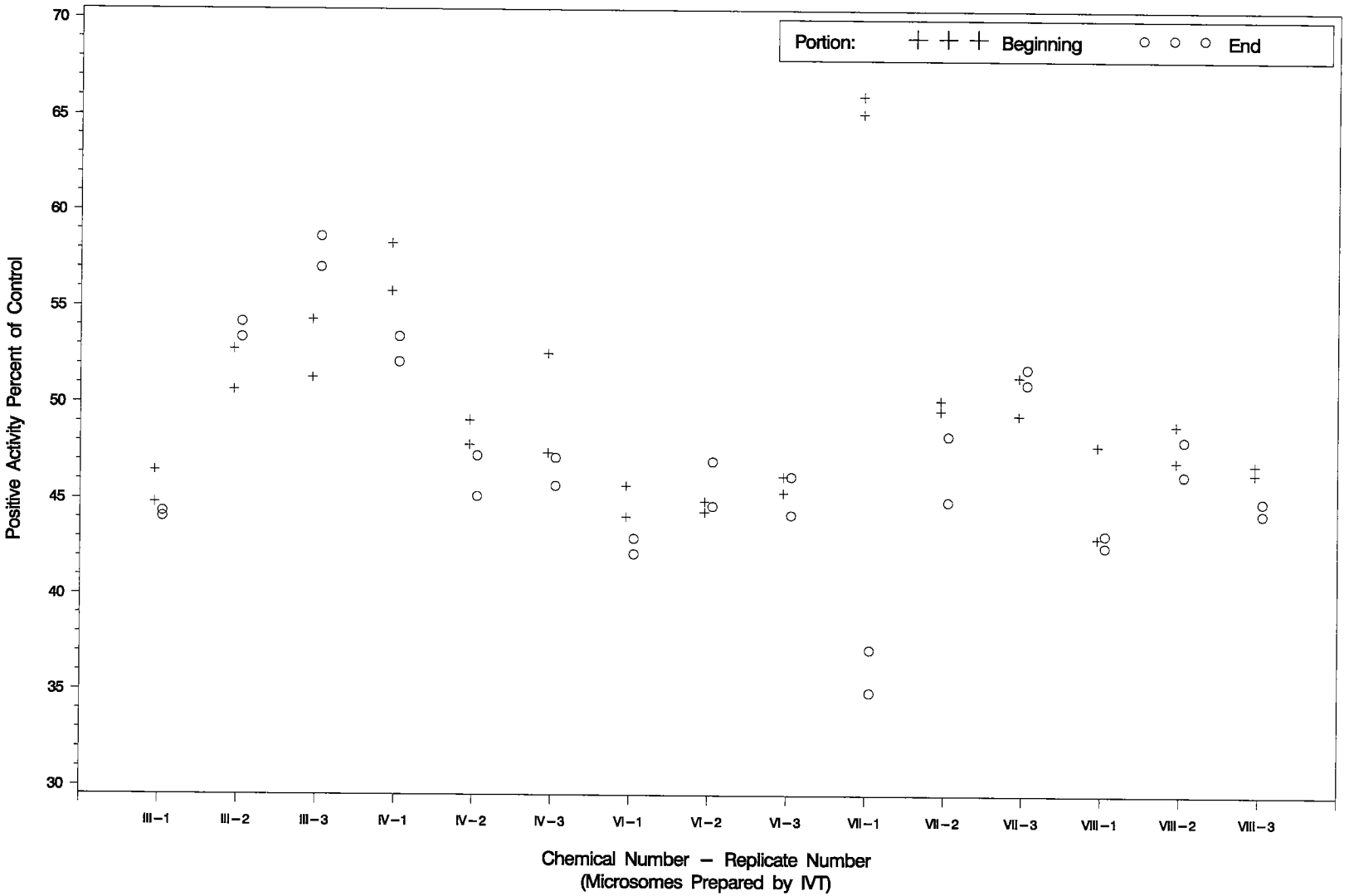


Figure 13b. **Positive Control Data. Microsomes Prepared by IVT. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay.**

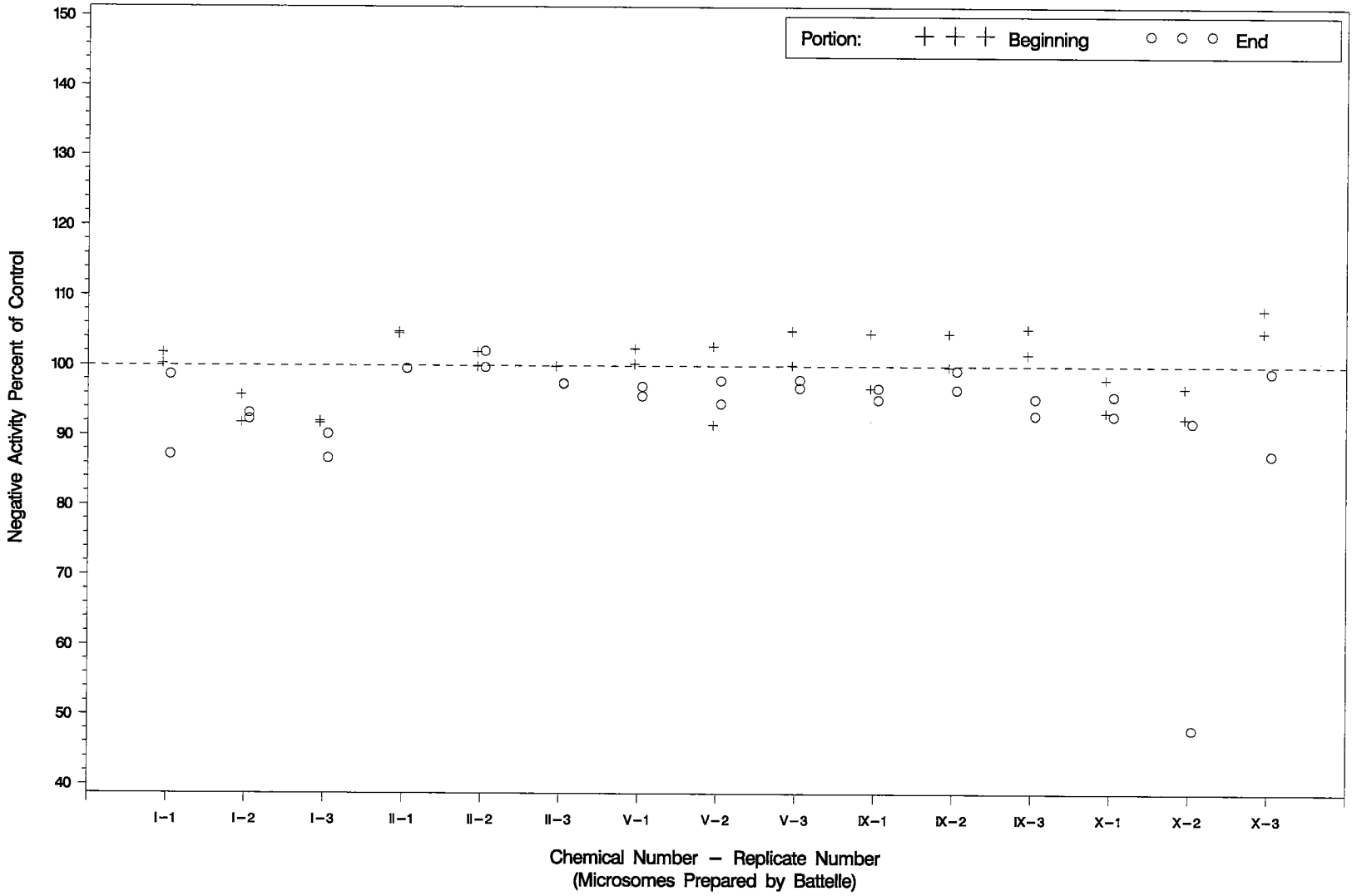


Figure 14a. **Negative Control Data. Microsomes Prepared by Battelle. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 100%.**

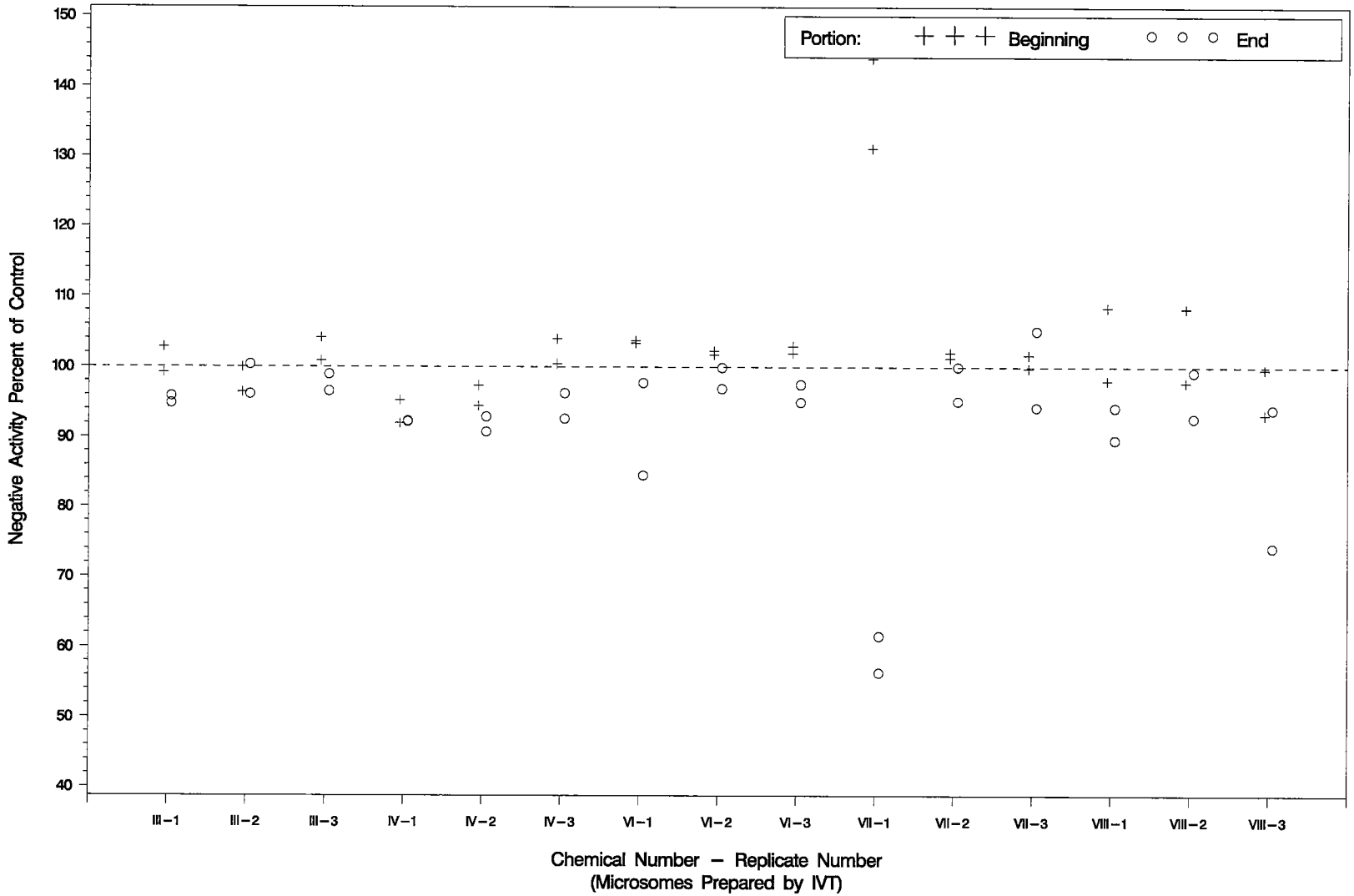


Figure 14b. **Negative Control Data. Microsomes Prepared by IVT. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 100%.**

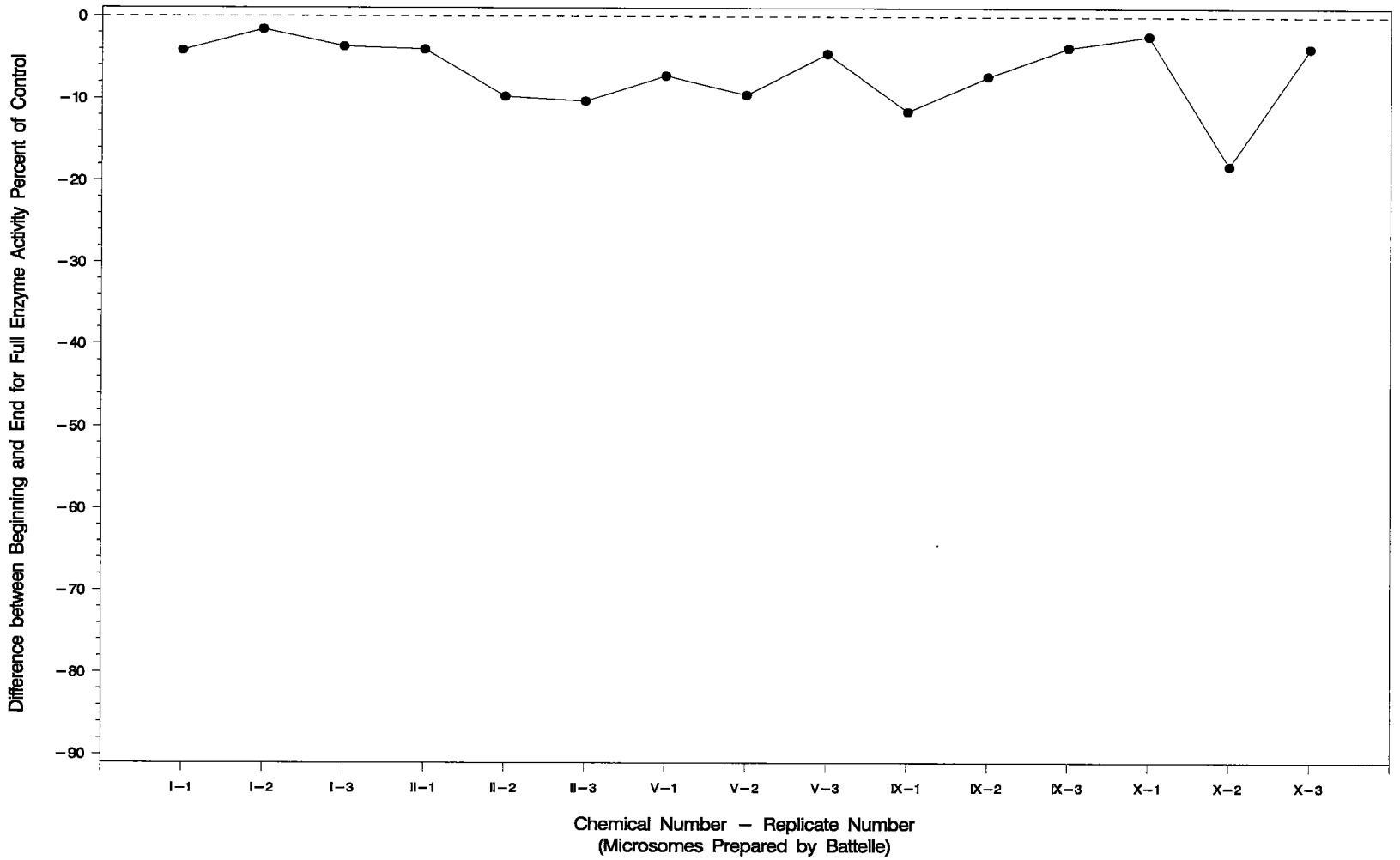


Figure 15a. **Full Enzyme Activity Control Data. Microsomes Prepared by Battelle. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

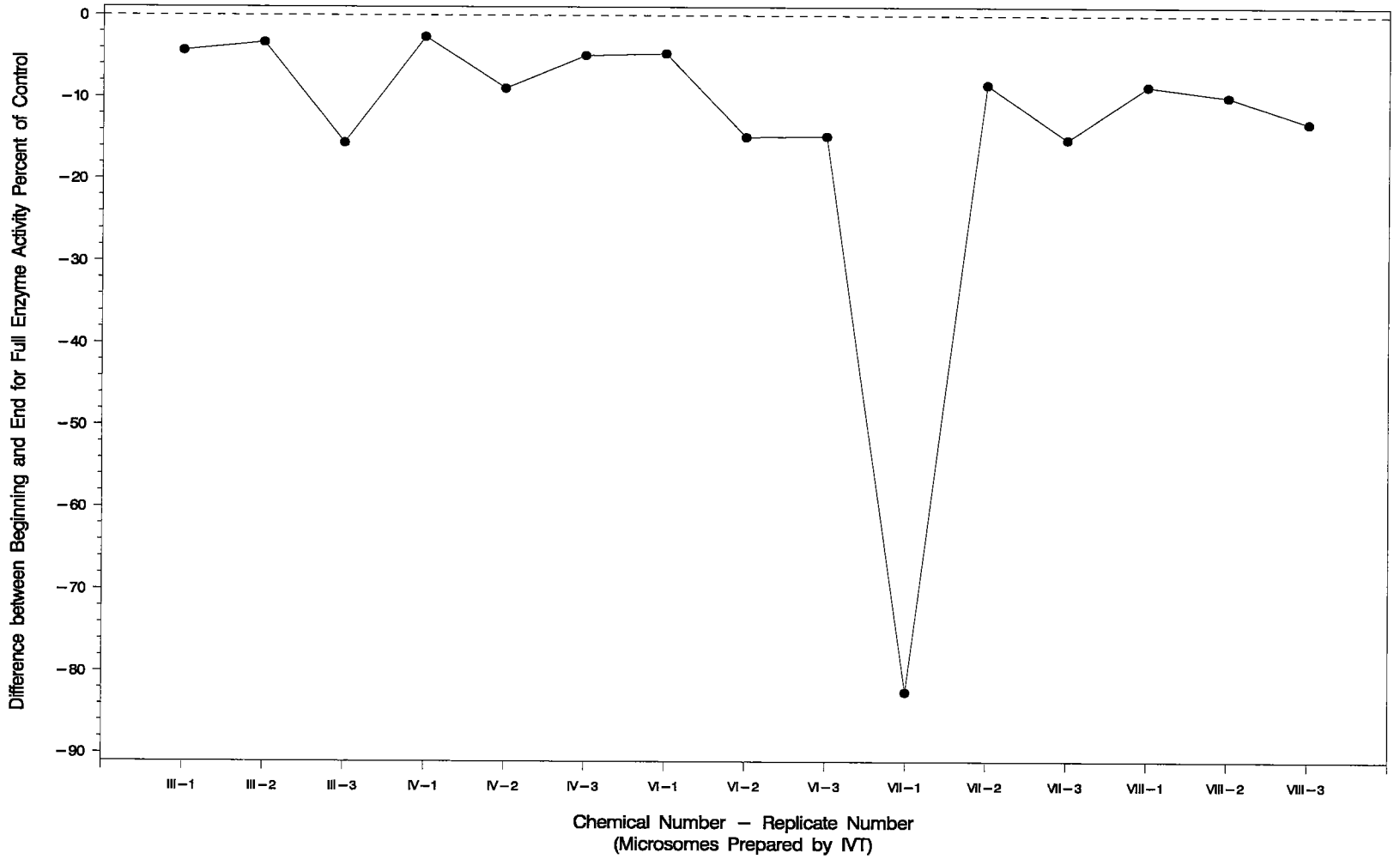


Figure 15b. **Full Enzyme Activity Control Data. Microsomes Prepared by IVT. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

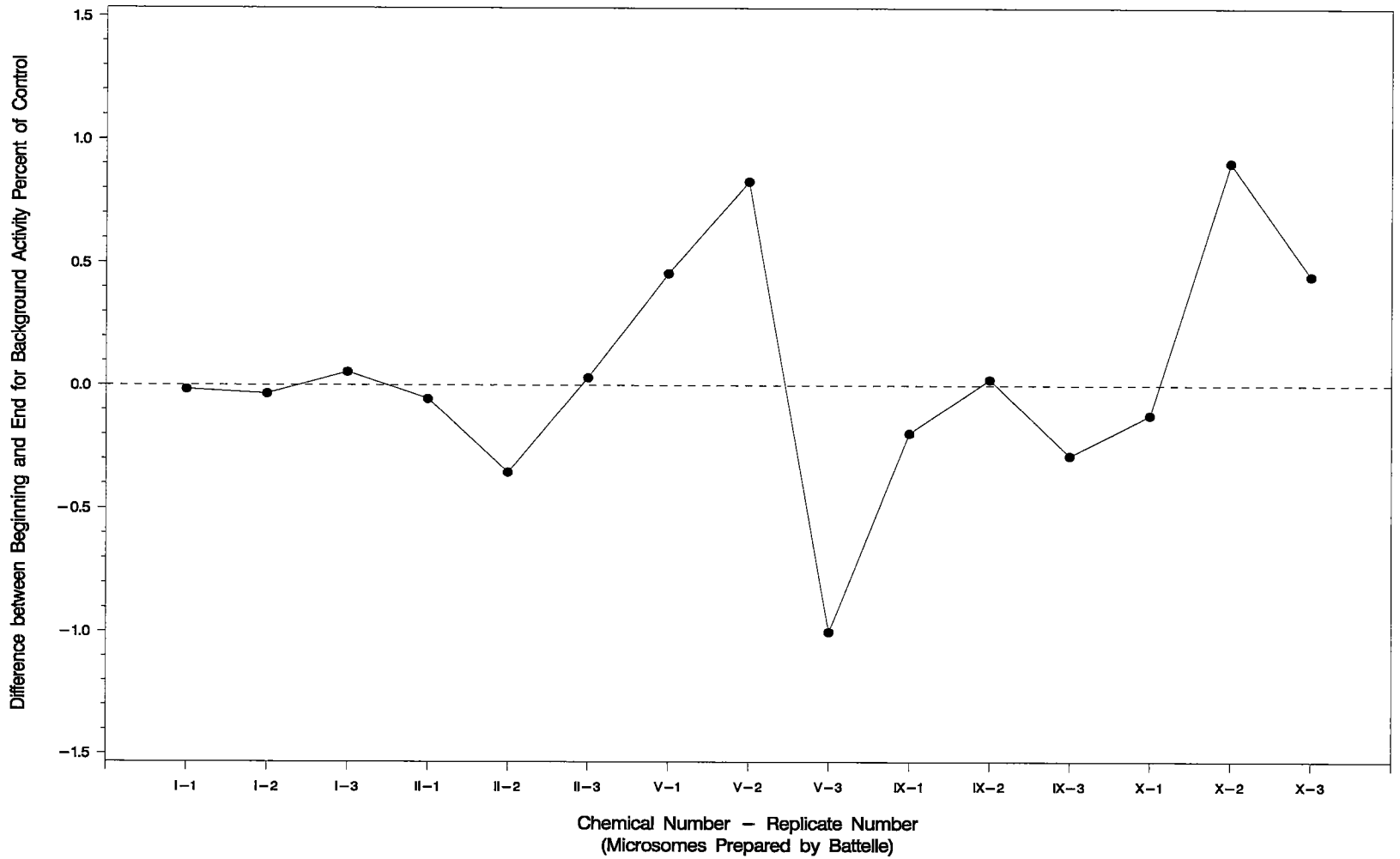


Figure 16a. **Background Activity Control Data. Microsomes Prepared by Battelle. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

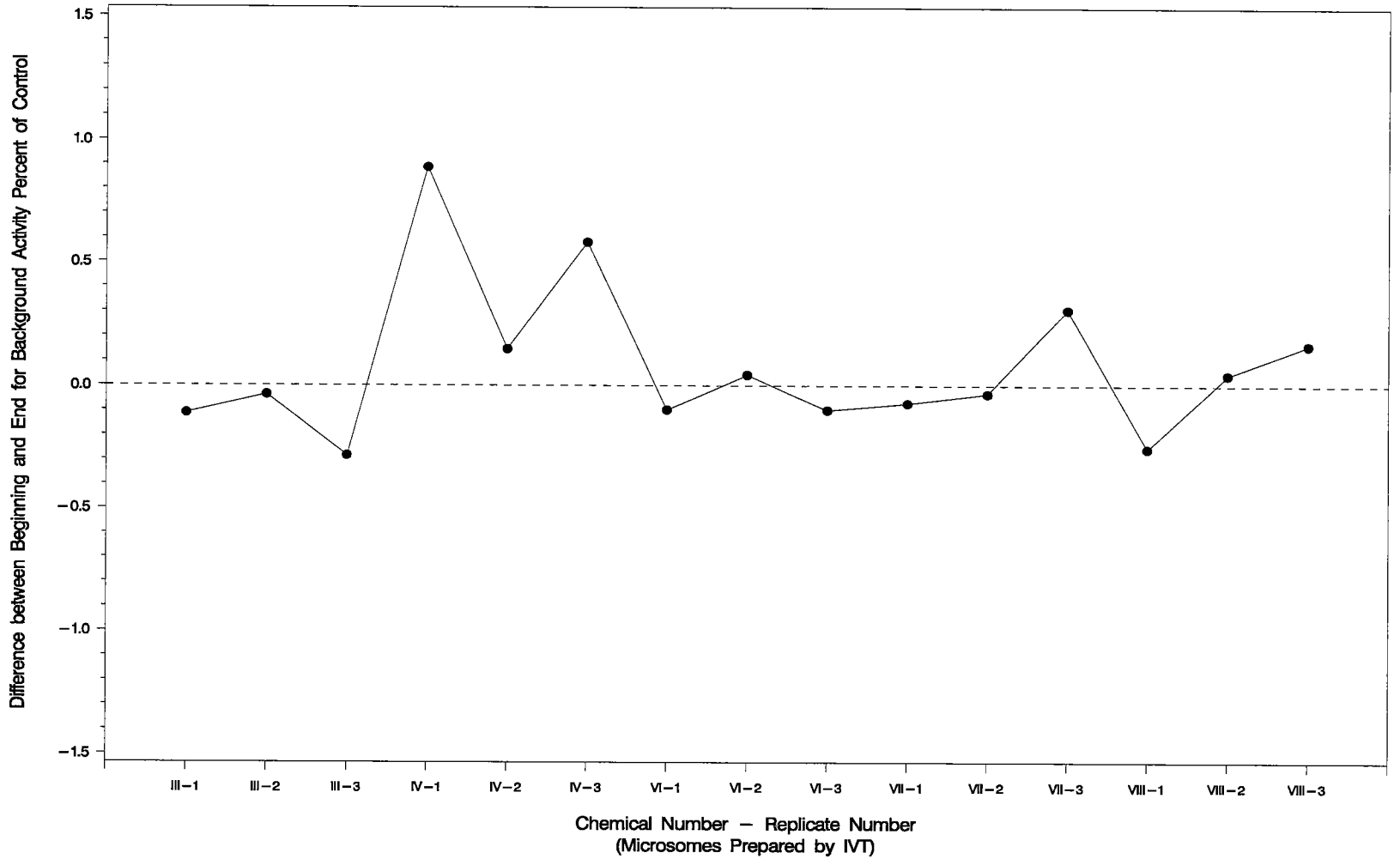


Figure 16b. **Background Activity Control Data. Microsomes Prepared by IVT. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

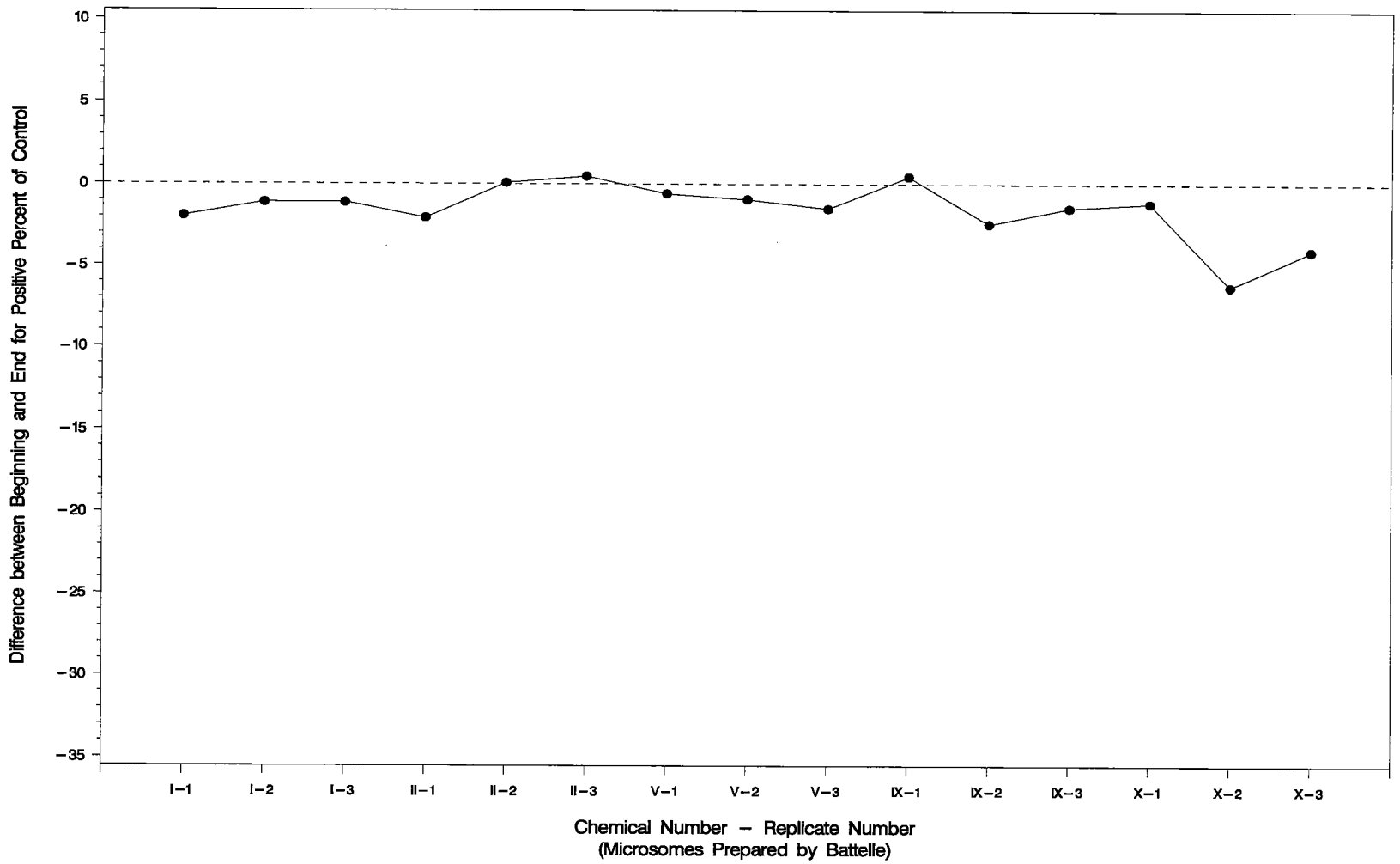


Figure 17a. **Positive Control Data. Microsomes Prepared by Battelle. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

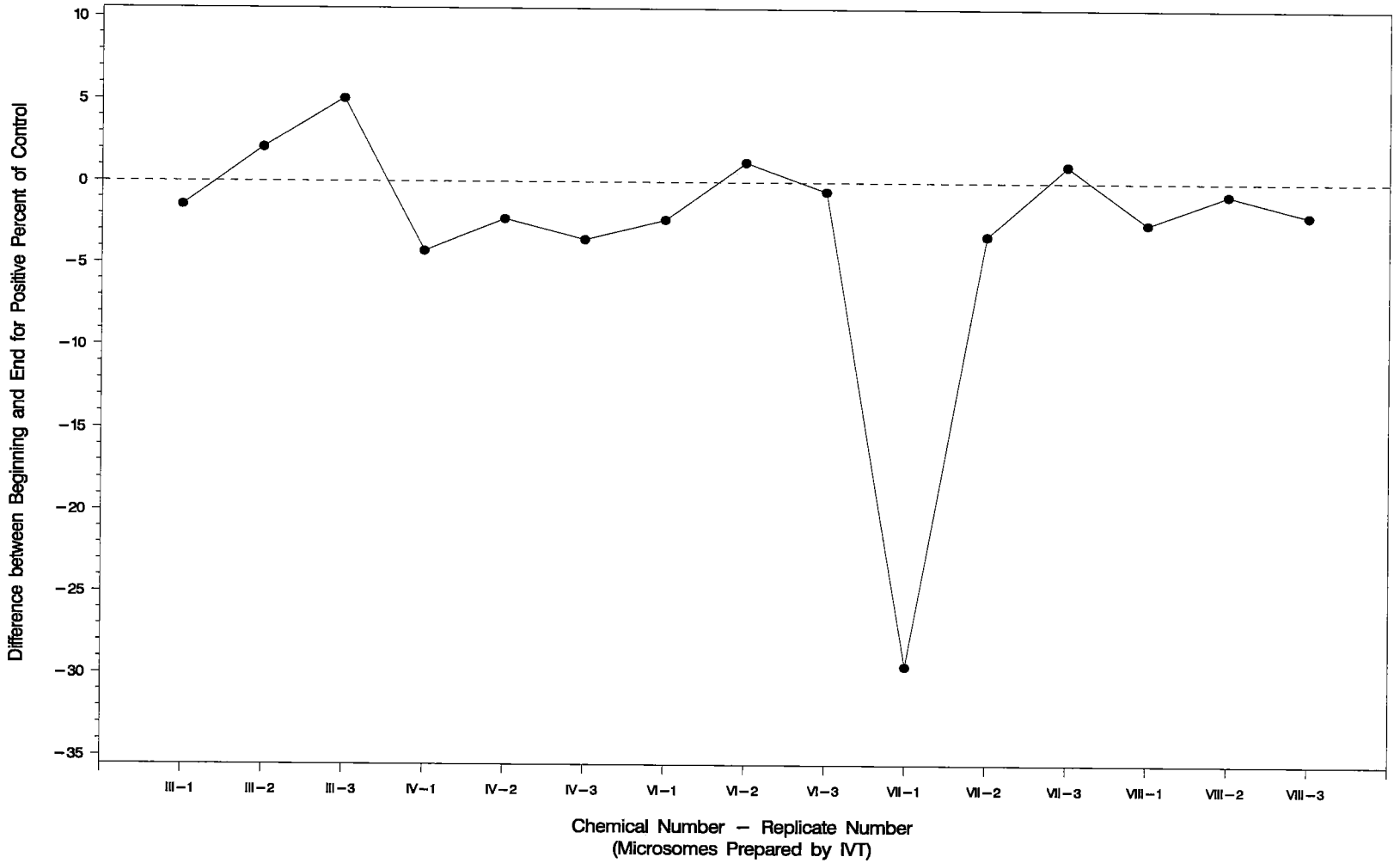


Figure 17b. **Positive Control Data. Microsomes Prepared by IVT. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

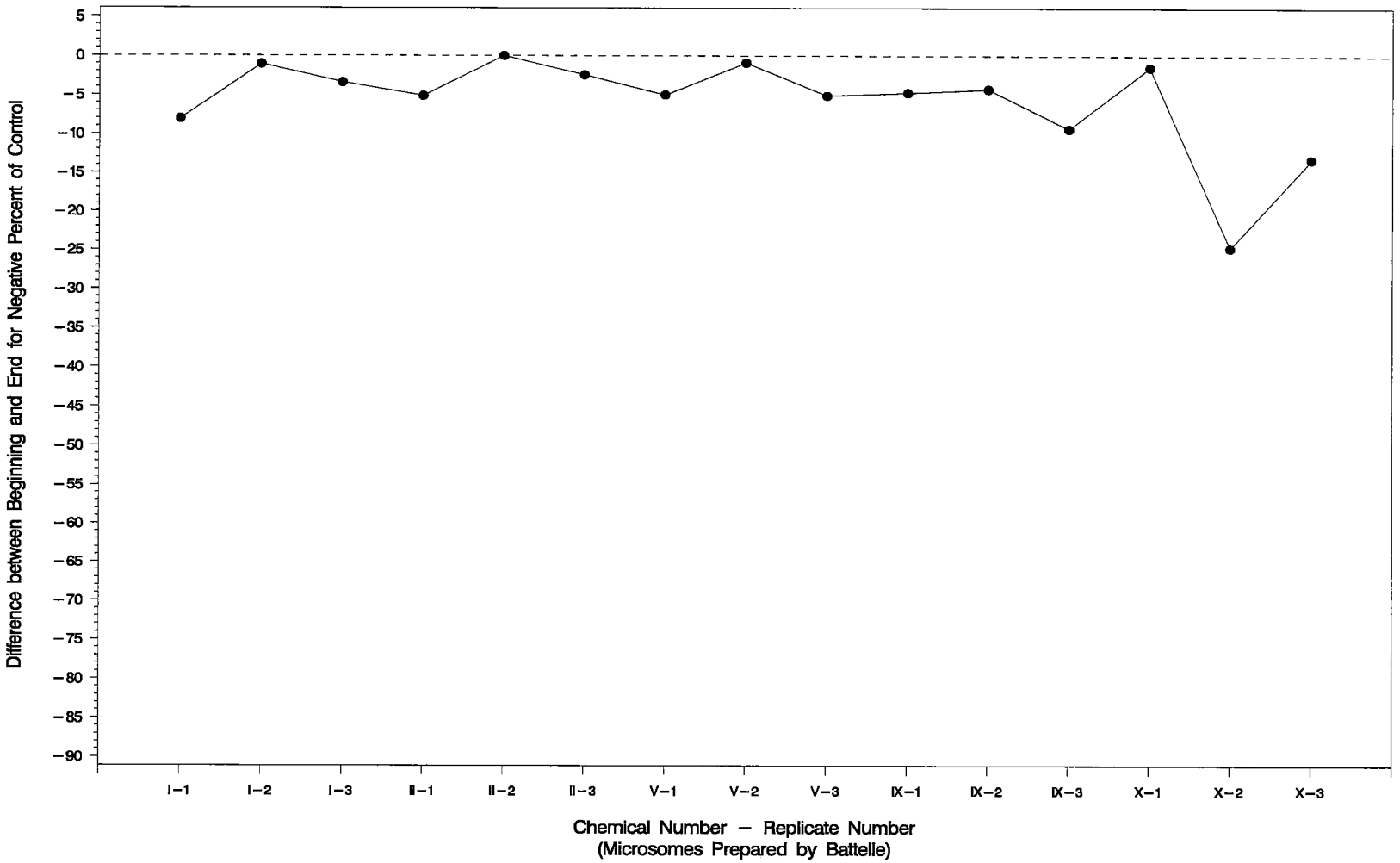


Figure 18a. **Negative Control Data. Microsomes Prepared by Battelle. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

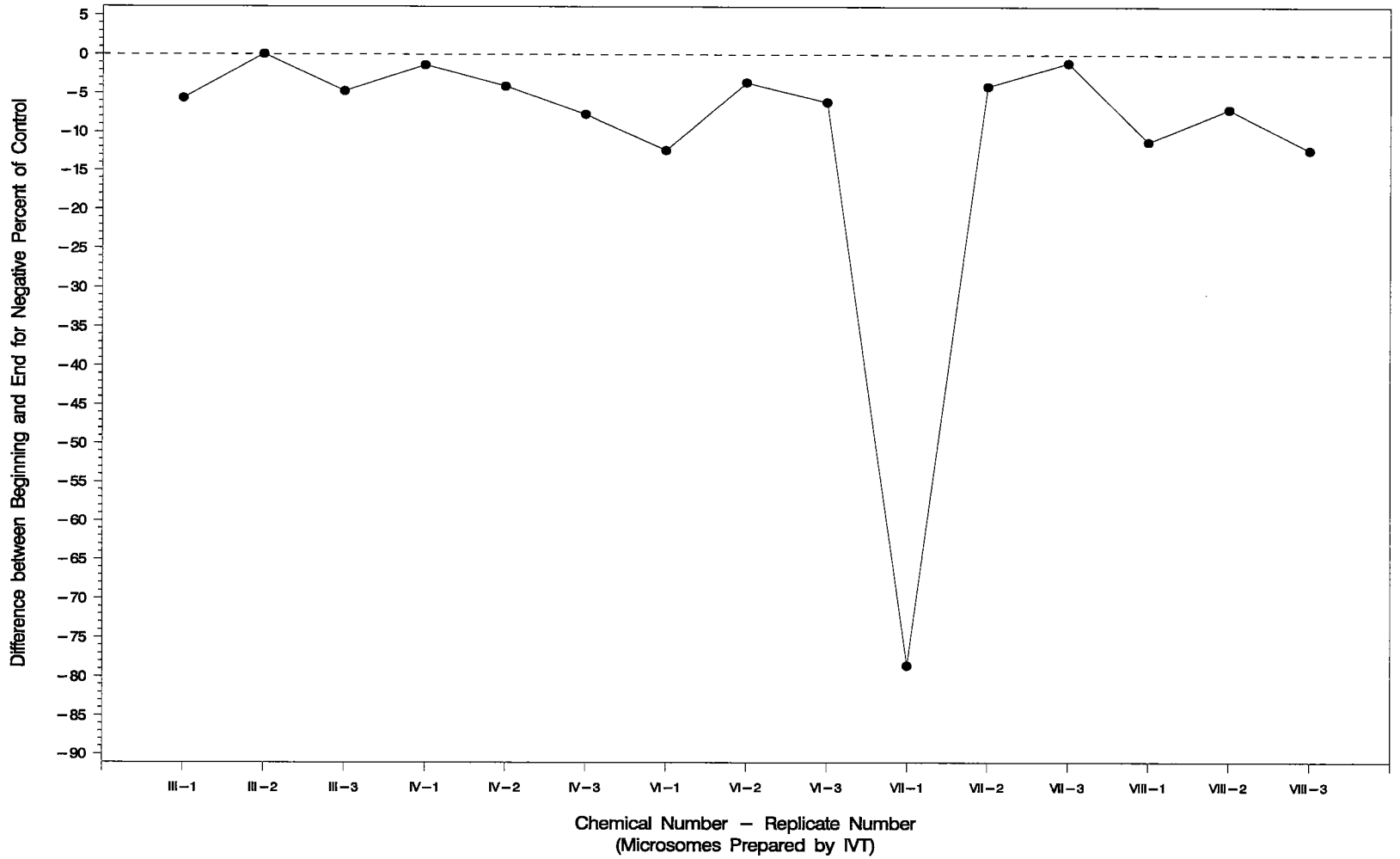


Figure 18b. **Negative Control Data. Microsomes Prepared by IVT. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

Table A-1a. Reference Chemical I: Aminoglutethimide
Microsomes Prepared by: Battelle

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Aminoglutethimide]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	36.012	36.808	36.456
	-5.0000	85.775	85.882	84.075
	-6.0000	97.888	98.329	96.288
	-7.0000	97.776	97.674	97.050
	-8.0000	98.381	100.013	97.301
	-9.0000	98.677	98.372	99.650
	-10.0000	99.023	101.949	101.751
	-11.0000	98.200	101.095	96.831
2	-3.0000	6.052	6.254	6.316
	-3.6021	20.216	20.739	19.907
	-4.0000	38.479	39.249	39.201
	-4.3010	55.194	56.756	55.973
	-4.6021	70.964	71.045	70.998
	-5.0000	82.663	84.804	85.262
	-5.6021	90.567	91.082	87.942
	-6.0000	95.567	90.293	95.771
3	-3.0000	5.203	4.894	4.525
	-3.6021	19.482	19.176	19.199
	-4.0000	38.502	38.153	37.965
	-4.3010	54.709	53.351	54.019
	-4.6021	70.165	69.228	68.745
	-5.0000	85.861	83.417	82.883
	-5.6021	93.867	91.838	91.625
	-6.0000	94.249	97.630	96.825

Table A-1b. Reference Chemical II: Ketoconazole
Microsomes Prepared by: Battelle

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Ketoconazole]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	0.985	0.509	0.406
	-5.0000	4.178	4.036	4.024
	-6.0000	29.995	30.993	29.897
	-6.6021	65.225	62.688	63.330
	-7.0000	79.091	79.074	75.888
	-8.0000	100.537	98.521	94.563
	-9.0000	101.232	100.097	100.344
	-10.0000	100.734	99.888	98.368
2	-4.0000	0.637	-0.156	-0.150
	-5.0000	4.262	3.737	4.277
	-6.0000	32.274	31.661	31.464
	-6.3010	47.763	46.318	47.071
	-6.6021	64.019	64.876	63.110
	-7.0000	81.669	82.055	78.973
	-8.0000	95.024	95.287	94.803
	-9.0000	99.660	98.689	98.399
3	-4.0000	0.294	0.360	0.361
	-5.0000	5.149	5.415	5.208
	-6.0000	34.861	36.068	35.587
	-6.3010	50.711	51.536	51.343
	-6.6021	68.863	63.111	68.667
	-7.0000	82.272	83.239	82.017
	-8.0000	95.589	95.544	82.614
	-9.0000	98.201	97.184	94.276

Table A-1c. Reference Chemical III: Prochloraz
Microsomes Prepared by: In Vitro Technologies

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Prochloraz]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	0.293	-0.002	-0.078
	-5.0000	0.493	0.179	0.152
	-6.0000	2.429	2.179	2.275
	-7.0000	18.414	18.914	17.335
	-8.0000	65.098	66.918	65.656
	-9.0000	92.772	93.704	96.384
	-10.0000	95.858	95.039	97.696
	-11.0000	96.593	96.475	97.056
2	-5.0000	0.153	-0.064	0.121
	-6.0000	2.599	2.415	2.584
	-7.0000	22.180	22.460	21.576
	-7.6021	47.614	49.997	51.413
	-8.0000	71.745	71.243	71.973
	-8.6021	89.050	89.330	87.070
	-9.0000	95.874	95.300	94.351
	-10.0000	99.212	99.063	98.220
3	-5.0000	-0.046	0.080	0.223
	-6.0000	3.240	3.152	3.233
	-7.0000	24.597	23.972	24.189
	-7.6021	56.573	54.050	54.196
	-8.0000	75.662	71.974	73.674
	-8.6021	87.053	89.327	91.555
	-9.0000	93.513	96.354	94.027
	-10.0000	100.162	94.503	96.656

Table A-1d. Reference Chemical IV: 4-Nonylphenol
Microsomes Prepared by: In Vitro Technologies

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [4-Nonylphenol]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-3.0000	3.811	-1.578	-0.497
	-4.0000	17.683	15.468	17.087
	-5.0000	96.334	96.367	96.636
	-6.0000	101.512	100.025	98.716
	-7.0000	107.224	102.076	106.407
	-8.0000	93.185	93.098	96.170
	-9.0000	80.557	96.547	93.558
	-10.0000	94.813	92.808	96.558
2	-3.0000	1.511	0.012	-0.766
	-4.0000	11.992	11.117	13.101
	-4.3010	26.661	27.962	26.710
	-4.6021	45.805	46.131	46.381
	-5.0000	83.983	85.928	82.093
	-6.0000	94.077	95.594	95.937
	-7.0000	88.005	93.521	91.243
	-8.0000	90.806	91.217	95.464
3	-3.0000	0.291	0.314	0.406
	-4.0000	12.943	13.901	15.364
	-4.3010	32.577	32.195	33.634
	-4.6021	46.977	48.248	50.003
	-5.0000	87.593	85.672	88.606
	-6.0000	99.412	99.134	96.448
	-7.0000	95.536	96.661	102.947
	-8.0000	98.489	98.382	96.405

Table A-1e. Reference Chemical V: Dibenz[a,h]anthracene
Microsomes Prepared by: Battelle

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Dibenz[a,h]anthracene]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	99.723	98.943	102.328
	-5.0000	105.918	104.934	105.147
	-6.0000	107.501	107.768	110.723
	-6.6021	107.451	104.555	104.154
	-7.0000	106.334	98.217	104.733
	-8.0000	102.854	100.761	104.850
	-9.0000	105.472	101.824	100.072
	-10.0000	97.587	105.466	101.183
2	-4.0000	107.576	102.698	108.555
	-5.0000	100.693	106.429	103.511
	-6.0000	102.218	107.975	105.997
	-6.6021	106.801	108.950	106.644
	-7.0000	103.330	98.565	109.086
	-8.0000	100.440	94.403	103.212
	-9.0000	107.453	103.194	100.767
	-10.0000	93.478	101.719	102.082
3	-4.0000	100.894	97.585	100.590
	-5.0000	95.467	96.495	98.991
	-6.0000	101.718	102.459	96.640
	-6.6021	96.787	100.396	101.809
	-7.0000	97.864	99.461	99.945
	-8.0000	99.336	99.682	101.844
	-9.0000	97.749	99.667	96.204
	-10.0000	97.755	96.304	74.540

Table A-1f. Reference Chemical VI: Fenarimol
Microsomes Prepared by: In Vitro Technologies

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Fenarimol]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-3.0000	1.260	1.064	1.319
	-4.0000	6.416	6.053	5.875
	-5.0000	33.804	39.368	39.614
	-6.0000	83.565	84.496	85.598
	-7.0000	97.858	102.564	99.853
	-8.0000	97.693	95.754	98.977
	-9.0000	97.460	99.511	100.180
	-10.0000	96.981	100.076	99.677
2	-3.0000	1.453	1.715	1.624
	-4.0000	6.402	6.132	5.847
	-5.0000	36.623	38.253	37.415
	-5.6021	68.350	67.037	71.031
	-6.0000	79.096	82.582	80.906
	-7.0000	97.382	93.817	93.361
	-8.0000	95.444	100.797	96.297
	-9.0000	96.693	98.328	97.469
3	-3.0000	1.301	1.266	1.169
	-4.0000	5.767	5.661	5.903
	-5.0000	38.040	38.216	37.859
	-5.6021	70.271	73.373	66.496
	-6.0000	86.325	87.940	88.023
	-7.0000	96.730	98.053	97.211
	-8.0000	101.558	95.649	98.255
	-9.0000	98.232	101.286	99.363

Table A-1g. Reference Chemical VII: Econazole
Microsomes Prepared by: In Vitro Technologies

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Econazole]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-3.0000	-0.365	-0.397	-0.258
	-4.0000	-0.381	-0.664	-0.588
	-5.0000	0.104	-0.751	-0.373
	-6.0000	-0.398	0.068	-0.305
	-7.0000	1.294	1.247	1.253
	-8.0000	13.181	11.644	11.992
	-9.0000	40.685	43.030	44.078
	-10.0000	57.012	57.855	50.235
2	-4.0000	-0.387	0.088	-0.392
	-5.0000	-0.365	0.336	-0.216
	-6.0000	-0.329	-0.262	-0.479
	-7.0000	1.480	1.906	1.474
	-8.0000	16.066	16.437	16.278
	-8.6021	45.697	44.666	43.608
	-9.0000	63.509	65.023	67.620
	-10.0000	83.755	90.144	93.558
3	-4.0000	-0.106	-0.435	-0.377
	-5.0000	-0.360	-0.561	0.160
	-6.0000	-0.077	-0.324	-0.144
	-7.0000	2.284	2.150	1.787
	-8.0000	16.945	15.836	16.007
	-8.6021	45.078	41.789	45.583
	-9.0000	69.421	68.394	68.287
	-10.0000	94.063	100.475	96.439

Table A-1h. Reference Chemical VIII: Chrysin
Microsomes Prepared by: In Vitro Technologies

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Chrysin]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	20.536	20.275	25.137
	-5.0000	25.003	25.280	23.803
	-6.0000	71.556	76.561	76.276
	-6.6021	86.284	86.927	87.767
	-7.0000	92.203	90.661	88.600
	-8.0000	85.404	92.990	88.693
	-9.0000	88.705	93.985	94.511
	-10.0000	93.258	90.355	87.091
2	-4.0000	19.500	17.122	20.777
	-5.0000	27.723	26.707	27.423
	-5.3010	37.613	39.806	40.151
	-5.6021	51.059	52.961	53.794
	-6.0000	74.762	74.520	78.204
	-7.0000	98.235	88.773	88.732
	-8.0000	94.824	96.978	93.276
	-9.0000	76.978	102.133	104.567
3	-4.0000	19.830	20.790	20.757
	-5.0000	27.890	26.805	25.562
	-5.3010	39.977	39.889	39.680
	-5.6021	53.848	54.801	55.918
	-6.0000	72.262	68.659	70.285
	-7.0000	90.199	56.696	90.528
	-8.0000	94.699	88.104	95.793
	-9.0000	93.841	94.736	91.434

Table A-1i. Reference Chemical IX: Dicofol
Microsomes Prepared by: Battelle

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Dicofol]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-3.0000	2.559	2.628	3.683
	-4.0000	20.004	20.157	21.387
	-5.0000	61.778	59.692	54.701
	-6.0000	89.091	94.220	89.727
	-7.0000	95.137	98.502	102.650
	-8.0000	97.404	95.931	98.115
	-9.0000	94.889	96.204	96.282
	-10.0000	100.763	100.478	92.895
2	-3.0000	3.053	2.576	2.746
	-4.0000	26.026	21.584	20.714
	-4.3010	34.856	44.548	30.662
	-5.0000	62.187	60.336	63.592
	-6.0000	92.564	92.164	94.314
	-7.0000	96.356	97.804	97.369
	-8.0000	98.881	97.229	98.809
	-9.0000	94.734	96.801	96.842
3	-3.0000	2.274	1.581	2.747
	-4.0000	23.254	22.847	22.422
	-4.3010	28.431	29.712	29.949
	-5.0000	82.795	82.927	81.239
	-6.0000	91.551	88.887	90.181
	-7.0000	93.371	95.667	100.843
	-8.0000	92.623	102.072	98.560
	-9.0000	92.516	100.042	97.900

Table A-1j. Reference Chemical X: Atrazine
Microsomes Prepared by: Battelle

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Atrazine]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-3.0000	81.179	82.199	80.869
	-4.0000	93.398	95.390	95.879
	-5.0000	102.087	101.780	102.582
	-6.0000	100.553	99.611	101.266
	-7.0000	96.569	97.966	98.251
	-8.0000	95.668	99.695	98.295
	-9.0000	96.016	95.782	95.015
	-10.0000	91.107	95.003	95.008
2	-3.0000	79.188	83.471	84.216
	-3.0458	85.380	81.388	79.580
	-3.0969	76.454	75.597	81.687
	-3.1249	78.195	86.599	85.152
	-3.3010	82.943	82.319	85.304
	-4.0000	96.534	91.579	91.045
	-5.0000	90.653	95.893	96.413
	-6.0000	93.482	96.021	95.874
3	-3.0000	85.300	84.766	86.205
	-3.0458	83.187	85.173	87.053
	-3.0969	86.582	85.307	86.337
	-3.1249	84.402	84.056	86.852
	-3.3010	85.992	85.841	84.058
	-4.0000	93.854	93.291	92.744
	-5.0000	93.961	89.722	98.908
	-6.0000	94.464	97.886	96.973

Table A-2a. Reference Chemical I: Aminoglutethimide
Microsomes Prepared by: Battelle

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.09423	103.938
		Beginning	0.09086	100.226
		End	0.08804	97.109
		End	0.08950	98.727
	2	Beginning	0.06598	101.563
		Beginning	0.06498	100.033
		End	0.06582	101.322
		End	0.06307	97.0820
	3	Beginning	0.06409	99.2540
		Beginning	0.06744	104.435
		End	0.06493	100.551
		End	0.06183	95.76
Background Control	1	Beginning	0.00006	0.071
		Beginning	-0.00005	-0.053
		End	0.00007	0.074
		End	-0.00008	-0.091
	2	Beginning	-0.00001	-0.019
		Beginning	0.00004	0.054
		End	0.00003	0.043
		End	-0.00005	-0.078
	3	Beginning	0.00002	0.024
		Beginning	-0.00005	-0.076
		End	-0.00001	-0.016
		End	0.00004	0.068
Positive Control	1	Beginning	0.04166	45.951
		Beginning	0.04032	44.471
		End	0.03913	43.162
		End	0.03929	43.344
	2	Beginning	0.02957	45.524
		Beginning	0.02950	45.408
		End	0.02861	44.036
		End	0.02901	44.65
	3	Beginning	0.02814	43.583
		Beginning	0.02926	45.321
		End	0.02829	43.810
		End	0.02767	42.844

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Negative Control	1	Beginning	0.0909	100.267
		Beginning	0.09226	101.773
		End	0.08948	98.706
		End	0.07912	87.270
	2	Beginning	0.06223	95.785
		Beginning	0.05966	91.837
		End	0.05997	92.316
		End	0.06055	93.206
	3	Beginning	0.05923	91.719
		Beginning	0.05942	92.021
		End	0.05825	90.203
		End	0.05600	86.731

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2b. Reference Chemical II: Ketoconazole
Microsomes Prepared by: Battelle

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.07037	103.859
		Beginning	0.06788	100.173
		End	0.06668	98.408
		End	0.06611	97.560
	2	Beginning	0.06254	106.39
		Beginning	0.06075	103.336
		End	0.05775	98.239
		End	0.05410	92.035
	3	Beginning	0.05275	106.237
		Beginning	0.05168	104.067
		End	0.04864	97.944
		End	0.04556	91.752
Background Control	1	Beginning	-0.00001	-0.009
		Beginning	0.00004	0.064
		End	-0.00001	-0.010
		End	-0.00003	-0.046
	2	Beginning	-0.00015	-0.250
		Beginning	0.00036	0.605
		End	-0.00011	-0.190
		End	-0.00010	-0.165
	3	Beginning	-0.00001	-0.015
		Beginning	-0.00001	-0.016
		End	0.00004	0.071
		End	-0.00002	-0.041
Positive Control	1	Beginning	0.03021	44.585
		Beginning	0.03022	44.598
		End	0.02874	42.420
		End	0.02888	42.626
	2	Beginning	0.02589	44.043
		Beginning	0.02720	46.266
		End	0.02664	45.321
		End	0.02653	45.131
	3	Beginning	0.02648	53.335
		Beginning	0.02354	47.414
		End	0.02485	50.045
		End	0.02565	51.653

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Negative Control	1	Beginning	0.07084	104.546
		Beginning	0.07104	104.840
		End	0.06745	99.551
		End	0.06748	99.595
	2	Beginning	0.05991	101.911
		Beginning	0.05872	99.894
		End	0.05864	99.755
		End	0.05999	102.054
	3	Beginning	0.04960	99.883
		Beginning	0.04963	99.944
		End	0.04838	97.425
		End	0.04842	97.510

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2c. Reference Chemical III: Prochloraz
Microsomes Prepared by: In Vitro Technologies

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.06044	102.238
		Beginning	0.06034	102.076
		End	0.05861	99.154
		End	0.05706	96.533
	2	Beginning	0.04296	96.801
		Beginning	0.04727	106.512
		End	0.04401	99.180
		End	0.04327	97.507
	3	Beginning	0.04486	112.608
		Beginning	0.04103	102.979
		End	0.03809	95.611
		End	0.03538	88.802
Background Control	1	Beginning	0.00001	0.023
		Beginning	0.00005	0.089
		End	-0.00010	-0.165
		End	0.00003	0.053
	2	Beginning	-0.00012	-0.267
		Beginning	0.00013	0.304
		End	0.00000	0.000
		End	-0.00002	-0.037
	3	Beginning	0.00005	0.120
		Beginning	0.00007	0.164
		End	-0.00003	-0.086
		End	-0.00008	-0.198
Positive Control	1	Beginning	0.02647	44.782
		Beginning	0.02747	46.476
		End	0.02621	44.331
		End	0.02604	44.054
	2	Beginning	0.02341	52.753
		Beginning	0.02248	50.666
		End	0.02405	54.188
		End	0.02369	53.375
	3	Beginning	0.02163	54.291
		Beginning	0.02044	51.296
		End	0.02272	57.024
		End	0.02336	58.628

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Negative Control	1	Beginning	0.05862	99.161
		Beginning	0.06077	102.797
		End	0.05666	95.855
		End	0.05607	94.856
	2	Beginning	0.04278	96.402
		Beginning	0.04436	99.970
		End	0.04452	100.322
		End	0.04267	96.152
	3	Beginning	0.04148	104.108
		Beginning	0.04018	100.840
		End	0.03942	98.934
		End	0.03847	96.566

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2d. Reference Chemical IV: 4-Nonylphenol
Microsomes Prepared by: In Vitro Technologies

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.03734	103.34
		Beginning	0.03588	99.308
		End	0.03518	97.359
		End	0.03613	99.993
	2	Beginning	0.05285	105.608
		Beginning	0.05169	103.297
		End	0.04927	98.454
		End	0.04636	92.641
	3	Beginning	0.04070	97.446
		Beginning	0.04488	107.457
		End	0.04072	97.505
		End	0.04076	97.592
Background Control	1	Beginning	0.00034	0.929
		Beginning	-0.00066	-1.815
		End	0.00058	1.611
		End	-0.00026	-0.724
	2	Beginning	0.00030	0.598
		Beginning	-0.00037	-0.743
		End	0.00029	0.585
		End	-0.00022	-0.440
	3	Beginning	0.00018	0.440
		Beginning	-0.00043	-1.020
		End	0.00021	0.509
		End	0.00003	0.071
Positive Control	1	Beginning	0.02015	55.779
		Beginning	0.02105	58.258
		End	0.01930	53.412
		End	0.01883	52.107
	2	Beginning	0.02458	49.115
		Beginning	0.02394	47.842
		End	0.02259	45.132
		End	0.02365	47.266
	3	Beginning	0.02195	52.549
		Beginning	0.01980	47.423
		End	0.01970	47.166
		End	0.01908	45.698

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Negative Control	1	Beginning	0.03324	92.001
		Beginning	0.03442	95.261
		End	0.03336	92.329
		End	0.03332	92.207
	2	Beginning	0.04728	94.474
		Beginning	0.04872	97.347
		End	0.04650	92.912
		End	0.04542	90.758
	3	Beginning	0.04193	100.411
		Beginning	0.04342	103.965
		End	0.03869	92.641
		End	0.04021	96.279

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2e. Reference Chemical V: Dibenz[a,h]anthracene
Microsomes Prepared by: Battelle

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.05479	102.841
		Beginning	0.05562	104.398
		End	0.05170	97.033
		End	0.05100	95.728
	2	Beginning	0.06192	115.260
		Beginning	0.05065	94.282
		End	0.05120	95.300
		End	0.05112	95.157
	3	Beginning	0.05799	100.410
		Beginning	0.06012	104.102
		End	0.05598	96.926
		End	0.05692	98.563
Background Control	1	Beginning	-0.00019	-0.356
		Beginning	-0.00005	-0.101
		End	0.00024	0.446
		End	0.00001	0.011
	2	Beginning	-0.00006	-0.106
		Beginning	-0.00039	-0.722
		End	0.00088	1.632
		End	-0.00043	-0.804
	3	Beginning	-0.00004	-0.066
		Beginning	0.00062	1.067
		End	0.00000	-0.007
		End	-0.00057	-0.994
Positive Control	1	Beginning	0.02416	45.352
		Beginning	0.02747	51.564
		End	0.02544	47.740
		End	0.02558	48.002
	2	Beginning	0.02925	54.441
		Beginning	0.02874	53.497
		End	0.02786	51.849
		End	0.02913	54.226
	3	Beginning	0.03039	52.612
		Beginning	0.02894	50.117
		End	0.02817	48.768
		End	0.02943	50.966

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Negative Control	1	Beginning	0.05458	102.429
		Beginning	0.05341	100.250
		End	0.05172	97.066
		End	0.05098	95.684
	2	Beginning	0.05521	102.770
		Beginning	0.04918	91.542
		End	0.05082	94.594
		End	0.05260	97.908
	3	Beginning	0.06063	104.978
		Beginning	0.05780	100.087
		End	0.05660	98.007
		End	0.05595	96.874

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2f. Reference Chemical VI: Fenarimol
Microsomes Prepared by: In Vitro Technologies

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.04925	103.549
		Beginning	0.04808	101.102
		End	0.04647	97.715
		End	0.04643	97.634
	2	Beginning	0.05629	106.532
		Beginning	0.05724	108.322
		End	0.04967	94.011
		End	0.04815	91.135
	3	Beginning	0.05380	109.949
		Beginning	0.05128	104.802
		End	0.04460	91.133
		End	0.04606	94.116
Background Control	1	Beginning	0.00005	0.111
		Beginning	-0.00001	-0.012
		End	0.00001	0.026
		End	-0.00006	-0.125
	2	Beginning	-0.00006	-0.109
		Beginning	0.00004	0.066
		End	-0.00003	-0.050
		End	0.00005	0.093
	3	Beginning	-0.00003	-0.062
		Beginning	0.00008	0.162
		End	-0.00001	-0.012
		End	-0.00004	-0.088
Positive Control	1	Beginning	0.02096	44.078
		Beginning	0.02173	45.692
		End	0.02005	42.155
		End	0.02044	42.972
	2	Beginning	0.02372	44.901
		Beginning	0.02343	44.340
		End	0.02483	46.995
		End	0.02360	44.659
	3	Beginning	0.02261	46.198
		Beginning	0.02219	45.356
		End	0.02163	44.203
		End	0.02261	46.207

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Negative Control	1	Beginning	0.04932	103.704
		Beginning	0.04916	103.366
		End	0.04648	97.733
		End	0.04020	84.517
	2	Beginning	0.05375	101.735
		Beginning	0.05404	102.278
		End	0.05122	96.930
		End	0.05279	99.909
	3	Beginning	0.04988	101.930
		Beginning	0.05036	102.910
		End	0.04772	97.516
		End	0.04649	95.010

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2g. Reference Chemical VII: Econazole
Microsomes Prepared by: In Vitro Technologies

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.05139	140.410
		Beginning	0.05200	142.089
		End	0.02074	56.656
		End	0.02227	60.845
	2	Beginning	0.05352	108.268
		Beginning	0.04951	100.154
		End	0.04717	95.433
		End	0.04752	96.145
	3	Beginning	0.04917	112.230
		Beginning	0.04508	102.896
		End	0.03563	81.328
		End	0.04536	103.545
Background Control	1	Beginning	0.00000	-0.004
		Beginning	0.00003	0.076
		End	-0.00001	-0.040
		End	-0.00001	-0.032
	2	Beginning	0.00003	0.061
		Beginning	-0.00001	-0.028
		End	0.00004	0.073
		End	-0.00005	-0.106
	3	Beginning	-0.00009	-0.217
		Beginning	-0.00004	-0.089
		End	0.00015	0.353
		End	-0.00002	-0.048
Positive Control	1	Beginning	0.02416	66.021
		Beginning	0.02383	65.114
		End	0.01359	37.138
		End	0.01277	34.901
	2	Beginning	0.02454	49.655
		Beginning	0.02481	50.192
		End	0.02219	44.896
		End	0.02389	48.338
	3	Beginning	0.02252	51.405
		Beginning	0.02165	49.424
		End	0.02271	51.836
		End	0.02236	51.032

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Negative Control	1	Beginning	0.04798	131.084
		Beginning	0.05270	143.982
		End	0.02253	61.549
		End	0.02062	56.340
	2	Beginning	0.05045	102.059
		Beginning	0.05005	101.256
		End	0.04703	95.145
		End	0.04943	99.998
	3	Beginning	0.04372	99.786
		Beginning	0.04454	101.666
		End	0.04130	94.266
		End	0.04604	105.097

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2h. Reference Chemical VIII: Chrysin
Microsomes Prepared by: In Vitro Technologies

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.04993	104.830
		Beginning	0.04943	103.779
		End	0.04530	95.103
		End	0.04586	96.288
	2	Beginning	0.04457	107.555
		Beginning	0.04241	102.341
		End	0.04271	103.063
		End	0.03607	87.042
	3	Beginning	0.05387	108.047
		Beginning	0.05238	105.054
		End	0.04459	89.428
		End	0.04860	97.471
Background Control	1	Beginning	0.00031	0.643
		Beginning	-0.00018	-0.387
		End	0.00013	0.267
		End	-0.00025	-0.523
	2	Beginning	0.00001	0.013
		Beginning	-0.00002	-0.055
		End	-0.00007	-0.158
		End	0.00008	0.200
	3	Beginning	0.00006	0.128
		Beginning	-0.00014	-0.290
		End	0.00006	0.115
		End	0.00002	0.047
Positive Control	1	Beginning	0.02049	43.007
		Beginning	0.02279	47.837
		End	0.02027	42.558
		End	0.02058	43.197
	2	Beginning	0.01948	47.000
		Beginning	0.02027	48.914
		End	0.01993	48.105
		End	0.01919	46.303
	3	Beginning	0.02313	46.388
		Beginning	0.02336	46.846
		End	0.02207	44.274
		End	0.02239	44.916

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Negative Control	1	Beginning	0.05163	108.400
		Beginning	0.04670	98.053
		End	0.04488	94.227
		End	0.04269	89.621
	2	Beginning	0.04052	97.775
		Beginning	0.04487	108.28
		End	0.04111	99.218
		End	0.03842	92.709
	3	Beginning	0.04968	99.641
		Beginning	0.04648	93.221
		End	0.03697	74.144
		End	0.04685	93.962

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2i. Reference Chemical IX: Dicofol
Microsomes Prepared by: Battelle

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.05633	110.703
		Beginning	0.05132	100.851
		End	0.04898	96.251
		End	0.04691	92.196
	2	Beginning	0.04853	105.231
		Beginning	0.04707	102.056
		End	0.04414	95.700
		End	0.04474	97.013
	3	Beginning	0.04816	103.593
		Beginning	0.04657	100.171
		End	0.04493	96.648
		End	0.04629	99.588
Background Control	1	Beginning	-0.00005	-0.101
		Beginning	0.00015	0.296
		End	-0.00011	-0.212
		End	0.00001	0.017
	2	Beginning	-0.00006	-0.133
		Beginning	0.00005	0.109
		End	0.00010	0.210
		End	-0.00009	-0.186
	3	Beginning	0.00005	0.102
		Beginning	0.00009	0.184
		End	-0.00003	-0.057
		End	-0.00011	-0.229
Positive Control	1	Beginning	0.02732	53.684
		Beginning	0.02472	48.584
		End	0.02658	52.228
		End	0.02593	50.961
	2	Beginning	0.02435	52.796
		Beginning	0.02547	55.232
		End	0.02373	51.461
		End	0.02385	51.705
	3	Beginning	0.02306	49.611
		Beginning	0.02398	51.585
		End	0.02280	49.054
		End	0.02289	49.242

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Negative Control	1	Beginning	0.05324	104.636
		Beginning	0.04927	96.828
		End	0.04928	96.835
		End	0.04844	95.194
	2	Beginning	0.04826	104.628
		Beginning	0.04605	99.851
		End	0.04457	96.634
		End	0.04582	99.347
	3	Beginning	0.04725	101.648
		Beginning	0.04895	105.301
		End	0.04432	95.341
		End	0.04321	92.945

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2j. Reference Chemical X: Atrazine
Microsomes Prepared by: Battelle

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.04434	96.013
		Beginning	0.04911	106.347
		End	0.04521	97.898
		End	0.04606	99.742
	2	Beginning	0.04390	109.369
		Beginning	0.04365	108.766
		End	0.03741	93.218
		End	0.03558	88.646
	3	Beginning	0.04389	98.818
		Beginning	0.04664	105.012
		End	0.04426	99.662
		End	0.04286	96.508
Background Control	1	Beginning	0.00004	0.078
		Beginning	0.00002	0.044
		End	-0.00001	-0.022
		End	-0.00005	-0.101
	2	Beginning	-0.00062	-1.554
		Beginning	0.00026	0.649
		End	-0.00016	-0.403
		End	0.00053	1.308
	3	Beginning	-0.00018	-0.396
		Beginning	-0.00002	-0.051
		End	0.00018	0.411
		End	0.00002	0.037
Positive Control	1	Beginning	0.02302	49.855
		Beginning	0.02363	51.169
		End	0.02268	49.105
		End	0.02291	49.602
	2	Beginning	0.02231	55.593
		Beginning	0.02165	53.935
		End	0.01883	46.925
		End	0.02007	49.995
	3	Beginning	0.02855	64.276
		Beginning	0.02795	62.931
		End	0.02759	62.127
		End	0.02527	56.893

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Negative Control	1	Beginning	0.04531	98.127
		Beginning	0.04311	93.352
		End	0.04288	92.868
		End	0.04420	95.709
	2	Beginning	0.03887	96.843
		Beginning	0.03712	92.482
		End	0.01924	47.941
		End	0.03689	91.921
	3	Beginning	0.04655	104.821
		Beginning	0.04800	108.082
		End	0.04403	99.126
		End	0.03876	87.265

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.