DRAFT FINAL REPORT

RECOMBINANT AROMATASE VALIDATION STUDY: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES
(Volume I-Overall Summary, Appendices A and B)

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Gary E. Timm
Work Assignment Manager
U.S. Environmental Protection Agency
Endocrine Disruptor Screening Program
Washington, D.C.

Battelle
505 King Avenue
Columbus, Ohio 43201

RECOMBINANT AROMATASE VALIDATION STUDY: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES

WA 4-17, Task 4

OVERALL TASK DRAFT FINAL REPORT

Authors (Overall Task Report):

Jerry D. Johnson, Ph.D.
Diplomate, A.B.T.
Work Assignment Leader
Battelle Memorial Institute

Zhenxu J. Ma, Ph.D.
Statistician
EDSP Data Coordination Center
Battelle Memorial Institute

Paul I. Feder, Ph.D.
Senior Statistician
EDSP Data Coordination Center
Battelle Memorial Institute

Performing Laboratory:

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

Sponsor:

Environmental Protection Agency
Endocrine Disruptor Screening Program
Washington, D.C.

Sponsor's Representatives:

David P. Houchens, Ph.D.
EDSP Program Manager
Battelle Memorial Institute

Jerry D. Johnson, Ph.D.
Diplomate, A.B.T.
Work Assignment Leader
Battelle Memorial Institute

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ERRATA

Draft Final Report
For WA 4-17, Task 4

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: Recombinant Aromatase Validation Study: Conduct Multiple Chemical Studies with Recombinant Microsomes

Overall Task Report Authors/Lab:

Jerry D. Johnson, Ph.D.
Diplomate, A.B.T.
Work Assignment Leader
Battelle Memorial Institute

Zhenxu J. Ma, Ph.D.
Statistician
EDSP Data Coordination Center
Battelle Memorial Institute

Paul I. Feder, Ph.D.
Senior Statistician
EDSP Data Coordination Center
Battelle Memorial Institute

Lead Laboratory: RTI International

Participating Labs:
Battelle Memorial Institute
In Vitro Technologies, Inc.
WIL Research Laboratories, LLC

Sponsor:
Environmental Protection Agency
Endocrine Disruptor Screening Program
Washington, D.C.

Sponsor's Representatives:

David P. Houchens, Ph.D.
EDSP Program Manager
Battelle Memorial Institute

Jerry D. Johnson, Ph.D.
Diplomate, A. B.T.
Work Assignment Leader
Battelle Memorial Institute

Authors:

_____
Jerry D. Johnson, Ph.D., D.A.B.T.
Signature / Date

_____
Zhenxu J. Ma, Ph.D.
Signature / Date

_____
Paul I. Feder, Ph.D.
Signature / Date

Approved:

_____
David P. Houchens, Ph.D.
Signature / Date

Quality Assurance Statement

Study Number: G608317

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

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Audit Overall Interlaboratory draft final report	6/23/06	6/23/06

Quality Assurance Unit **Date**

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EXECUTIVE SUMMARY

The objectives of this task were to evaluate the responsiveness of recombinant microsomes 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. In addition, the results of the present study were compared to the results obtained using human placental microsomes (WA 4-16, Task 7).

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). Recombinant microsomes were used as the aromatase source. RTI obtained the recombinant microsomes from a commercial supplier and distributed them to the other laboratories. Each laboratory used the recombinant microsomal aromatase assay to evaluate the assay's response to the following reference chemicals: aminoglutethimide, atrazine, chrysin, dibenz [a,h] anthracene, dicofol, econazole, fenarimol, ketoconazole, 4-nonylphenol, and prochloraz. In addition, 4-hydroxyandrostenedione and lindane were included in the assay design as the positive and negative controls, respectively. 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 protein concentration of the recombinant microsomes as specified by the supplier was 4.9 mg/mL. The laboratory group mean (± Standard Error of the Mean, SEM) protein concentration values were 4.7 ± 0.2, 5.2 ± 0.0, 3.3 ± 0.4, and 6.6 ± 0.4 mg/mL for RTI, Battelle, In Vitro, and WIL, respectively. The precision (% CV) ranged from 4.2 to 51.9 percent. The accuracy (% RE) ranged from -33.6 to 35.5 percent. The overall task mean ± SEM protein concentration was 4.9 ± 0.7 mg/mL with a % CV of 28.3 percent.

Protein QC samples (target concentrations of 10 and 100 µg/mL), had acceptable accuracy (±15 % RE) for two of four laboratories for the low QC (range was -39.7 to 16.3 percent) and all laboratories for the high QC (range was -0.7 to 14.7 percent). Precision was acceptable (±15 % CV) for one of four laboratories for the low QC (range was 10.9 to 67.9 percent) and all laboratories for the high QC (range was 2.4 to 7.6 percent).

For the full enzyme activity control, the laboratory overall average ± SEM (% CV) values were 0.378 ± 0.029 (18.6 %), 0.312 ± 0.016 (16.1 %), 1.258 ± 0.400 (100.4 %) and 0.340 ± 0.052 (37.3 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The higher activity and low precision for In Vitro was attributed to a few inaccurate protein concentration determinations. The overall task mean ± SEM full enzyme activity control value was 0.343 ± 0.019 nmol/mg protein/min with a percent CV of 9.6 percent (without In Vitro) and 0.572 ± 0.229 nmol/mg protein/min with a percent CV of 80.1 percent (with In Vitro).

Background enzyme activity control values 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 0.216 ± 0.017 (19.8 %), 0.169 ± 0.008 (14.9 %), 0.642 ± 0.226 (111.0 %), and 0.166 ± 0.019 (27.5 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The higher activity and low precision for In Vitro was attributed to a few inaccurate protein concentration determinations. The overall task mean ± SEM full enzyme activity control value was 0.183 ± 0.016 nmol/mg protein/min with a percent CV of 15.3 percent (without In Vitro) and 0.298 ± 0.115 nmol/mg protein/min and 77.4 percent (with In Vitro). In regard to percent inhibition, the laboratory overall group mean ± SEM (% CV) inhibition values for 4-OH ASDN (as a percent of control) were 57.0 ± 0.9 (4.1 %), 54.2 ± 0.8 (4.7 %), 48.5 ± 2.1 (13.7 %), and 49.7 ± 1.7 (8.4 %) percent for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM percent of control value was 52.4 ± 2.0 percent with a percent CV of 7.5.

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 0.373 ± 0.032 (20.9 %), 0.305 ± 0.016 (16.8 %), 1.083 ± 0.379 (110.7 %), and 0.328 ± 0.048 (36.1 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively. The higher activity and low precision for In Vitro was attributed to a few inaccurate protein concentration determinations. The overall task mean ± SEM negative control value was 0.335 ± 0.020 nmol/mg protein/min with a percent CV of 10.2 percent (without In Vitro) and 0.522 ± 0.187 nmol/mg protein/min and 71.8 percent (with In Vitro). In regard to percent inhibition, the laboratory overall group mean ± SEM (% CV) inhibition values for lindane (as a percent of control) were 98.3 ± 1.3 (3.2 %), 97.7 ± 1.1 (3.4 %), 81.0 ± 3.8 (14.9 %), and 96.8 ± 2.2 (5.5 %) percent for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM percent of control value was 93.4 ± 4.2 percent with a percent CV of 8.9.

For aminoglutethimide, increasing concentrations decreased the activity of the recombinant 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 91 percent. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-7} M were 0.38 ± 0.05 and 97.17 ± 3.19 percent, respectively. The overall task assay precision ranged from 5 to 12 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 24 percent. The IC_{50} values for aminoglutethimide (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 5.23 ± 0.45 (15.0), 5.71 ± 0.63 (19.2), 3.89 ± 0.28 (12.3), and 4.22 ± 0.85 (34.7) µM, respectively. The overall task group mean ± SEM IC_{50} value was 4.76 ± 0.43 µM and the percent CV was 17.9 percent.

For atrazine, increasing concentrations did <u>not</u> decrease the activity of the recombinant microsomal aromatase activity. At an atrazine concentration of 10^{-3} M (3 of 4 labs), aromatase activity was 86 to 92 percent of control, which was similar to the values obtained at 10^{-10} M when 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 88.06 ± 2.02 and 96.46 ± 3.51 percent, respectively. The overall task assay precision ranged from 4 to 8 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 recombinant 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 15 to 25 percent. In contrast, at chrysin concentrations $\leq 10^{-8}$ M, most laboratories reported percent of control values greater than or equal to 85 percent. The overall task mean ± SEM percent of control values at 10^{-4} and 10^{-8} M were 20.84 ± 2.20 and 91.23 ± 2.16 percent, respectively. The overall task assay precision ranged from 5 to 11 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 21 percent. IC_{50} values (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 2.18 ± 0.17 (13.1), 1.48 ± 0.36 (42.0), 3.02 ± 0.46 (26.5), and 3.41 ± 0.20 (10.2) µM, respectively. The overall task group mean ± SEM IC_{50} value was 2.52 ± 0.43 µM and the percent CV was 34.3 percent.

For dicofol, increasing concentrations decreased the activity of the recombinant 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 2 to 3 percent. In contrast, at a dicofol concentration of approximately 10^{-7} 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^{-7} M were 2.65 ± 0.32 and 93.32 ± 1.66 percent, respectively. The overall task assay precision ranged from 4 to 13 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 21 and 33 percent, respectively. IC_{50} values (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 35.77 ± 0.79 (3.8), 17.54 ± 2.13 (21.0), 6.71 ± 1.95 (50.3), and 39.96 ± 6.55 (28.4) µM, respectively. The overall task group mean ± SEM IC_{50} value was 25.00 ± 7.80 µM and the percent CV was 62.4 percent.

For dibenz[a,h]anthracene, increasing concentrations did <u>not</u> decrease the activity of the recombinant microsomal aromatase activity. At a dibenz[a,h]anthracene concentration of 10^{-4} M 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 95.98 ± 3.83 and 96.49 ± 4.24 percent, respectively. The overall task assay precision ranged from 5 to 9 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 recombinant microsomal aromatase activity and the decrease was concentration-dependent. At an econazole concentration of 10^{-7} M, percent of control values were less than or equal 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 all four labs. The overall task mean ± SEM percent of control values at 10^{-7} and 10^{-10} M were 1.76 ± 0.17 and 94.21 ± 2.33 percent, respectively. The overall task assay precision ranged from 5 to 19 percent for those concentrations that were tested by 3 or more labs, except at concentrations $\geq 10^{-5}$ 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 2.47 ± 0.05 (3.6), 2.39 ± 0.34 (24.7),

1.63 ± 0.03 (3.6), and 2.38 ± 0.09 (6.4) nM, respectively. The overall task group mean ± SEM IC_{50} value was 2.22 ± 0.20 nM and the percent CV was 17.9 percent.

For fenarimol, increasing concentrations decreased the activity of the recombinant microsomal aromatase activity and the decrease was concentration-dependent. At a fenarimol concentration of 10^{-3} M, percent of control values were equal to or less than 8 percent for three of four labs. In contrast, at a fenarimol concentration of approximately 10^{-7} M, there was little to no aromatase inhibition with percent of control values being greater than 90 percent. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-7} M were 3.52 ± 2.32 and 95.67 ± 1.90 percent, respectively. The overall task assay precision ranged from 3 to 30 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 114 and 94 percent, respectively. The IC_{50} values for fenarimol (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 6.38 ± 0.33 (9.1), 5.42 ± 0.13 (4.3), 3.79 ± 1.01 (46.1), and 6.95 ± 0.77 (15.7) µM, respectively. The overall task group mean ± SEM IC_{50} value was 5.63 ± 0.69 µM and the percent CV was 24.6 percent.

For ketoconozole, increasing concentrations decreased the activity of the recombinant microsomal aromatase activity and the decrease was concentration-dependent. At a ketoconazole concentration of 10^{-4} M, percent of control values ranged from 4 to 8 percent. In contrast, at a concentration of approximately 10^{-7} M, there was little to no aromatase inhibition with percent of control values being greater than 92 percent. The overall task mean ± SEM percent of control values at 10^{-4} and 10^{-7} M were 6.01 ± 1.01 and 98.30 ± 2.10 percent, respectively. The overall task assay precision ranged from 1 to 8 percent for those concentrations that were tested by 3 or more labs, except at concentrations of 10^{-4} and 10^{-5} M where the % CV values were 18 and 34 percent, respectively. The IC_{50} values for ketoconazole (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 9.38 ± 0.73 (13.5), 11.07 ± 3.03 (47.5), 3.34 ± 0.67 (34.7), and 6.73 ± 1.05 (27.0) µM, respectively. The overall task group mean ± SEM IC_{50} value was 7.63 ± 1.69 µM and the percent CV was 44.2 percent.

For 4-nonylphenol, increasing concentrations decreased the activity of the recombinant 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. In contrast, at a 4-nonylphenol concentration of approximately 10^{-6} M, there was little to no aromatase inhibition with percent of control values being greater than or equal to 94 percent. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-6} M were 0.34 ± 0.22 and 97.03 ± 1.62 percent, respectively. The overall task assay precision ranged from 3 to 15 percent for those concentrations that were tested by 3 or more labs, except at concentrations equal to or greater than 5 x 10^{-5} where the % CV values were equal to or greater than 33 percent. The IC_{50} values for 4-nonylphenol (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 20.98 ± 0.77 (6.4), 20.21 ± 0.84 (7.2), 18.7 ± 2.06 (19.1), and 15.59 ± 1.33 (14.8) µM, respectively. The overall task group mean ± SEM IC_{50} value was 18.87 ± 1.19 µM and the percent CV was 12.6 percent.

For prochloraz, increasing concentrations decreased the activity of the recombinant 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. In contrast, at a prochloraz concentration of approximately 10^{-9} M, there was little to no

aromatase inhibition with percent of control values being equal or greater than 93 percent. The overall task mean ± SEM percent of control values at 10^{-6} and 10^{-9} M were 7.26 ± 4.27 and 96.33 ± 2.77 percent, respectively. The overall task assay precision ranged from 5 to 11 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 46 to 189 percent. The IC_{50} values for prochloraz (average ± SEM, % CV) for RTI, Battelle, In Vitro, and WIL were 0.0334 ± 0.0007 (3.5), 0.0325 ± 0.0047 (25.2), 0.0198 ± 0.0009 (6.1), and 0.0284 ± 0.0021 (10.5) μM, respectively. The overall task group mean ± SEM IC_{50} value was 0.0285 ± 0.0031 μM and the percent CV was 21.9 percent.

Inhibition curves were characterized as "complete" by all laboratories for all reference chemicals, except for atrazine and dibenz[a,h]anthracene, which were characterized as 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 reference substances. Two reference substances, dibenz[a,h]anthracene and atrazine, were noninhibitors and concentration response relation fits were not carried out.

1. Differences among laboratories for $\log_{10}LC_{50}$. The estimates for $\log_{10}IC_{50}$ were in general consistent among the four laboratories for most chemicals. The In Vitro estimates were slightly lower than the other three laboratories for ketoconazole, econazole, and dicofol. The ratios of the among laboratory variance to the pooled within laboratory variance were 6.4 for ketoconazole, 4.9 for econazole, 9.5 for dicofol, and less than 2.7 for the other chemicals. The CVs for the IC_{50} were relatively high: 39.5 percent for ketoconazole, 70.6 percent for dicofol, 26.9 percent for chrysin, 29.2 percent for prochloraz, and greater than 11.4 percent for the other chemicals.

2. Differences among laboratories for slope. The estimated slopes were similar among the four laboratories for most chemicals. The In Vitro slope was more negative than those of the other laboratories for ketoconazole and dicofol and was less negative for 4-nonylphenol and econazole. The CV values for the slope were 28.1 percent for dicofol, 14.5 percent for 4-nonylphenol, and less than 10 percent for the other six chemicals. The ratios of the among laboratory variance to the pooled within laboratory variance were 2.5 or less for all the chemicals.

3. Differences among laboratories for top threshold. The estimated top thresholds varied between 85 and 111 percent, and they were in general consistent among the four laboratories for most chemicals, except for aminoglutethimide. For aminoglutethimide the estimated top threshold for In Vitro was higher than those for the other three laboratories (approximately 110 percent compared to approximately 99 percent or less). The CVs for the top thresholds were less than or equal to six percent for all chemicals. The ratios of the among laboratory variances to the pooled within laboratory variance ratios were 7.1 for aminoglutethimide and 3.9 or less for the other chemicals.

4. Differences among laboratories for bottom threshold. The estimated bottom thresholds were between -2.5 and 11 percent for all chemicals except chrysin and dicofol. For chrysin the bottom thresholds were between 12.8 and 22.6 percent and were consistent among laboratories. For dicofol In Vitro had a bottom threshold of 41.1 percent, which was inconsistent with those of the other laboratories (bottom thresholds between -6.0 and 2.1 percent). The ratios of the among laboratory variances to the pooled within laboratory variance were 21.4 for dicofol and less than 1.4 for the other chemicals.

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.

1. For the background activity control, there were no differences between the end and the beginning of replicates. The among laboratory variance was zero.

2. For the full enzyme activity controls, the end portion was statistically significantly lower than the beginning portion for each individual laboratory and on average across laboratories. The difference between the two portions for RTI was more than two times of those for the other three laboratories. The estimated variance among the laboratories was about eight times of the pooled within laboratory variance.

3. For the negative control, the results for In Vitro differed from those of the other laboratories. The end portion was (nearly significantly) higher than the beginning portion for In Vitro, and statistically significantly lower than the beginning for the other laboratories. As a result of this inconsistency, the estimated variance among the laboratories was about 4.6 times the average within laboratory variance. The end and the beginning portions did not differ significantly on average across laboratories.

4. For the positive controls, the result for In Vitro differed slightly from the other three laboratories. The end portion was slightly higher than the beginning portion for In Vitro, but was statistically significantly lower than the beginning for the other laboratories, and on average across laboratories. The estimated variance among the laboratories was about 1.6 times of the average within laboratory variance.

C. Interlaboratory Statistical Analysis Summary and Conclusions

In Vitro's results differed slightly from the other laboratories with respect to $\log_{10}IC_{50}$ and slope for ketoconazole, econazole and dicofol, for slope for 4-nonylphenol, for bottom for dicofol, for top for aminoglutethimide, and for differences between end and beginning portions for the negative and positive 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 while those at the other laboratories decreased.

The experimental designs for Work Assignments 4-17, Task 4 and 4-16, Task 7 differed only in the types of microsomes used for testing. The overall group mean $Log_{10}IC_{50}$s and slopes for a given reference chemical and overall control values were very similar for both types of microsomes.

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 recombinant 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 recombinant microsomal aromatase assay protocol, 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). RTI obtained the recombinant microsomes and then distributed them to the other laboratories. Each laboratory conducted at least three independent replicates of the recombinant 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 recombinant microsomes 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. In addition, the results from using recombinant versus placental microsomes were compared.

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 5×10^{-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 RECOMBINANT MICROSOMES

Recombinant microsomes (Human CYP19 + P450 Reductase SUPERSOMES) were provided to each of the laboratories by RTI (Lot # 5, 4.9 mg protein/mL, purchased from Gentest, Woburn, MA). 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 0.004 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 involved a serial dilution of 1:10 and then 1:60 (or 1:50 and then 1:12) for a total dilution of 1:600. Battelle's dilution scheme involved a serial dilution of 1:60 and then 1:10.5 for a total dilution scheme of 1:630, except for the first two replicates of ketoconazole, which involved a serial dilution scheme of 1:50 and then 1:12.5 for a total dilution scheme of 1:625. In Vitro's dilution scheme involved a single direct dilution of 1:600 except atrazine, dicofol, 4-nonylphenol, chrysin (rep 4), dibenz [a,h], anthracene, which were 1:61, then 1:10 for a total dilution of 1:610. WIL's dilution scheme involved a single direct dilution of 1:612.

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 2. The CR obtained the DMSO and ethanol and distributed it to the participating laboratories. The laboratories purchased their own propylene glycol and buffer materials.

Table 2. 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[d]	Mallinkrodt and EMD	Battelle CR	Battelle CR
95% Ethanol[c]	Battelle CR	Battelle CR	Battelle CR	Battelle CR

a. Two different lots of NADPH were used.
b. Various lots of DMSO were used.
c. A single lot of ethanol was used except for In Vitro, which used a different lot.
d. A lot of DMSO from an unspecified supplier was also 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, 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 3.

Table 3. 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 4. 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 4. 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	0.004 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 reference chemical and replicate within reference chemical a concentration response curve was fitted to the percent of control activity values at the three repetitions at each of the eight graded reference chemical 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 reference chemical is a non-inhibitor, and parameter estimates are not calculated.

For each of the reference chemicals 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 replicate. The random effect was replicate within reference chemical. 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 reference chemicals 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 reference chemical 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 reference chemicals 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 was 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 Reference Chemicals and Replicates Within Reference Chemicals

Within each reference chemical and replicate within reference chemical, 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 reference chemicals. 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. 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.

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 reference chemical, portion (beginning or end), and portion by reference chemical interaction. The random effects were replicate nested within reference chemical and portion by replicate within reference chemical 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 eight concentration response relation and control endpoints discussed above in the Test Organization section: $\log_{10}IC_{50}$, slope, top, bottom, and portion effects (end minus beginning) for the background activity, full enzyme activity, 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. The analyses for the controls were performed combining all chemicals.

For each endpoint, 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 four 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 was 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 means for the $\log_{10}IC_{50}$, the slope, the top and the bottom threshold parameters across replicates, all four laboratories incorporated the replicate-to-replicate component of variation into the standard errors of the averages. However, as discussed previously the four laboratories reported different summary statistics for the background activity, the full enzyme activity, the negative activity and positive controls. The differences in activity between the beginning and the end portions were therefore necessarily determined differently for each of the laboratories.

The differences between the beginning and the end (end minus beginning) were determined for each control endpoint as follows:

1. For RTI, the differences (Diff) between the end and the beginning were first calculated for each chemical. The associated standard errors for these differences were calculated as $|\text{Diff}|/(\text{F-value})^{1/2}$, where the F-values were associated with test results for the portion effects for chemicals, as provided in the intralaboratory analysis reports. The differences between the beginning and the end portions were averaged across all chemicals resulting in the overall average differences between the end and the beginning portions. 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 i^{th} chemical.

2. For WIL Laboratories, the ten chemicals were combined into subsets of size one or two. The subsets of chemicals were tested simultaneously, with common controls. This resulted in a total of six sets of independent controls. For each subset of chemical combinations, the mean values and the associated standard errors at the beginning and at the end portions, as well as the averages across the two portions were reported. The differences between the end and beginning were first calculated for each chemical. The associated standard errors for these differences were calculated as $(2(S_e^2+S_b^2)-4S_s^2)^{1/2}$, where S_e, S_b, and S_s were the standard errors associated with the end, the beginning, and the averages across the end and the beginning respectively, as provided in the intralaboratory analysis report. The differences between the beginning and the end portions (end minus beginning) were averaged across the chemical combinations. The associated standard errors were calculated as $(1/6)(\sum s_i^2)^{1/2}$, where s_i is the standard error associated with the difference for the i^{th} chemical combination.

3. Results for Battelle and In Vitro were as reported in the intralaboratory report, averaged across chemicals with associated standard errors.

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 the IC_{50} in the original domain, rather than $\log_{10}IC_{50}$.) The coefficient of variation is defined as the standard deviation of the effect response divided by its mean. The methods for calculating the CV and the associated 95 percent CI differed, depending on the underlying assumption about the distribution of the endpoint parameter.

The measurements for IC_{50} were assumed to be approximately log normally distributed. The CV for IC_{50} therefore was expressed as

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

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 ($\log_{10}IC_{50}$) estimate. This expression

would be exact if the within laboratory variances were equal across laboratories. The 95 percent confidence interval associated with the CV is based on the chi square distribution and is calculated as

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

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

For the slope (β) and the top (T), the measurements are assumed to be approximately normal. The CV therefore 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 the 95 percent confidence interval for the CV are 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 ($\equiv M/(S/\sqrt{4})$) and degrees of freedom df is the estimated degrees of freedom among the four laboratories. 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 iterative procedure is used to solve for the noncentrality parameters in these equations (Lehmann, 1986).

CVs were not calculated for the bottom threshold parameter, and for the differences between the end and the beginning for the background activity, full enzyme activity, negative and positive controls. The ranges of the mean values for these endpoints include zero and so the estimates fluctuate between positive and negative values. CV is therefore not stable for these endpoint parameters and could be very large or even infinite.

To describe the variability among laboratories relative to the 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 at the four laboratories. A confidence interval for this ratio is based on the F-distribution with (ν_{lab}, ν_{wi}) degree of freedom

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

where $\nu_{lab}=3$ 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 eight endpoint parameters.

In several places entries in the tables 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 body and Appendix A of the Interlaboratory Statistical Analysis Report (Appendix E of the overall report) includes a number of figures, to be discussed in greater detail in the results section. The plotting symbols are circles. Some of the circles are filled while others are empty. This is due to an issue in the graphing program. For purposes of interpretation, it should be disregarded whether the circle is filled or is empty.

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. Food and Drug Administration 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 Eric Balsley, B.S. – 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 5). 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 two and one days beyond the 20 day stability, respectively. 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 5. 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.

For In Vitro, there were four instances when the determined protein concentration of the final dilution was very near or below the lowest standard. As a result, the determined values were very low and, in at least one instance, the value was a negative number, thereby suggesting that the determined protein concentrations were inaccurate. For this reason, the laboratory was directed to use an intermediate dilution sample to determine the protein concentration. However, the four instances when the final dilutions were analyzed prior to this directive were used by the laboratory and are included in the results reported in Table 6, except for the one negative value. Inclusion of these values accounts for the much of the disparity reported for In Vitro.

The supplier documented protein concentration provided by RTI at the time of distribution was 4.9 mg/mL. The laboratory group mean (± Standard Error of the Mean, SEM) protein concentration values were 4.7 ± 0.2, 5.2 ± 0.0, 3.3 ± 0.4, and 6.6 ± 0.4 mg/mL for RTI, Battelle, In Vitro, and WIL, respectively (Table 6). The precision (% CV) ranged from 4.2 to 27.1 percent, except for In Vitro which had a % CV value of 51.9 percent. A comparison of the original reported protein concentration to the values determined by the laboratories resulted in a percent relative error (%RE) of -4.1, 5.5, -33.6, and 35.5 percent for RTI, Battelle, In Vitro, and WIL, respectively. If a comparison is made to the results obtained by the lead laboratory (RTI), then the %RE was 10.0, -30.8, and 41.2 percent for Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM protein concentration was 4.9 ± 0.7 mg/mL with a percent CV of 28.3 percent.

Table 6. Recombinant Microsomal Protein Concentration[a]

Parameter	RTI	Battelle	In Vitro[b]	WIL
Average (mg/mL)	4.7	5.2	3.3	6.6
sd	1.1	0.2	1.7	1.8
SEM	0.2	0.0	0.4	0.4
Minimum (mg/mL)	2.9	4.6	0.1	3.8
Maximum (mg/mL)	6.3	5.6	5.4	9.5
% CV	22.6	4.2	51.9	27.1

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).

b. Includes aberrant values (see text for explanation) attributed to low sample concentration. Aberrant values were 0.261, 0.570, and 0.142 mg/mL, as well as one negative value that was not included in the summary calculations.

Protein QCs were analyzed with the unknowns during the study (Table 7). The target QC concentrations were 10 and 100 µg/mL (RTI's reported concentrations were 12 and 110 µg/mL). Accuracy (% RE) ranged from -2.3 to 16.3 percent for the low QC, except for In Vitro which had a value of -39.7 percent. Accuracy ranged from -0.7 to 14.7 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 27.2 percent for the low QC, except for In Vitro that reported a value of 67.9 percent, whereas the precision ranged from 2.4 to 7.6 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 7. Protein QC Standards[a]

Parameter	RTI	Battelle	In Vitro	WIL
Low Standard (10 µg/mL)				
Overall Average	10	11	6	12
Overall sd	3	1	4	3
% CV	27.2	10.9	67.9	23.1
% RE[b]	-2.3	6.3	-39.7	16.3
High Standard (100 µg/mL)				
Overall Average	115	107	103	99
Overall sd	5	3	8	4
% CV	4.8	2.4	7.6	4.0
% RE[b]	14.7	6.8	2.9	-0.7

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.

A comparison of the before and ending activity tubes indicated that activity changed. For all four laboratories, the activity for the before tubes was higher than it was for the ending tubes. The statistical analysis provides a measure of this finding.

The laboratory overall average ± SEM (% CV) full enzyme activity control values were 0.378 ± 0.029 (18.6 %), 0.312 ± 0.016 (16.1 %), 1.258 ± 0.400 (100.4 %) and 0.340 ± 0.052 (37.3 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively (Table 8). The higher activity and lack of precision for In Vitro was attributed to the inaccurate protein concentration determinations (see protein concentration section). The slightly lower precision for WIL was attributed to low activity for replicate #2 for prochloraz and fenarimol. The overall task mean ± SEM full enzyme activity control value (excluding In Vitro) was 0.343 ± 0.019 nmol/mg protein/min with a percent CV of 9.6 percent. [If In Vitro is included, the overall task mean ± SEM and % CV were 0.572 ± 0.229 nmol/mg protein/min and 80.1 percent.] Comparison of the lead laboratory's results relative to the individual laboratory results produced % RE values of -17.4, 232.8, and -10.1 percent for Battelle, In Vitro, and WIL, respectively.

Table 8. Recombinant Full Enzyme Activity Control Determinations[a]

Parameter	Aromatase Activity (nmol/mg protein/min)			
	RTI	Battelle	In Vitro[b]	WIL
Overall Average	0.378	0.312	1.258	0.340
Overall sd	0.070	0.050	1.264	0.127
Overall SEM	0.029	0.016	0.400	0.052
% CV	18.6	16.1	100.4	37.3

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

b. Includes aberrant values (see text for explanation) attributed to low protein concentration determinations. Aberrant values were 2.905, 2.850, and 3.446 nmol/mg protein/min.

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.

The laboratory overall average ± SEM (% CV) enzyme activity values in the presence of 4-OH ASDN were 0.216 ± 0.017 (19.8 %), 0.169 ± 0.008 (14.9 %), 0.642 ± 0.226 (111.0 %), and 0.166 ± 0.019 (27.5 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively (Table 9). Comparison of the lead laboratory to the individual laboratories resulted in % RE values of -21.7, 198.0, and -23.2 percent for Battelle, In Vitro, and WIL, respectively. The higher activity and lack of precision for In Vitro was attributed to the inaccurate protein concentration determinations (see protein concentration section). The overall task mean ± SEM full enzyme activity control value (excluding In Vitro) was 0.183 ± 0.016 nmol/mg protein/min with a percent CV of 15.3 percent. [If In Vitro is included, the overall task mean ± SEM and % CV were 0.298 ± 0.115 nmol/mg protein/min and 77.4 percent.]

Table 9. Recombinant Enzyme Activity in the Presence of 4-OH ASDN (Positive-Control)[a]

Parameter	Aromatase Activity (nmol/mg protein/min)			
	RTI	Battelle	In Vitro[b]	WIL
Overall Average	0.216	0.169	0.642	0.166
Overall sd	0.043	0.025	0.713	0.046
Overall SEM	0.017	0.008	0.226	0.019
% CV	19.8	14.9	111.0	27.5

a. The overall average value for the laboratory was calculated using the mean values for the ten reference chemicals, (mean values from paired reference chemicals were only used once).
b. Includes aberrant values (see text for explanation) attributed to low protein concentration determinations. Aberrant values were 1.517, 1.192, and 2.148 nmol/mg protein/min.

The laboratory overall group mean ± SEM (% CV) inhibition values for 4-OH ASDN (as a percent of control) were 57.0 ± 0.9 (4.1 %), 54.2 ± 0.8 (4.7 %), 48.5 ± 2.1 (13.7 %), and 49.7 ± 1.7 (8.4 %) percent for RTI, Battelle, In Vitro, and WIL, respectively (Table 10). Comparison of the lead laboratory to the individual laboratories resulted in % RE values of -4.9, -14.8, and -12.7 percent for Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM percent of control value was 52.4 ± 2.0 percent with a percent CV of 7.5.

Table 10. Recombinant Enzyme Activity Inhibition by 4-OH ASDN (Positive Control)[a]

Parameter	Percent of Control			
	RTI	Battelle	In Vitro	WIL
Overall Average	57.0	54.2	48.5	49.7
Overall sd	2.3	2.6	6.7	4.2
Overall SEM	0.9	0.8	2.1	1.7
% CV	4.1	4.7	13.7	8.4

a. The overall average value for the laboratory was calculated using the mean values for the ten reference chemicals, (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.

The laboratory overall average ± SEM (% CV) enzyme activity values in the presence of lindane were 0.373 ± 0.032 (20.9 %), 0.305 ± 0.016 (16.8 %), 1.083 ± 0.379 (110.7 %), and 0.328 ± 0.048 (36.1 %) nmol/mg protein/min for RTI, Battelle, In Vitro, and WIL, respectively (Table 11). Comparison of the lead laboratory to the individual laboratories resulted in % RE values of -18.1, 190.6, and -12.0 percent for Battelle, In Vitro, and WIL, respectively. The higher activity and lack of precision for In Vitro was attributed to the inaccurate protein

concentration determinations (see protein concentration section). The overall task mean ± SEM full enzyme activity control value (excluding In Vitro) was 0.335 ± 0.020 nmol/mg protein/min with a percent CV of 10.2 percent. [If In Vitro is included, the overall task mean ± SEM and % CV were 0.522 ± 0.187 nmol/mg protein/min and 71.8 percent.] This enzyme activity value is similar to that measured in the absence of any inhibitor (full enzyme activity control value).

Table 11. Recombinant Enzyme Activity in the Presence of Lindane (Negative Control)[a]

Parameter	Aromatase Activity (nmol/mg protein/min)			
	RTI	Battelle	In Vitro[b]	WIL
Overall Average	0.373	0.305	1.083	0.328
Overall sd	0.078	0.051	1.199	0.119
Overall SEM	0.032	0.016	0.379	0.048
% CV	20.9	16.8	110.7	36.1

a. The overall average value for the laboratory was calculated using the mean values for the ten reference chemicals, (mean values from paired reference chemicals were only used once).
b. Includes aberrant values (see text for explanation) attributed to low protein concentration determinations. Aberrant values were 2.502, 2.096, and 3.597 nmol/mg protein/min.

The laboratory overall group mean ± SEM (% CV) inhibition values for lindane (as a percent of control) were 98.3 ± 1.3 (3.2 %), 97.7 ± 1.1 (3.4 %), 81.0 ± 3.8 (14.9 %), and 96.8 ± 2.2 (5.5 %) percent for RTI, Battelle, In Vitro, and WIL, respectively (Table 12). It is interesting to note that In Vitro reported a decrease in enzyme activity of approximately 20 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 -0.6, -17.6, and -1.5 percent for Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM percent of control value was 93.4 ± 4.2 percent with a percent CV of 8.9.

Table 12. Recombinant Enzyme Activity Inhibition by Lindane (Negative Control)[a]

Parameter	Percent of Control			
	RTI	Battelle	In Vitro	WIL
Overall Average	98.3	97.7	81.0	96.8
Overall sd	3.2	3.4	12.1	5.4
Overall SEM	1.3	1.1	3.8	2.2
% CV	3.2	3.4	14.9	5.5

a. The overall average value for the laboratory was calculated using the mean values for the ten reference chemicals, (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 13 and by overall task in Table 14. 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 13). 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 13. 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	0.30	0.21	0.12	68.23
	-4.00	3	4.47	0.36	0.21	7.98
	-5.00	3	32.20	1.70	0.98	5.27
	-5.30	3	47.05	1.14	0.66	2.42
	-6.00	3	77.80	3.01	1.74	3.87
	-7.00	3	91.09	5.48	3.17	6.02
	-8.00	3	92.84	5.82	3.36	6.27
	-9.00	3	93.44	4.01	2.31	4.29
Battelle	-3.00	3	0.51	0.12	0.07	23.94
	-4.00	3	5.41	0.79	0.45	14.57
	-5.00	3	34.82	3.44	1.98	9.87
	-5.30	2	52.33	NC[a]	NC	NC
	-5.60	3	67.62	2.83	1.64	4.19
	-6.00	3	82.10	2.44	1.41	2.97
	-7.00	3	94.77	3.55	2.05	3.74
	-8.00	3	97.88	3.02	1.75	3.09
	-9.00	1	103.34	NC	NC	NC
In Vitro	-3.00	3	0.34	0.11	0.06	31.4
	-4.00	3	4.40	0.22	0.13	5.0
	-5.00	3	32.17	1.36	0.79	4.2
	-6.00	3	85.74	3.05	1.76	3.6
	-7.00	3	106.05	2.77	1.60	2.6
	-8.00	3	110.06	6.24	3.60	5.7
	-9.00	3	111.16	1.76	1.01	1.6
	-10.00	3	109.98	5.99	3.46	5.4
WIL	-3.00	3	0.38	0.11	0.06	27.75
	-4.00	3	4.21	1.17	0.67	27.66
	-4.30	3	7.99	2.60	1.50	32.57
	-5.00	3	29.12	7.71	4.45	26.46
	-6.00	3	78.42	5.37	3.10	6.85
	-7.00	3	96.78	1.84	1.06	1.90
	-8.00	3	99.54	0.36	0.21	0.37
	-9.00	3	97.54	1.04	0.60	1.07

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

Table 14. 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	0.38	0.09	0.05	23.8
-4.00	4	4.62	0.54	0.27	11.6
-4.30	1	7.99	NC[b]	NC	NC
-5.00	4	32.08	2.33	1.17	7.3
-5.30	2	49.69	NC	NC	NC
-5.60	1	67.62	NC	NC	NC
-6.00	4	81.02	3.68	1.84	4.5
-7.00	4	97.17	6.37	3.19	6.6
-8.00	4	100.08	7.24	3.62	7.2
-9.00	4	101.37	7.69	3.84	7.6
-10.00	1	109.98	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.

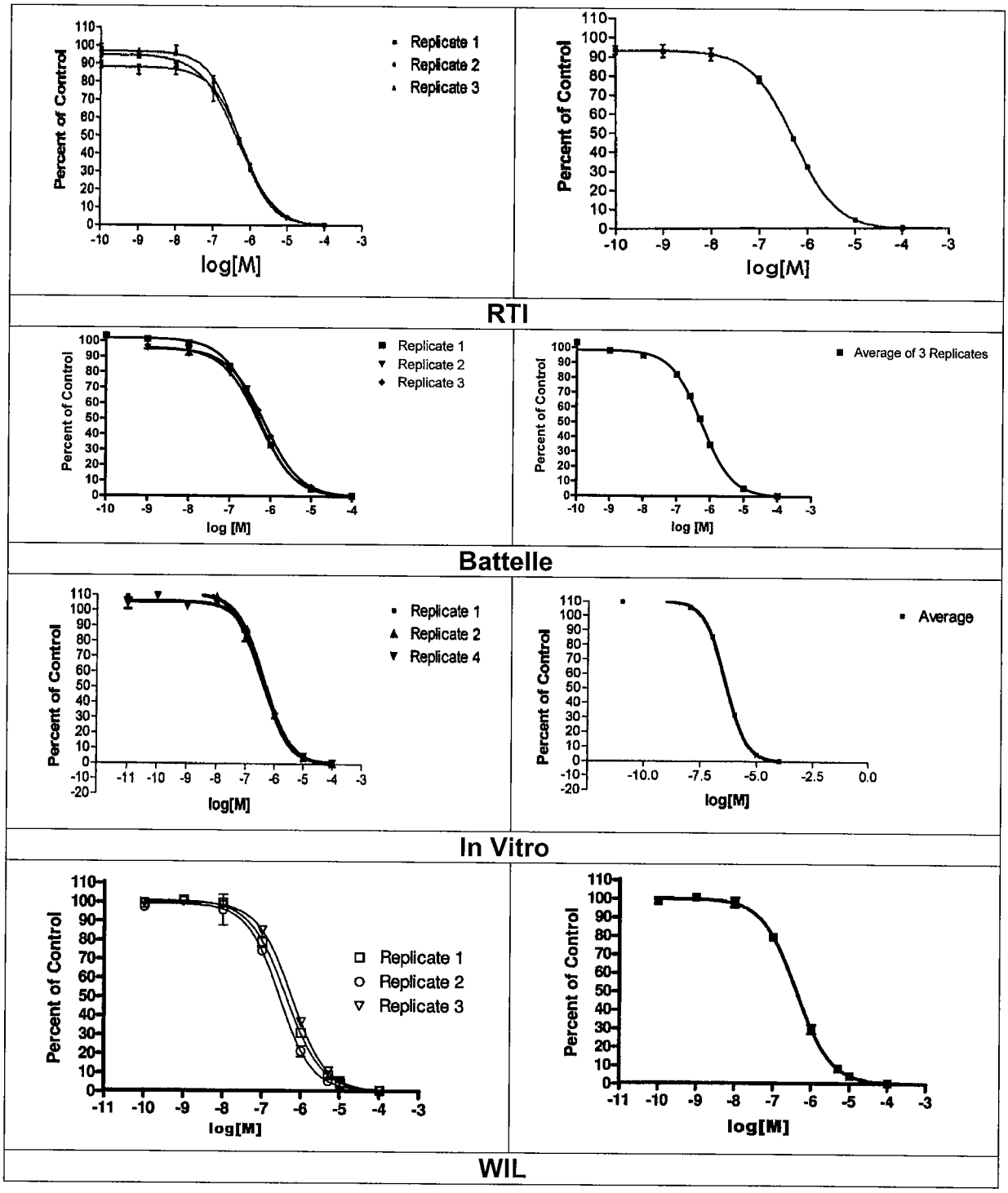

Figure 1. **Individual Replicate and Average Aminoglutethimide Inhibition Response Curves (percent of control) by Laboratory**

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

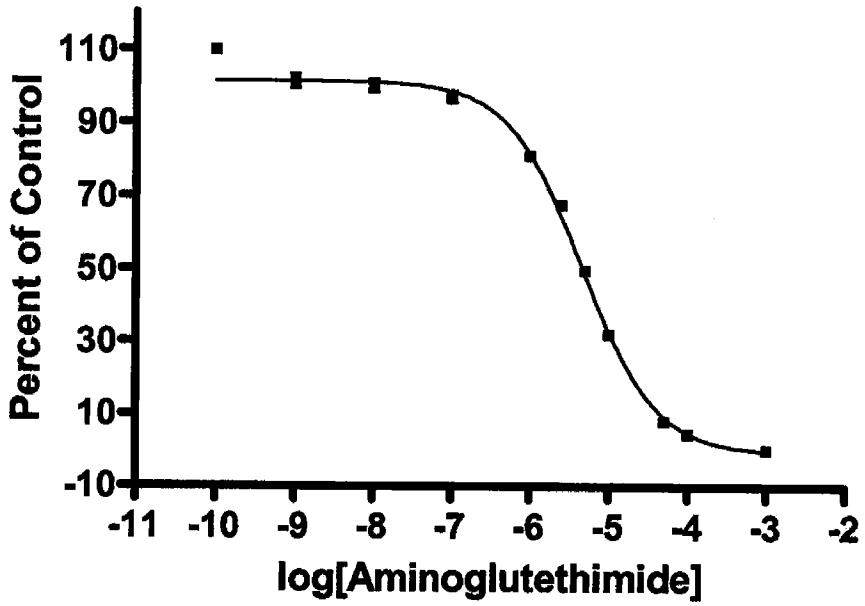

Figure 2. Overall Task Aminoglutethimide Inhibition Response Curve (percent of control)

For all four laboratories, increasing concentrations of aminoglutethimide decreased the activity of the recombinant microsomal aromatase activity and the decrease was concentration-dependent (Table 13). The shapes of the percent of control vs. aminoglutethimide concentration 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 91 percent for all labs. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-7} M were 0.38 ± 0.05 and 97.17 ± 3.19 percent, respectively (Table 14).

All labs reported assay precision values (% CV) that were less than or equal to 15 percent for those concentrations used in at least three replicates, except at a concentration of 10^{-3} M (and to 10^{-5} M for WIL) where the precision varied widely, i.e. 24 to 68 percent (Table 13). The overall task assay precision ranged from 5 to 12 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 24 percent (Table 14).

3.8.2 Atrazine

The effect of increasing concentrations of atrazine on aromatase activity is reported by laboratory in Table 15 and by overall task in Table 16. 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 15). 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 recombinant microsomal aromatase activity, although there was a trend toward inhibition at the highest concentration (Table 15). The shapes of the percent of control vs atrazine concentration curves approached a horizontal line (Figure 3). At an atrazine concentration of 10^{-3} M (or 10^{-4} M for In Vitro), aromatase activity approximated 86 to 92 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 88.06 ± 2.02 and 96.46 ± 3.51 percent, respectively (Table 16).

All labs reported assay precision values (% CV) that were less than 9 percent for those concentrations used in at least three replicates (Table 15). The overall task assay precision ranged from 4 to 8 percent for those concentrations that were tested by 3 or more labs (Table 16).

Table 15. 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	85.74	4.50	2.60	5.25
	-3.30	2	87.59	4.18	2.95	4.77
	-3.60	2	90.43	1.81	1.28	2.00
	-4.00	3	96.23	1.61	0.93	1.68
	-4.30	2	95.94	5.39	3.81	5.62
	-5.00	3	96.71	1.73	1.00	1.79
	-6.00	3	96.44	3.71	2.14	3.85
	-7.00	3	98.92	7.32	4.23	7.40
	-8.00	1	96.03	NC	NC	NC
	-9.00	1	95.46	NC	NC	NC
	-10.00	1	90.15	NC	NC	NC
Battelle	-3.00	3	92.09	5.81	3.35	6.31
	-3.05	2	90.66	NC	NC	NC
	-3.10	2	88.29	NC	NC	NC
	-3.12	2	89.41	NC	NC	NC
	-3.30	2	90.15	NC	NC	NC
	-4.00	3	101.05	8.86	5.12	8.77
	-5.00	3	105.36	5.11	2.95	4.85
	-6.00	3	104.89	6.64	3.83	6.33
	-7.00	1	110.10	NC	NC	NC
	-8.00	1	107.15	NC	NC	NC
	-9.00	1	105.84	NC	NC	NC
	-10.00	1	105.10	NC	NC	NC
In Vitro	-4.00	3	87.86	3.23	1.87	3.7
	-4.48	3	92.14	2.13	1.23	2.3
	-5.00	3	92.70	2.61	1.51	2.8
	-6.00	3	88.93	4.33	2.50	4.9
	-7.00	3	92.41	0.45	0.26	0.5
	-8.00	3	89.87	3.30	1.90	3.7
	-9.00	3	91.89	3.42	1.97	3.7
	-10.00	3	91.36	4.39	2.54	4.8
WIL	-3.00	3	86.34	2.93	1.69	3.40
	-4.00	3	97.24	1.96	1.13	2.02
	-5.00	3	101.36	2.82	1.63	2.78
	-6.00	3	100.67	3.15	1.82	3.13
	-7.00	3	100.90	1.04	0.60	1.03
	-8.00	3	101.19	2.77	1.60	2.74
	-9.00	3	100.83	1.73	1.00	1.72
	-10.00	3	99.21	1.68	0.97	1.69

a. NC – value **N**ot **C**alculated when n $\leq$2.

Table 16. 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	88.06	3.51	2.02	4.0
-3.05	1	90.66	NC[b]	NC	NC
-3.10	1	88.29	NC	NC	NC
-3.12	1	89.41	NC	NC	NC
-3.30	2	88.87	NC	NC	NC
-3.60	1	90.43	NC	NC	NC
-4.00	4	95.60	5.56	2.78	5.8
-4.30	1	95.94	NC	NC	NC
-4.48	1	92.14	NC	NC	NC
-5.00	4	99.03	5.51	2.75	5.6
-6.00	4	97.73	6.81	3.40	7.0
-7.00	4	100.58	7.31	3.65	7.3
-8.00	4	98.56	7.36	3.68	7.5
-9.00	4	98.51	6.12	3.06	6.2
-10.00	4	96.46	7.02	3.51	7.3

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.

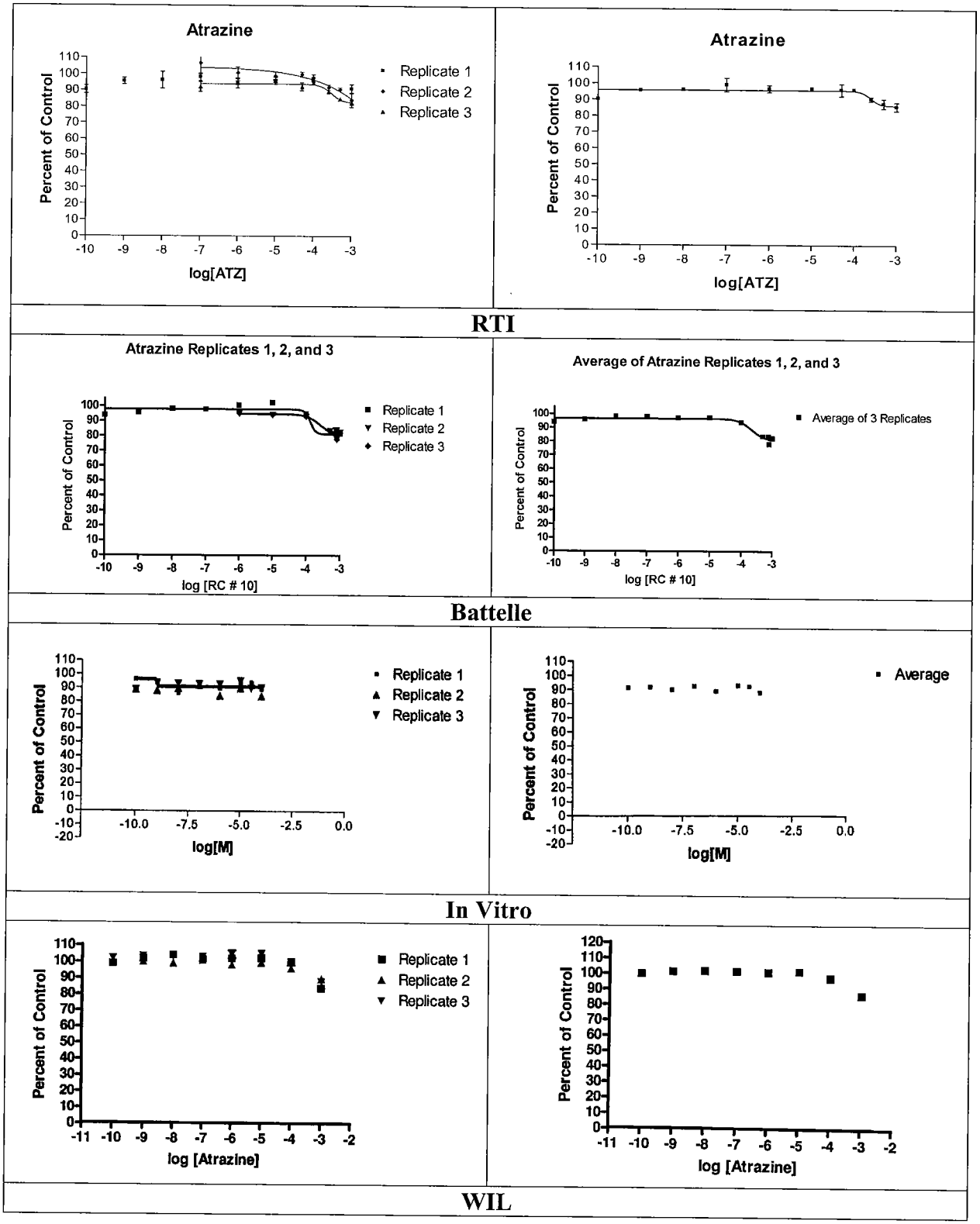

Figure 3. **Individual Replicate and Average Atrazine Inhibition Response Curves (percent of control) by Laboratory**

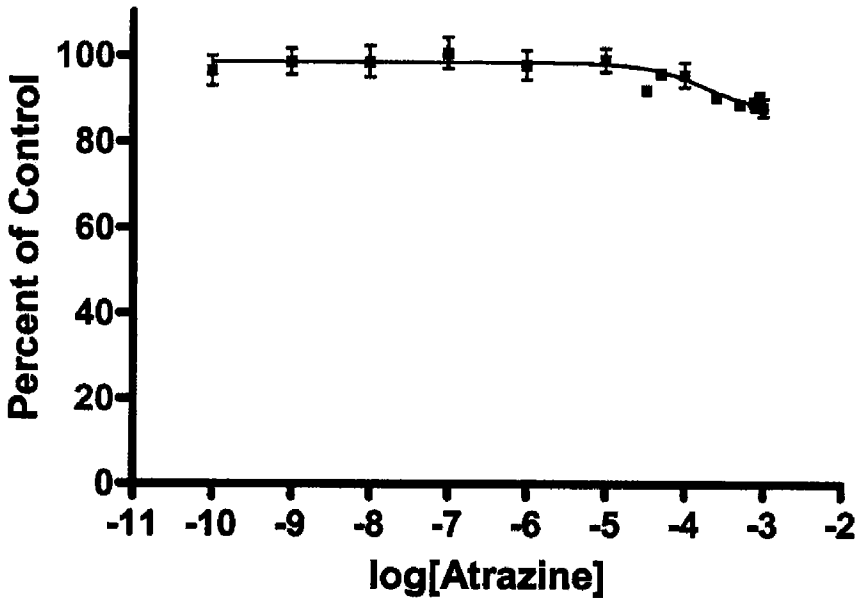

Figure 4. Overall Task Atrazine Inhibition Response Curve (percent of control)

3.8.3 Chrysin

The effect of increasing concentrations of chrysin on aromatase activity is reported by laboratory in Table 17 and by overall task in Table 18. 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 three of four laboratories modified the chrysin concentrations selected for testing in replicates 2 and 3 (Table 17). RTI tested the same concentrations for all three replicates (a fourth replicate was used when the first replicate did not show any dose dependence). The modifications involved including additional mid-concentrations between 10^{-5} and 10^{-6} M and using them in place of concentrations at the high end, i.e. 10^{-4} M (In Vitro), or the low end, i.e. 10^{-9} to 10^{-10} M (Battelle and WIL), of the concentrations that were tested in replicate 1.

For all four laboratories, increasing concentrations of chrysin decreased the activity of the recombinant microsomal aromatase activity and the decrease was concentration-dependent (Table 17). The shapes of the percent of control vs chrysin concentration 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 approximately 15 to 25 percent. It is important to note that the highest chrysin concentrations tested did not result in a 0 percent response. In contrast, at chrysin concentrations $\leq 10^{-8}$ M there was little aromatase inhibition with most laboratories reporting percent of control values greater than or equal to approximately 85 percent. The overall task mean ± SEM percent of control values at 10^{-4} and 10^{-8} M were 20.84 ± 2.20 and 91.23 ± 2.16 percent, respectively (Table 18).

Assay precision values (% CV) were less than or equal to 11 percent for those concentrations used in at least three replicates for RTI and WIL, ranged from 4 to 17 percent for Battelle, and ranged from 6 to 62 percent for In Vitro (Table 17). The overall task assay precision ranged from 5 to 11 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 21 percent (Table 18).

Table 17. 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	24.83	2.23	1.29	8.98
	-5.00	3	26.89	2.03	1.17	7.53
	-5.30	3	40.68	4.28	2.47	10.52
	-5.60	3	56.60	3.97	2.29	7.01
	-6.00	3	71.20	1.79	1.04	2.52
	-6.30	3	82.17	2.83	1.64	3.45
	-7.00	3	91.73	3.51	2.03	3.82
	-8.00	3	91.77	1.74	1.00	1.90
Battelle	-4.00	3	23.97	2.88	1.66	12.00
	-5.00	3	25.84	1.09	0.63	4.23
	-5.30	2	37.53	NC[a]	NC	NC
	-5.60	2	53.06	NC	NC	NC
	-6.00	3	59.56	10.39	6.00	17.45
	-6.60	1	64.93	NC	NC	NC
	-7.00	3	80.97	8.05	4.65	9.94
	-8.00	3	85.30	10.33	5.96	12.11
	-9.00	3	87.37	12.58	7.26	14.40
	-10.00	1	75.02	NC	NC	NC
In Vitro	-4.00	1	19.16	NC	NC	NC
	-4.48	3	18.13	11.18	6.45	61.6
	-5.00	3	25.19	4.54	2.62	18.0
	-5.48	2	67.65	NC	NC	NC
	-6.00	3	71.48	4.09	2.36	5.7
	-7.00	3	90.10	8.09	4.67	9.0
	-8.00	3	92.16	5.60	3.23	6.1
	-9.00	3	86.88	9.97	5.76	11.5
	-10.00	3	91.13	9.94	5.74	10.9
WIL	-4.00	3	15.39	1.74	1.01	11.33
	-5.00	3	30.27	1.07	0.62	3.53
	-5.30	3	45.51	2.53	1.46	5.56
	-5.60	2	61.92	3.12	2.21	5.04
	-6.00	3	78.63	3.43	1.98	4.36
	-7.00	3	96.10	3.48	2.01	3.63
	-8.00	3	95.68	3.59	2.07	3.75
	-9.00	1	100.36	NC	NC	NC
	-10.00	3	88.87	8.50	4.91	9.56

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

Table 18. 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	20.84	4.41	2.20	21.1
-4.48	1	18.13	NC[b]	NC	NC
-5.00	4	27.05	2.26	1.13	8.4
-5.30	3	41.24	4.02	2.32	9.7
-5.48	1	67.65	NC	NC	NC
-5.60	3	57.19	4.46	2.57	7.8
-6.00	4	70.22	7.89	3.95	11.2
-6.30	1	82.17	NC	NC	NC
-6.60	1	64.93	NC	NC	NC
-7.00	4	89.73	6.36	3.18	7.1
-8.00	4	91.23	4.33	2.16	4.7
-9.00	3	91.54	7.65	4.41	8.4
-10.00	3	85.01	8.72	5.04	10.3

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because three of four laboratories 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. RTI used the same concentrations for all three replicates.

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

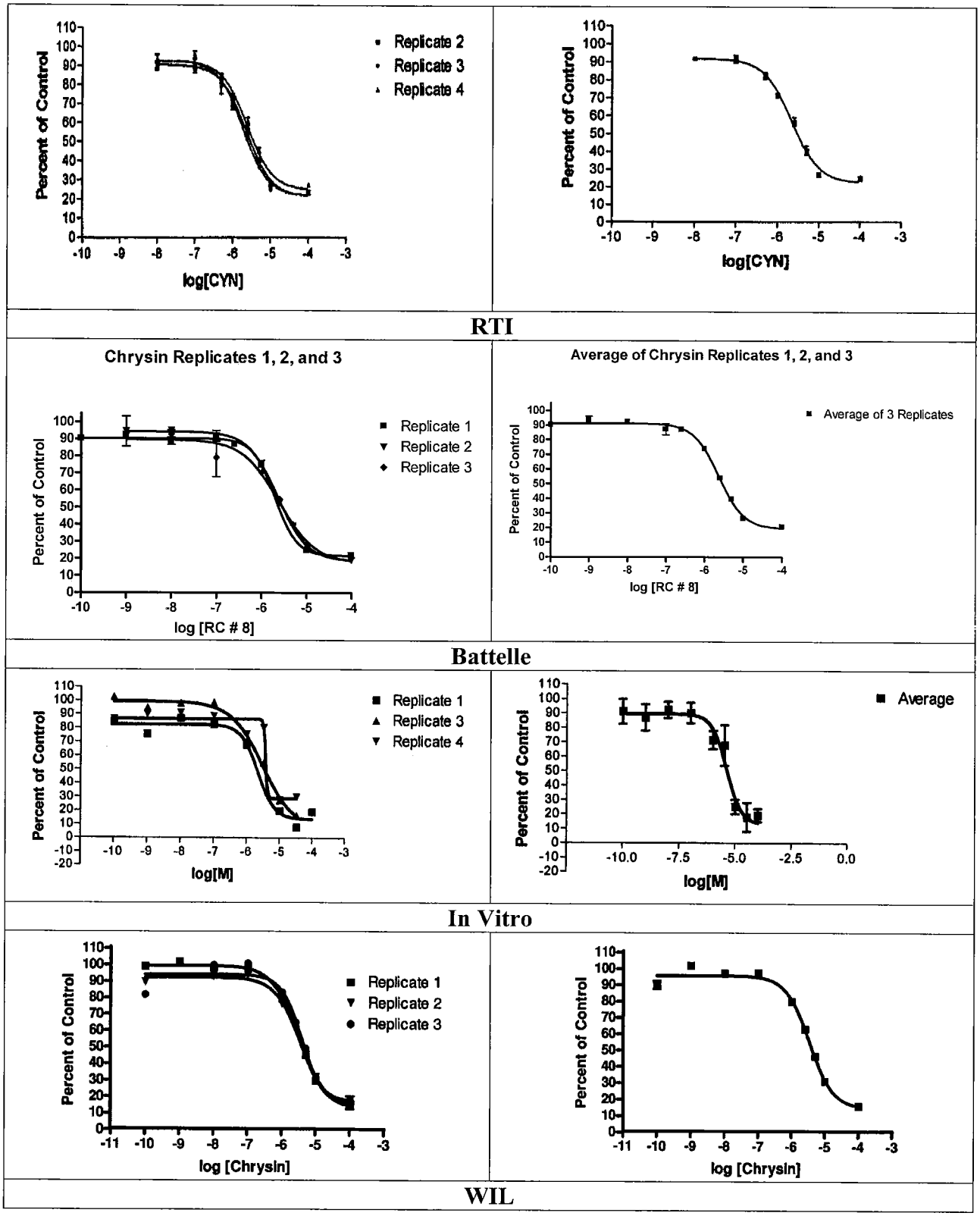

Figure 5. **Individual Replicate and Average Chrysin Inhibition Response Curves (percent of control) by Laboratory**

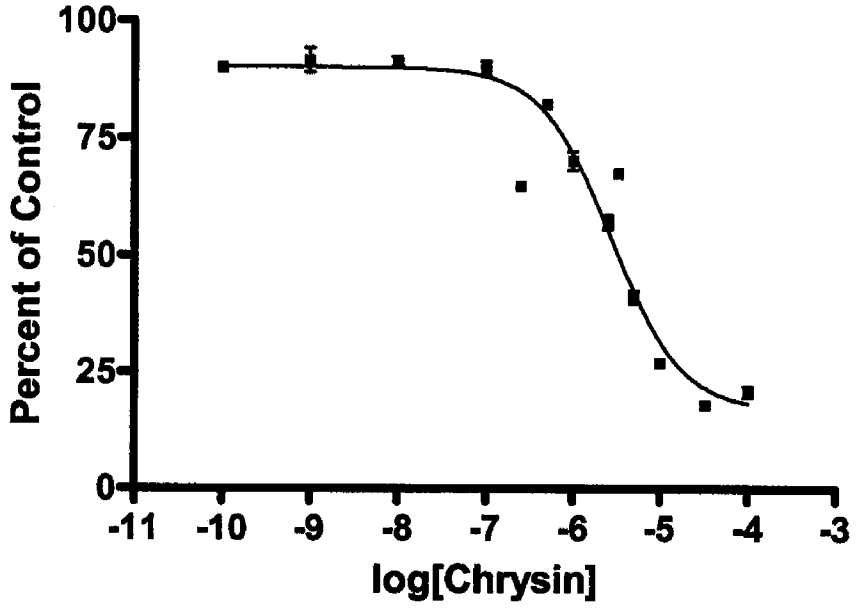

Figure 6. Overall Task Chrysin Inhibition Response Curve (percent of control)

3.8.4 Dicofol

The effect of increasing concentrations of dicofol on aromatase activity is reported by laboratory in Table 19 and by overall task in Table 20. 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 19). In Vitro used the same dicofol concentrations for all three replicates and may have done so based on information from the previous work assignment. It is not clear why In Vitro's highest concentration tested was 10^{-4} M, rather than 10^{-3} M. In general, the modifications involved including additional mid-concentrations between 10^{-3} and 10^{-6} M, which replaced concentrations at the low end, i.e.10^{-7} to 10^{-10} M (RTI, Battelle, and WIL) of the concentration range that was tested in replicate 1.

For all four laboratories, increasing concentrations of dicofol decreased the activity of the recombinant microsomal aromatase activity and the decrease was concentration-dependent (Table 19). The shapes of the percent of control vs dicofol concentration curves were sigmoidal (Figure 7). 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 2 to 3 percent at 10^{-3} M. It is important to note that the curves for In Vitro did not result in achieving activity that was 0 percent of control at the highest concentration tested, i.e. the percent of control value at 10^{-4} M was 38 percent. In contrast, at a dicofol concentration of approximately 10^{-7} 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^{-7} M were 2.65 ± 0.32 and 93.32 ± 1.66 percent, respectively (Table 20).

Assay precision values (% CV) at concentrations of 10^{-5} M or lower were equal to or less than 10 percent for all labs, whereas for concentrations higher than 10^{-5} M the precision varied widely, i.e. 1 to 30 percent (Table 19). The overall task assay precision ranged from 4 to 13 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 21 and 33 percent, respectively (Table 20).

Table 19. 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	2.32	0.12	0.07	5.09
	-3.30	2	6.25	0.96	0.68	15.37
	-3.60	2	12.42	0.15	0.10	1.19
	-4.00	3	25.69	3.02	1.75	11.77
	-5.00	3	76.12	3.24	1.87	4.25
	-6.00	3	96.78	5.83	3.37	6.02
	-7.00	3	95.64	4.90	2.83	5.12
	-8.00	3	101.80	6.53	3.77	6.41
	-9.00	1	98.19	NC[a]	NC	NC
	-10.00	1	93.21	NC	NC	NC
Battelle	-3.00	3	2.35	0.44	0.25	18.76
	-4.00	3	16.54	0.72	0.42	4.37
	-4.30	2	22.95	NC	NC	NC
	-5.00	3	61.72	6.09	3.51	9.86
	-6.00	3	92.58	9.20	5.31	9.94
	-7.00	3	91.02	4.53	2.62	4.98
	-8.00	3	94.43	3.14	1.81	3.32
	-9.00	3	95.84	5.04	2.91	5.26
	-10.00	1	93.55	NC	NC	NC
In Vitro	-4.00	3	38.10	8.34	4.81	21.9
	-4.48	3	47.10	14.21	8.21	30.2
	-5.00	3	56.91	5.31	3.06	9.3
	-6.00	3	87.98	2.24	1.29	2.5
	-7.00	3	89.97	3.39	1.96	3.8
	-8.00	3	85.46	6.76	3.90	7.9
	-9.00	3	89.45	1.82	1.05	2.0
	-10.00	3	91.58	3.53	2.04	3.9
WIL	-3.00	3	3.29	0.64	0.37	19.48
	-3.52	2	12.10	0.36	0.26	3.00
	-4.00	3	27.92	3.01	1.74	10.77
	-4.52	2	59.51	3.26	2.31	5.48
	-5.00	3	69.51	2.60	1.50	3.74
	-5.52	2	86.35	1.43	1.01	1.65
	-6.00	3	94.72	2.00	1.16	2.11
	-7.00	1	96.65	NC	NC	NC
	-8.00	3	101.38	2.37	1.37	2.34
	-9.00	1	103.12	NC	NC	NC
	-10.00	1	102.13	NC	NC	NC

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

Table 20. 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	2.65	0.55	0.32	20.8
-3.30	1	6.25	NC[b]	NC	NC
-3.52	1	12.10	NC	NC	NC
-3.60	1	12.42	NC	NC	NC
-4.00	4	27.06	8.85	4.43	32.7
-4.30	1	22.95	NC	NC	NC
-4.48	1	47.10	NC	NC	NC
-4.52	1	59.51	NC	NC	NC
-5.00	4	66.07	8.48	4.24	12.8
-5.52	1	86.35	NC	NC	NC
-6.00	4	93.02	3.77	1.88	4.1
-7.00	4	93.32	3.32	1.66	3.6
-8.00	4	95.77	7.66	3.83	8.0
-9.00	4	96.65	5.68	2.84	5.9
-10.00	4	95.12	4.75	2.38	5.0

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.

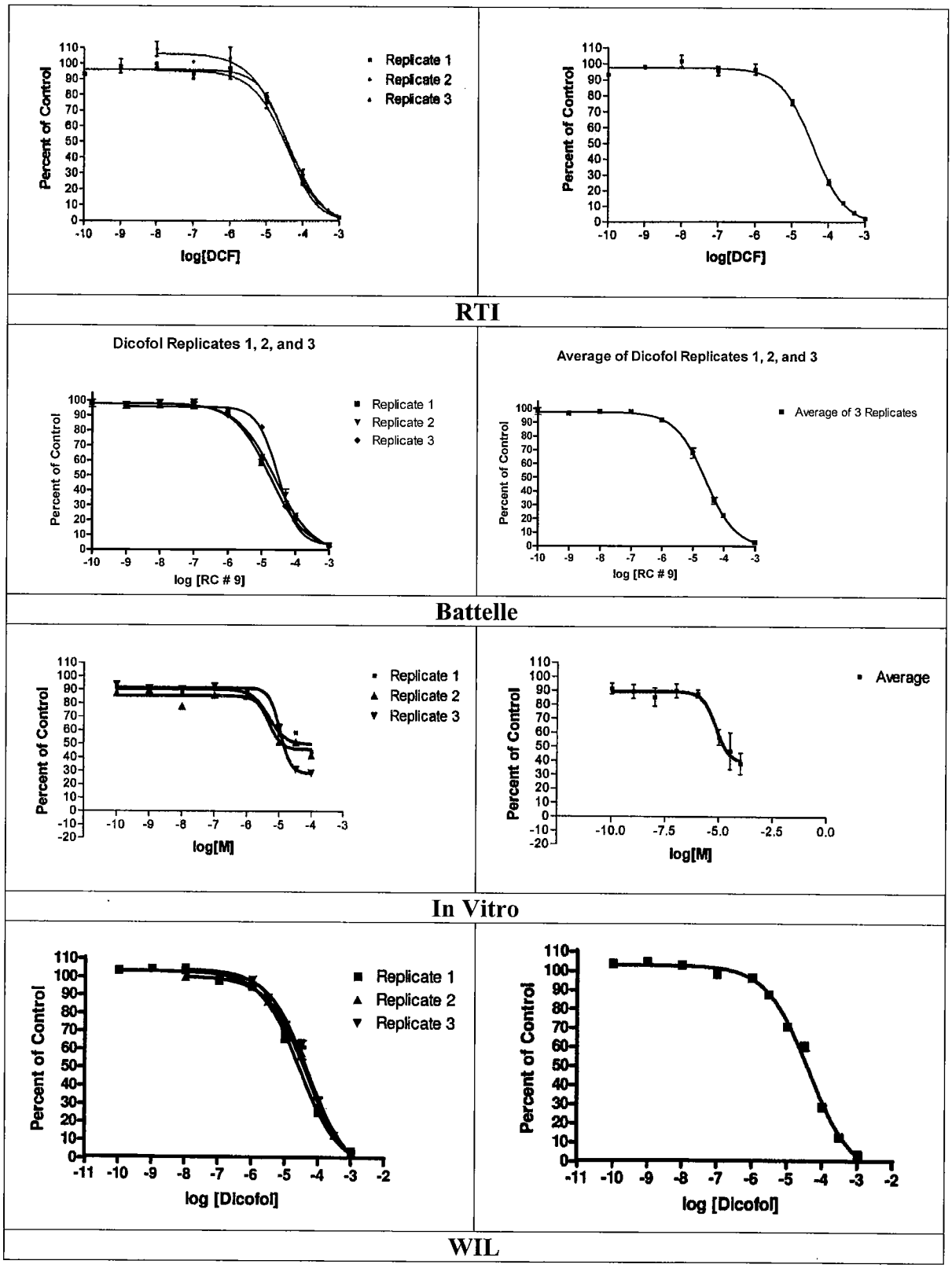

Figure 7. **Individual Replicate and Average Dicofol Inhibition Response Curves (percent of control) by Laboratory**

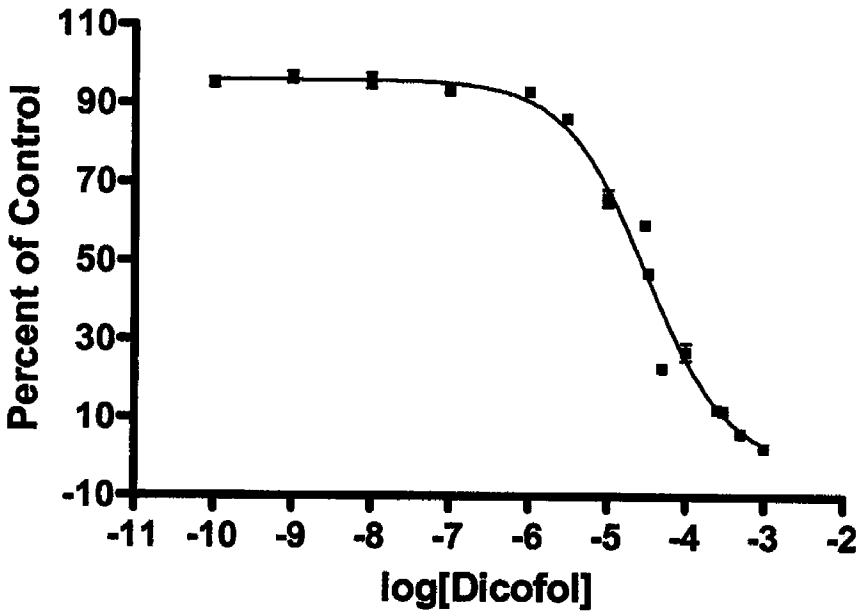

Figure 8. Overall Task Dicofol Inhibition Response Curve (percent of control)

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 21 and by overall task in Table 22. 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 from three of four laboratories did not make any modifications of the dibenz[a,h]anthracene concentrations selected for testing in replicates 2 and 3 (Table 21). In Vitro modified the replicate 1 concentrations tested by deleting the 3.3×10^{-6} M concentration and adding a high-dilution concentration, i.e. 10^{-4} M. Based on the replicate 1 results and the absence of any inhibition, the other laboratories apparently didn't believe it was necessary to modify the replicate 1 concentrations.

For all four laboratories, increasing concentrations of dibenz[a,h]anthracene did not decrease the activity of the recombinant microsomal aromatase activity (Table 21). The shapes of the percent of control vs dibenz[a,h]anthracene concentration curves approached a horizontal line (Figure 9). At a dibenz[a,h]anthracene concentration of 10^{-4} M 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 95.98 ± 3.83 and 96.49 ± 4.24 percent, respectively (Table 22).

All labs had assay precision values (% CV) that were less than or equal to 9 percent for those concentrations used in at least three replicates (Table 21). The overall task assay precision ranged from 5 to 9 percent for those concentrations that were tested by 3 or more labs (Table 22).

Table 21. 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	93.49	4.34	2.51	4.64
	-5.00	3	96.24	4.60	2.66	4.78
	-6.00	3	94.46	3.78	2.18	4.00
	-6.30	3	94.48	2.45	1.42	2.60
	-7.00	3	95.72	5.10	2.95	5.33
	-8.00	3	94.76	7.00	4.04	7.39
	-9.00	3	96.30	2.13	1.23	2.22
	-10.00	3	94.64	0.76	0.44	0.80
Battelle	-4.00	3	106.44	4.51	2.60	4.24
	-5.00	3	103.50	7.26	4.19	7.01
	-6.00	3	106.03	8.37	4.83	7.89
	-6.60	3	102.86	5.10	2.94	4.95
	-7.00	3	101.57	6.97	4.02	6.86
	-8.00	3	101.88	4.48	2.59	4.40
	-9.00	3	102.11	5.70	3.29	5.58
	-10.00	3	102.58	3.39	1.95	3.30
In Vitro	-4.00	2	88.24	NC[a]	NC	NC
	-4.48	3	89.56	1.52	0.88	1.7
	-5.00	3	91.20	1.24	0.72	1.4
	-5.48	1	94.50	NC	NC	NC
	-6.00	3	89.80	1.26	0.73	1.4
	-7.00	3	90.80	4.67	2.69	5.1
	-8.00	3	89.93	3.51	2.03	3.9
	-9.00	3	84.35	6.17	3.56	7.3
	-10.00	3	85.26	3.50	2.02	4.1
WIL	-4.00	3	95.75	2.09	1.21	2.18
	-5.00	3	103.31	1.06	0.61	1.03
	-5.30	3	104.28	1.44	0.83	1.38
	-6.00	3	104.26	1.64	0.95	1.57
	-7.00	3	95.06	8.21	4.74	8.63
	-8.00	3	105.61	1.47	0.85	1.39
	-9.00	3	103.93	0.85	0.49	0.82
	-10.00	3	103.47	2.35	1.36	2.27

a. NC – Value Not Calculated when n $\leq$2.

Table 22. 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	4	95.98	7.65	3.83	8.0
-4.48	1	89.56	NC[b]	NC	NC
-5.00	4	98.56	5.96	2.98	6.0
-5.30	1	104.28	NC	NC	NC
-5.48	1	94.50	NC	NC	NC
-6.00	4	98.64	7.78	3.89	7.9
-6.30	1	94.48	NC	NC	NC
-6.60	1	102.86	NC	NC	NC
-7.00	4	95.79	4.43	2.21	4.6
-8.00	4	98.05	7.04	3.52	7.2
-9.00	4	96.67	8.84	4.42	9.1
-10.00	4	96.49	8.47	4.24	8.8

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.

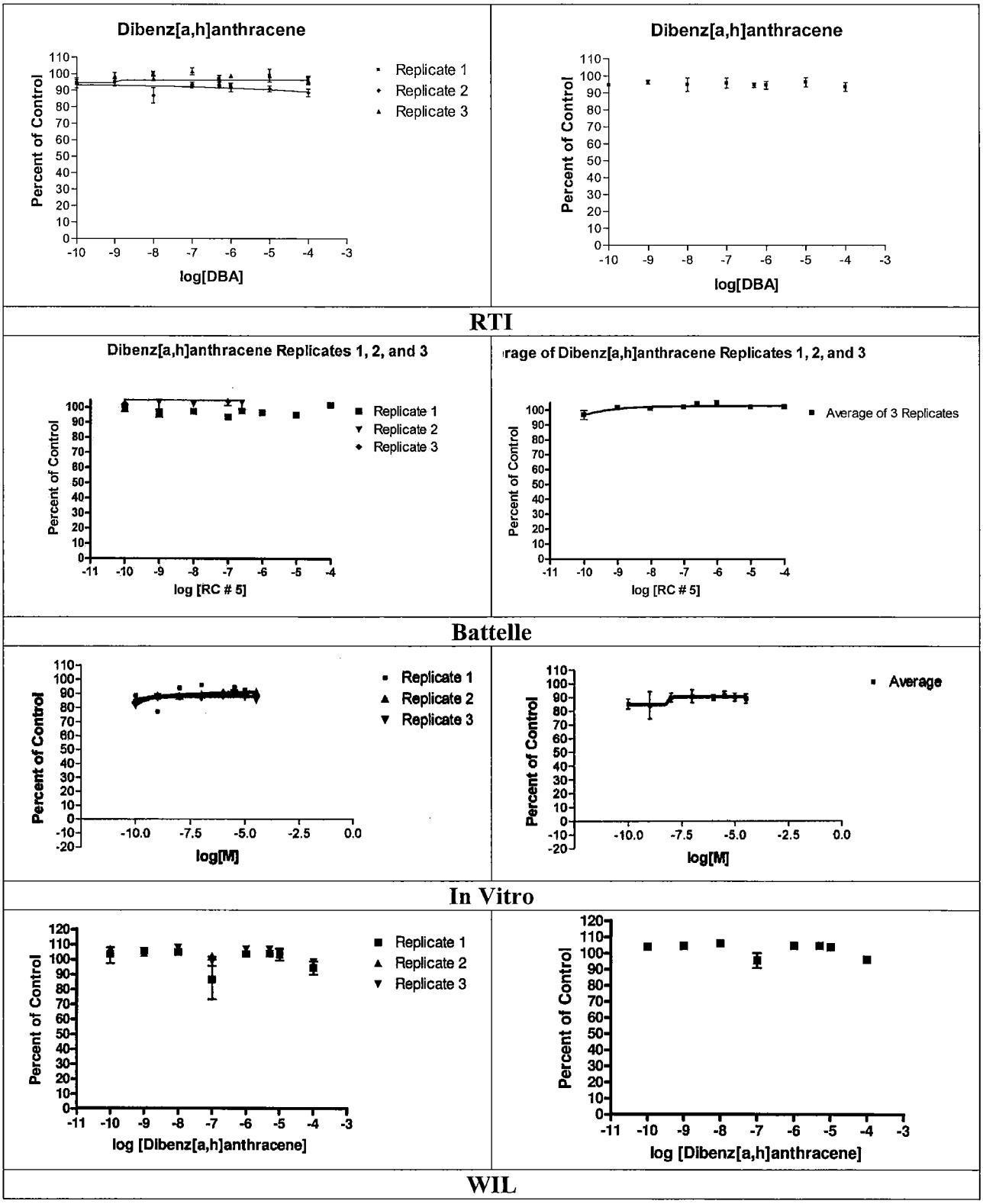

Figure 9. **Individual Replicate and Average Dibenz[a,h]anthracene Inhibition Response Curves (percent of control) by Laboratory**

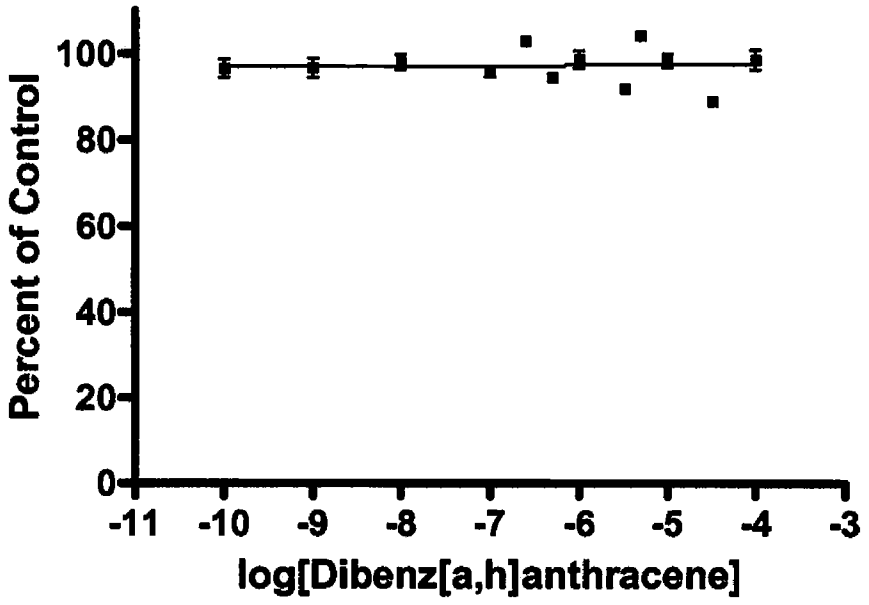

Figure 10. Overall Task Dibenz[a,h]anthracene Inhibition Response Curve (percent of control)

3.8.6 Econazole

The effect of increasing concentrations of econazole on aromatase activity is reported by laboratory in Table 23 and by overall task in Table 24. 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 three of four laboratories modified the econazole concentrations selected for testing in replicates 2 and 3 (Table 23). 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^{-5} M that were tested in replicate 1. RTI used the same concentrations for all three replicates and tested between the range of 10^{-6} M and 10^{-10} M, presumably because these were the concentrations found to be optimal for WA 4-16, Task 7. (RTI replaced the first replicate because it didn't show concentration dependence.)

For all laboratories, increasing concentrations of econazole decreased the activity of the recombinant aromatase activity and the decrease was concentration-dependent (Table 23). The shapes of the percent of control vs. econazole concentration 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. Less than or equal to 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 all four labs. The overall task mean ± SEM percent of control values at 10^{-7} and 10^{-10} M were 1.76 ± 0.17 and 94.21 ± 2.33 percent, respectively (Table 24).

The precision values (% CV) were less than or equal to approximately 20 percent at $\leq 10^{-7}$ M (for those concentrations with at least three replicates), but at a concentration of 10^{-6} M or higher the % CV values ranged from 11 to 998 (Table 23). The overall task assay precision ranged from 5 to 19 percent for those concentrations that were tested by 3 or more labs, except at concentrations $\geq 10^{-5}$ M where the % CV values were very large (Table 24). The large variability at the highest econazole 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.

Table 23. 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	-6.00	3	0.26	0.04	0.02	13.59
	-7.00	3	2.12	0.13	0.08	6.34
	-8.00	3	17.61	1.30	0.75	7.38
	-8.30	3	31.41	2.49	1.44	7.94
	-9.00	3	73.59	2.13	1.23	2.89
	-9.30	3	87.85	4.31	2.49	4.91
	-9.60	3	93.78	1.98	1.14	2.11
	-10.00	3	97.77	2.80	1.62	2.86
Battelle	-3.00	1	0.14	NC[a]	NC	NC
	-4.00	3	0.07	0.03	0.02	45.33
	-5.00	3	-0.01	0.08	0.05	-998.02
	-6.00	3	0.20	0.07	0.04	34.69
	-7.00	3	1.79	0.26	0.15	14.79
	-8.00	3	15.43	2.42	1.40	15.69
	-8.60	2	49.15	NC	NC	NC
	-9.00	3	68.20	10.73	6.19	15.73
	-10.00	3	90.12	8.14	4.70	9.03
In Vitro	-4.00	1	-0.01	NC	NC	NC
	-4.48	1	-0.06	NC	NC	NC
	-5.00	3	0.20	0.35	0.20	178.0
	-6.00	3	0.25	0.17	0.10	69.0
	-7.00	3	1.30	0.18	0.11	14.2
	-8.00	3	12.92	2.52	1.46	19.5
	-8.48	2	32.94	NC	NC	NC
	-9.00	3	58.72	3.43	1.98	5.8
	-9.48	2	84.05	NC	NC	NC
	-10.00	3	90.26	6.85	3.95	7.6
WIL	-3.00	1	0.01	NC	NC	NC
	-4.00	1	0.00	NC	NC	NC
	-5.00	3	0.04	0.03	0.02	89.54
	-6.00	3	0.21	0.02	0.01	10.92
	-7.00	3	1.83	0.11	0.06	5.96
	-8.00	3	16.17	0.60	0.35	3.73
	-8.52	2	43.76	3.26	2.30	7.44
	-9.00	3	74.78	2.74	1.58	3.67
	-9.52	2	94.03	2.72	1.92	2.89
	-10.00	3	98.68	1.72	0.99	1.74

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

Table 24. 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	2	0.08	NC[b]	NC	NC
-4.00	3	0.02	0.04	0.03	217.9
-4.48	1	-0.06	NC	NC	NC
-5.00	3	0.08	0.11	0.06	143.1
-6.00	4	0.23	0.03	0.01	12.8
-7.00	4	1.76	0.34	0.17	19.3
-8.00	4	15.53	1.96	1.96	12.6
-8.30	1	31.41	NC	NC	NC
-8.48	1	32.94	NC	NC	NC
-8.52	1	43.76	NC	NC	NC
-8.60	1	49.15	NC	NC	NC
-9.00	4	68.82	7.32	3.66	10.6
-9.30	1	87.85	NC	NC	NC
-9.48	1	84.05	NC	NC	NC
-9.52	1	94.03	NC	NC	NC
-9.60	1	93.78	NC	NC	NC
-10.00	4	94.21	4.65	2.33	4.9

a. Number of labs that conducted the assay at the cited reference chemical concentration. The replicate number varied because three out of the four laboratories 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. RTI used the same concentrations for all three replicates.

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

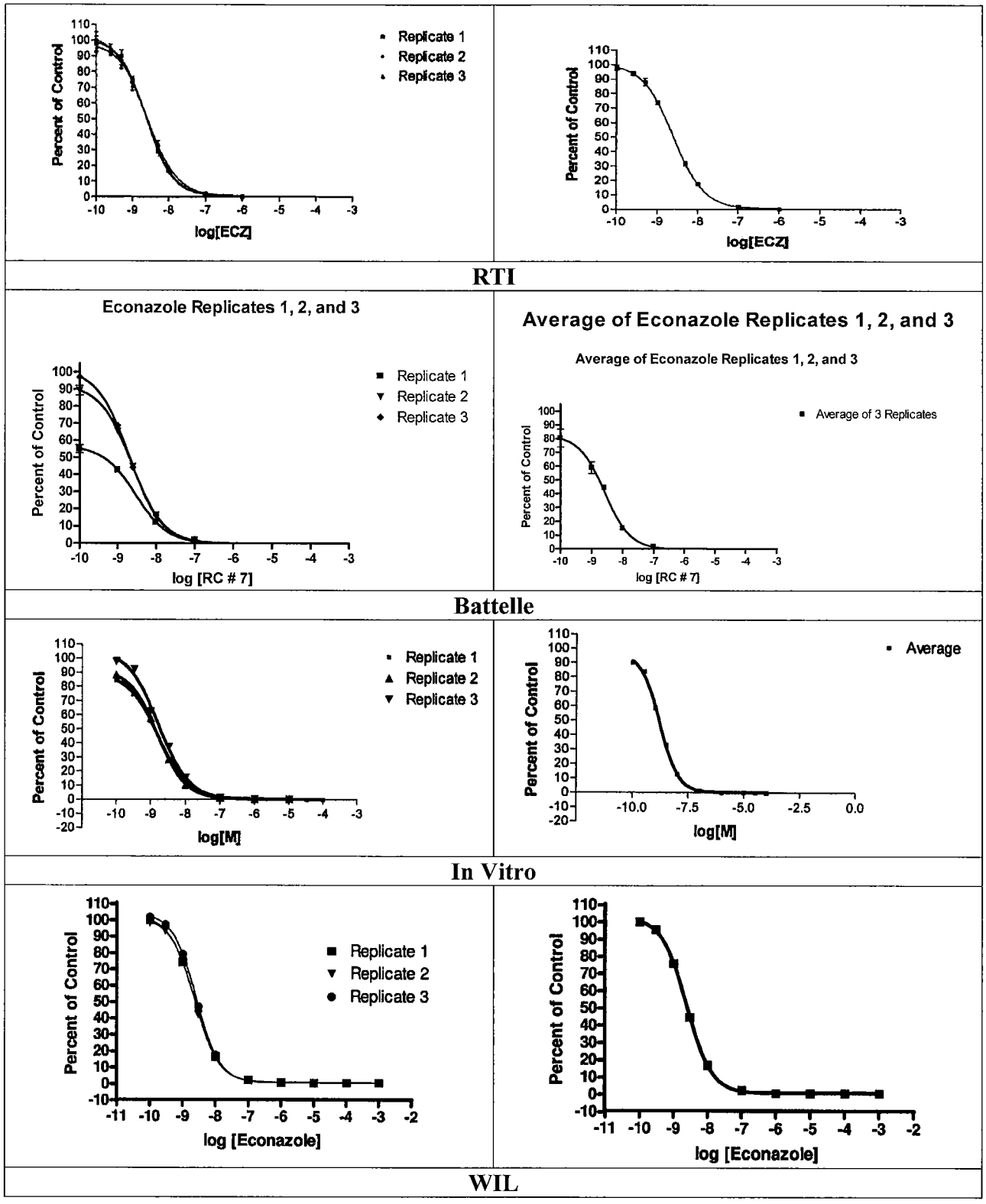

Figure 11. **Individual Replicate and Average Econazole Inhibition Response Curves (percent of control) by Laboratory**

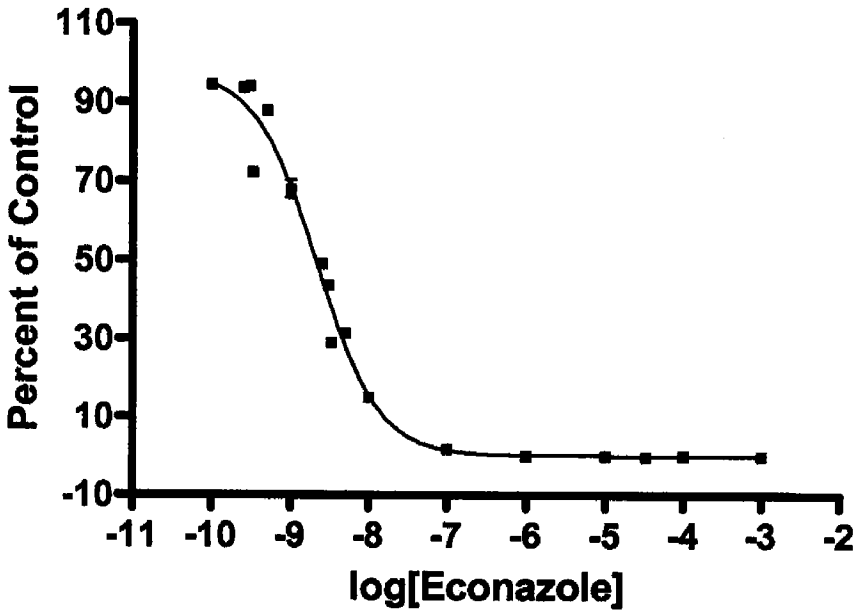

Figure 12. Overall Task Econazole Inhibition Response Curve (percent of control)

3.8.7 Fenarimol

The effect of increasing concentrations of fenarimol on aromatase activity is reported by laboratory in Table 25 and by overall task in Table 26. 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 25). 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 3.3×10^{-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. Also, In Vitro used the same concentrations as was used for WA 4-16, Task 7, which explains why no adjustments were made after the first replicate.

For all four laboratories, increasing concentrations of fenarimol decreased the activity of the recombinant microsomal aromatase activity and the decrease was concentration-dependent (Table 25). The shapes of the percent of control vs fenarimol concentration curves were sigmoidal (Figure 13). At a fenarimol concentration of 10^{-3} M, aromatase inhibition was almost complete; the laboratory percent of control values for RTI and Battelle were approximately 1 percent. For WIL, two of three replicates had percent of control values of approximately 1 percent, but the low enzyme activity measured for the second replicate resulted in a higher percent of control value at 10^{-3} M, i.e. 23 percent, which resulted in an average percent of control at this concentration of 8 percent. For In Vitro at its highest concentration tested (3.3×10^{-5} M), the percent of control value was 20 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. The overall task mean ± SEM percent of control values at 10^{-3} and 10^{-7} M were 3.52 ± 2.32 and 95.67 ± 1.90 percent, respectively (Table 26).

Table 25. 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	0.95	0.08	0.05	8.38
	-4.00	3	6.29	0.12	0.07	1.96
	-4.60	2	19.64	0.48	0.34	2.44
	-5.00	3	35.58	1.94	1.12	5.44
	-5.30	2	49.42	0.70	0.49	1.41
	-6.00	3	80.47	2.62	1.51	3.26
	-7.00	3	90.28	1.74	1.00	1.93
	-8.00	3	90.15	0.47	0.27	0.52
	-9.00	1	91.26	NC[a]	NC	NC
	-10.00	1	89.84	NC	NC	NC
Battelle	-3.00	3	1.45	0.12	0.07	8.17
	-4.00	3	5.66	0.10	0.06	1.84
	-5.00	3	36.03	1.38	0.80	3.84
	-5.60	2	68.00	NC	NC	NC
	-6.00	3	81.88	0.73	0.42	0.89
	-7.00	3	97.75	2.63	1.52	2.69
	-8.00	3	100.56	0.30	0.17	0.30
	-9.00	3	97.49	0.82	0.48	0.84
	-10.00	1	99.01	NC	NC	NC
In Vitro	-4.48	3	20.45	11.70	6.75	57.2
	-5.00	3	32.23	4.13	2.38	12.8
	-5.48	3	53.86	11.42	6.59	21.2
	-6.00	3	79.07	9.25	5.34	11.7
	-7.00	3	95.86	11.40	6.58	11.9
	-8.00	3	94.10	14.22	8.21	15.1
	-9.00	3	95.51	13.20	7.62	13.8
	-10.00	3	94.54	10.39	6.00	11.0
WIL	-3.00	3	8.16	12.69	7.33	155.58
	-4.00	3	27.40	37.29	21.53	136.13
	-4.52	2	55.70	49.79	35.21	89.40
	-5.00	3	59.17	35.57	20.54	60.12
	-5.52	2	81.53	20.60	14.57	25.27
	-6.00	3	90.00	5.81	3.35	6.45
	-7.00	3	98.80	3.41	1.97	3.45
	-8.00	1	103.78	NC	NC	NC
	-9.00	3	96.36	7.57	4.37	7.85
	-10.00	1	98.95	NC	NC	NC

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

Table 26. 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	3.52	4.03	2.32	114.4
-4.00	3	13.12	12.37	7.14	94.3
-4.48	1	20.45	NC[b]	NC	NC
-4.52	1	55.70	NC	NC	NC
-4.60	1	19.64	NC	NC	NC
-5.00	4	40.75	12.39	6.20	30.4
-5.30	1	49.42	NC	NC	NC
-5.48	1	53.86	NC	NC	NC
-5.52	1	81.53	NC	NC	NC
-5.60	1	68.00	NC	NC	NC
-6.00	4	82.86	4.90	2.45	5.9
-7.00	4	95.67	3.80	1.90	4.0
-8.00	4	97.15	6.16	3.08	6.3
-9.00	4	95.16	2.72	1.36	2.9
-10.00	4	95.59	4.36	2.18	4.6

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.

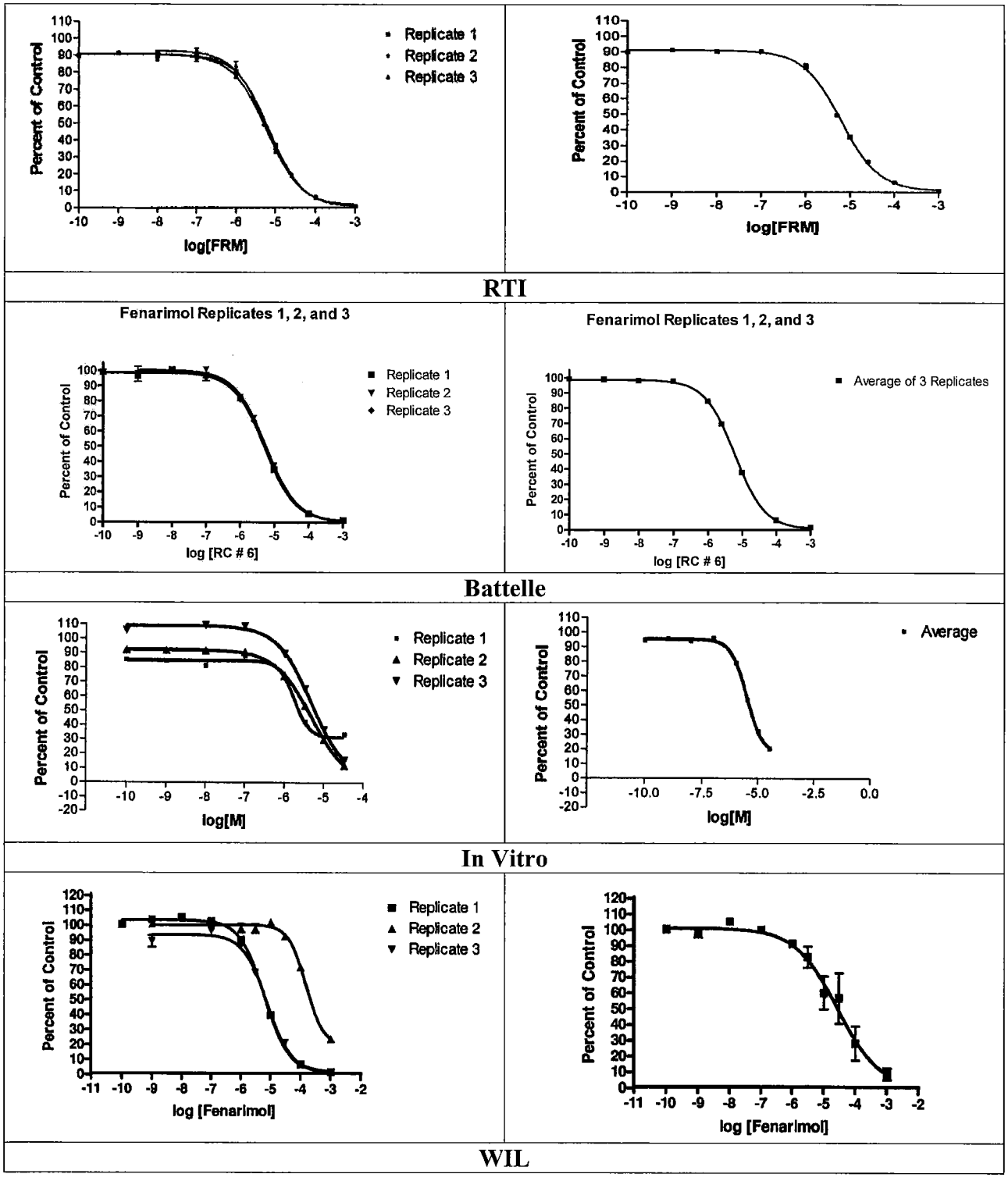

Figure 13. **Individual Replicate and Average Fenarimol Inhibition Response Curves (percent of control) by Laboratory**

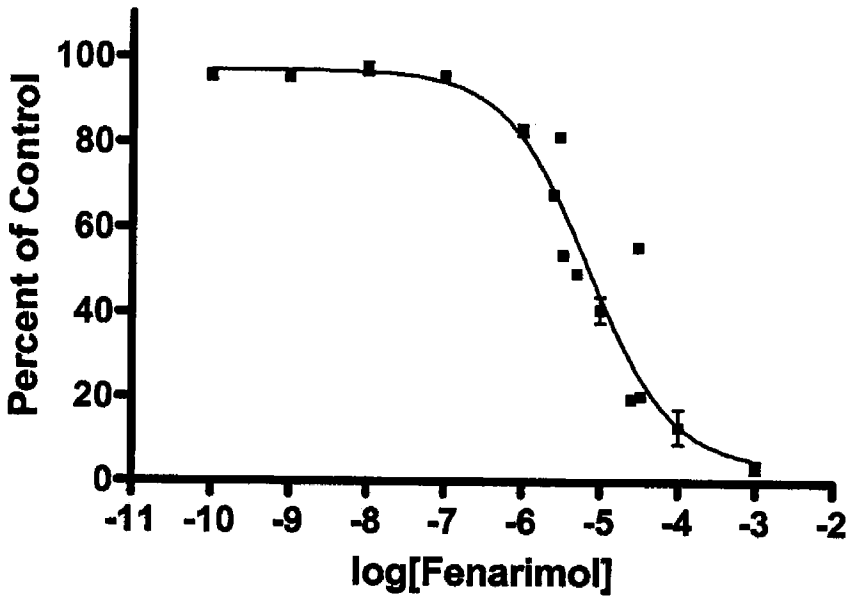

Figure 14. Overall Task Fenarimol Inhibition Response Curve (percent of control)

RTI and Battelle had assay precision values (% CV) that were less than or equal to 8 percent for those concentrations used in at least three replicates. For In Vitro and WIL, % CV values were less than or equal to 15 percent at concentrations of 10^{-6} M or less, whereas at higher concentrations the precision varied widely, i.e. 13 to 156 percent (Table 26). The overall task assay precision ranged from 3 to 30 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 114 and 94 percent, respectively (Table 26).

3.8.8 Ketoconazole

The effect of increasing concentrations of ketoconazole on aromatase activity is reported by laboratory in Table 27 and by overall task in Table 28. 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 27). In general, the modifications involved including additional mid-concentrations between 10^{-4} and 10^{-7} M, which replaced the concentrations tested in replicate 1 from 10^{-8} to 10^{-12} M, except for In Vitro that replaced 3 x 10^{-5} M. 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 recombinant microsomal aromatase activity and the decrease was concentration-dependent (Table 27). The shapes of the percent of control vs ketoconazole concentration 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 4 to 8 percent. 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 92 percent for all labs. The overall task mean ± SEM percent of control values at 10^{-4} and 10^{-7} M were 6.01 ± 1.01 and 98.30 ± 2.10 percent, respectively (Table 28).

RTI and Battelle had assay precision values (% CV) that were less than or equal to 12 percent for those concentrations used in at least three replicates. In Vitro and WIL had % CV values that were less than 19 percent at concentrations of 10^{-5} M or less but at higher concentrations the precision ranged from 25 to 32 percent (Table 27). The overall task assay precision ranged from 1 to 8 percent for those concentrations that were tested by 3 or more labs, except at concentrations of 10^{-4} and 10^{-5} M where the % CV values were 18 and 34 percent, respectively (Table 28).

Table 27. 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	8.13	1.01	0.59	12.47
	-4.30	2	14.60	0.39	0.28	2.67
	-4.60	2	27.38	0.84	0.60	3.08
	-5.00	3	45.54	2.02	1.17	4.44
	-5.30	2	60.82	2.88	2.04	4.74
	-6.00	3	86.61	1.48	0.85	1.70
	-7.00	3	92.04	3.73	2.15	4.05
	-8.00	3	96.45	1.86	1.07	1.93
	-9.00	1	99.02	NC[a]	NC	NC
	-10.00	1	94.84	NC	NC	NC
	-11.00	1	95.35	NC	NC	NC
Battelle	-4.00	2	7.10	NC	NC	NC
	-4.60	2	25.44	NC	NC	NC
	-5.00	3	44.05	4.77	2.75	10.82
	-5.30	2	63.44	NC	NC	NC
	-5.60	2	79.55	NC	NC	NC
	-6.00	3	87.96	7.29	4.21	8.29
	-6.60	2	101.75	NC	NC	NC
	-7.00	3	99.79	7.34	4.24	7.36
	-8.00	1	91.26	NC	NC	NC
	-9.00	1	95.64	NC	NC	NC
	-10.00	1	94.72	NC	NC	NC
	-11.00	1	94.49	NC	NC	NC
	-12.00[b]	1	91.39	NC	NC	NC
In Vitro	-4.00	3	3.59	0.91	0.52	25.4
	-4.52	1	17.88	NC	NC	NC
	-5.00	3	29.48	5.27	3.04	17.9
	-5.48	2	37.41	NC	NC	NC
	-6.00	3	87.88	9.76	5.64	11.1
	-7.00	3	100.93	13.18	7.61	13.1
	-8.00	3	94.99	11.61	6.70	12.2
	-9.00	3	105.05	9.20	5.31	8.8
	-10.00	3	102.94	10.59	6.12	10.3
WIL	-4.00	3	5.20	1.30	0.75	25.04
	-4.30	2	11.63	3.75	2.65	32.26
	-5.00	3	40.30	6.22	3.59	15.44
	-5.30	2	54.53	10.18	7.20	18.67
	-6.00	3	86.48	4.96	2.87	5.74
	-7.00	3	100.44	1.19	0.69	1.19
	-8.00	3	102.87	0.57	0.33	0.55
	-9.00	1	104.52	NC	NC	NC
	-10.00	3	101.52	2.55	1.47	2.51
	-11.00	1	103.08	NC	NC	NC

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

Table 28. 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	6.01	2.02	1.01	33.6
-4.30	2	13.12	NC[b]	NC	NC
-4.52	1	17.88	NC	NC	NC
-4.60	2	26.41	NC	NC	NC
-5.00	4	39.84	7.25	3.63	18.2
-5.30	3	59.60	4.58	2.64	7.7
-5.48	1	37.41	NC	NC	NC
-5.60	1	79.55	NC	NC	NC
-6.00	4	87.23	0.80	0.40	0.9
-6.60	1	101.75	NC	NC	NC
-7.00	4	98.30	4.20	2.10	4.3
-8.00	4	96.39	4.84	2.42	5.0
-9.00	4	101.06	4.53	2.26	4.5
-10.00	4	98.51	4.34	2.17	4.4
-11.00	3	97.64	4.73	2.73	4.8
-12.00	1	91.39	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 $n \leq 2$, then the sd, SEM, and %CV were not calculated.

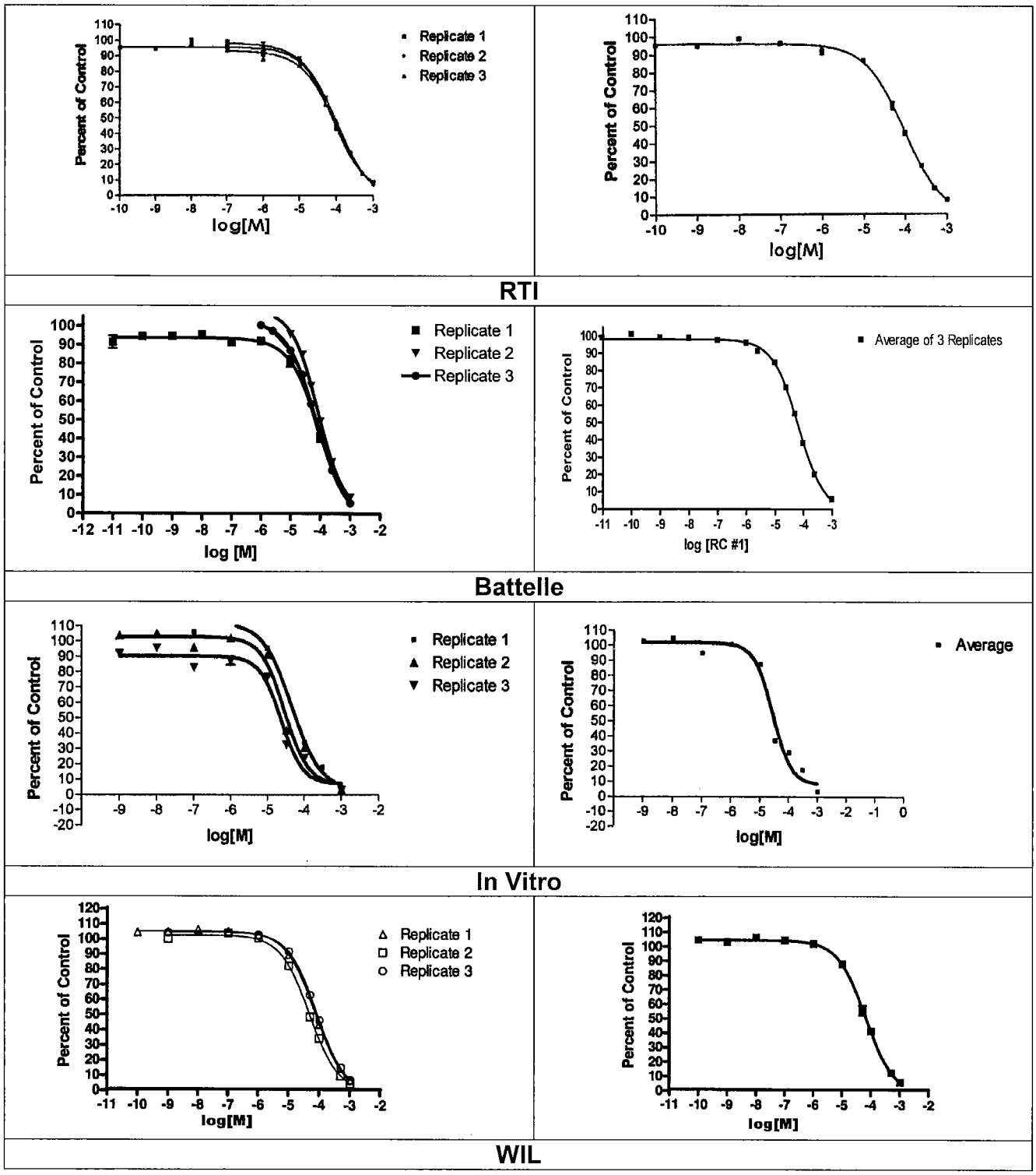

Figure 15. **Individual Replicate and Average Ketoconazole Inhibition Response Curves (percent of control) by Laboratory**
[Note: x-axis values are 10-fold high, e.g., 10^{-4} M should actually be 10^{-5} M.]

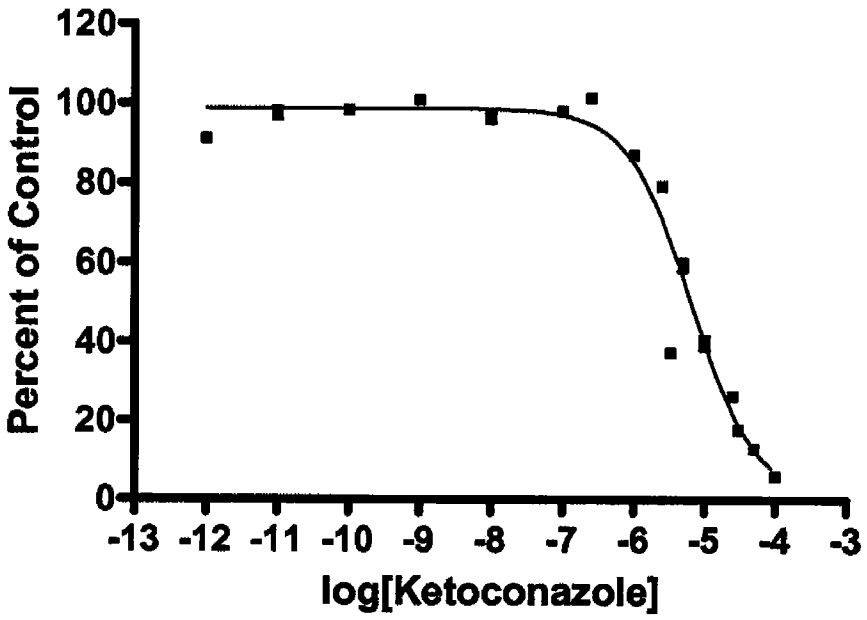

Figure 16. Overall Task Ketoconazole Inhibition Response Curve (percent of control)

3.8.9 4-Nonylphenol

The effect of increasing concentrations of 4-nonylphenol on aromatase activity is reported by laboratory in Table 29 and by overall task in Table 30. In addition, the results are graphically presented by laboratory (individual replicates and average) and overall task in Figures 17 and 18.

Table 29. 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	0.10	0.01	0.01	13.72
	-4.00	3	3.57	1.58	0.91	44.22
	-4.30	2	20.67	0.90	0.64	4.37
	-4.60	2	40.62	0.12	0.09	0.30
	-5.00	3	85.47	1.61	0.93	1.89
	-5.30	2	101.41	6.94	4.91	6.85
	-6.00	3	101.14	3.60	2.08	3.55
	-7.00	3	100.53	3.00	1.73	2.98
	-8.00	1	93.65	NC	NC	NC
	-9.00	1	98.57	NC	NC	NC
	-10.00	1	102.63	NC	NC	NC
Battelle	-3.00	3	0.78	0.25	0.15	32.10
	-4.00	3	4.04	1.20	0.69	29.58
	-4.30	2	10.84	NC	NC	NC
	-4.60	2	35.75	NC	NC	NC
	-5.00	3	78.70	7.40	4.27	9.40
	-6.00	3	93.93	12.03	6.94	12.80
	-7.00	3	95.99	6.62	3.82	6.90
	-8.00	3	101.60	7.65	4.42	7.53
	-9.00	1	106.82	NC	NC	NC
	-10.00	1	105.53	NC	NC	NC
In Vitro	-4.00	3	4.38	2.34	1.35	53.3
	-4.48	3	27.11	8.09	4.67	29.8
	-5.00	3	60.67	5.15	2.97	8.5
	-6.00	3	95.01	0.81	0.47	0.9
	-7.00	3	95.27	1.34	0.77	1.4
	-8.00	3	91.21	3.63	2.10	4.0
	-9.00	3	91.17	0.87	0.50	1.0
	-10.00	3	90.00	5.55	3.20	6.2
WIL	-3.00	3	0.13	0.04	0.02	31.39
	-4.00	3	1.79	0.21	0.12	11.89
	-4.30	2	12.15	2.56	1.81	21.09
	-5.00	3	67.82	4.46	2.57	6.57
	-6.00	3	98.02	2.77	1.60	2.83
	-7.00	3	99.68	5.67	3.27	5.68
	-8.00	3	99.04	5.13	2.96	5.18
	-9.00	3	97.47	2.56	1.48	2.63
	-10.00	1	97.65	NC	NC	NC

a. NC – value **N**ot **C**alculated when n $\leq$2.

Table 30. 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	0.34	0.38	0.22	114.1
-4.00	4	3.45	1.15	0.58	33.4
-4.30	3	14.55	5.34	3.08	36.7
-4.48	1	27.11	NC[b]	NC	NC
-4.60	2	38.19	NC	NC	NC
-5.00	4	73.17	11.06	5.53	15.1
-5.30	1	101.41	NC	NC	NC
-6.00	4	97.03	3.24	1.62	3.3
-7.00	4	97.87	2.62	1.31	2.7
-8.00	4	96.38	4.78	2.39	5.0
-9.00	4	98.51	6.43	3.21	6.5
-10.00	4	98.95	6.80	3.40	6.9

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.

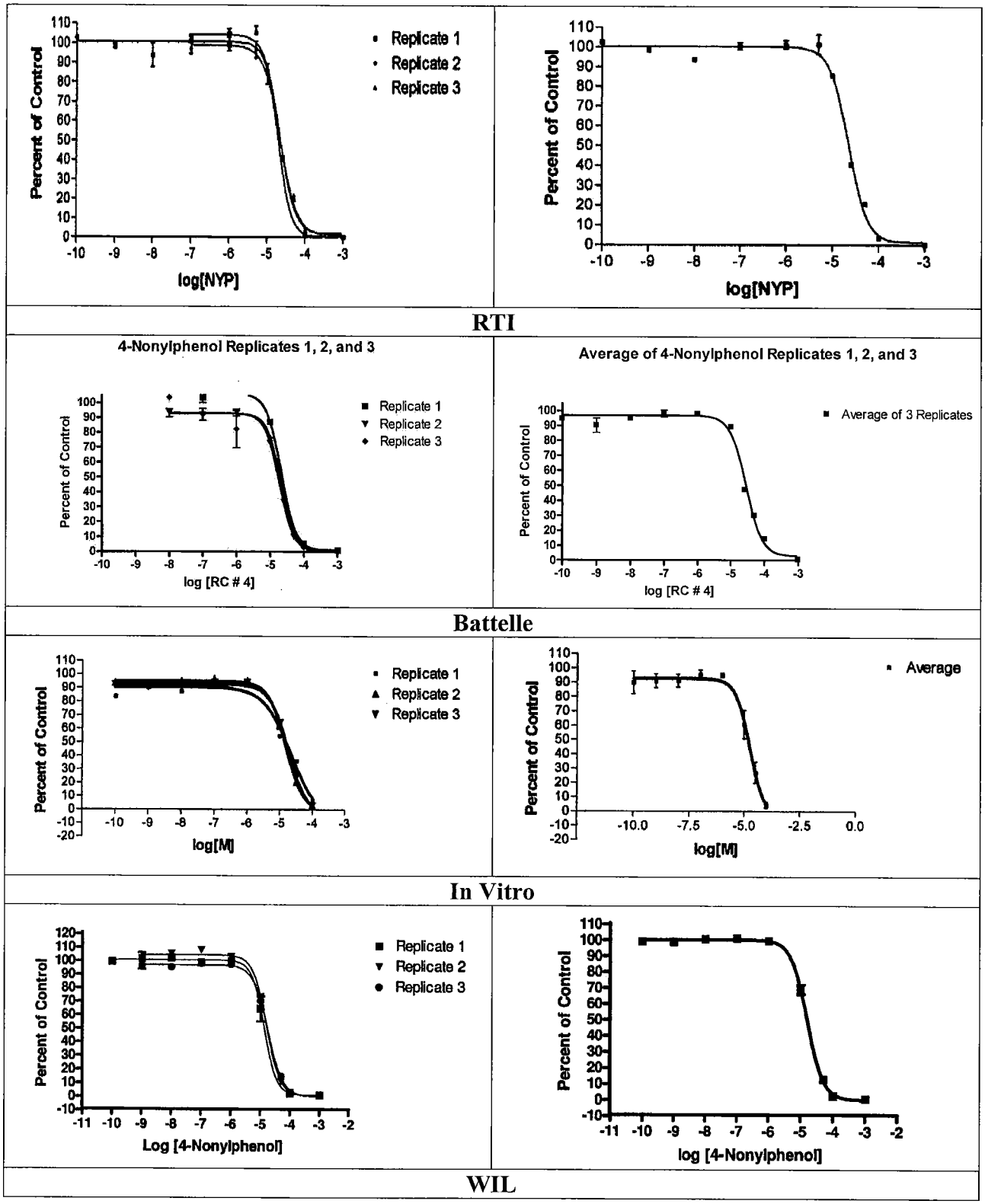

Figure 17. **Individual Replicate and Average 4-Nonylphenol Inhibition Response Curves (percent of control) by Laboratory**

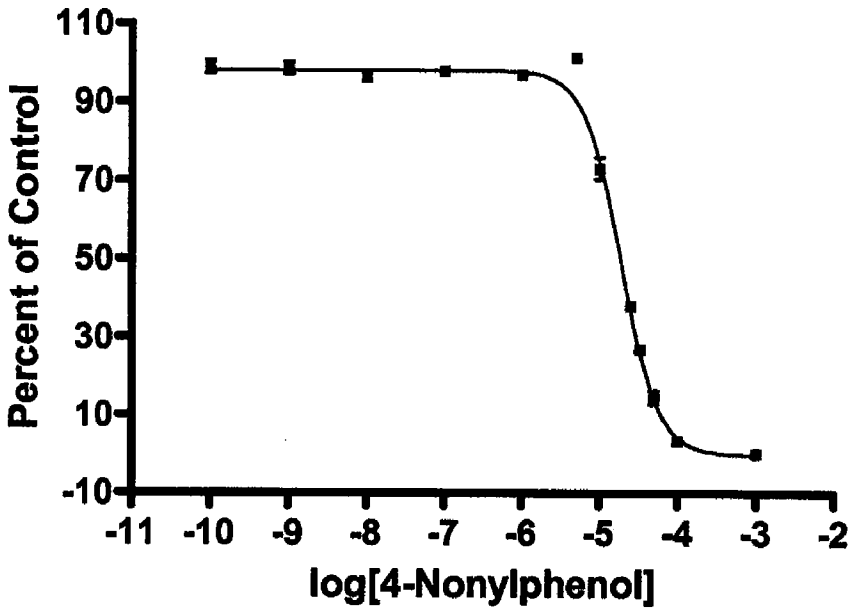

Figure 18. Overall Task 4-Nonylphenol Inhibition Response Curve (percent of control)

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 29). 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. The concentration(s) that were replaced by these modifications ranged from 10^{-8} to 10^{-10} M.

For all four laboratories, increasing concentrations of 4-nonylphenol decreased the activity of the recombinant aromatase activity and the decrease was concentration-dependent (Table 29). The shapes of the percent of control vs 4-nonylphenol concentration 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 4 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 $\geq$ 94 percent. The overall task mean $\pm$ SEM percent of control values at 10^{-3} and 10^{-6} M were 0.34 $\pm$ 0.22 and 97.03 $\pm$ 1.62 percent, respectively (Table 30).

All labs have assay precision values (% CV) that were less than 13 percent for concentrations less than or equal to 10^{-5} M and used in at least three replicates. The precision varied widely at a concentration of 10^{-3} M for three of four labs, i.e. 14 to 32 percent (In Vitro did not test at this concentration, Table 29); and at a concentration of 10^{-4} M for all four labs, i.e. 12 to 53 percent of 10^{-4} M. The overall task assay precision ranged from 3 to 15 percent for those concentrations that were tested by 3 or more labs, except at concentrations of 10^{-3}, 10^{-4}, and 5 x 10^{-5} M where the % CV values were 114, 33, and 37 percent, respectively (Table 30).

3.8.10 Prochloraz

The effect of increasing concentrations of prochloraz on aromatase activity is reported by laboratory in Table 31 and by overall task in Table 32. 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 all four laboratories modified the prochloraz concentrations selected for testing in latter replicates (Table 31). In general, the modifications involved including additional mid-concentrations between 10^{-7} and 10^{-9} M, which replaced the concentrations of 10^{-3} and 10^{-4} M 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 recombinant microsomal aromatase activity and the decrease was concentration-dependent (Table 31). The shapes of the percent of control vs prochloraz concentration 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 due to a low enzyme activity value for replicate 2 had a high percent of control value for this one replicate, i.e. 53 percent, but values of approximately 3 to 4 percent for the other two replicates, thereby resulting in an average percent of control value of 20 percent. 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 $\geq$93 percent for all four labs. The overall task mean $\pm$ SEM percent of control values at 10^{-6} and 10^{-9} M were 7.26 ± 4.27 and 96.33 ± 2.77 percent, respectively (Table 32).

Assay precision values for prochloraz at concentrations $\leq 10^{-6}$ M were less than 22 percent for three of four labs but at higher concentrations ranged from 13 to 134 percent (Table 31). For WIL, the % CV values were generally higher over the concentration range due to the low enzyme activity for replicate 2, i.e. 1 to 156 percent. The overall task assay precision ranged from 5 to 11 percent for concentrations $\leq 10^{-8}$ M that were tested by 3 or more labs, whereas at higher concentrations, the overall task assay precision ranged from 46 to 189 percent (Table 32).

Table 31. 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	-0.05	NC[a]	NC	NC
	-4.00	1	-0.01	NC	NC	NC
	-5.00	3	0.43	0.14	0.08	31.04
	-6.00	3	3.35	0.21	0.12	6.26
	-7.00	3	24.92	1.45	0.83	5.80
	-7.60	2	53.79	1.34	0.95	2.49
	-8.00	3	72.33	1.41	0.81	1.94
	-8.30	2	83.06	2.72	1.92	3.28
	-9.00	3	92.99	1.56	0.90	1.68
	-10.00	3	94.24	2.45	1.42	2.60
Battelle	-4.00	1	0.12	NC	NC	NC
	-5.00	3	0.35	0.04	0.03	12.83
	-6.00	3	3.32	0.48	0.28	14.54
	-7.00	3	23.98	2.62	1.51	10.91
	-7.60	2	57.80	NC	NC	NC
	-8.00	3	73.97	5.12	2.96	6.92
	-8.60	2	92.89	NC	NC	NC
	-9.00	3	93.13	6.71	3.88	7.21
	-10.00	3	97.66	1.12	0.65	1.15
	-11.00[b]	1	100.41	NC	NC	NC
In Vitro	-4.00	3	0.24	0.32	0.18	133.6
	-5.00	3	0.41	0.12	0.07	29.4
	-6.00	3	2.31	0.51	0.30	22.1
	-7.00	3	18.81	2.20	1.27	11.7
	-7.48	1	82.69	NC	NC	NC
	-8.00	3	67.45	8.45	4.88	12.5
	-8.48	2	87.75	NC	NC	NC
	-9.00	3	94.62	13.09	7.56	13.8
	-10.00	3	98.37	13.52	7.81	1.37
WIL	-3.00	1	0.08	NC	NC	NC
	-4.00	1	0.22	NC	NC	NC
	-5.00	3	4.12	6.43	3.71	156.03
	-6.00	3	20.05	28.60	16.51	142.65
	-7.00	3	48.59	42.67	24.64	87.83
	-7.52	2	76.98	31.32	22.15	40.69
	-8.00	3	86.92	13.47	7.78	15.50
	-8.52	2	100.92	5.95	4.21	5.89
	-9.00	3	104.56	1.40	0.81	1.34
	-10.00	3	104.79	4.51	2.60	4.30

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

Table 32. 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	3	0.05	0.09	0.05	177.8
-4.00	4	2.64	4.99	2.49	188.7
-5.00	4	1.33	1.86	0.93	140.3
-6.00	4	7.26	8.54	4.27	117.7
-7.00	4	29.08	13.28	6.64	45.7
-7.48	1	82.69	NC[b]	NC	NC
-7.52	1	76.98	NC	NC	NC
-7.60	2	55.80	NC	NC	NC
-8.00	4	75.17	8.31	4.15	11.1
-8.30	1	83.06	NC	NC	NC
-8.48	1	87.75	NC	NC	NC
-8.52	1	100.92	NC	NC	NC
-8.60	1	92.89	NC	NC	NC
-9.00	4	96.33	5.54	2.77	5.8
-10.00	4	98.77	4.40	2.20	4.5
-11.00	1	100.41	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 $\leq$2, then the sd, SEM, and %CV were not calculated.

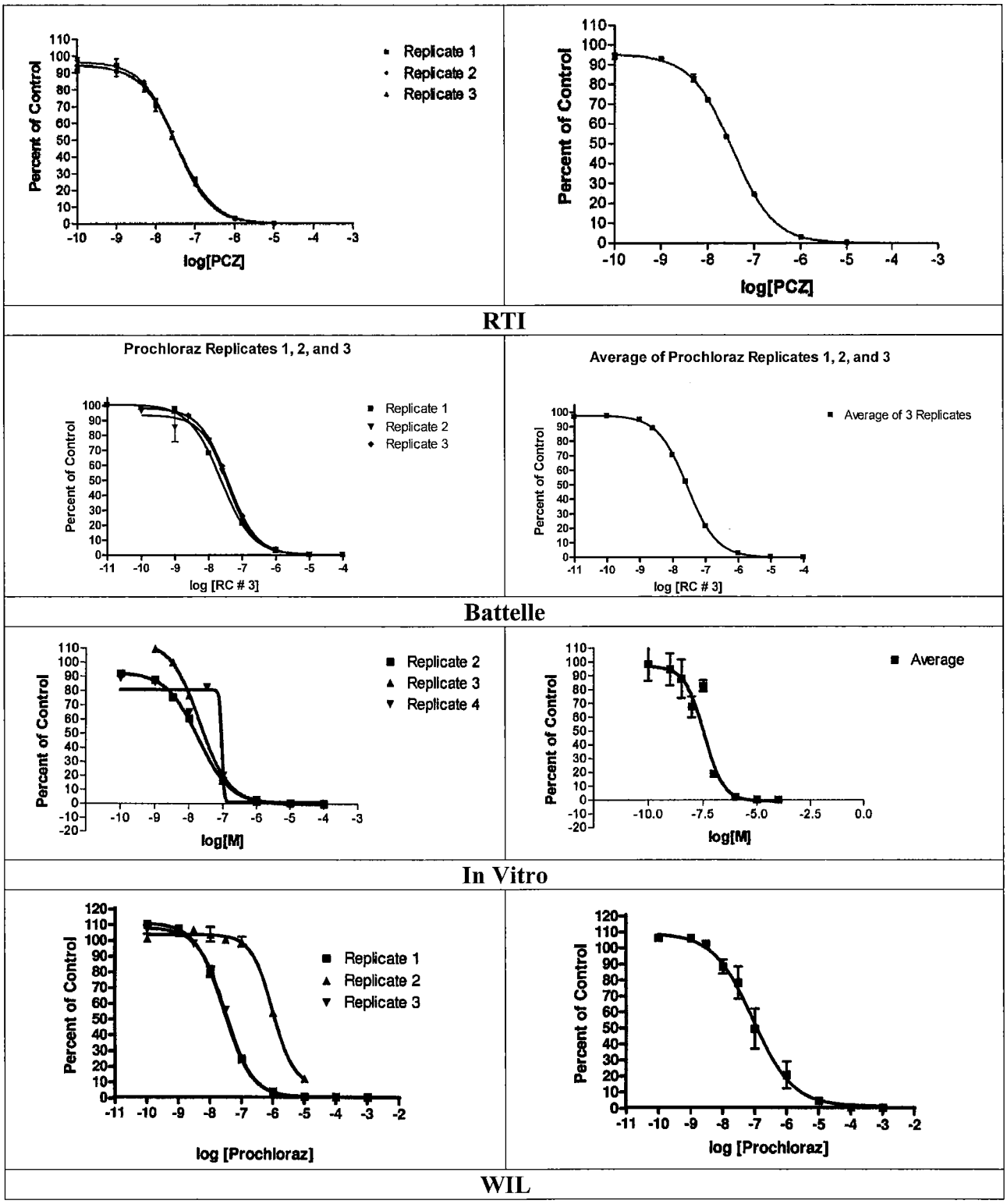

Figure 19. **Individual Replicate and Average Prochloraz Inhibition Response Curves (percent of control) by Laboratory**

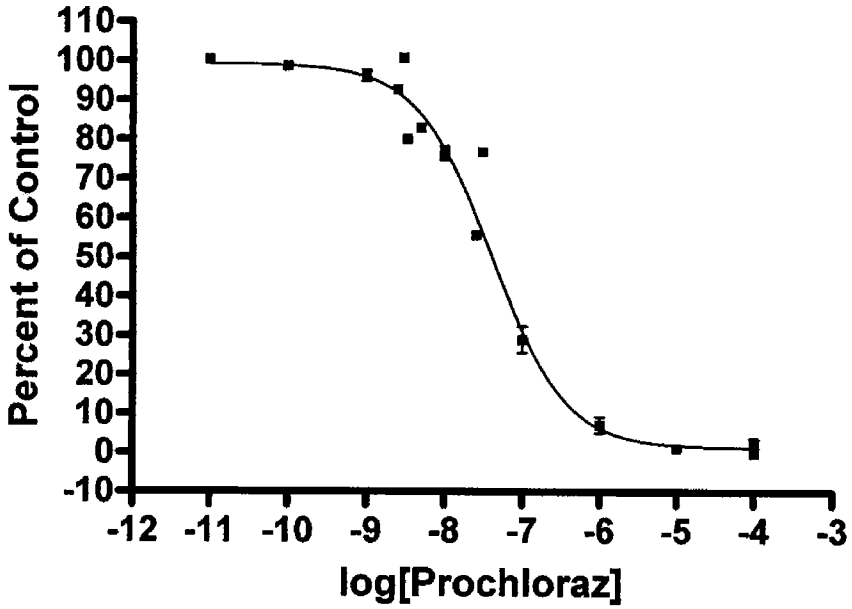

Figure 20. Overall Task Prochloraz Inhibition Response Curve (percent of control)

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 33.

Table 33. Reference Chemical IC_{50} Values

Replicate	IC_{50} Values			
	RTI	Battelle	In Vitro	WIL
Aminoglutethimide (µM)				
1	4.656	4.969	4.22	4.105
2	6.124	5.196	3.34	2.821
3	4.920	6.972	4.10	5.745
Average	5.23	5.71	3.89	4.22
Sd	0.78	1.10	0.48	1.47
SEM	0.45	0.63	0.28	0.85
% CV	15.0	19.2	12.3	34.7
Atrazine (µM)				
1	no inhibition	no inhibition[a]	no inhibition[a]	no inhibition
2	no inhibition[a]	no inhibition[a]	no inhibition	no inhibition
3	no inhibition	no inhibition[a]	no inhibition	no inhibition
Chrysin (µM)				
1	2.265	0.8299	2.19	3.030
2	1.862	1.536	3.09	3.472
3	2.415	2.066	3.79	3.713
Average	2.18	1.48	3.02	3.41
Sd	0.29	0.62	0.80	0.35
SEM	0.17	0.36	0.46	0.20
% CV	13.1	42.0	26.5	10.2
Dibenz[a,h]anthracene (µM)				
1	no inhibition	no inhibition	no inhibition	no inhibition
2	no inhibition[a]	no inhibition	no inhibition[a]	no inhibition
3	no inhibition	no inhibition[a]	no inhibition[a]	no inhibition
Dicofol (µM)				
1	37.33	15.25	4.74	27.22
2	35.24	15.57	4.78	43.67
3	34.75	21.79	10.6	49.00
Mean	35.77	17.54	6.71	39.96
Sd	1.37	3.69	3.37	11.35
SEM	0.79	2.13	1.95	6.55
% CV	3.8	21.0	50.3	28.4
Econazole (µM)				
1	0.002432	0.001901	0.00165	0.002268
2	0.002576	0.003049	0.00156	0.002314
3	0.002415	0.002226	0.00167	0.002551
Mean	0.00247	0.00239	0.00163	0.00238
Sd	0.00009	0.00059	0.00006	0.00015
SEM	0.00005	0.00034	0.00003	0.00009
% CV	3.6	24.7	3.6	6.4
Fenarimol (µM)				
1	7.047	5.165	1.77	6.178
2	6.039	5.626	4.83	150.5
3	6.053	5.454	4.76	7.718
Mean	6.38	5.42	3.79	6.95
Sd	0.58	0.23	1.75	1.09
SEM	0.33	0.13	1.01	0.77
% CV	9.1	4.3	46.1	15.7
Ketoconazole (µM)				
1	8.017	17.12	4.64	7.272

Replicate	IC_{50} Values			
	RTI	Battelle	In Vitro	WIL
2	10.52	8.503	2.95	4.703
3	9.594	7.599	2.42	8.215
Mean	9.38	11.07	3.34	6.73
Sd	1.27	5.26	1.16	1.82
SEM	0.73	3.03	0.67	1.05
% CV	13.5	47.5	34.7	27.0
4-Nonylphenol (µM)				
1	19.54	21.11	22.5	13.20
2	22.18	18.52	15.4	15.79
3	21.23	20.99	18.2	17.79
Mean	20.98	20.21	18.7	15.59
Sd	1.34	1.46	3.58	2.30
SEM	0.77	0.84	2.06	1.33
% CV	6.4	7.2	19.1	14.8
Prochloraz (µM)				
1	0.03475	0.02307	0.0189	0.0263
2	0.03258	0.03697	0.0206	0.9492*
3	0.03296	0.03749	0.0913	0.0305
Mean	0.0334	0.0325	0.0198	0.0284
Sd	0.0012	0.0082	0.0012	0.0030
SEM	0.0007	0.0047	0.0009	0.0021
% CV	3.5	25.2	6.1	10.5

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 5.23 ± 0.45, 5.71 ± 0.63, 3.89 ± 0.28, and 4.22 ± 0.85 µM, respectively. The percent CV values were 15.0, 19.2, 12.3, and 34.7 percent, respectively. Only one of the laboratories modified the concentrations tested after the first replicate, thereby suggesting that the initial range of concentrations selected were adequate to characterize the curve. The overall task group mean ± SEM IC_{50} value was 4.76 ± 0.43 µM and the percent CV was 17.9 percent.

For atrazine, the % of control curves for each replicate and all laboratories indicated that no graded inhibition occurred, thereby precluding any determination of a valid IC_{50} value. Although there were IC_{50} values determined for some of the laboratories, the Prism analysis for these replicates indicated that the error associated with all of the parameter estimates was so large that the results were considered unreliable. Thus, atrazine did not inhibit aromatase.

For chrysin, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 2.18 ± 0.17, 1.48 ± 0.36, 3.02 ± 0.46, and 3.41 ± 0.20 µM, respectively. The percent CV values were 13.1, 42.0, 26.5, and 10.2 percent, respectively. The higher % CV values for Battelle and In Vitro were attributed to the IC_{50} value obtained from one of the three replicates. For Battelle, replicate 1 produced a relatively lower IC_{50} value, which was attributed to the lower concentrations producing a higher level of inhibition that was not observed for replicates 2 and 3. This is a good example of how the variability was increased by including the results from the first replicate in the average. For In Vitro, replicate 3 produced a relatively higher IC_{50} value, which was attributed to an oddly shaped % of control curve with a very steep slope, i.e. almost perpendicular with the x-axis. It appears that the IC_{50} could be adequately estimated without modifying the concentrations tested, although the data from WIL is the only laboratory that can

be used to draw such a conclusion because of the issues addressed above for Battelle and In Vitro, as well as the fact that RTI used the same concentrations for all three replicates. It is also important to note that for all of the laboratories, the percent of control values at the highest chrysin concentrations tested did not achieve a zero percent response. Rather, the overall task average percent of control value achieved was approximately 21 percent. The overall task group mean ± SEM IC_{50} value was 2.52 ± 0.43 μM and the percent CV was 34.3 percent.

For dicofol, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 35.77 ± 0.79, 17.54 ± 2.13, 6.71 ± 1.95, and 39.96 ± 6.55 μM, respectively. The percent CV values were 3.8, 21.0, 50.3, and 28.4 percent, respectively. The higher % CV value for In Vitro was attributed to the response curves for all three replicates. Unlike the other laboratories, the percent of control values at the higher concentrations did not achieve a zero percent response for In Vitro, presumably because they didn't test at 10^{-3}M, like the other labs. Not only was there a larger measure of variability in the IC_{50} value, but it was also several fold lower than what was estimated by the other laboratories. It is unclear why In Vitro's results differed at the higher concentrations tested. The results from the other laboratories indicate that the IC_{50} can be precisely estimated using the replicate 1 starting concentrations. However, the results also indicated 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 25.00 ± 7.80 μM and the percent CV was 62.4 percent.

For dibenz[a,h]anthracene, the % of control curves for each replicate and all laboratories indicated that no graded inhibition occurred, thereby precluding any determination of a valid IC_{50} value. Although there were IC_{50} values determined for some of the laboratories, the Prism analysis for these replicates indicated that the error associated with all of the parameter estimates was so large that the results were considered unreliable. Thus, dibenz[a,h]anthracene did not inhibit aromatase.

For econazole, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 2.47 ± 0.05, 2.39 ± 0.34, 1.63 ± 0.03, and 2.38 ± 0.09 nM, respectively. The percent CV values were 3.6, 24.7, 3.6, and 6.4 percent, respectively. Only Battelle's IC_{50} estimate had a relative low measure of precision (% CV). However, a review of Battelle's % of control curves indicated that there wasn't a replicate that was disparate from the others; rather, the three replicates simply varied to the extent determined. The high precision measured by RTI may be attributed to using information from the previous work assignment in determining the concentrations tested (see percent of control results). However, In Vitro and WIL modified their concentrations after replicate 1 and still achieved a measure of precision that approximated 4 to 6 percent, thereby indicating that a high degree of precision was still attainable by following the study design. The overall task group mean ± SEM IC_{50} value was 2.22 ± 0.20 nM and the percent CV was 17.9 percent.

For fenarimol, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 6.38 ± 0.33, 5.42 ± 0.13, 3.79 ± 1.01, and 6.95 ± 0.77 μM, respectively. The percent CV values were 9.1, 4.3, 46.1, and 15.7 percent, respectively. The average reported for WIL was based on results only from replicates 1 and 3 because WIL's percent of control curve for replicate 2 was determined using an aberrant full enzyme activity control value, which resulted in an IC_{50} value of 150.5 (average ± SEM, % CV, n = 54.80 ± 47.85 μM, 151.3 %, 3). The higher % CV value for In Vitro was attributed to the IC_{50} value obtained from replicate 1, which did not have % of

control values that approached 0 or 100 percent at the higher and lower concentrations. Although In Vitro did not modify their concentrations after replicate 1, the % of control values obtained for replicates 2 and 3 resulted in responses that were much closer to 0 and 100 percent, thereby resulting in better curves and parameter estimates. The results from RTI and Battelle indicated that the IC_{50} could be precisely estimated using the replicate 1 starting concentrations. However, these two labs also showed that modifications to the replicate 1 concentrations tested did improve the characterization of the % of control curve and IC_{50} estimate. The overall task group mean ± SEM IC_{50} value was 5.63 ± 0.69 µM and the percent CV was 24.6 percent.

For ketoconazole, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 9.38 ± 0.73, 11.07 ± 3.03, 3.34 ± 0.67, and 6.73 ± 1.05 µM, respectively. The percent CV values were 13.5, 47.5, 34.7, and 27.0 percent, respectively. In general, the precision was low for most of the laboratories (RTI was the exception). 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 and, for Battelle, estimates by RTI and WIL. The results from RTI and WIL indicate that the IC_{50} could be precisely estimated using the replicate 1 starting concentrations. However, RTI and Battelle 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 7.63 ± 1.69 µM and the percent CV was 44.2 percent.

For 4-nonylphenol, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 20.98 ± 0.77, 20.21 ± 0.84, 18.7 ± 2.06, and 15.59 ± 1.33 µM, respectively. The percent CV values were 6.4, 7.2, 19.1, and 14.8 percent, respectively. A review of the % of control curves for each laboratory indicated that there were no overt aberrant curves. Modifications to the concentrations tested replicate 1 did not appreciably alter the initially estimated IC_{50} values. The results from all of the laboratories indicated that the IC_{50} could be precisely estimated using the replicate 1 starting concentrations. The overall task group mean ± SEM IC_{50} value was 18.87 ± 1.19 µM and the percent CV was 12.6 percent.

For prochloraz, the average ± SEM IC_{50} values for RTI, Battelle, In Vitro, and WIL were 0.0334 ± 0.0007, 0.0325 ± 0.0047, 0.0198 ± 0.0009, and 0.0284 ± 0.0021 µM, respectively. The percent CV values were 3.5, 25.2, 6.1, and 10.5 percent, respectively. The average reported for In Vitro and WIL were based on results from only 2 of 3 replicates because In Vitro's third replicate (replicate 4) had a very skewed % of control curve and WIL's % of control curve for replicate 2 was determined using an aberrant full enzyme activity control value. If all three replicates were used, then the average ± SEM and % CV for In Vitro were 0.0436 ± 0.0239 µM and 94.8 % and for WIL were 0.3353 ± 0.3069 µM and 158.5 %. The slightly higher % CV value for Battelle was attributed to the IC_{50} value obtained from replicate 1. The results from all the labs indicate that the IC_{50} could be precisely estimated using the replicate 1 starting concentrations. However, the results also showed that modifications to the replicate 1 concentrations, as was made by Battelle, could 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 0.0285 ± 0.0031 µM and the percent CV was 21.9 percent (does not include the aberrant values for In Vitro and WIL).

3.9.2 Laboratory Slope Values

The slope values by replicate and laboratory are summarized in Table 34 for each reference chemical.

Table 34. Reference Chemical Slope Values

Replicate	Slope Values			
	RTI	Battelle	In Vitro	WIL
Aminoglutethimide				
1	-0.8562	-0.9977	-0.9324	-0.9349
2	-1.020	-0.9779	-0.8409	-1.0425
3	-1.056	-0.9826	-1.010	-0.9907
Average	-0.9774	-0.9861	-0.9278	-0.9894
sd	0.1065	0.0103	0.0846	0.0538
SEM	0.0615	0.0060	0.0489	0.0311
% CV	10.9	1.0	9.1	5.4
Atrazine				
1	no inhibition	no inhibition[a]	no inhibition[a]	no inhibition
2	no inhibition[a]	no inhibition[a]	no inhibition	no inhibition
3	no inhibition	no inhibition[a]	no inhibition	no inhibition
Chrysin				
1	-1.295	-1.426	-1.691	-1.1072
2	-1.321	-0.8334	-0.8879	-1.1553
3	-1.298	-1.145	-15.72[b]	-1.4316
Average	-1.305	-1.135	-6.100	-1.231
sd	0.014	0.296	8.341	0.175
SEM	0.008	0.171	4.816	0.101
% CV	1.1	26.1	136.7	14.2
Dibenz[a,h]anthracene				
1	no inhibition	no inhibition	No inhibition	no inhibition
2	no inhibition[a]	no inhibition	No inhibition[a]	no inhibition
3	no inhibition	no inhibition[a]	No inhibition[a]	no inhibition
Dicofol				
1	-1.159	-1.246	-1.664	-0.7327
2	-0.8375	-0.8659	-2.236	-0.7303
3	-0.9354	-1.321	-2.570	-0.7148
Mean	-0.9773	-1.1443	-2.1567	-0.7259
sd	0.1648	0.2440	0.4582	0.0097
SEM	0.0951	0.1409	0.2645	0.0056
% CV	16.9	21.3	21.2	1.3
Econazole				
1	-1.216	-1.046	-0.9904	-1.1489
2	-1.176	-1.297	-1.080	-1.1875
3	-1.043	-1.112	-0.9838	-1.2174
Mean	-1.1450	-1.1517	-1.0181	-1.1846
sd	0.0906	0.1301	0.0537	0.0343
SEM	0.0523	0.0751	0.0310	0.0198
% CV	7.9	11.3	5.3	2.9
Fenarimol				
1	-1.041	-0.9738	-2.269[b]	-1.0523
2	-1.049	-0.9460	-0.9373	-1.4869
3	-0.9949	-0.9191	-0.9985	-1.0769
Mean	-1.0283	-0.9463	-1.4016	-1.2054

Replicate	Slope Values			
	RTI	Battelle	In Vitro	WIL
sd	0.0292	0.0274	0.7518	0.2441
SEM	0.0169	0.0158	0.4341	0.1409
% CV	2.8	2.9	53.6	20.3
Ketoconazole				
1	-1.036	-0.8087	-1.108	-0.8992
2	-0.9035	-1.009	-1.364	-0.9173
3	-0.9384	-0.8902	-1.491	-0.9315
Mean	-0.9593	-0.9026	-1.3210	-0.9160
sd	0.0687	0.1007	0.1951	0.0162
SEM	0.0397	0.0582	0.1126	0.0093
% CV	7.2	11.2	14.8	1.8
4-Nonylphenol				
1	-2.588	-2.014	-1.039	-2.0442
2	-2.018	-2.096	-1.622	-1.8805
3	-2.186	-2.112	-1.520	-1.7222
Mean	-2.264	-2.074	-1.394	-1.8823
sd	0.2929	0.0526	0.3114	0.1610
SEM	0.1691	0.0304	0.1798	0.0930
% CV	12.9	2.5	22.3	8.6
Prochloraz				
1	-0.9214	-0.9271	-0.9053	-0.9744
2	-0.9868	-1.101	-1.012	-1.2731
3	-0.9968	-1.021	-12.77[b]	-1.0019
Mean	-0.9683	-1.016	-4.896	-1.0831
sd	0.0410	0.0870	6.819	0.1651
SEM	0.0236	0.0503	3.937	0.0953
% CV	4.2	8.6	139.3	15.2

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

b. Considered an aberrant value. See text for results without the value included.

For aminoglutethimide, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -0.9774 ± 0.0615, -0.9861 ± 0.0060, -0.9278 ± 0.0489, and -0.9894 ± 0.0311, respectively. The percent CV values were 10.9, 1.0, 9.1, and 5.4 percent, respectively. Only one of the laboratories modified the concentrations tested after the first replicate, thereby suggesting that the initial range of concentrations selected were adequate to characterize the curve. The slopes of the curves were determined with a high degree of precision for all laboratories. The overall task group mean ± SEM slope value was -0.9702 ± 0.0144 and the percent CV was 3.0 percent.

For atrazine, the % of control curves had no valid measurable slopes. For the most part, the curves appeared as horizontal lines. There was no indication that any inhibition of aromatase by atrazine occurred.

For chrysin, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -1.305 ± 0.008, -1.135 ± 0.171, -1.289 ± 0.402, and -1.231 ± 0.101, respectively. The percent CV values were 1.1, 26.1, 44.0, and 14.2 percent, respectively. The higher % CV value for Battelle was attributed to the shape of the replicate 1 curve, which did not produce a full response at the low and high concentrations. As for In Vitro, the values reported above were

calculated only using replicates 1 and 2 because the shape of the curve for the last replicate was very skewed, i.e. the slope was virtually perpendicular to the x-axis. If all three replicates were used, then the average ± SEM slope and % CV values for In Vitro were -6.100 ± 4.816 and 136.7 percent. The relatively high degree of precision achieved by RTI was attributed to their use of the same concentrations for all three replicates. Regardless, it appears that the slope of the curve was precisely determined over the concentration range tested. The overall task group mean ± SEM slope value was -1.240 ± 0.038 and the percent CV was 6.2 percent. [Note: overall values do not include the aberrant value for In Vitro.]

For dicofol, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -0.9773 ± 0.0951, -1.1443 ± 0.1409, -2.1567 ± 0.2645, and -0.7259 ± 0.0056, respectively. The percent CV values were 16.9, 21.3, 21.2, and 1.3 percent, respectively. The shapes of the curves were similar for RTI, Battelle, and WIL but dissimilar for In Vitro in that the higher concentrations did not result in a 0 percent response for any of the replicates. This finding led to a steeper slope estimate for In Vitro than for the other laboratories. Although three of the four laboratories modified the concentrations tested after replicate 1, there wasn't an obvious improvement in characterizing the curve or estimating the slope. Thus, the initial concentrations tested were adequate in providing this information. The overall task group mean ± SEM slope value was -1.2511 ± 0.3139 and the percent CV was 50.2 percent.

For dibenz[a,h]anthracene, the % of control curves had no valid measurable slopes. For the most part, the curves appeared as horizontal lines. There was no indication that any inhibition of aromatase by dibenz[a,h]anthracene occurred.

For econazole, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -1.1450 ± 0.0523, -1.1517 ± 0.0751, -1.0181 ± 0.0310, and -1.1846 ± 0.0198, respectively. The percent CV values were 7.9, 11.3, 5.3, 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 affect on being able to characterize the curve or estimate the slope values. The overall task group mean ± SEM slope value was -1.1248 ± 0.0366 and the percent CV was 6.5 percent.

For fenarimol, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -1.0283 ± 0.0169, -0.9463 ± 0.0158, -1.4016 ± 0.4341, and -1.2054 ± 0.1409, respectively. The percent CV values were 2.8, 2.9, 53.6, and 20.3 percent, respectively. The low precision for In Vitro was attributed to the curve for replicate 1, which did not have 0 and 100 percent of control responses at the high and low concentrations like were achieved for replicates 2 and 3 (same concentrations were tested for all three replicates). Although the precision for WIL was relatively high, the relatively slightly lower precision was attributed to the results from replicate 2, which had a low full enzyme activity control value and shifted the curve to the right. Since the shape of the curve was very similar to the other two replicates, the aberrant full enzyme activity value had a greater affect on the IC_{50} value than on the slope. For RTI and Battelle, modifications to concentrations after replicate 1 did not seem to result in any appreciable improvement on estimating the slope values, thereby indicating that the initial concentrations tested produced a reliable estimate of the parameter. The overall task group mean ± SEM slope value was -1.1454 ± 0.1011 and the percent CV was 17.6 percent.

For ketoconazole, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -0.9593 ± 0.0397, -0.9026 ± 0.0582, -1.3210 ± 0.1126, and -0.9160 ± 0.0093, respectively. The percent CV values were 7.2, 11.2, 14.8, and 1.8 percent, respectively. The replicates for a given lab and between labs were in close agreement. Modifications to concentrations did not seem to result in any appreciable improvement on estimating the slope values, thereby indicating that the initial concentrations tested produced a reliable estimate of the parameter. The overall task group mean ± SEM slope value was -1.0247 ± 0.0995 and the percent CV was 19.4 percent.

For 4-nonylphenol, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -2.264 ± 0.1691, -2.0740 ± 0.0304, -1.394 ± 0.1798, and -1.8823 ± 0.0930, respectively. The percent CV values were 12.9, 2.5, 22.3, and 8.6 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 to result in any appreciable improvement on estimating the slope values, thereby indicating that the initial concentrations tested produced a reliable estimate of the parameter. The overall task group mean ± SEM slope value was -1.9035 ± 0.1870 and the percent CV was 19.6 percent.

For prochloraz, the average ± SEM slope values for RTI, Battelle, In Vitro, and WIL were -0.9683 ± 0.0236, -1.016 ± 0.0503, -0.9587 ± 0.0534 and -1.0831 ± 0.0953, respectively. The percent CV values were 4.2, 8.6, 7.9, and 15.2 percent, respectively. For In Vitro, the values reported above were calculated only using replicates 1 and 2 because the shape of the curve for the third replicate was very skewed, i.e. the slope was virtually perpendicular to the x-axis. If all three replicates were used, then the average ± SEM slope and % CV values for In Vitro were -4.896 ± 3.937 and 139.3 percent. Although the precision for WIL was relatively high, the relatively slighty lower precision was attributed to the results from replicate 2, which had a low full enzyme activity control value and shifted the curve to the right. Since the shape of the curve was very similar to the other two replicates, the aberrant full enzyme activity value had a greater affect on the IC_{50} value than on the slope. For RTI and Battelle, modifications to concentrations after replicate 1 did not seem to result in any appreciable improvement on estimating the slope values, thereby indicating that the initial concentrations tested produced a reliable estimate of the parameter. The overall task group mean ± SEM slope value was -1.0066 ± 0.0569 and the percent CV was 5.7 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:

> Concentration response relations could not be determined for dibenz[a,h]anthracene and for atrazine due to the low aromatase inhibition. These two chemicals were categorized as non-inhibitors. Among the other eight chemicals, the estimated $\log_{10}IC_{50}$s were: -8.60 for econazole, -7.48 for prochloraz, -6.28 for aminoglutethimide, and from -5.66 to -4.03 for the remainder. Thus econazole, prochloraz, and to a lesser extent aminoglutethimide were relatively strong inhibitors. The most negative slope was observed for 4-nonylphenol (-2.26). The slopes for the other chemicals were about -1.0. The bottom thresholds were close to zero, except for chrysin (22.58). The top thresholds were about 91 to 100 for all eight chemicals. There was little variance in the IC_{50}, slope, top and bottom estimates over the three replicates for any of the eight chemicals for which concentration response relations were fitted.
>
> Aromatase activity decreased significantly between the beginnings and the ends of replicates for the full enzyme activity, the negative, and the positive controls.

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.

Dibenz[a,h]anthracene and atrazine were non-inhibitors. For dibenz[a,h]anthracene all responses were above 90 percent of control. For atrazine all but two responses were above 80 percent of control. The other two responses were at 78.65 and 77.95 percent of control.

For chrysin the low threshold of responses was in the range of 18 percent to 26 percent.

Among the four parameters, the top parameter had the most replicate-to-replicate variation. The other three parameters had replicate-to-replicate variation that was small or close to zero.

The overall standard errors of the mean of the weighted average estimates of the $\log_{10}IC_{50}$ and the slope were close to zero. For 4-nonylphenol – slope; chrysin – $\log_{10}IC_{50}$ and slope; and dicofol – slope; the standard errors were several times larger than for the other chemicals.

For the full enzyme activity, positive, and negative controls 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.

For all the control types, the control averages did not differ significantly across chemicals and the portion effects (i.e. end minus beginning) did not differ significantly across chemicals.

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 the positive control the majority of variation was from replicate-to-replicate within chemicals.

There is some ambiguity in the $\log_{10}IC_{50}$ estimate. The "$\log_{10}IC_{50}$" concentration in fact corresponds to the (B+T)/2 percentile, which for some chemicals (e.g. chrysin) deviate somewhat from 50 percent. Such variation in percent inhibition can inflate the replicate-to-replicate variation among the $\log_{10}IC_{50}$ estimates. An alternative (nonstandard) parameterization of the model might be adopted in which the "$\log_{10}IC_{50}$" concentration corresponds to the 50 percentile, irrespective of the top and bottom thresholds.

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.

Dibenz[a,h]anthracene and atrazine were non-inhibitors. For dibenz[a,h]anthracene all responses but one (59.4 percent) were above 79 percent of control. For atrazine all responses were above 80 percent of control.

Among the four concentration response curve parameters the top threshold had the most replicate-to-replicate variation within chemicals.

For most of the reference chemicals there was considerable replicate-to-replicate variation within chemicals for at least some of the concentration response curve parameters:

- Aminoglutethimide – Top
- Ketoconazole – Top
- Prochloraz – Top, IC_{50}, Slope
- Fenarimol – Bottom, Top
- Econazole – Top
- Chrysin – Bottom, Top, Slope
- Dicofol – Bottom, Top

For dicofol the bottom threshold was in the range 28 percent to 50 percent.

The overall standard errors of the mean of the weighted average estimates of the $\log_{10}IC_{50}$ and the slope were small.

For the full enzyme activity controls 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.

For the negative control the differences between the beginning and the end portions, when averaged across replicates, approached significance (p=0.054). The end portion was significantly higher than the beginning portion. This implies an increase in aromatase activity between the beginning and the end of a replicate. It is not understood why there is a difference in the direction of change of activity between the negative controls and the full enzyme activity controls.

For the background activity controls and the positive controls the differences between the beginning and the end portions, when averaged across replicates, were not significant.

For all control types the average control results did not vary significantly across chemicals. The portion effects within chemicals also did not vary significantly across chemicals

There is some ambiguity in the $\log_{10}IC_{50}$ estimate. The "$\log_{10}IC_{50}$" concentration in fact corresponds to the (B+T)/2 percentile, which for some chemicals (e.g. fenarimol – (B+T)/2 = 58.1, 45.5, 55.7) deviate somewhat from 50 percent. Such variation in percent inhibition can inflate the replicate-to-replicate variation among the $\log_{10}IC_{50}$ estimates. An alternative (nonstandard) parameterization of the model might be adopted in which the "$\log_{10}IC_{50}$" concentration corresponds to the 50 percentile, irrespective of the top and bottom thresholds.

Variation in aromatase activity during the course of a replicate run can result in potential bias or potential loss of precision due to decrease or increase of aromatase activity within replicates. If the inhibition concentration tubes are tested in order of inhibitor concentration, the change in aromatase activity across the replicate can accentuate or can lessen the apparent inhibition, depending on the ordering of the reference chemical concentrations. If the 24 tubes (8 inhibitor concentrations × 3 repetitions per concentration) were tested in randomized order then the change 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.

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:

> Concentration response relations could not be determined for dibenz[a,h]anthracene and atrazine due to their low aromatase inhibition. Econazole, prochloraz, and ketoconazole were relatively strong inhibitors. The estimated $\log_{10}IC_{50}$s for these chemicals were -8.63 for econazole, -7.04 for prochloraz, and -6.39 for ketoconazole. For the other chemicals the $\log_{10}IC_{50}$s ranged from -5.48 to -4.19. The most negative slope was observed for 4-nonylphenol (-1.87) and the least negative slope was observed for dicofol (-0.73). The slopes for the other chemicals were distributed around -1.0. Chrysin had a relatively high

bottom threshold, 12.79. For the other chemicals the bottom thresholds varied between -6.00 and 5.73. The top thresholds ranged from 94.19 to 106.25.

Prochloraz showed some variation among the three replicates for the $\log_{10}IC_{50}$ and the bottom threshold parameters. Fenarimol showed some variation among the three replicates for the $\log_{10}IC_{50}$, the top threshold, and the bottom threshold parameters. For the other chemicals there was little variation in the IC_{50}, slope, top and bottom threshold parameter estimates over the three replicates.

Aromatase activity decreased significantly between the beginnings and the ends of replicates for the full enzyme activity, the negative, and the positive controls.

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 relation fits for the two non-inhibitor chemicals dibenz[a,h]anthracene and atrazine in all four intralaboratory analyses of the individual laboratory data. Therefore the interlaboratory analyses for the concentration response relation parameters were restricted to the other eight chemicals.

Table 35 displays the estimated $\log_{10}IC_{50}$ and the slope within each laboratory and the associated 95 percent confidence intervals for each chemical[1]. This table also displays 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. 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 35.

Table 36 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 36 also displays the laboratory-to-laboratory random variations, the p-values associated with the test 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

[1] The confidence intervals in Table 35 were calculated for the interlaboratory analysis 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 35 thus may differ in the low significant digits from those displayed in the intralaboratory analysis reports due to round off error in intermediate calculations.

[2] Degrees of freedom within laboratories (Table 36) 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 was assigned. The degrees of freedom for RTI were based on an analysis of variance model for all four chemicals combined. There were 24 observations (8 chemicals × 3 replicates per chemical) and 8 effects estimated, leaving 16 degrees of freedom for residual.

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 appendix of the Interlaboratory Statistical Analysis Report (Appendix E of the overall report). Notice that CV was not calculated for the bottom parameter since its distribution straddles zero.

The estimates for $\log_{10}IC_{50}$ (Table 35) were generally consistent among the four laboratories for most chemicals. The In Vitro estimates were slightly lower than those for the other three laboratories for ketoconazole, econazole, and dicofol. The ratios of the among laboratory variance to the pooled within laboratory variance were relatively large for dicofol (9.5), ketoconazole (6.4), and econazole (4.9) and were less than 2.7 for the other chemicals (Table 36). The CVs for the IC_{50} were 39.5 percent for ketoconazole, 70.6 percent for dicofol, 26.9 percent for chrysin, 29.2 for prochloraz, and between 11.4 and 18.3 percent for the other chemicals (Table 35). All the CVs exceeded 10 percent.

The slope estimates (Table 35) were similar among the four laboratories for most chemicals. The In Vitro slope estimates were more negative than those for the other three laboratories for ketoconazole (Figure 24) and dicofol (Figure 36) and were less negative than those for the other three laboratories for 4-nonylphenol (Figure 28) and econazole (Figure 32). The slope CVs were 28.1 percent for dicofol, 14.5 percent for 4-nonylphenol, 7.4 percent for econazole, and less than 6.1 percent for the other five chemicals (Table 35). The ratios of the among laboratory variance to the pooled within laboratory variance were 2.5 or less for all of the chemicals (Table 36).

The estimates for the top thresholds varied between 85 and 111 percent. They were in general consistent among the four laboratories for most chemicals except for aminoglutethimide. The estimated top threshold for In Vitro was higher than the other three laboratories for aminoglutethimide (110.3 compared to 99 percent or less). The top threshold CVs were 6.0 percent for aminoglutethimide and less than 5.4 percent for the other seven chemicals. The ratios of the among laboratory variances to the pooled within laboratory variance ratios were 7.1 for aminoglutethimide and 3.9 or less for the other seven chemicals.

The estimated bottom thresholds were between approximately -2.5 and 11 for all chemicals except for chrysin and dicofol. The bottom thresholds were between 12.8 and 22.6 percent for chrysin, and consistent among laboratories. In Vitro had a bottom threshold of 41.1 percent for dicofol, which was inconsistent with those of the other three laboratories (-6.0 to 2.1 percent). The 95 percent 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 21.4 for dicofol and less than 1.4 for any of the other seven chemicals.

3.11.2 Control Activity: Differences Between the Beginning and the End Portions

Table 37 displays the estimated parameter values and the associated within laboratory 95 percent confidence intervals for the differences between the beginning and the end portions[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 (end minus beginning) and confidence intervals are displayed in Figures 37 to 40, one for each control endpoint. Each figure includes a reference line corresponding to the overall average.

Table 38 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 38 also displays the laboratory-to-laboratory random variation and the p-values associated with their significance, 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 differences between the end and the beginning of replicates. The among laboratory variance was zero.

For the full enzyme activity controls, the end portion was statistically significantly lower than the beginning portion for each individual laboratory and on average across laboratories. The difference between the two portions for RTI was more than two times those for the other laboratories. The estimated variance among the laboratories was about eight times the pooled within laboratory variance.

For the negative 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 statistically significantly lower than the beginning for the other three laboratories. As a result of this disagreement, the estimated variance among the laboratories was about 4.6 times of the average within laboratory variance. The two portions did not differ significantly on average across laboratories.

For the positive controls, the result for In Vitro differed slightly from the other three laboratories. The end portion was slightly higher than the beginning portion for In Vitro, but statistically significantly lower than the beginning portion for the other laboratories, and on average across laboratories. The estimated variance among laboratories was about 1.6 times that of the pooled average within laboratory variance.

[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.

Table 35. Parameter Estimates and 95 Percent Confidence Intervals for the $Log_{10}IC_{50}$ and Slope Parameters of the Concentration Response Relations for the Recombinant Aromatase Assay. Estimated by Chemical.

Chemical[4]	Parameter	Estimate and 95 Percent Confidence Interval					CV(percent) and 95 percent CI[3]
		RTI[1]	Battelle[1]	WIL[1]	In Vitro[1]	Overall[2]	
AG	Log_IC50	-5.279(-5.358,-5.201)	-5.250(-5.449,-5.051)	-5.385(-5.759,-5.011)	-5.393(-5.441,-5.345)	-5.325(-5.411,-5.239)	15.647(9.763,39.077)
AG	Slope	-0.980(-1.108,-0.853)	-0.987(-1.039,-0.935)	-0.970(-1.086,-0.854)	-0.939(-1.017,-0.861)	-0.972(-1.005,-0.939)	3.345(2.726,4.329)
KCZ	Log_IC50	-5.031(-5.111,-4.952)	-5.097(-5.147,-5.047)	-5.185(-5.503,-4.867)	-5.492(-5.852,-5.132)	-5.186(-5.428,-4.943)	39.467(22.510,185.087)
KCZ	Slope	-0.937(-1.037,-0.836)	-0.943(-1.064,-0.822)	-0.916(-1.032,-0.800)	-1.248(-1.641,-0.855)	-0.934(-0.986,-0.881)	4.592(2.955,10.168)
PCZ	Log_IC50	-7.481(-7.520,-7.443)	-7.503(-7.787,-7.219)	-7.040(-9.226,-4.854)	-7.704(-7.742,-7.666)	-7.562(-7.772,-7.353)	29.237(16.024,174.277)
PCZ	Slope	-0.973(-1.048,-0.899)	-0.981(-1.079,-0.883)	-0.991(-1.137,-0.845)	-0.957(-1.068,-0.846)	-0.979(-1.019,-0.938)	4.029(3.231,5.354)
NYP	Log_IC50	-4.666(-4.702,-4.630)	-4.721(-4.772,-4.670)	-4.782(-4.902,-4.662)	-4.781(-4.854,-4.708)	-4.731(-4.798,-4.665)	11.409(6.913,31.719)
NYP	Slope	-2.264(-2.620,-1.907)	-2.095(-2.355,-1.835)	-1.874(-2.747,-1.001)	-1.576(-1.738,-1.414)	-1.930(-2.295,-1.565)	14.548(8.941,38.504)
FRM	Log_IC50	-5.195(-5.243,-5.147)	-5.269(-5.297,-5.241)	-4.717(-6.636,-2.798)	-5.452(-6.101,-4.803)	-5.242(-5.334,-5.150)	11.832(6.432,58.984)
FRM	Slope	-1.023(-1.094,-0.951)	-0.954(-1.008,-0.900)	-1.069(-1.280,-0.858)	-0.952(-1.031,-0.873)	-0.990(-1.053,-0.926)	4.320(2.514,14.136)
ECZ	Log_IC50	-8.604(-8.641,-8.566)	-8.630(-8.888,-8.372)	-8.625(-8.694,-8.556)	-8.786(-8.818,-8.754)	-8.664(-8.768,-8.561)	18.347(11.300,48.541)
ECZ	Slope	-1.174(-1.262,-1.085)	-1.152(-1.475,-0.829)	-1.177(-1.272,-1.082)	-0.998(-1.056,-0.940)	-1.121(-1.228,-1.014)	7.418(4.622,18.391)
CYN	Log_IC50	-5.663(-5.736,-5.590)	-5.862(-6.340,-5.384)	-5.481(-5.593,-5.369)	-5.599(-5.751,-5.447)	-5.616(-5.773,-5.458)	26.852(15.982,85.968)
CYN	Slope	-1.304(-1.485,-1.123)	-1.074(-1.648,-0.500)	-1.132(-1.304,-0.960)	-1.209(-2.029,-0.389)	-1.158(-1.231,-1.086)	6.052(4.711,8.475)
DCF	Log_IC50	-4.446(-4.516,-4.376)	-4.762(-4.958,-4.566)	-4.421(-4.778,-4.064)	-5.151(-5.582,-4.720)	-4.676(-5.081,-4.272)	70.615(38.533,776.709)
DCF	Slope	-0.974(-1.158,-0.791)	-1.113(-1.565,-0.661)	-0.728(-0.883,-0.573)	-2.179(-3.062,-1.296)	-1.024(-1.511,-0.536)	28.065(15.240,159.525)

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 were estimated using a random effects analysis of variance, with laboratory as a random effect and with heterogeneous variances among the four laboratories. The variance for each laboratory was specified as the square of the within laboratory standard error. The degrees of freedom are given in Table 36.
3. CV was calculated for the IC_{50} and the slope parameters based on average results. CVs shown as associated with $log_{10}IC_{50}$ are actually CVs of the IC_{50}s.
4. Concentration response relations were not fitted for dibenz[a,h]anthracene and for atrazine, since they resulted in very little aromatase inhibition (i.e. they were noninhibitors).

Table 36. Variance Components and Ratio of Between and Within Laboratories Variances for the $Log_{10}IC_{50}$ and Slope Parameter of the Concentration Response Relations for the Recombinant Aromatase Assay. Estimated by Chemical.

Chemical[7]	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]			
AG	Log_IC50	0.001/df=16.0	0.002/df=2.0	0.008/df=2.0	0.001/df=44.3	0.003/df=4.4	0.003 (0.17921)	0.001/df=5.1	0.896 (0.100, 13.458)
AG	Slope	0.004/df=16.0	0.001/df=56.1	0.001/df=2.0	0.002/df=53.2	0.002/df=37.7	0.000 (1.000)	0.000/df=37.7	0.000 (0.000, 0.000)
KCZ	Log_IC50	0.001/df=16.0	0.001/df=20.0	0.005/df=2.0	0.007/df=2.1	0.004/df=5.3	0.024 (0.12436)	0.007/df=3.5	6.431 (0.874, 95.409)
KCZ	Slope	0.002/df=16.0	0.003/df=20.6	0.001/df=2.0	0.016/df=3.1	0.005/df=6.0	0.000 (1.000)	0.000/df=6.0	0.000 (0.000, 0.000)
PCZ	Log_IC50	0.000/df=16.0	0.005/df=2.2	0.258/df=2.0	0.000/df=20.0	0.066/df=2.1	0.010 (0.12884)	0.004/df=2.7	0.155 (0.004, 2.471)
PCZ	Slope	0.001/df=16.0	0.002/df=8.6	0.001/df=2.0	0.003/df=20.0	0.002/df=31.9	0.000 (1.000)	0.000/df=31.9	0.000 (0.000, 0.000)
NYP	Log_IC50	0.000/df=16.0	0.000/df=5.1	0.001/df=2.0	0.001/df=19.8	0.001/df=17.3	0.002 (0.14043)	0.001/df=4.3	2.694 (0.675, 38.267)
NYP	Slope	0.028/df=16.0	0.017/df=44.2	0.041/df=2.0	0.006/df=26.8	0.023/df=9.4	0.058 (0.12870)	0.020/df=4.8	2.505 (0.504, 36.191)
FRM	Log_IC50	0.001/df=16.0	0.000/df=44.9	0.199/df=2.0	0.019/df=1.8	0.055/df=2.4	0.001 (0.24440)	0.001/df=2.5	0.019 (0.001, 0.295)
FRM	Slope	0.001/df=16.0	0.001/df=46.2	0.002/df=2.0	0.001/df=20.6	0.001/df=10.6	0.001 (0.34020)	0.000/df=3.5	0.401 (0.085, 5.776)
ECZ	Log_IC50	0.000/df=16.0	0.004/df=2.0	0.000/df=2.0	0.000/df=47.2	0.001/df=3.0	0.005 (0.09468)	0.002/df=4.8	4.873 (0.317, 75.225)
ECZ	Slope	0.002/df=16.0	0.006/df=2.0	0.000/df=2.0	0.001/df=43.5	0.002/df=4.7	0.005 (0.12045)	0.002/df=5.0	2.389 (0.287, 35.709)
CYN	Log_IC50	0.001/df=16.0	0.015/df=2.2	0.001/df=2.0	0.005/df=20.0	0.006/df=4.8	0.009 (0.16991)	0.003/df=4.1	1.670 (0.206, 24.922)
CYN	Slope	0.007/df=16.0	0.022/df=2.3	0.002/df=2.0	0.154/df=20.0	0.046/df=24.3	0.000 (1.000)	0.001/df=24.3	0.000 (0.000, 0.000)
DCF	Log_IC50	0.001/df=16.0	0.003/df=2.5	0.007/df=2.0	0.018/df=3.0	0.007/df=6.2	0.070 (0.11404)	0.019/df=3.5	9.519 (1.473, 140.080)
DCF	Slope	0.007/df=16.0	0.024/df=3.6	0.001/df=2.0	0.190/df=37.0	0.056/df=43.4	0.058 (0.27558)	0.021/df=2.7	1.038 (0.302, 14.554)

1. The within laboratory variance for each laboratory is the square of the standard error associated with the 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 random effects analysis of variance model with heterogeneous variances among the individual laboratories equal to the squares of the within laboratory standard errors. P-values are associated with the test that the among laboratory variation is zero.
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 to 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 very little aromatase inhibition (i.e. they were noninhibitors).

Table 37. Parameter Estimate and the 95 Percent Confidence Intervals for Differences Between the Beginning and the End Portions for the Percent of Control Responses for the Recombinant Aromatase Assay.

Parameter	Estimate and 95 Percent Confidence Interval				
	RTI[1,2]	Battelle[1,2]	WIL[1,2]	In Vitro[1,2]	Mean[3]
Background Activity Control	0.007 (-0.017, 0.031)	-0.159 (-0.405, 0.087)	0.040 (-0.083, 0.163)	-0.049 (-0.193, 0.095)	0.005 (-0.017, 0.027)
Full Enzyme Activity Control	-14.225 (-16.008, -12.442)	-5.878 (-8.381, -3.375)	-5.244 (-7.189, -3.299)	-6.354 (-10.330, -2.378)	-8.032 (-13.160, -2.903)
Negative Control	-8.049 (-10.727, -5.372)	-4.115 (-6.300, -1.930)	-6.467 (-9.558, -3.376)	7.479 (-0.152, 15.110)	-3.732 (-12.321, 4.858)
Positive Control	-4.407 (-5.527, -3.287)	-2.795 (-4.246, -1.344)	-4.214 (-5.366, -3.061)	0.619 (-2.587, 3.825)	-3.250 (-5.945, -0.555)

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 given in Appendix E.
3. The overall (mean) effects and confidence intervals were estimated using a one-way random effects analysis of variance, with laboratory as a random effect, and with heterogeneous variances among the individual laboratories. The variances for each laboratory were specified as the squares of the within laboratory standard errors.

Table 38. Variance Components and Ratio of Among and Within Laboratories Variances for Differences Between the Beginning and the End Portions for the Percent of Control Responses for the Recombinant 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]			
Background Activity Control	0.000/df=20.0	0.013/df=16.0	0.003/df=12.0	0.004/df=14.0	0.005/df=33.2	0.000 (1.00)	0.000/df=33.2	0.000 (0.000, 0.000)
Full Enzyme Activity Control	0.730/df=20.0	1.523/df=35.9	0.797/df=12.0	3.698/df=23.2	1.687/df=62.0	13.112 (0.09369)	3.677/df=4.4	7.772 (2.331, 108.729)
Negative Control	1.648/df=20.0	1.100/df=20.4	2.012/df=12.0	12.982/df=16.2	4.435/df=28.7	20.380 (0.17558)	6.003/df=2.6	4.595 (1.272, 64.732)
Positive Control	0.288/df=20.0	0.448/df=12.5	0.280/df=12.0	2.307/df=16.9	0.831/df=32.4	1.314 (0.25770)	0.477/df=2.2	1.581 (0.445, 22.244)

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 of 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.

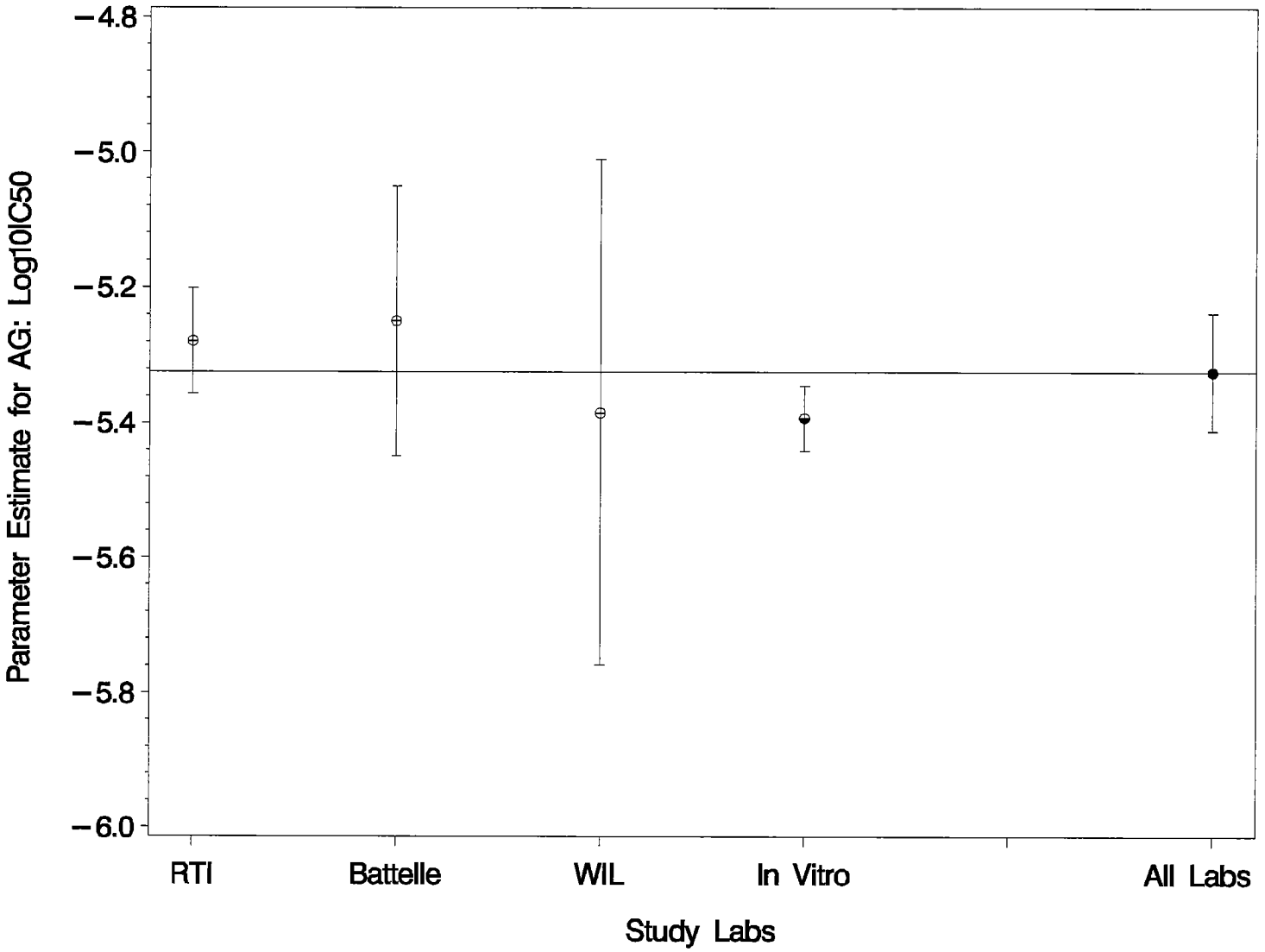

Figure 21. **Aminoglutethimide: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories**

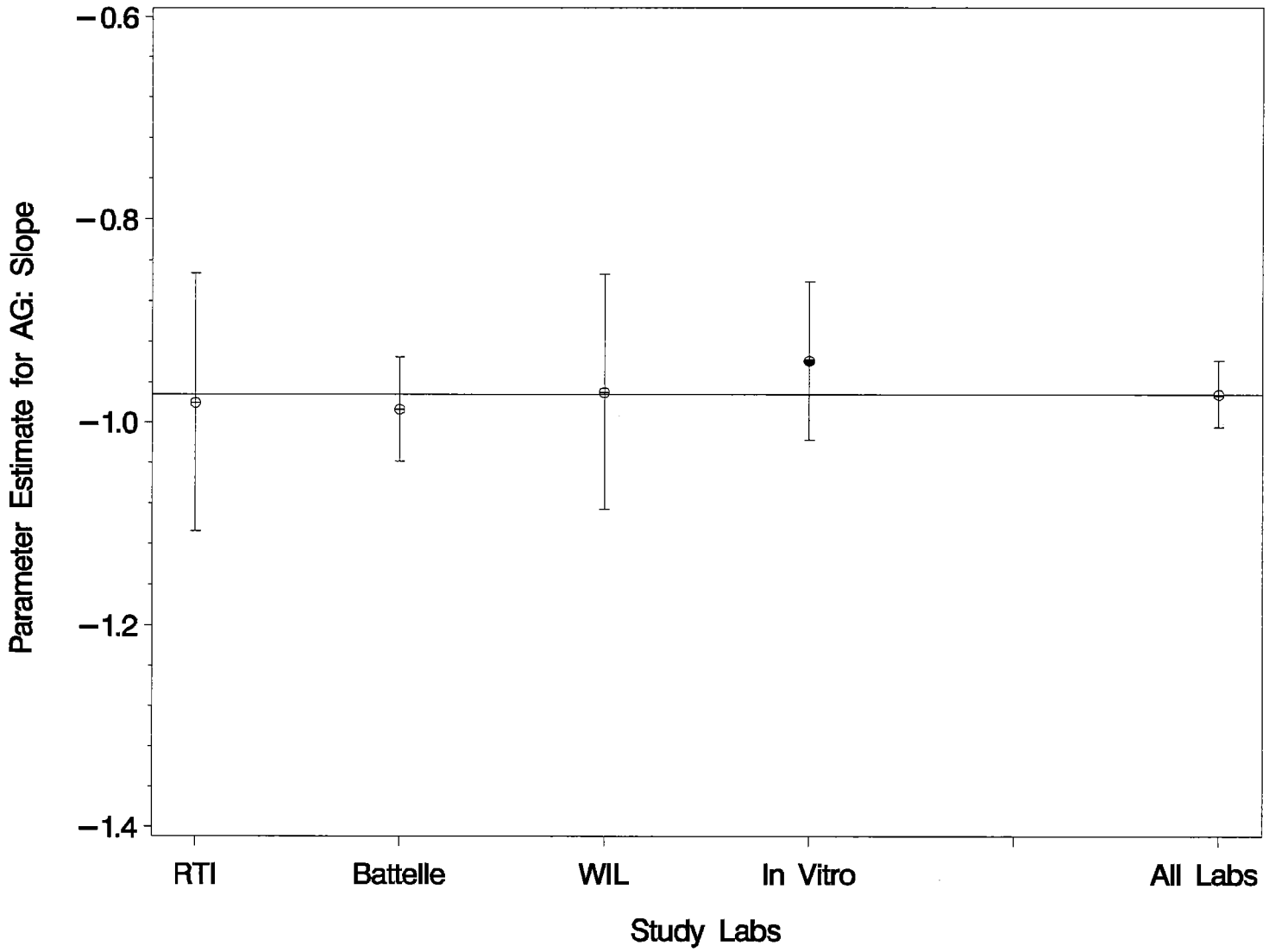

Figure 22. **Aminoglutethimide: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories**

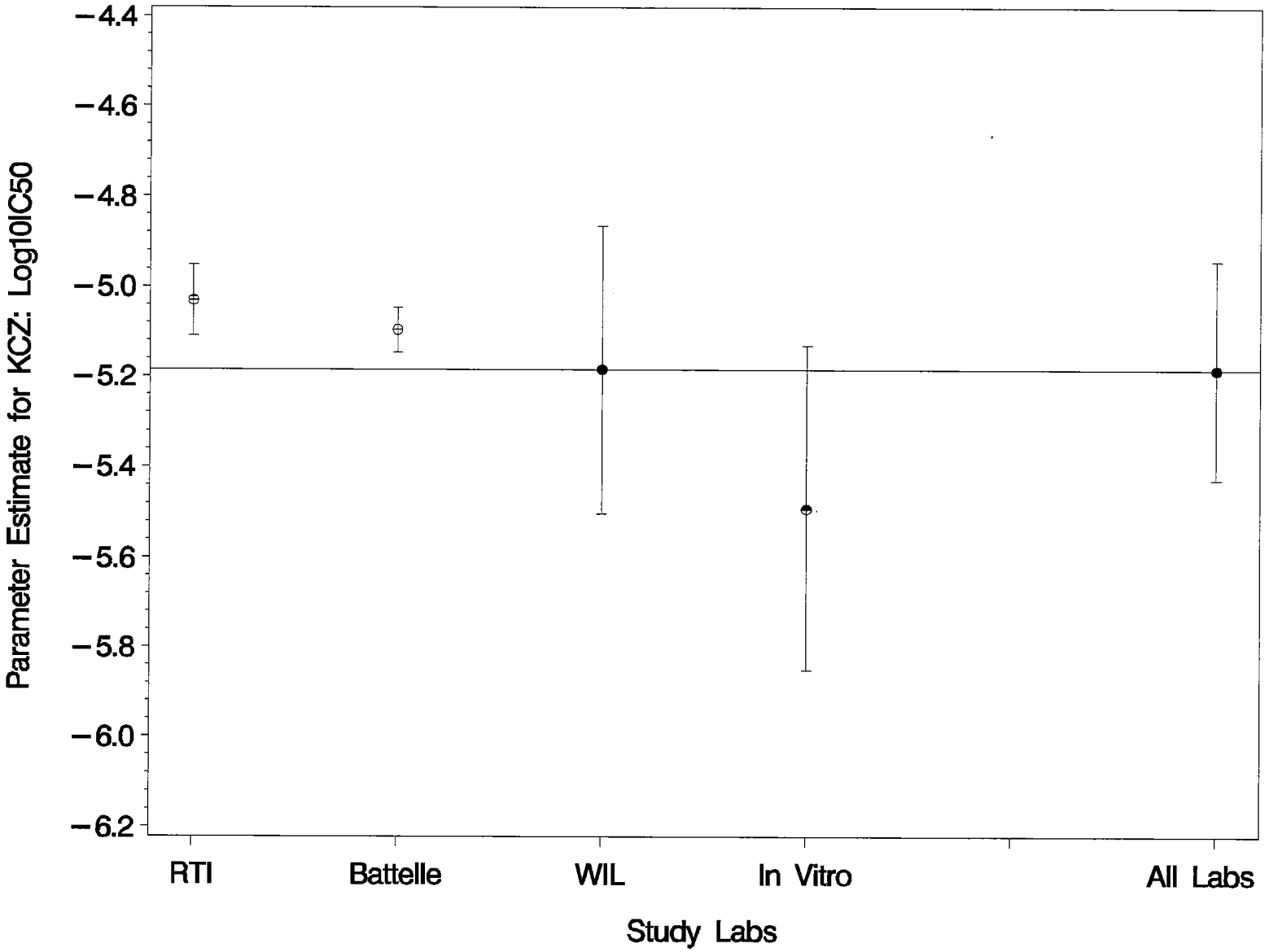

Figure 23. Ketoconazole: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories

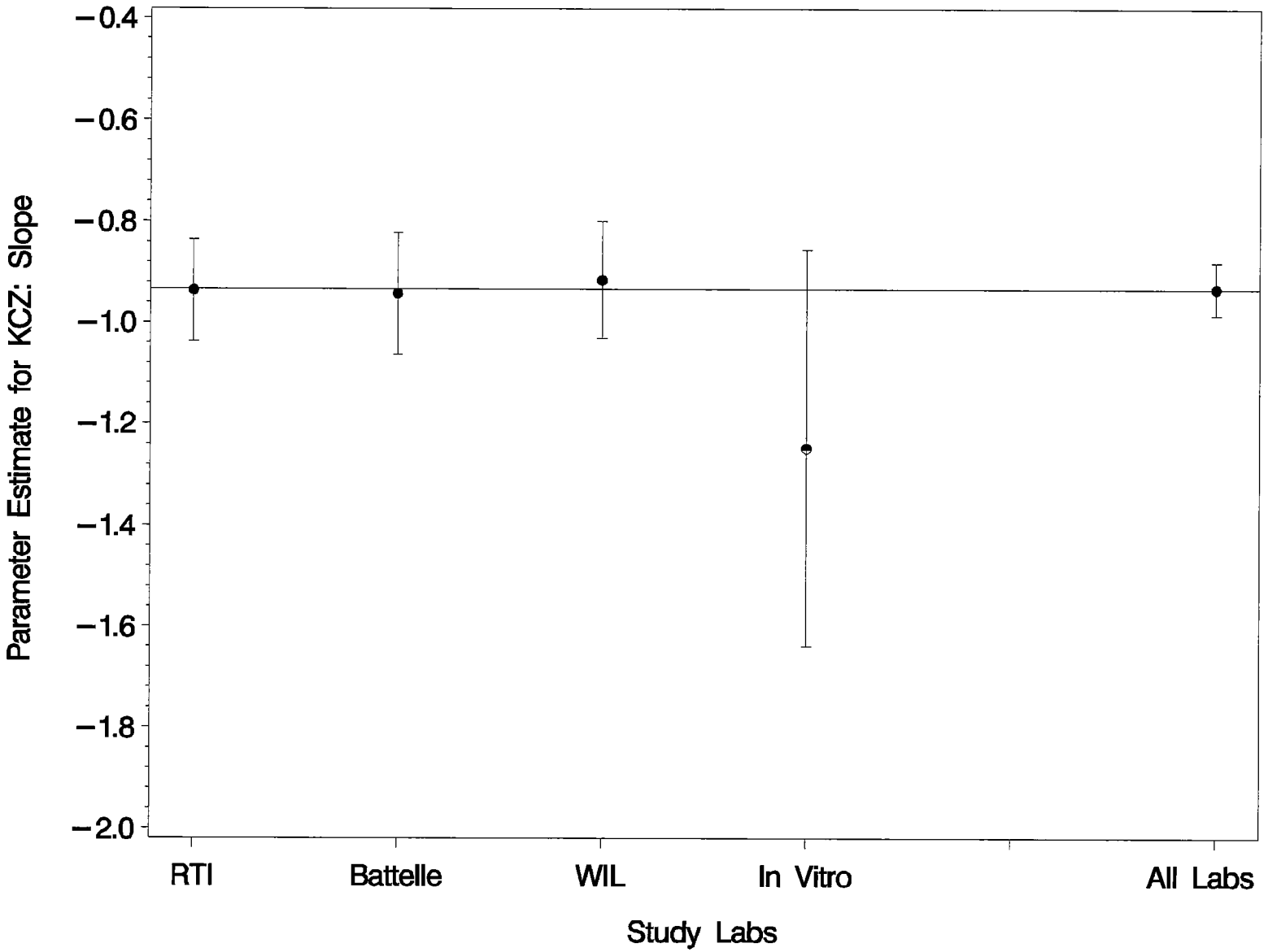

Figure 24. **Ketoconazole: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

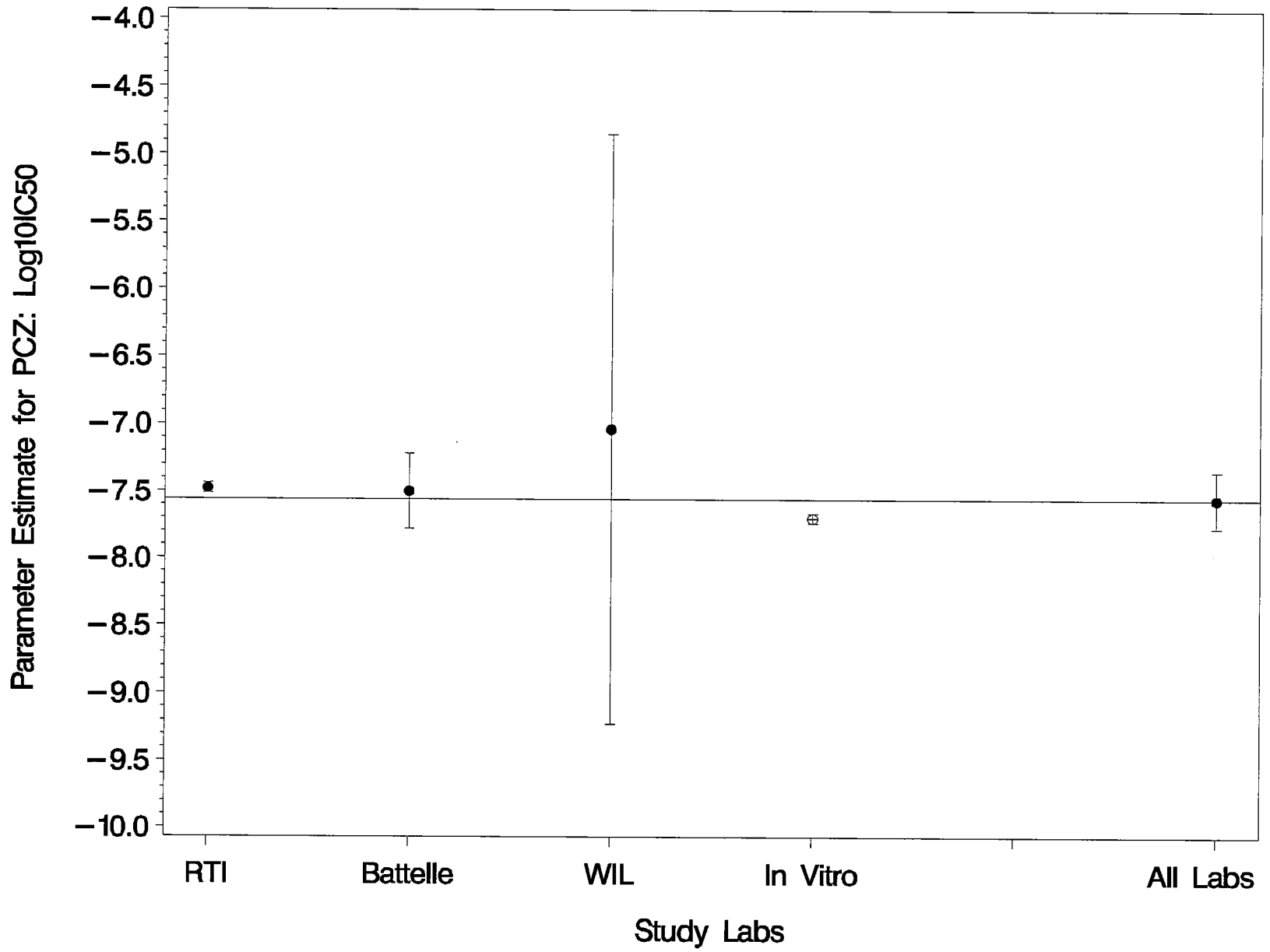

Figure 25. **Prochloraz: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

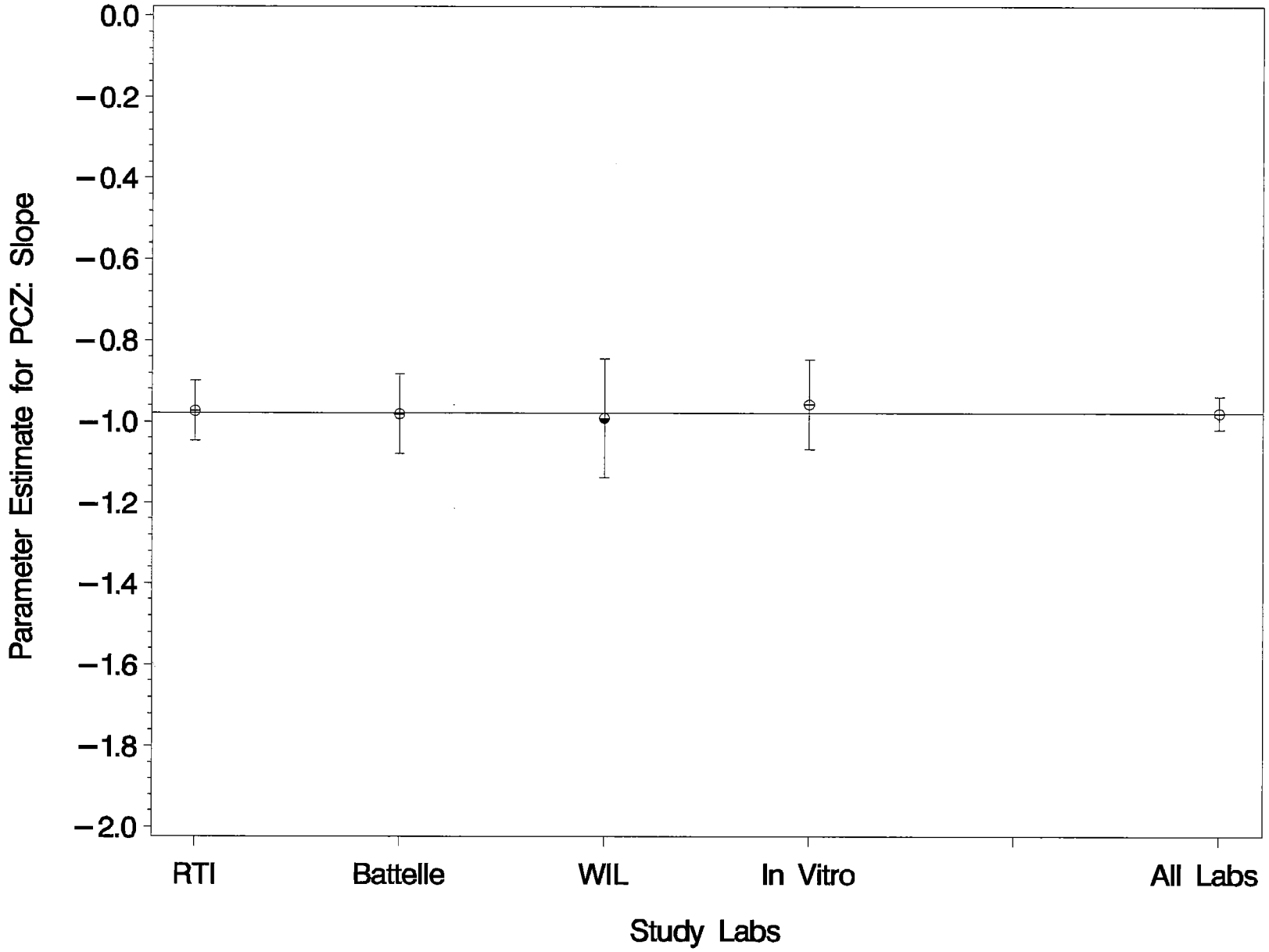

Figure 26. **Prochloraz: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

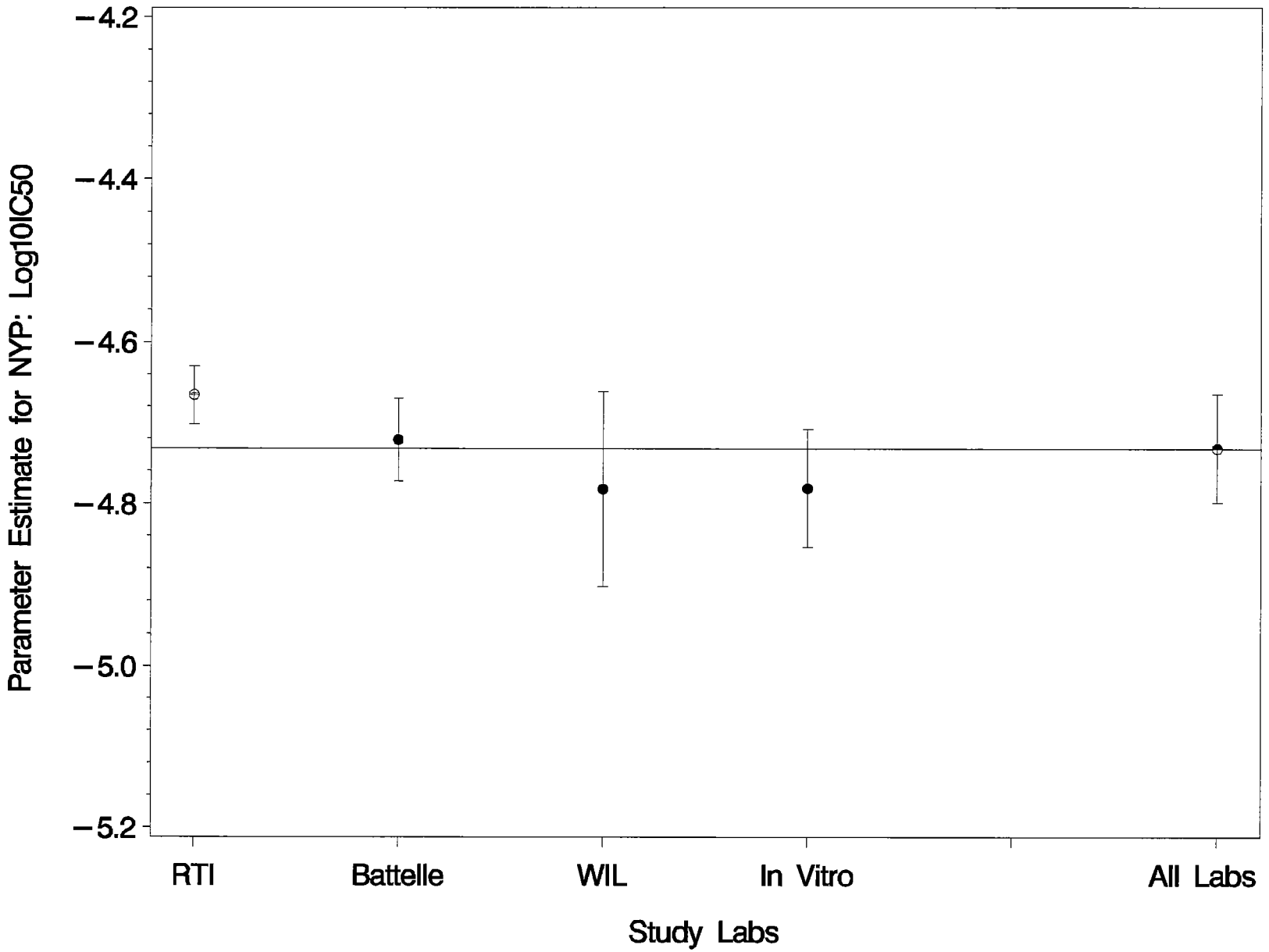

Figure 27. **4-Nonylphenol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

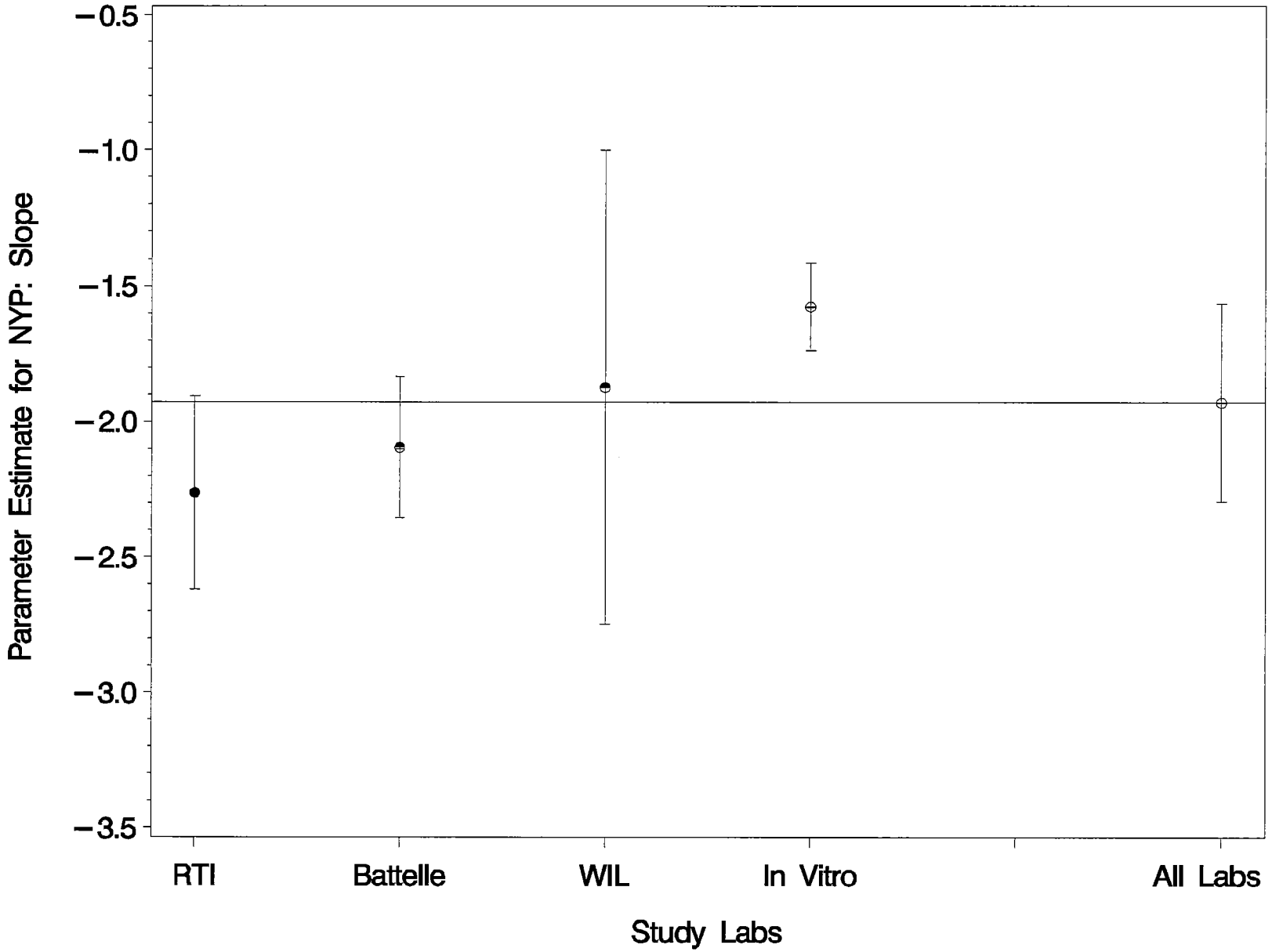

Figure 28. **4-Nonylphenol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

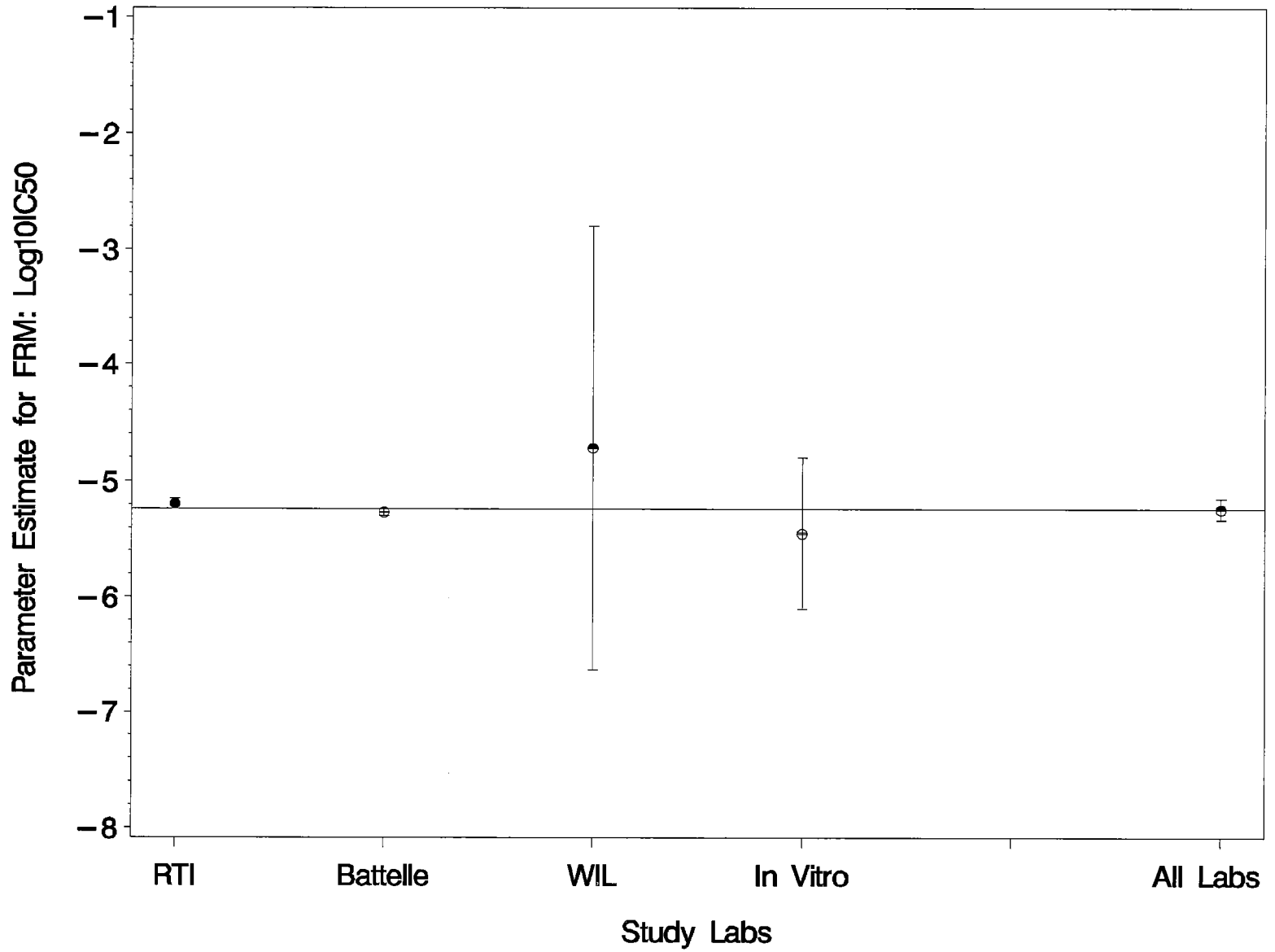

Figure 29. **Fenarimol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

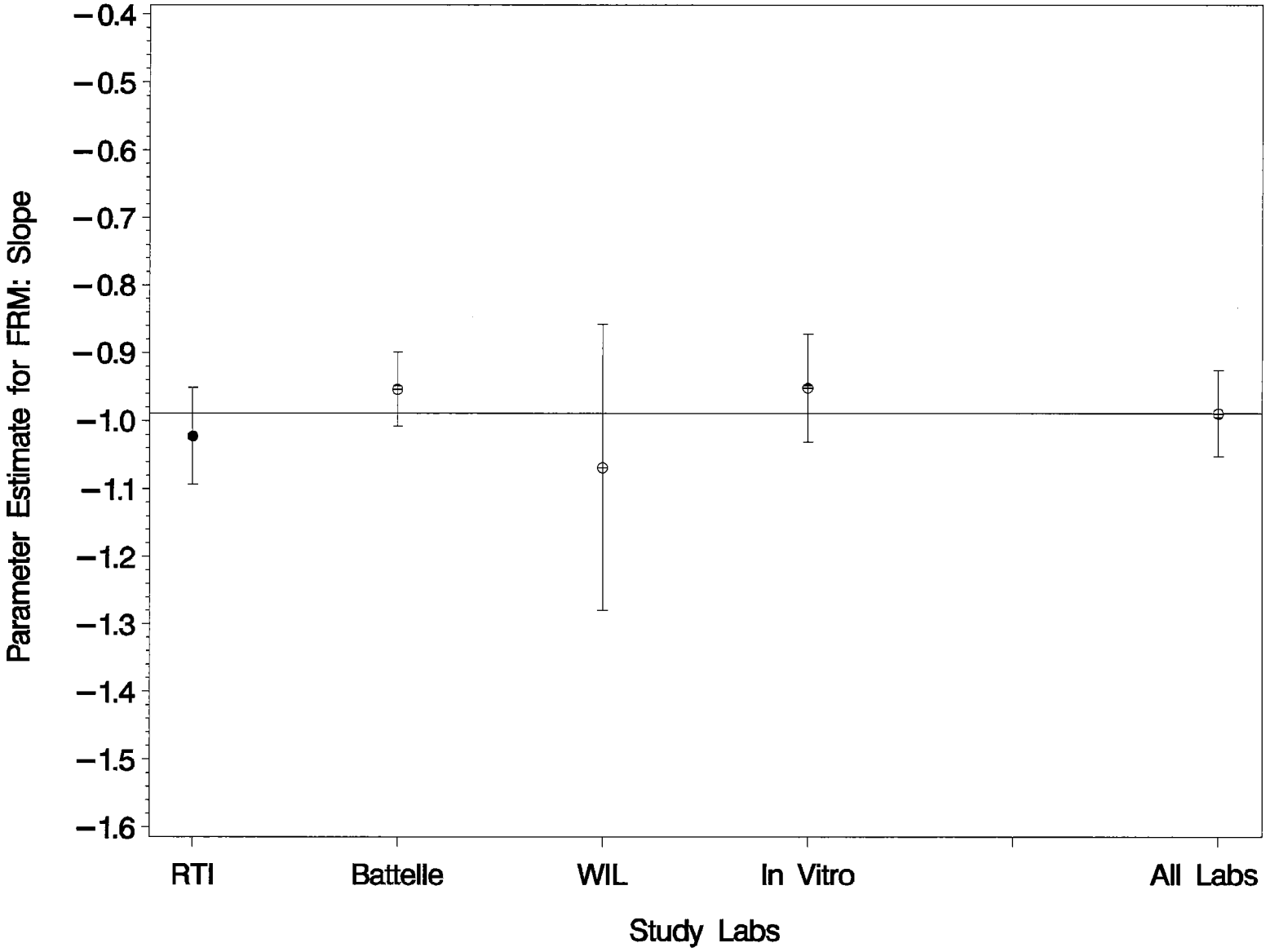

Figure 30. **Fenarimol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

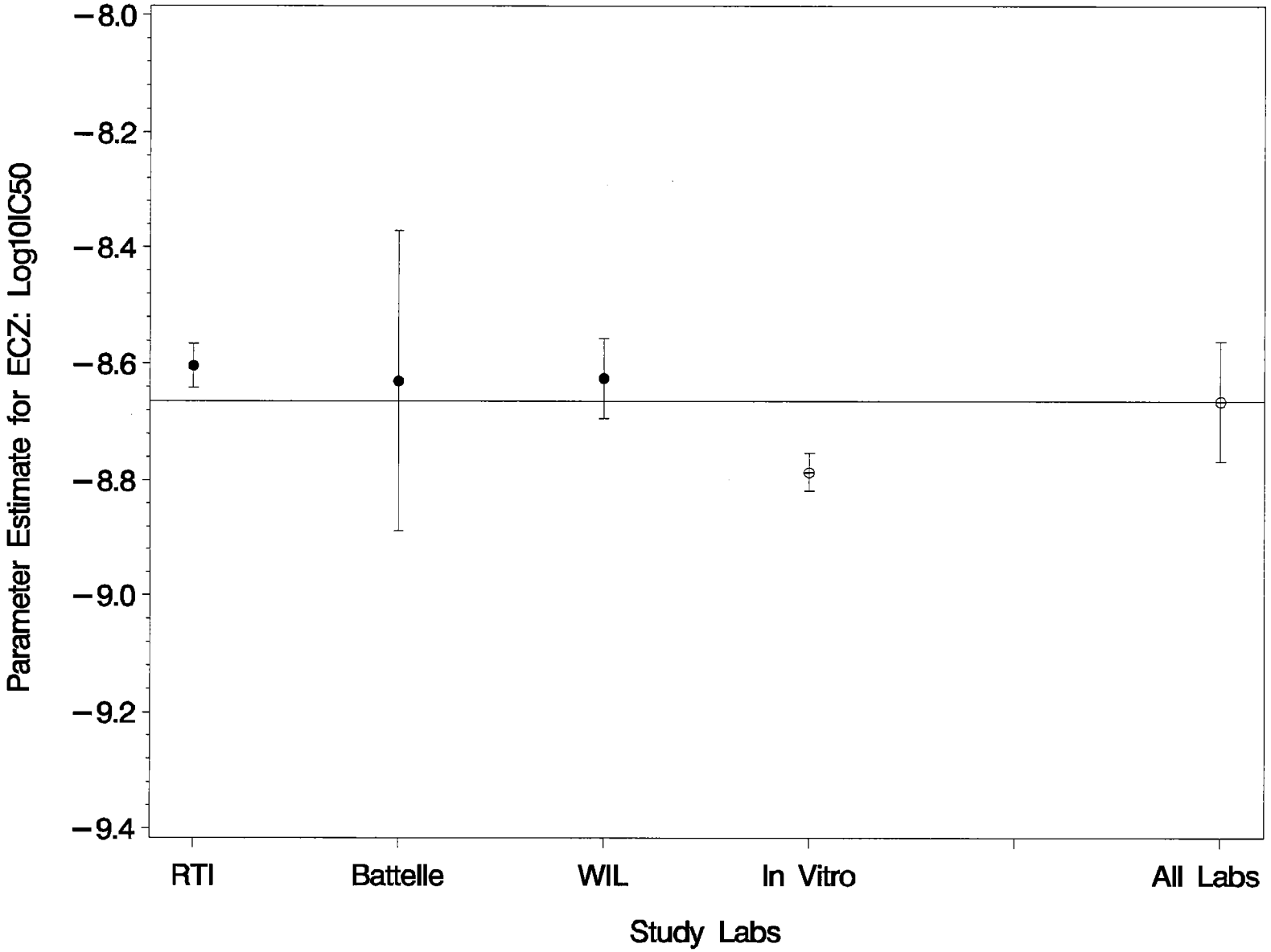

Figure 31. **Econazole: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

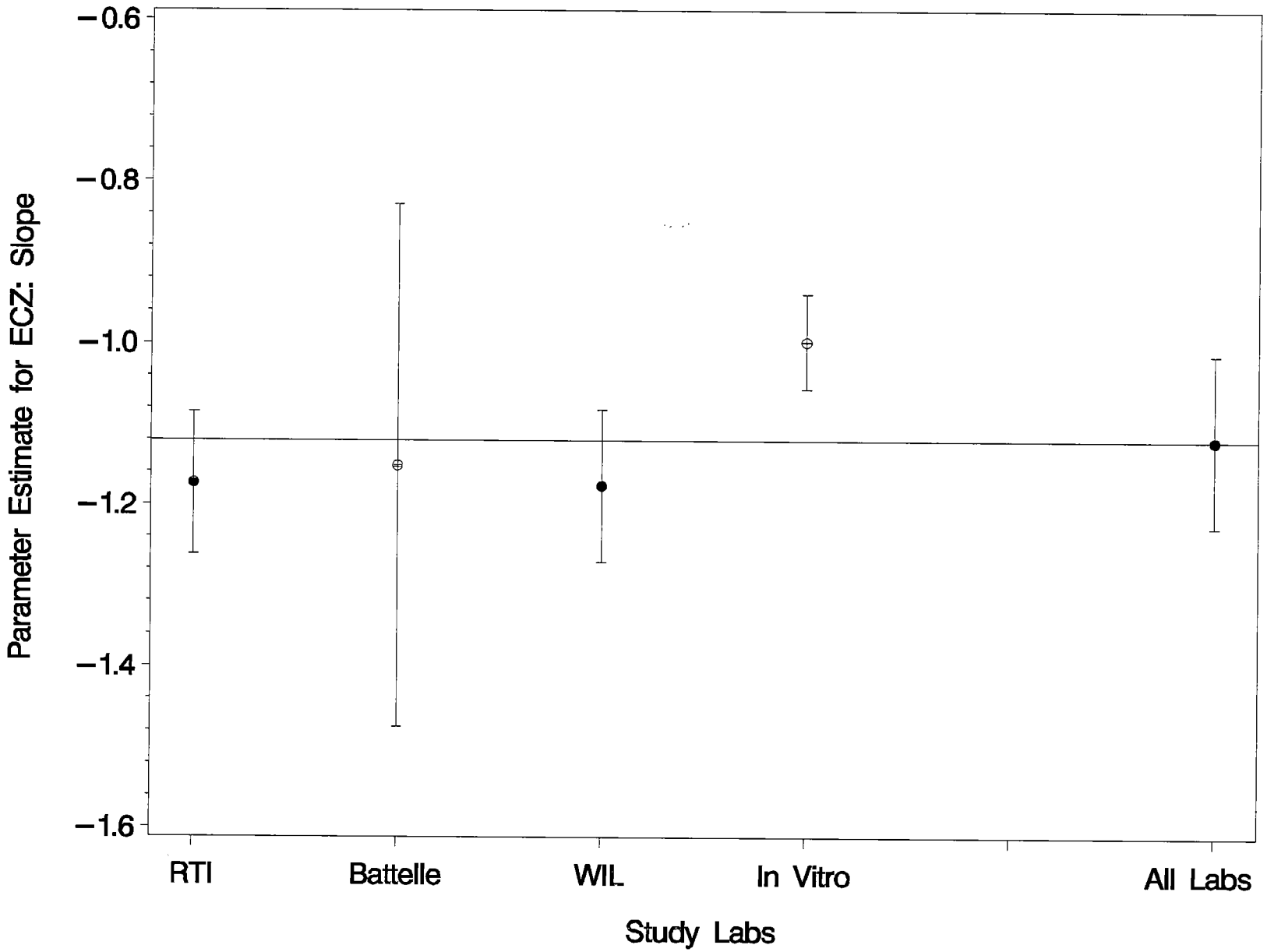

Figure 32. **Econazole: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

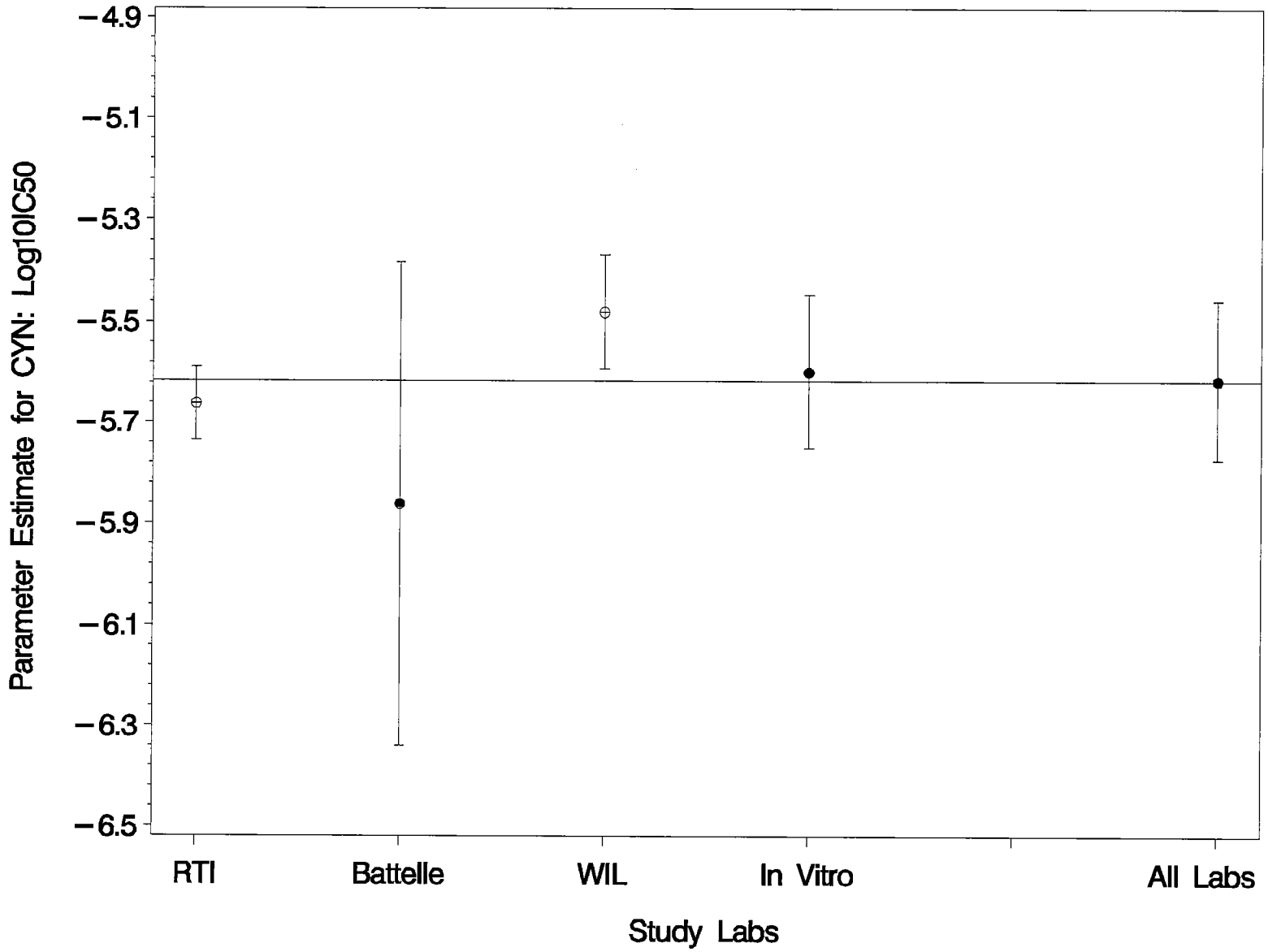

Figure 33. Chrysin: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.

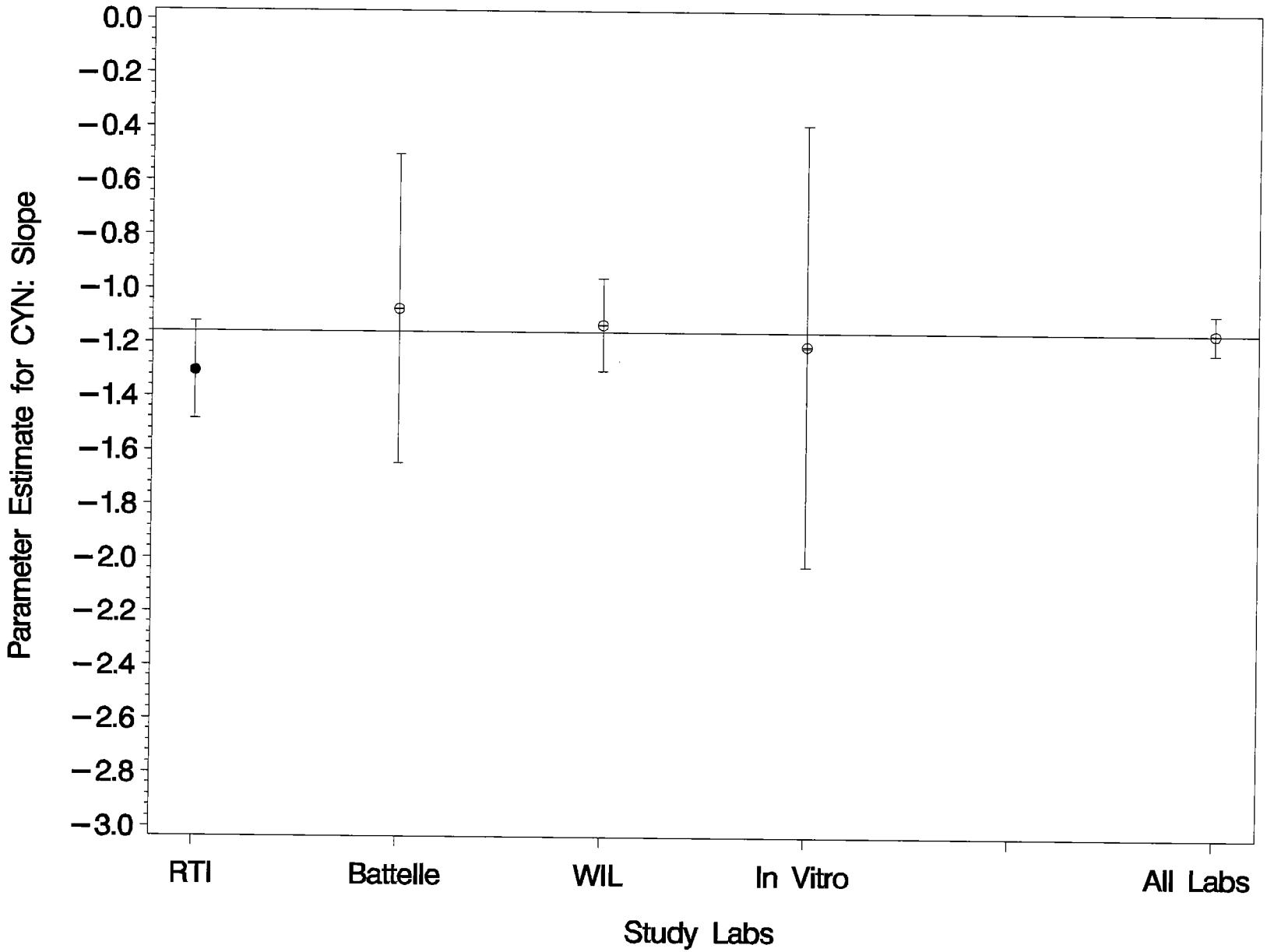

Figure 34. **Chrysin: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

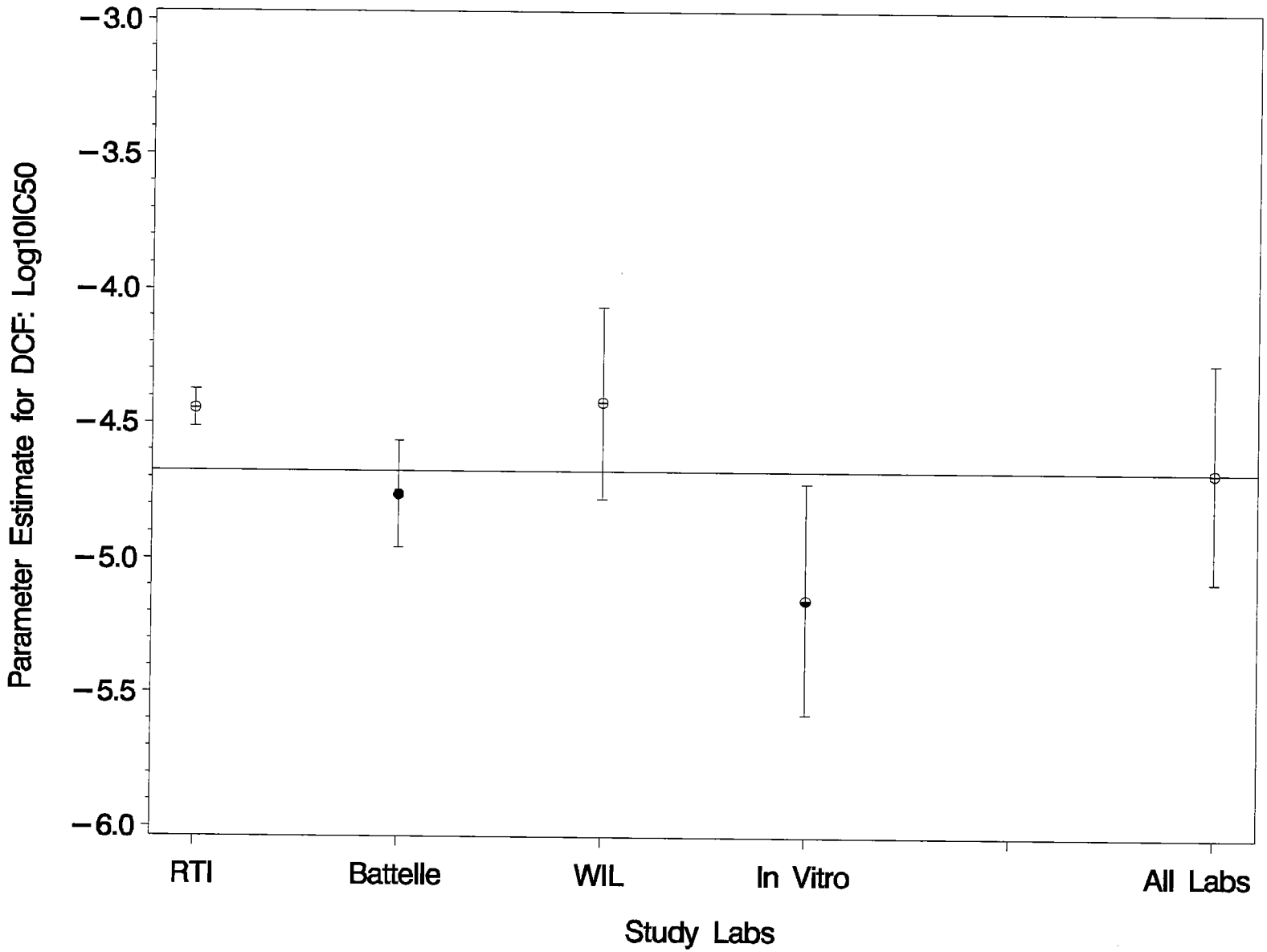

Figure 35. **Dicofol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

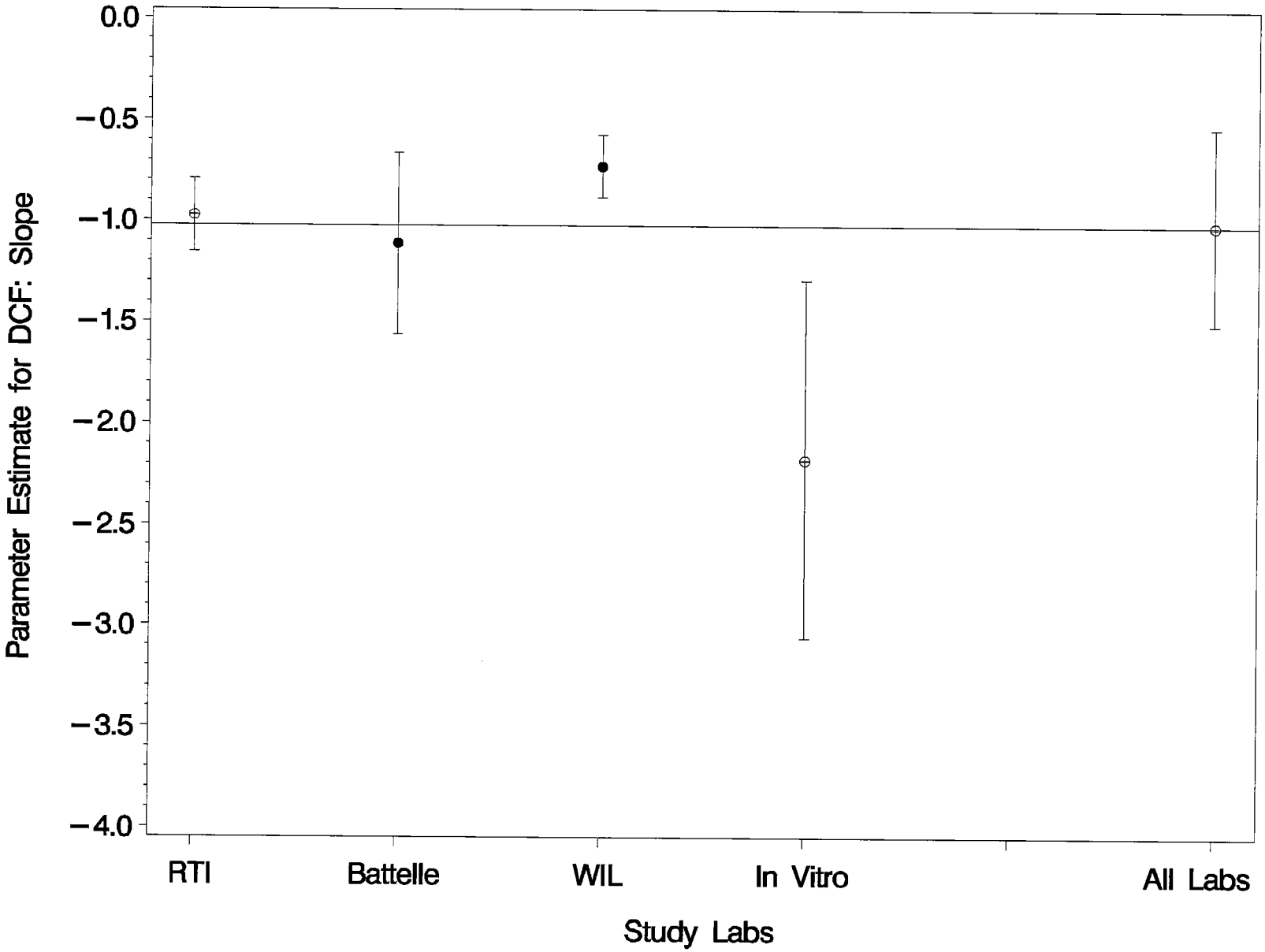

Figure 36. **Dicofol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

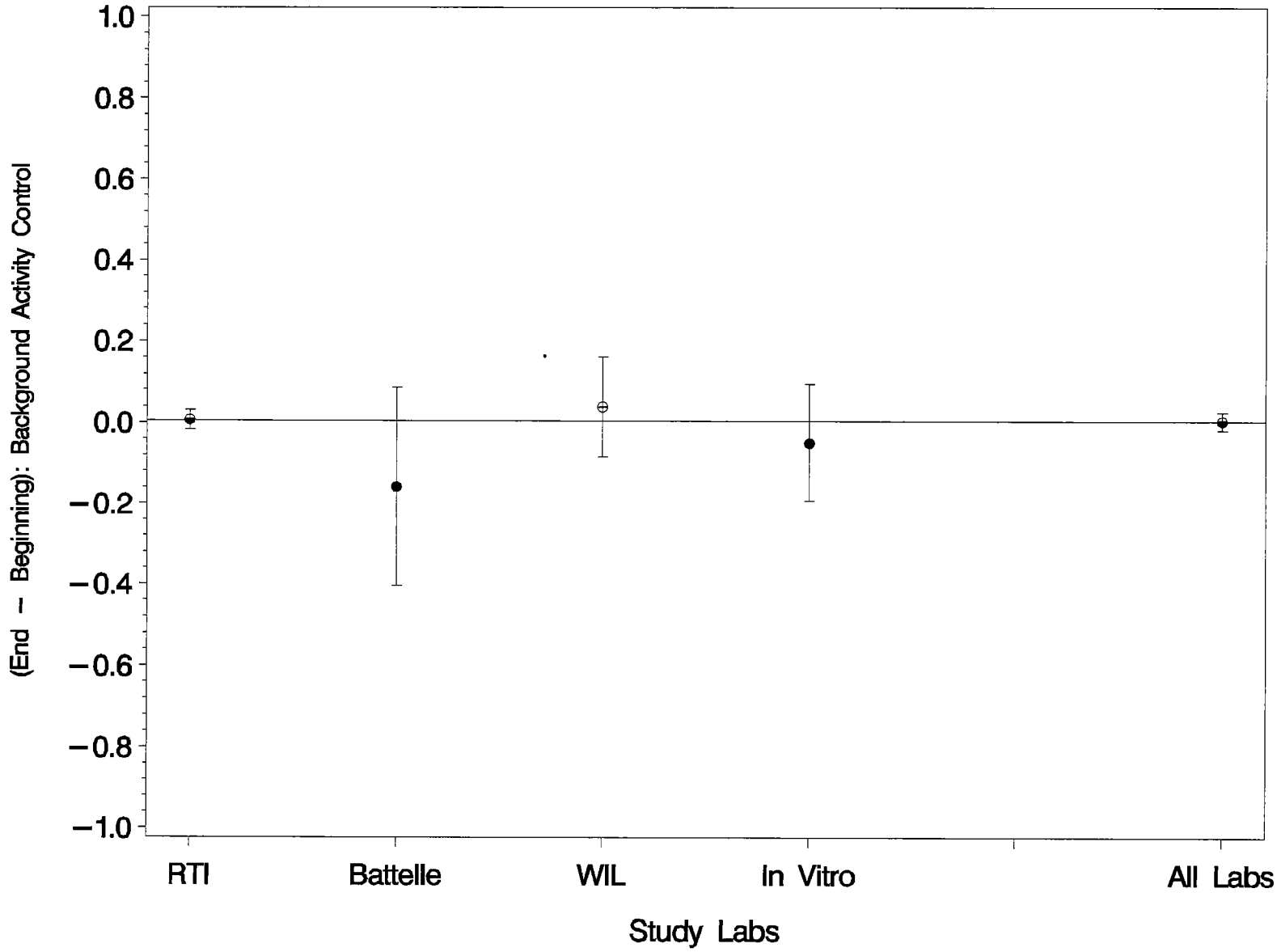

Figure 37. **Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Difference (End Minus Beginning) for Background Activity Controls in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

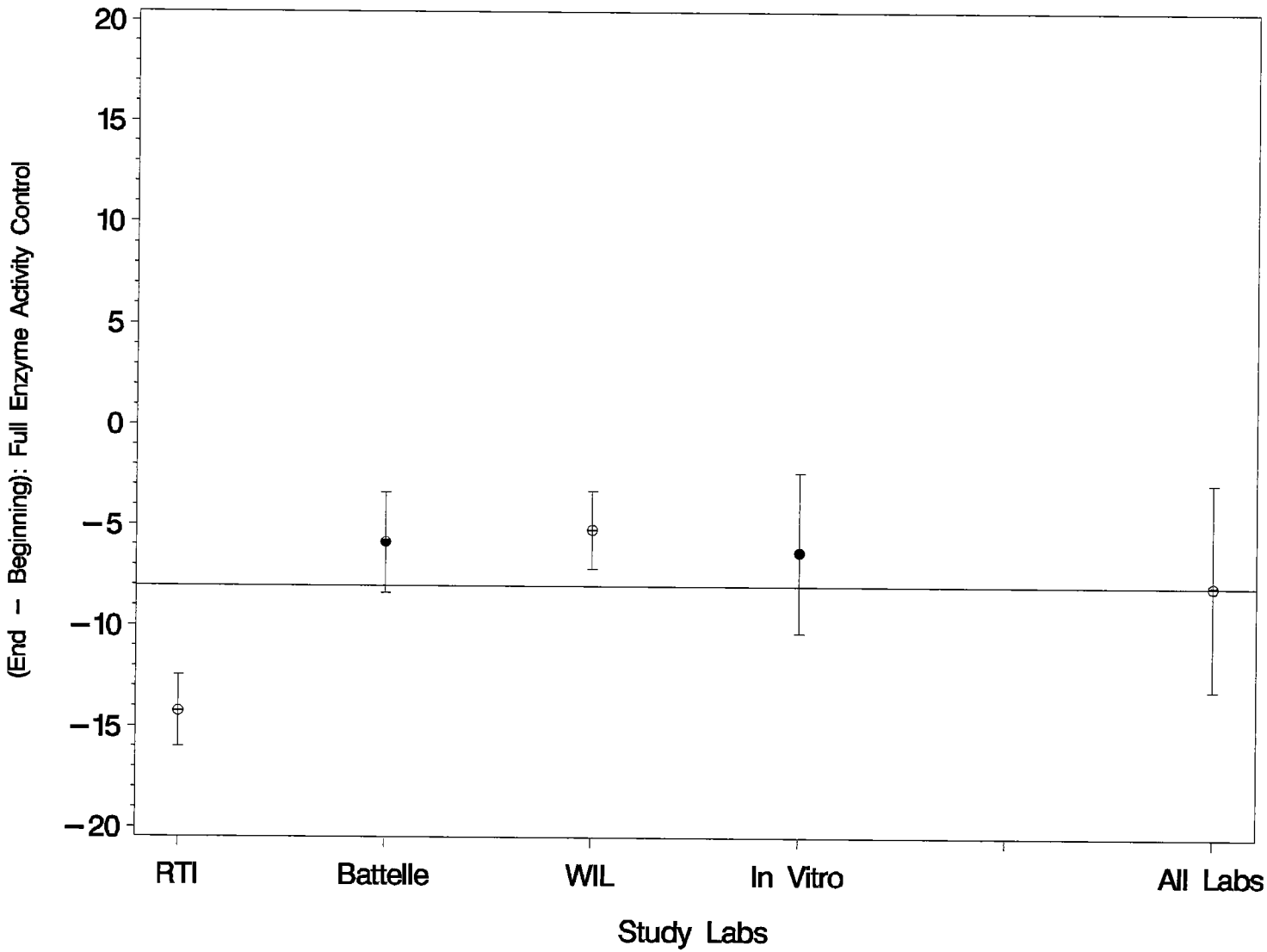

Figure 38. **Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Difference (End Minus Beginning) for Full Enzyme Activity Controls in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

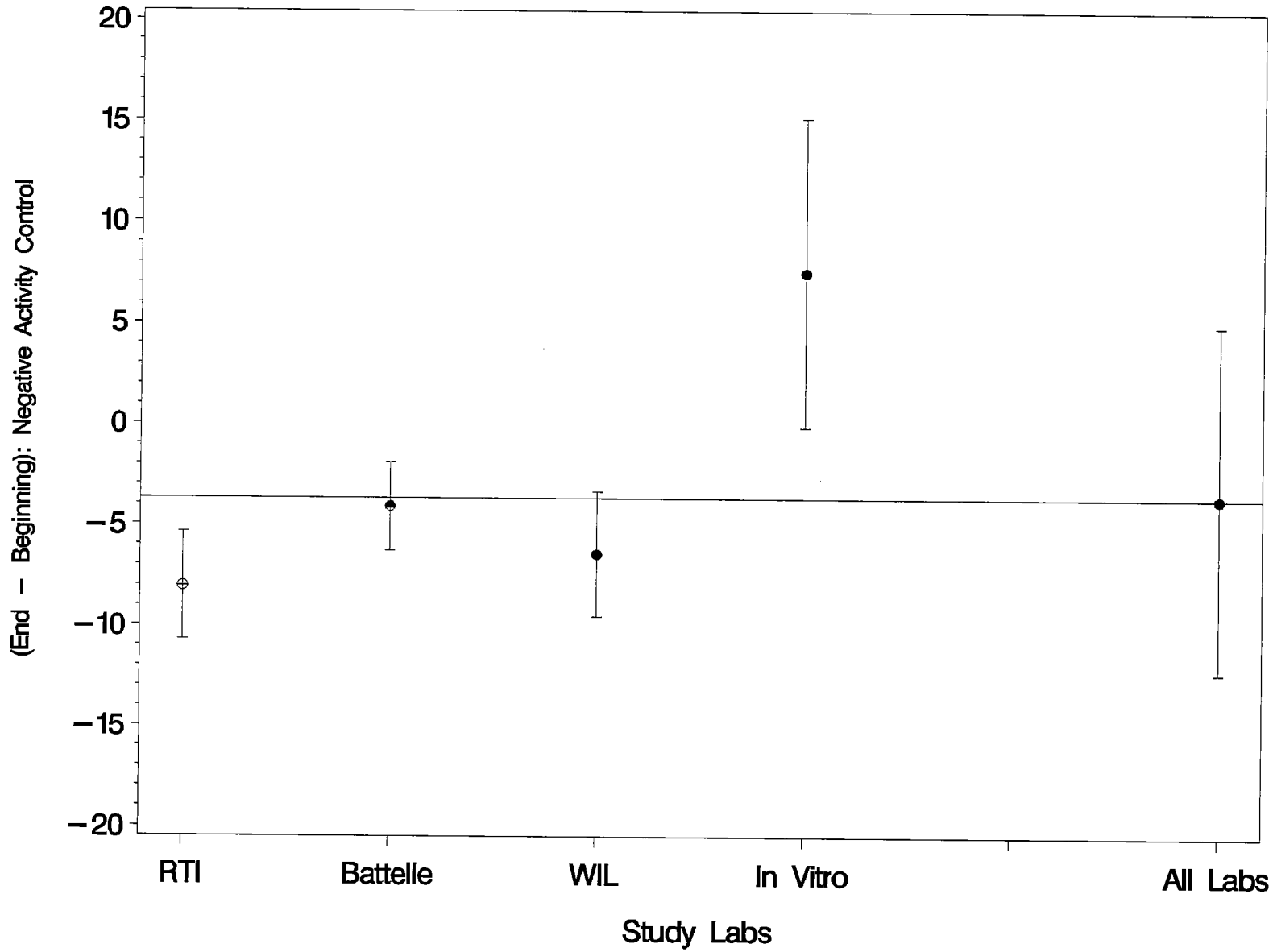

Figure 39. **Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Difference (End Minus Beginning) for Negative Controls in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

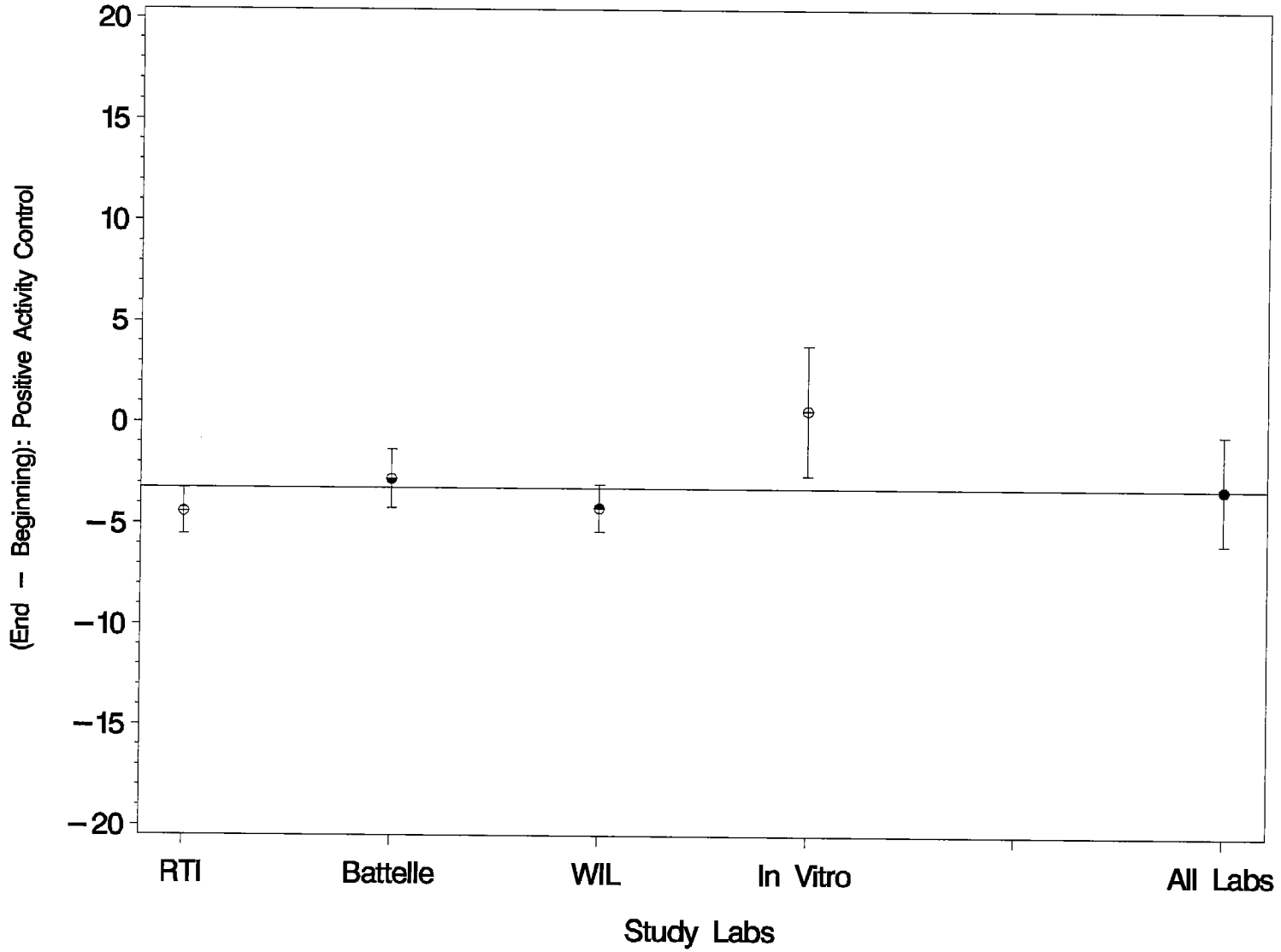

Figure 40. **Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Difference (End Minus Beginning) for Positive Controls in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

4.0 DISCUSSION

4.1 RECOMBINANT MICROSOMAL TASK

In this task, the responsiveness of the recombinant microsomal aromatase assay to ten reference chemicals and positive and negative control reference chemicals (4-OH ASDN and lindane) were evaluated. The assay was conducted by a lead laboratory (RTI) and three participating laboratories using microsomes obtained by RTI from a commercial supplier and then distributed by RTI to the participating laboratories. 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. In addition, this discussion section presents a comparison of the results from the present study with those obtained from using human placental microsomes (WA 4-16, Task 7).

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 that were within or near ± 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. Finally, the use of a 5-point standard curve ranging from 5 to 125 µg/mL will also improve the accuracy and precision of the assay.

The full enzyme activity control value for this assay was required to be at least 0.03 nmol/mg protein/min according to the acceptance criteria in the study plan. The enzyme activity for the recombinant microsomes was approximately 10-fold higher than what was required. It is also interesting to note that the enzyme activity for the recombinant microsomes was approximately 10-fold higher than what was measured for the human placental microsomes, i.e.

overall full enzyme activity for the recombinant and placental microsomes was 0.343 ± 0.019 and 0.054 ± 0.005 nmol/mg protein/min, respectively (the placental microsomal value included both sources of microsomes used in WA 4-16, Task 7). A possible explanation for the greater enzyme activity of the recombinant microsomes is that the reductase may be linked with the CYP19, whereas this is not the case for the placental microsomes (personal communication from RTI). Regardless, the level of enzyme activity needed to conduct this task was achieved. 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 only a single replicate when one laboratory had a much higher % CV for a given replicate, e.g. >25 percent, 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 lower. The lead laboratory had an overall inter-assay precision value of 19 percent, and the results for Battelle, In Vitro, and WIL were 16, 100, and 37 percent (the high value for In Vitro was attributed to the inaccurate protein concentration determinations for a few replicates). These results indicated that the assay was also consistent from day to day for multiple laboratories. Using RTI's results as the standard, a measure of accuracy (as measured by % RE) was able to be determined, which for Battelle, In Vitro, and WIL were -17.4, 232.8, and -10.1 percent, respectively. Again, the high value for In Vitro was attributed to a few aberrant samples. Thus, more than one of the laboratories demonstrated that assay precision at a level of approximately 15 to 20 percent could be achieved and, for accuracy, a level of approximately 10 to 20 percent was attainable.

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 about 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 57.0 ± 0.9, 54.2 ± 0.8, 48.5 ± 2.1, and 49.7 ± 1.7 percent for RTI, Battelle, In Vitro, and WIL, respectively. The overall task mean ± SEM percent of control value was 52.4 ± 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 4.1, 4.7, 13.7, and 8.4 percent, respectively (Table 10). Using RTI for comparison in order to obtain a measure of accuracy, resulted in % RE values of -4.9, -14.8, and -12.7 percent for Battelle, In Vitro, and WIL, respectively. Thus, the precision and accuracy of the assay to measure 4-OH ASDN inhibition was 15 percent or better for all laboratories.

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 80 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 3.2, 3.4, 14.9, and 5.5 percent, respectively (Table 12). Using RTI for comparison in order to obtain a measure of accuracy, resulted in % RE values of -0.6, -17.6, and -1.5 percent for Battelle, In Vitro, and WIL, respectively. Thus, with the exception of one high % RE value, the precision and accuracy of the assay to measure 4-OH ASDN inhibition was 15 percent or better for all laboratories.

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 39. 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 by 4-nonylphenol 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. When 4-nonylphenol was tested using human placental microsomes (WA 4-16, Task 7), a similar degree of inhibition occurred as was observed in the present study. 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 reference chemical on aromatase activity.

Further explanation is required for the error described at the beginning of this report. An examination of the individual laboratory reports indicated 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 39. Summary of Previous and Present Task and Literature IC_{50} Values

Reference Compound	IC_{50} Values (μM or nM)[a]				Literature Reference
	Recombinant Microsomes[b]	Human Placental Microsomes[b,c]	Other Previous Tasks[d]	Literature Values	
Aminoglutethimide	4.8 ± 0.4	4.3 ± 0.2	4.4 ± 0.1 5.6 ± NA[e]	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	No inhibition	--	None	Roberge, et. al., 2004
Chrysin	2.5 ± 0.4	2.5 ± 0.3	4.1 ± 0.3 3.2 ± NA[e]	0.7 4.4 11.0	LeBail, et. al., 2001 Jeong, et. al., 1999 Chen, et. al., 1997
Dibenz[a,h]anthracene	No inhibition	No inhibition	--	--	--
Dicofol	25.0 ± 7.8	29.1 ± 8.6	--	>50	Vinggaard, et. al., 2000
Econazole	2.2 ± 0.2	1.6 ± 0.3	2.1 ± 0.1 2.8 ± NA[e]	0.03	Mason, et. al., 1987
Fenarimol	5.6 ± 0.7	5.8 ± 0.6	--	10	Vinggaard, et. al., 2000
Ketoconazole	7.6 ± 1.7	6.5 ± 0.7	7.5 ± 0.6 15.0 ± NA[e]	6 15 60 7.3	White, et. al., 1999 Rowlands, et al., 1995 Mason, et. al., 1987 Ayub and Levell, 1988
4-Nonylphenol	18.9 ± 1.2	29.1 ± 3.0	No inhibition	None	Laws, et. al., 2000
Prochloraz	28.5 ± 3.1	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. WA 4-16, Task 7
d. Previous tasks include WA 2-24, WA 4-10, and WA 4-16 (Tasks 4, 5, and 6).
e. 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 been 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. The subsequent adjustment in concentrations contributed to 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 use of three replicates where the concentrations have been settled upon, may substantially improve the precision and accuracy obtained for the assay. In addition, there were a few instances in the present study when a laboratory used information from the human placental study to decide upon concentrations to be tested in the recombinant assay for all three replicates, which resulted in less variability for its three replicates relative to the other laboratories that did not do so. Consequently, the interlaboratory variability estimates in such instances may be underestimated, assuming that the "insider" information used increased the variability for the one laboratory relative to the other laboratories.

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.

4.2 RECOMBINANT AND HUMAN PLACENTAL MICROSOMAL TASKS – A COMPARISON

The experimental designs for Work Assignments 4-17, Task 4 and 4-16, Task 7 differed only in the types of microsomes used for testing the reference chemicals and positive and negative controls. This section examines a few of the comparisons that can now be made when the aromatase assay is conducted using recombinant or human placental microsomes. Table 40 summarizes and Figures 41 through 44 illustrates the recombinant and placental $\log_{10}IC_{50}$ and slope overall mean and %CV values (with 95% confidence intervals for each) by reference

chemical. Table 41 summarizes and Figure 45 illustrates the recombinant and placental parameter estimates (with their corresponding 95% confidence intervals) for the controls.

The $log_{10}IC_{50}$ values were very similar for both types of microsomes, especially if the associated 95% confidence intervals were taken into consideration (Figure 41). If just the means are evaluated, there were approximately as many recombinant values above as there were below the placental values. Average % CV values were also very similar, although the means for the recombinant microsomes were generally higher than what was determined for the placental microsomes (Figure 43). The 95% confidence intervals were larger in every instance for the recombinant microsomes, except for econozole. Also, for ketoconazole, prochloraz, and dicofol, the 95% confidence intervals were much larger than what was determined for the placental microsomes. The reasons for this greater variability in these few instances were attributed to a few aberrant values for one or two laboratories as described in the results and discussion sections for the recombinant microsomes.

The slope values were very similar for both types of microsomes, especially if the associated 95% confidence intervals were taken into consideration (Figure 42). If just the means are evaluated, there were approximately as many recombinant values above as there were below the placental values. Average % CV values were also very similar, except for 4-nonylphenol and dicofol where the % CV was higher (Figure 44). However, even for these two reference chemicals, there was no difference when the 95% confidence intervals were taken into consideration.

The control values were very similar for both types of microsomes (Figure 45). The relatively large 95% confidence interval for the recombinant full enzyme activity control was attributed to the aberrant values in a few replicates as was described in the report. For the 95% confidence intervals associated with the negative control of both microsomal types, it will be necessary to determine why there was such an opposite response obtained for In Vitro in comparison to the other three laboratories. At the time this report was prepared, an explanation had not been found.

Table 40. Comparison of Recombinant and Human Placental Microsome Assays $Log_{10}IC_{50}$ and Slope Values

Obs	Assay	Reference chemical	Parameter	Overall Mean with 95% C.I.	CV (percent) with 95% C.I.
1	Recombinant	AG	Log_IC50	-5.325 (-5.411,-5.239)	15.647 (9.763,39.077)
2	Recombinant	AG	Slope	-0.972 (-1.005,-0.939)	3.345 (2.726,4.329)
3	Recombinant	KCZ	Log_IC50	-5.186 (-5.428,-4.943)	39.467 (22.51,185.087)
4	Recombinant	KCZ	Slope	-0.934 (-0.986,-0.881)	4.592 (2.955,10.168)
5	Recombinant	PCZ	Log_IC50	-7.562 (-7.772,-7.353)	29.237 (16.024,174.277)

Obs	Assay	Reference chemical	Parameter	Overall Mean with 95% C.I.	CV (percent) with 95% C.I.
6	Recombinant	PCZ	Slope	-0.979 (-1.019,-0.938)	4.029 (3.231,5.354)
7	Recombinant	NYP	Log_IC50	-4.731 (-4.798,-4.665)	11.409 (6.913,31.719)
8	Recombinant	NYP	Slope	-1.93 (-2.295,-1.565)	14.548 (8.941,38.504)
9	Recombinant	FRM	Log_IC50	-5.242 (-5.334,-5.15)	11.832 (6.432,58.984)
10	Recombinant	FRM	Slope	-0.99 (-1.053,-0.926)	4.32 (2.514,14.136)
11	Recombinant	ECZ	Log_IC50	-8.664 (-8.768,-8.561)	18.347 (11.3,48.541)
12	Recombinant	ECZ	Slope	-1.121 (-1.228,-1.014)	7.418 (4.622,18.391)
13	Recombinant	CYN	Log_IC50	-5.616 (-5.773,-5.458)	26.852 (15.982,85.968)
14	Recombinant	CYN	Slope	-1.158 (-1.231,-1.086)	6.052 (4.711,8.475)
15	Recombinant	DCF	Log_IC50	-4.676 (-5.081,-4.272)	70.615 (38.533,776.709)
16	Recombinant	DCF	Slope	-1.024 (-1.511,-0.536)	28.065 (15.24,159.525)
17	Placental	AG	Log_IC50	-5.391 (-5.422,-5.359)	5.765 (3.621,13.865)
18	Placental	AG	Slope	-0.987 (-1.037,-0.936)	3.751 (2.268,10.388)
19	Placental	KCZ	Log_IC50	-5.181 (-5.202,-5.161)	4.616 (3.667,6.23)
20	Placental	KCZ	Slope	-0.957 (-1.017,-0.898)	6.017 (4.68,8.439)
21	Placental	PCZ	Log_IC50	-7.651 (-7.736,-7.566)	14.682 (8.903,41.008)
22	Placental	PCZ	Slope	-0.97 (-1.004,-0.935)	3.317 (2.398,5.381)
23	Placental	NYP	Log_IC50	-4.583 (-4.596,-4.57)	2.845 (2.181,4.092)
24	Placental	NYP	Slope	-1.902 (-2.002,-1.803)	5.225 (4.357,6.534)
25	Placental	FRM	Log_IC50	-5.194 (-5.235,-5.153)	5.504 (3.038,23.906)
26	Placental	FRM	Slope	-0.992 (-1.038,-0.946)	4.434 (3.363,6.516)
27	Placental	ECZ	Log_IC50	-8.818 (-8.953,-8.682)	22.545 (13.342,72.698)
28	Placental	ECZ	Slope	-1.003 (-1.131,-0.875)	8.824 (5.181,27.943)
29	Placental	CYN	Log_IC50	-5.627 (-5.763,-5.49)	22.583 (13.335,73.684)

Obs	Assay	Reference chemical	Parameter	Overall Mean with 95% C.I.	CV (percent) with 95% C.I.
30	Placental	CYN	Slope	-1.324 (-1.487,-1.16)	10.199 (6.58,22.526)
31	Placental	DCF	Log_IC50	-4.549 (-4.78,-4.318)	47.367 (30.42,114.548)
32	Placental	DCF	Slope	-0.673 (-0.795,-0.552)	15.242 (9.95,32.692)

Table 41. Comparison of Recombinant and Human Placental Microsome Assays Control Values

Obs	Portion Effect	Assay	Estimate with 95% C.I.
1	BAC	Placental	0.071 (-0.061,0.203)
2	FEAC	Placental	-9.543 (-10.552,-8.535)
3	Negative	Placental	-3.611 (-19.896,12.674)
4	Positive	Placental	-2.086 (-6.538,2.366)
5	BAC	Recombinant	0.005 (-0.017,0.027)
6	FEAC	Recombinant	-8.032 (-13.16,-2.903)
7	Negative	Recombinant	-3.732 (-12.321,4.858)
8	Positive	Recombinant	-3.25 (-5.945,-0.555)

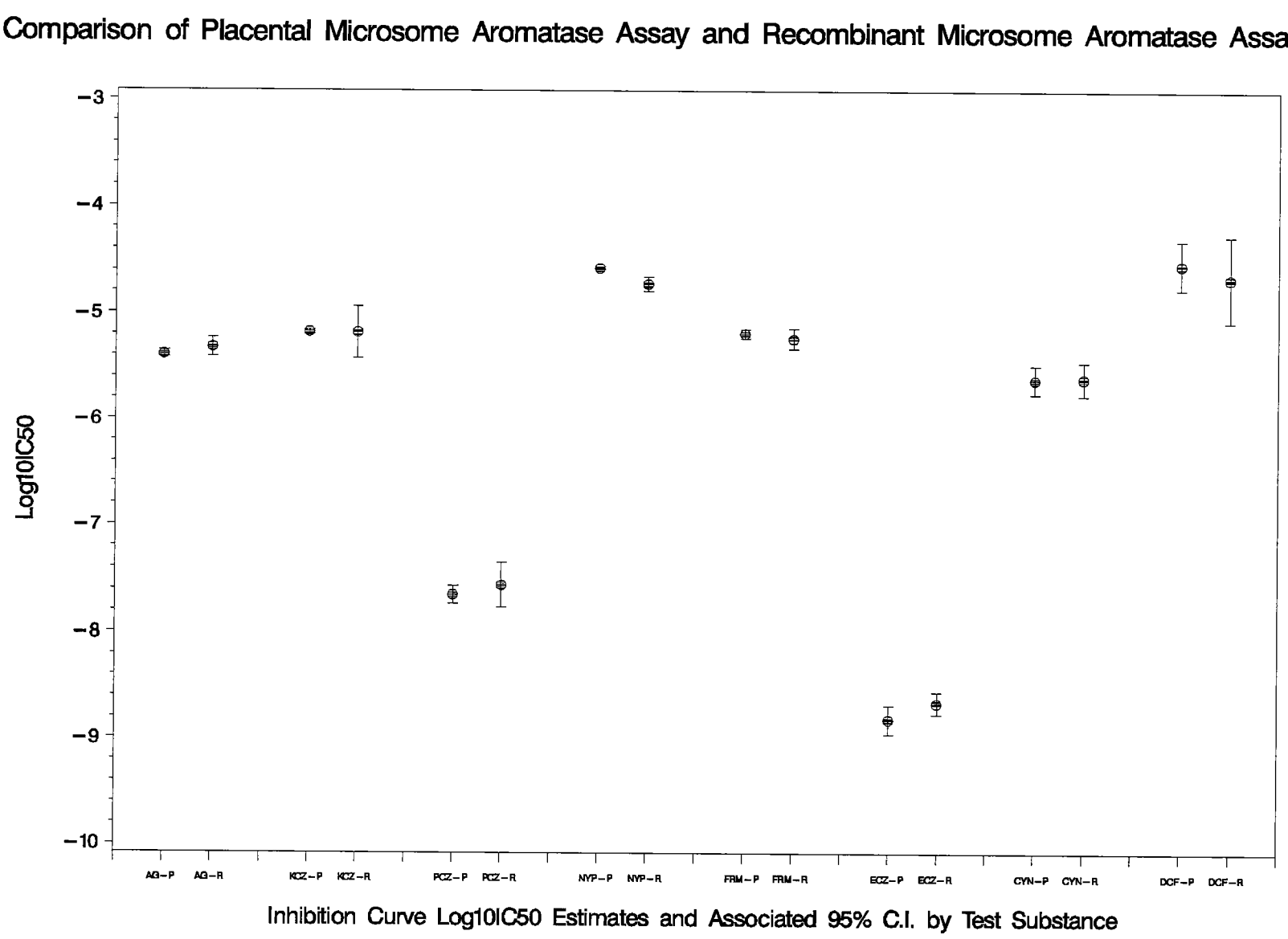

Figure 41. Comparison of Placental Microsome Aromatase Assay and Recombinant Microsome Aromatase Assay: $Log_{10}IC_{50}$ Values

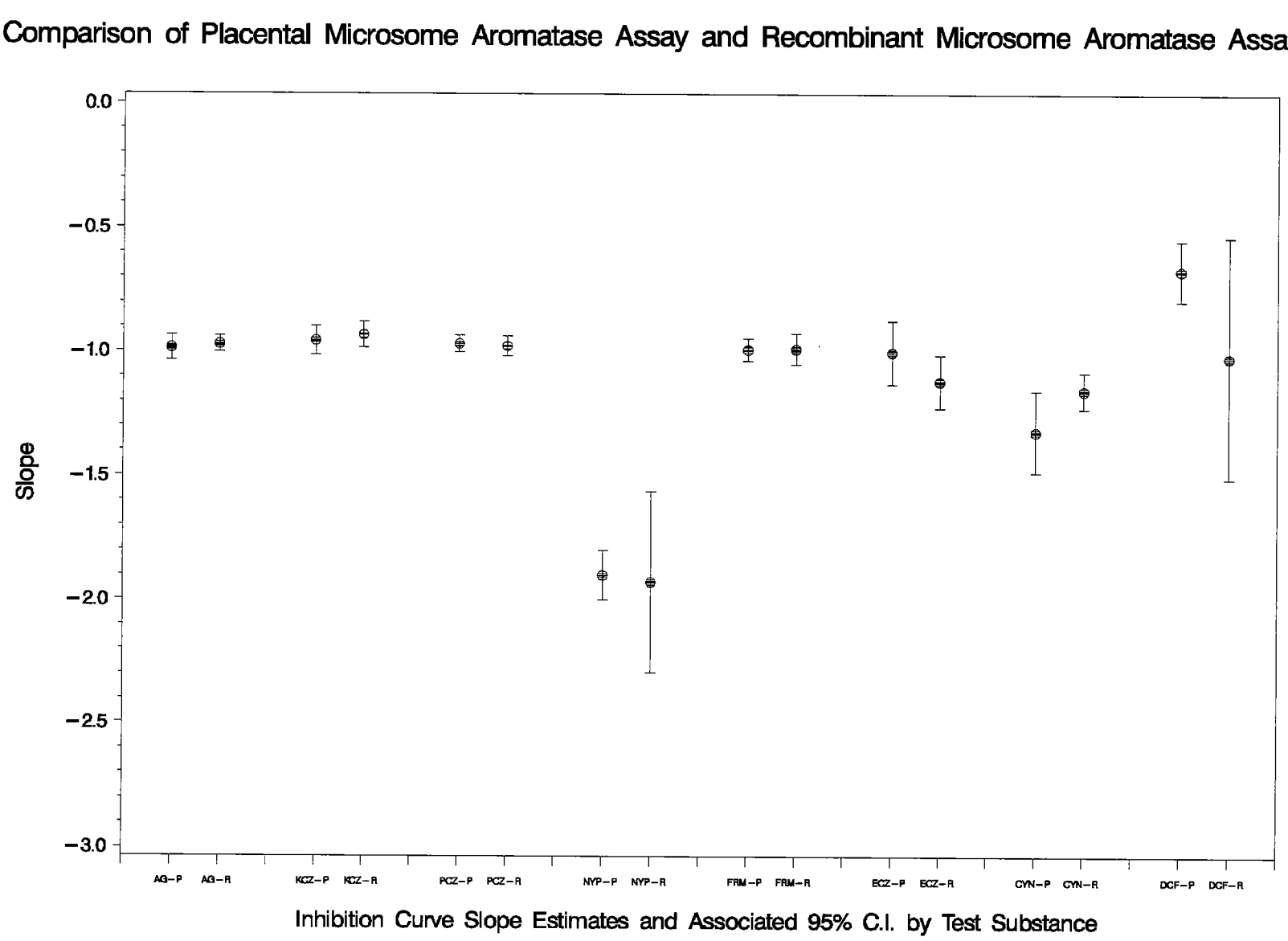

Figure 42. Comparison of Placental Microsome Aromatase Assay and Recombinant Microsome Aromatase Assay: Slope Estimates

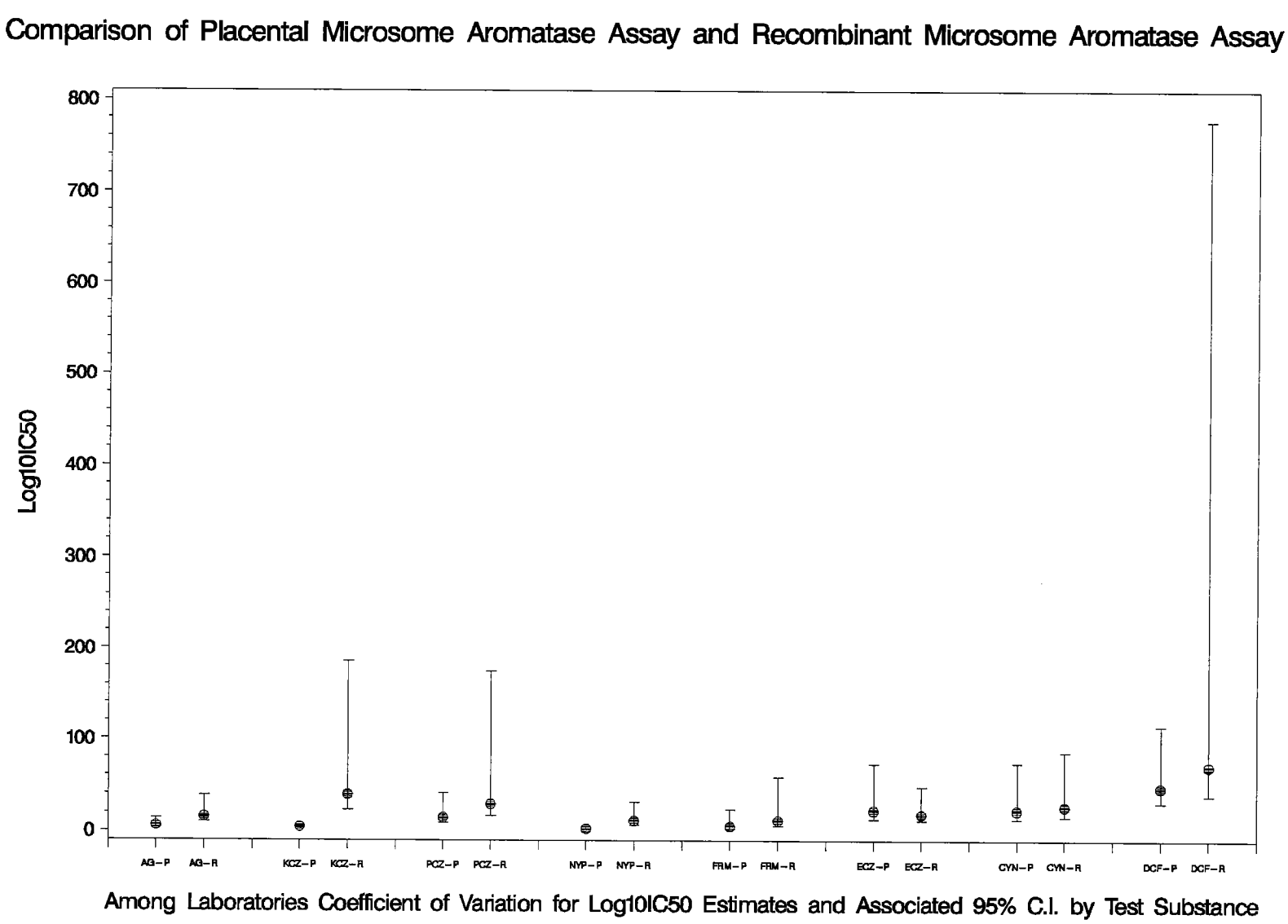

Figure 43. Comparison of Placental Microsome Aromatase Assay and Recombinant Microsome Aromatase Assay: Coefficient of Variation for $Log_{10}IC_{50}$

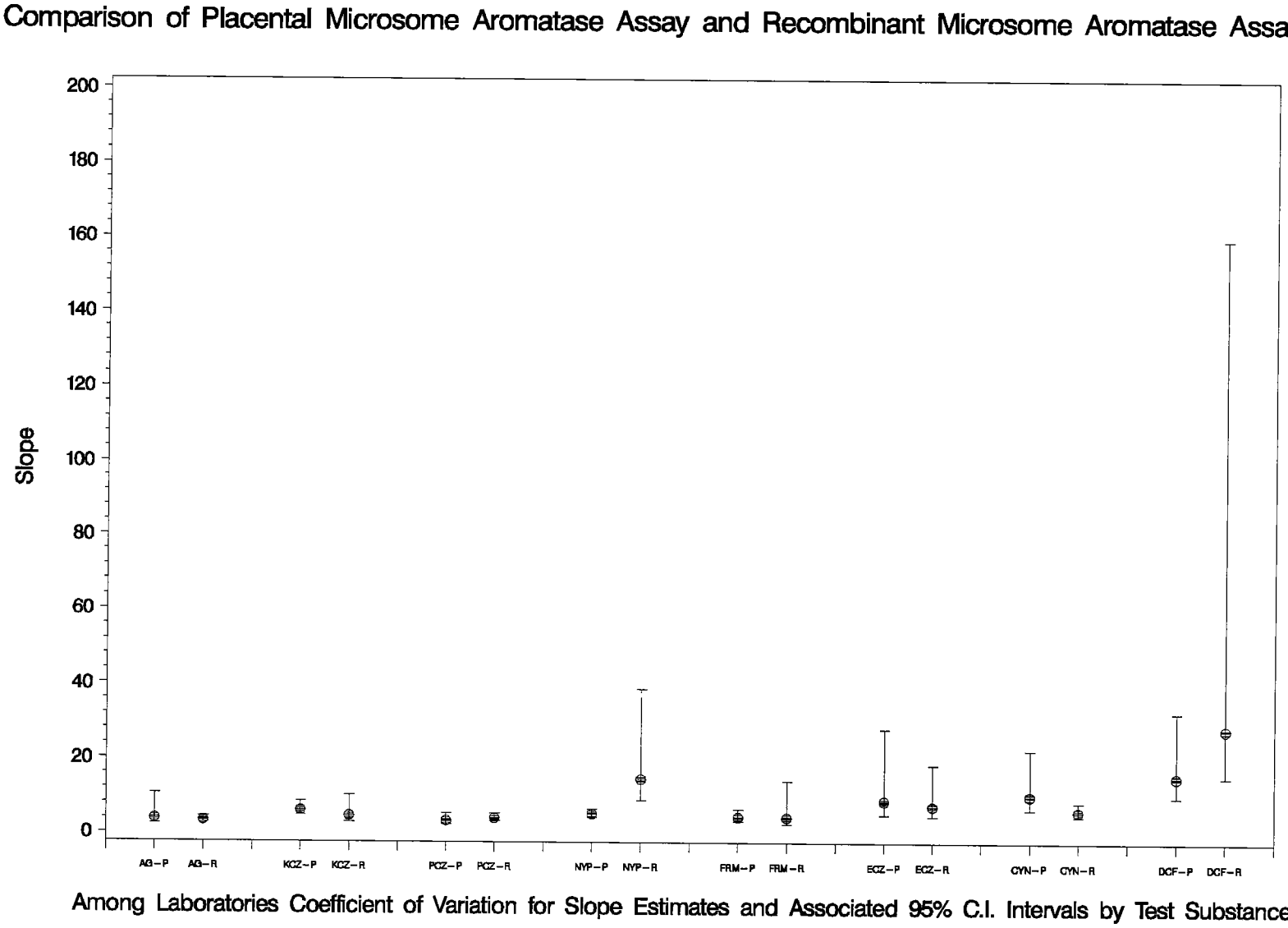

Figure 44. Comparison of Placental Microsome Aromatase Assay and Recombinant Microsome Aromatase Assay: Coefficient of Variation for Slope Estimates

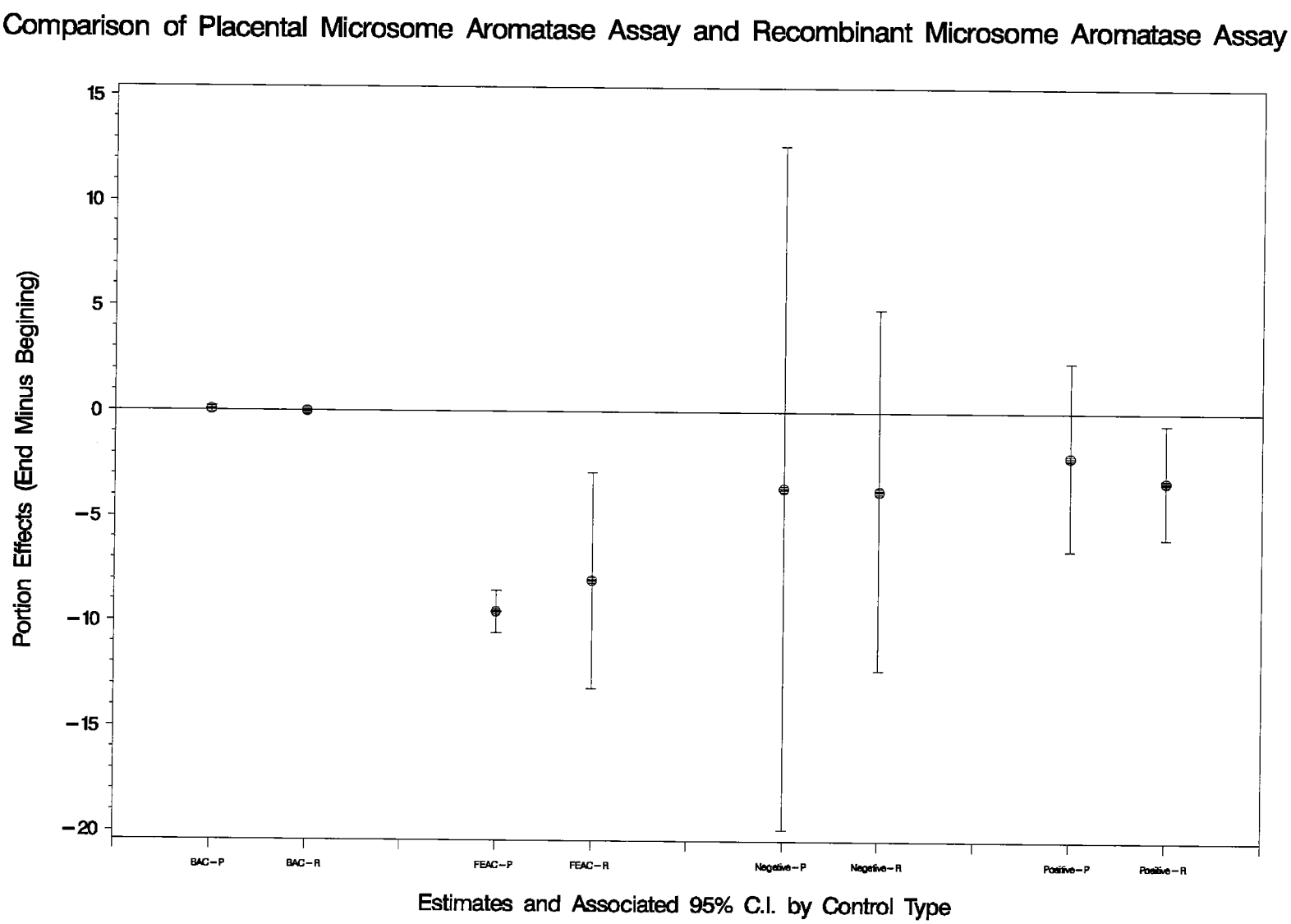

Figure 45. Comparison of Placental Microsome Aromatase Assay and Recombinant Microsome Aromatase Assay: Control Estimates

5.0 CONCLUSIONS

A lead laboratory and three participating laboratories used recombinant microsomes to validate the aromatase assay using ten reference chemicals. The reference chemicals and a positive and negative control were tested for their capacity to inhibit aromatase. 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

DRAFT TASK REPORT

RECOMBINANT AROMATASE VALIDATION STUDY

WA 4-17 Task 4: Conduct Multiple Chemical Studies with Recombinant Microsomes

EPA Contract Number 68-W-01-023
Work Assignment 4-17

Sponsor:

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

Performing Laboratory:

RTI International
Drug Metabolism and Pharmacokinetics
Science and Engineering Group
Health Sciences Unit
P.O. Box 12194
Research Triangle Park, NC 27709

DRAFT REPORT

Title: **WA-17 Task 4: Conduct Multiple Chemical Studies with Recombinant Microsomes**

Authors: Sherry Black, B.S.
James M. Mathews, Ph.D., DABT

Performing Laboratory: RTI International
Drug Metabolism and Pharmacokinetics
Science and Engineering Group
Health Sciences Unit
P.O. Box 12194
Research Triangle Park, NC 27709

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

Sponsor's Representatives: David P. Houchens, Ph.D.
EDSP Program Manager
Battelle Memorial Institute

Jerry D. Johnson, Ph.D., DABT
Work Assignment Leader
Battelle Memorial Institute

Study Initiation Date: July 6, 2005

Experimental Dates: July 14 – September 29, 2005

Draft Task Report Date: March 24, 2006

RTI Identification Number: 08055.004.039 – Task 4

Author:

James M. Mathews, Ph.D., DABT Date
Study Director
Director, Drug Metabolism and Pharmacokinetics
Health Sciences
RTI International

Approved:

Alan H. Staple, M.Sc. Date
Vice President
Health Sciences
RTI International

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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 a recombinant aromatase 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.

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 recombinant microsomes used in this task had a reported protein concentration of 4.9 mg/mL. The average measured protein content of the microsome stock was 4.7 ± 1.1 mg/mL (24.2% coefficient of variance [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 (4.1% relative error) of calculated and known amounts for the high QC standard, while the overall relative error for the low QC standard was -17.7%.

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 CVs on the order of 27%. The coefficient of variance for the protein concentration of the microsomes was 24.2%, 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 5-20%. 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 estimated for each reference chemical using a four-parameter equation. There was little variance (3-15%) 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 25-92%, 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 Disrupter 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 Disrupter Method 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 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 recombinant aromatase microsomal 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 (Work Assignment [WA] 2-24) were conducted to optimize the microsomal aromatase assay protocol for recombinant microsomes, 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 recombinant 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 recombinant microsomes to 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 at RTI in a previous Task. The results of this 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 formulations to the participating laboratories. These chemistry activities and results are described in the Sponsor's Chemistry reports.

3.2.1 Reference Chemicals

The reference chemical 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 reference chemical formulations were coded (Table 1) prior to distribution to the laboratory for use in the aromatase assay.

Table 1. Reference Chemicals for Task 4

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 7 (Section 3.4). The dilution scheme described in Table 2 was used in the preparation of dilutions for the first replicate of each reference chemical 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 reference chemical tested in all replicates are included in Table 13 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 7, 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 4

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

Recombinant microsomes (Human CYP19 + P450 Reductase SUPERSOMES™, Lot # 5, 4.9 mg protein/mL, purchased from Gentest, Woburn, MA) were used on this task. The microsomes were stored at approximately -70°C. Ten vials of the stock microsomes were thawed rapidly in a 37 ± 1°C water bath and immediately transferred to an ice bath. The microsomes were pooled, rehomogenized using a Potter Elvejhem homogenizer and then were divided into 200 µL aliquots, refrozen in liquid nitrogen and 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 (either 1:10 and then 1:60 or 1:50 and then 1:12, for a total 1:600 dilution) and maintained on ice

until used. The time between thawing of the microsomes and their use in the assay was limited to less than 1 h and, in most cases was about 45 min.

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 7. The lot numbers for the DMSO used in the preparation of the stock solutions for reference chemicals 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 7. 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, catalog # 4011-01, 137.99 g/mol) and sodium phosphate dibasic (JT

Baker, catalog # 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. Protein content of the final microsome dilution was determined for all assay days. Additionally, at the direction of the sponsor, the protein content of an intermediate dilution (1:50) of the stock microsomes was measured in conjunction with the assays for reference chemicals 4, 5, 9 and 10. 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 reference chemical with all replicates for a given reference chemical conducted by the same technician. In most cases, two references chemicals were tested on each day of assay and the two reference chemicals sets shared a set of control tubes. The following pairs of reference chemicals were run concurrently and shared control tubes: 1 and 2, 3 and 6, 4 and 5, 7 and 8, and 9 and 10. The first replicate of chemical 1 was run alone, as was the third replicate of reference chemical 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. 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 described in Table 8.

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 were in the full aromatase activity controls, except NADPH
- positive controls which contained: all components that were 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 were 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 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 8. Reference Chemical Study Design

Sample type	Repetitions (test tubes)	Description	Test 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}
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 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 reference chemical (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 9. 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 9. Optimized Aromatase Assay Conditions

Assay factor (units)	Recombinant Assay
Microsomal Protein (mg/mL)[a]	0.004
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 reference chemical 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 (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 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, full enzyme 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.

3.7.2.4 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 Radiochemical Purity

The [^{3}H] ASDN was found to be 97% radiochemically pure by HPLC analysis. The radiochemical purity report appears in Appendix B.

4.2 Stock Formulation Analysis

The formulation stability and formulation analysis results for all ten reference chemicals and the positive and negative control chemicals from the Battelle CR are included in their Chemistry reports. Some of the formulation analysis data are summarized in Table 10.

Table 10. 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 4-17, Task 4
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	9/29/2005
RC-5	Dibenz[a,h]anthracene	5	11007-132-02	97	9/13/2005	10/3/2005	9/29/2005
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	8/31/2005
RC-8	Chrysin	8	11007-122-03	98.2	8/9/2005	11/16/2005	8/31/2005
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	9/29/2005
N/A	4- OH ASDN	4-ASDN-1	11007-107-07	99	6/27/2005	12/16/2005	9/29/2005

4.3 Protein Analysis

Protein content of the recombinant 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 data from the intermediate dilutions of microsomes used in replicates 2 and 3 of chemicals 9 and 10 and replicate 3 of chemicals 4 and 5 was used in the calculation of protein content for these assays. The measured protein concentration for the recombinant microsomes for each day of use is presented in Table 12.

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 recombinant microsomes as determined using the 5-point standard curve were used in calculation of aromatase activity.

The QC results are presented in Table 11. The measured values for the high QC sample (110 μg/mL) showed an overall percent relative error of 4.1%, whereas, for the low QC sample (12 μg/mL) the overall percent relative error was -17.7%. There was a much higher variability in the values for the low QC (26.9% CV) than the high QC (4.9% 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 relative errors from the means of less than 10%.

Table 11. Protein QC Table

Reference Chemical	Rep	Date	Low QC					High QC				
			Measured Conc.[a]	Mean	SD	% RE	% CV	Measured Conc.[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	0.009	0.005	-27.8	59.2	0.120	0.116	0.003	5.5	3.0
	2	9/23/2005	0.010					0.114				
	3	9/29/2005	0.003					0.114				
ECZ, CYN	2[b]	8/24/2005	0.012	0.009	0.002	-22.2	24.7	0.110	0.120	0.009	8.8	7.1
	3[b]	8/26/2005	0.008					0.126				
	4[b]	8/31/2005	0.008					0.123				
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				**0.010**	**0.003**	**-17.7**	**26.9**		**0.115**	**0.006**	**4.1**	**4.9**

[a]Concentration units – mg/mL

[b]Data from replicate 1 of the assay run with ECZ/CYN was not used – substituted with a fourth replicate

The protein concentration measured for each day of assay for each reference chemical is presented in Table 12. The overall average measured protein concentration for the recombinant microsomes was 4.7 ± 1.1 mg/mL (24.2 % CV), which is near the 4.9 mg/mL concentration reported by the supplier of the microsomes.

Table 12. Measured Protein Concentrations

Reference Chemical	Replicate	Date	Protein Concentration (mg/mL)	Mean	Standard Deviation	% CV
AG	1	7/14/2005	4.9	4.7	0.2	4.7
	2	7/18/2005	4.6			
	3	7/20/2005	4.5			
KCZ	1	7/18/2005	4.6	4.8	0.5	10.7
	2	7/20/2005	4.5			
	3	7/22/2005	5.4			
PCZ, FRM	1	8/2/2005	4.5	3.7	0.8	22.2
	2	8/4/2005	3.7			
	3	8/5/2005	2.9			
NYP, DBA	1	9/21/2005	4.2	4.6	1.4	31.1
	2	9/23/2005	3.5			
	3	9/29/2005	6.2*			
ECZ, CYN	2[a]	8/24/2005	6.3	4.4	1.6	36.6
	3[a]	8/26/2005	3.4			
	4[a]	8/31/2005	3.6			
DCF, ATZ	1	9/8/2005	6.3	5.9	0.4	6.4
	2	9/13/2005	5.6*			
	3	9/15/2005	5.9*			
Overall				4.7	1.1	24.2

[a]Data from replicate 1 of the assay run with ECZ/CYN was not used – substituted with a fourth replicate
*Protein content calculated from intermediate dilution.

4.4 Aromatase Activity

Aromatase activity was measured in the presence of eight concentrations of each of ten RCs. Each assay set also included four types of controls (see Section 3.6). Three replicates were run of each RC. The aromatase activity data for RC- 7 and RC-8, replicate 1 showed no concentration dependence and were excluded from analysis. A fourth replicate was conducted for RC- 7 and RC-8 to replace the first replicate. Information regarding assay dates, protein concentration, substrate specific activity and tested RC concentration ranges is presented in Table 13. Radioactivity remaining in each assay tube after extraction of the unreacted substrate represented 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.

Table 13. 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	4.9	1.422	1.00E-03 to 1.00E-10
RC-1	Aminoglutethimide	2	7/18/2005	4.6	1.571	1.00E-03 to 1.00E-07
RC-1	Aminoglutethimide	3	7/20/2005	4.5	1.432	1.00E-03 to 1.00E-07
RC-2	Ketoconazole	1	7/18/2005	4.6	1.571	1.00E-04 to 1.00E-10
RC-2	Ketoconazole	2	7/20/2005	4.5	1.432	1.00E-04 to 1.00E-10
RC-2	Ketoconazole	3	7/22/2005	5.4	1.380	1.00E-04 to 1.00E-10
RC-3	Prochoraz	1	8/2/2005	4.5	1.377	1.00E-03 to 1.00E-10
RC-3	Prochoraz	2	8/4/2005	3.7	1.444	1.00E-05 to 1.00E-10
RC-3	Prochoraz	3	8/5/2005	2.9	1.369	1.00E-05 to 1.00E-10
RC-4	4-Nonylphenol	1	9/21/2005	4.2	1.379	1.00E-03 to 1.00E-10
RC-4	4-Nonylphenol	2	9/23/2005	3.5	1.244	1.00E-03 to 1.00E-07
RC-4	4-Nonylphenol	3	9/29/2005	6.2	1.218	1.00E-03 to 1.00E-07
RC-5	Dibenz[a,h]anthracene	1	9/21/2005	4.2	1.379	1.00E-04 to 1.00E-10
RC-5	Dibenz[a,h]anthracene	2	9/23/2005	3.5	1.244	1.00E-04 to 1.00E-10
RC-5	Dibenz[a,h]anthracene	3	9/29/2005	6.2	1.218	1.00E-04 to 1.00E-10
RC-6	Fenarimol	1	8/2/2005	4.5	1.377	1.00E-03 to 1.00E-10
RC-6	Fenarimol	2	8/4/2005	3.7	1.444	1.00E-03 to 1.00E-08
RC-6	Fenarimol	3	8/5/2005	2.9	1.369	1.00E-03 to 1.00E-08
RC-7	Econazole	2	8/24/2005	6.3	1.279	1.00E-06 to 1.00E-10
RC-7	Econazole	3	8/26/2005	3.4	1.457	1.00E-06 to 1.00E-10
RC-7	Econazole	4	8/31/2005	3.6	1.281	1.00E-06 to 1.00E-10
RC-8	Chrysin	2	8/24/2005	6.3	1.279	1.00E-04 to 1.00E-08
RC-8	Chrysin	3	8/26/2005	3.4	1.457	1.00E-04 to 1.00E-08
RC-8	Chrysin	4	8/31/2005	3.6	1.281	1.00E-04 to 1.00E-08
RC-9	Dicofol	1	9/8/2005	6.3	1.287	1.00E-03 to 1.00E-10
RC-9	Dicofol	2	9/13/2005	5.6	1.297	1.00E-03 to 1.00E-08
RC-9	Dicofol	3	9/15/2005	5.9	1.232	1.00E-03 to 1.00E-08
RC-10	Atrazine	1	9/8/2005	6.3	1.287	1.00E-03 to 1.00E-10
RC-10	Atrazine	2	9/13/2005	5.6	1.297	1.00E-03 to 1.00E-07
RC-10	Atrazine	3	9/15/2005	5.9	1.232	1.00E-03 to 1.00E-07

4.4.1 Control Results

Each replicate set for each RC included four types of controls, each run in quadruplicate. The control types were full aromatase activity, background activity, positive and negative controls. 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 portions and the overall means, SD, SEM and CVs across replicates and across chemicals are presented in Table 14.

Table 14. Control Activities[a]

Reference Chemical	Replicate	Mean Control Beginning	Mean Control End	Overall Mean	SD	SEM	%CV	Overall			
								Mean	SD	SEM	%CV
Full Aromatase Activity Control											
AG	1	0.3478	0.3082	0.3280	0.0257	0.0129	7.84	0.3416	0.0705	0.0407	20.63
	2	0.2936	0.2642	0.2789	0.0175	0.0088	6.29				
	3	0.4527	0.3830	0.4178	0.0406	0.0203	9.72				
KCZ	1	0.2936	0.2642	0.2789	0.0175	0.0088	6.29	0.3465	0.0714	0.0412	20.61
	2	0.4563	0.3860	0.4211	0.0410	0.0205	9.72				
	3	0.3411	0.3377	0.3394	0.0054	0.0027	1.59				
PCZ	1	0.4448	0.3775	0.3999	0.0445	0.0257	11.14	0.4981	0.1333	0.0770	26.76
	2	0.4950	0.3942	0.4446	0.0587	0.0293	13.19				
	3	0.6903	0.6095	0.6499	0.0496	0.0248	7.63				
NYP	1	0.4299	0.4029	0.4164	0.0160	0.0080	3.84	0.4193	0.1246	0.0720	29.72
	2	0.5700	0.5208	0.5454	0.0380	0.0190	6.96				
	3	0.3055	0.2869	0.2962	0.0115	0.0057	3.88				
DBA	1	0.4299	0.4029	0.4164	0.0160	0.0080	3.84	0.4193	0.1246	0.0720	29.72
	2	0.5700	0.5208	0.5454	0.0380	0.0190	6.96				
	3	0.3055	0.2869	0.2962	0.0115	0.0057	3.88				
FRM	1	0.4448	0.3775	0.3999	0.0445	0.0257	11.14	0.4981	0.1333	0.0770	26.76
	2	0.4950	0.3942	0.4446	0.0587	0.0293	13.19				
	3	0.6903	0.6095	0.6499	0.0496	0.0248	7.63				
ECZ	2	0.3171	0.2470	0.2821	0.0423	0.0212	15.00	0.3624	0.0707	0.0408	19.50
	3	0.4437	0.3863	0.4150	0.0388	0.0194	9.35				
	4	0.4377	0.3426	0.3901	0.0633	0.0317	16.23				
CYN	2	0.3171	0.2470	0.2821	0.0423	0.0212	15.00	0.3624	0.0707	0.0408	19.50
	3	0.4437	0.3863	0.4150	0.0388	0.0194	9.35				
	4	0.4377	0.3426	0.3901	0.0633	0.0317	16.23				
DCF	1	0.2935	0.2590	0.2763	0.0218	0.0109	7.90	0.3006	0.0245	0.0141	8.14
	2	0.3621	0.2883	0.3252	0.0486	0.0243	14.94				
	3	0.3132	0.2871	0.3002	0.0165	0.0083	5.51				
ATZ	1	0.2935	0.2590	0.2763	0.0218	0.0109	7.90	0.3006	0.0245	0.0141	8.14
	2	0.3621	0.2883	0.3252	0.0486	0.0243	14.94				
	3	0.3132	0.2871	0.3002	0.0165	0.0083	5.51				
Overall								0.3849	0.1043	0.0190	27.10

(continued)

Table 14. Control Activities[a] (continued)

Reference Chemical	Replicate	Mean Control Beginning	Mean Control End	Overall Mean	SD	SEM	%CV	Overall Mean	Overall SD	Overall SEM	Overall %CV
Positive Control											
AG	1	0.1853	0.1602	0.1727	0.0152	0.0076	8.80	0.1912	0.0338	0.0195	17.69
	2	0.1763	0.1649	0.1706	0.0067	0.0034	3.93				
	3	0.2333	0.2271	0.2302	0.0098	0.0049	4.25				
KCZ	1	0.1763	0.1649	0.1706	0.0067	0.0034	3.93	0.2027	0.0308	0.0178	15.21
	2	0.2352	0.2289	0.2320	0.0099	0.0049	4.25				
	3	0.2021	0.2085	0.2053	0.0106	0.0053	5.16				
PCZ	1	0.2574	0.2019	0.2297	0.0339	0.0169	14.76	0.2955	0.0762	0.0440	25.78
	2	0.2981	0.2577	0.2779	0.0275	0.0137	9.89				
	3	0.4099	0.3481	0.3790	0.0430	0.0215	11.34				
NYP	1	0.2339	0.2329	0.2334	0.0063	0.0031	2.69	0.2219	0.0515	0.0297	23.21
	2	0.2785	0.2551	0.2668	0.0154	0.0077	5.77				
	3	0.1725	0.1589	0.1657	0.0130	0.0065	7.85				
DBA	1	0.2339	0.2329	0.2334	0.0063	0.0031	2.69	0.2219	0.0515	0.0297	23.21
	2	0.2785	0.2551	0.2668	0.0154	0.0077	5.77				
	3	0.1725	0.1589	0.1657	0.0130	0.0065	7.85				
FRM	1	0.2574	0.2019	0.2297	0.0339	0.0169	14.76	0.2955	0.0762	0.0440	25.78
	2	0.2981	0.2577	0.2779	0.0275	0.0137	9.89				
	3	0.4099	0.3481	0.3790	0.0430	0.0215	11.34				
ECZ	2	0.1592	0.1479	0.1536	0.0083	0.0041	5.39	0.2107	0.0496	0.0286	23.54
	3	0.2545	0.2308	0.2427	0.0138	0.0069	5.67				
	4	0.2448	0.2269	0.2358	0.0169	0.0084	7.15				
CYN	2	0.1592	0.1479	0.1536	0.0083	0.0041	5.39	0.2107	0.0496	0.0286	23.54
	3	0.2545	0.2308	0.2427	0.0138	0.0069	5.67				
	4	0.2448	0.2269	0.2358	0.0169	0.0084	7.15				
DCF	1	0.1595	0.1667	0.1631	0.0063	0.0032	3.88	0.1715	0.0162	0.0093	9.43
	2	0.1900	0.1904	0.1902	0.0111	0.0055	5.82				
	3	0.1681	0.1546	0.1613	0.0095	0.0048	5.92				
ATZ	1	0.1595	0.1667	0.1631	0.0063	0.0032	3.88	0.1715	0.0162	0.0093	9.43
	2	0.1900	0.1904	0.1902	0.0111	0.0055	5.82				
	3	0.1681	0.1546	0.1613	0.0095	0.0048	5.92				
Overall								0.2193	0.0591	0.0108	26.93

(continued)

Table 14. Control Activities[a] (continued)

Reference Chemical	Replicate	Mean Control Beginning	Mean Control End	Overall Mean	SD	SEM	%CV	Overall Mean	Overall SD	Overall SEM	Overall %CV
Negative Control											
AG	1	0.3272	0.2754	0.3013	0.0317	0.0159	10.54	0.3209	0.0666	0.0385	20.76
	2	0.2755	0.2572	0.2663	0.0111	0.0055	4.16				
	3	0.3890	0.4013	0.3951	0.0173	0.0086	4.37				
KCZ	1	0.2755	0.2572	0.2663	0.0111	0.0055	4.16	0.3280	0.0664	0.0383	20.25
	2	0.3920	0.4045	0.3983	0.0174	0.0087	4.37				
	3	0.3209	0.3176	0.3193	0.0073	0.0037	2.29				
PCZ	1	0.4504	0.3550	0.4027	0.0593	0.0296	14.72	0.5069	0.1277	0.0737	25.20
	2	0.4991	0.4379	0.4685	0.0488	0.0244	10.42				
	3	0.6965	0.6022	0.6493	0.0559	0.0279	8.60				
NYP	1	0.4722	0.4137	0.4429	0.0397	0.0199	8.97	0.4193	0.0987	0.0570	23.54
	2	0.5279	0.4802	0.5040	0.0276	0.0138	5.48				
	3	0.3150	0.3068	0.3109	0.0256	0.0128	8.22				
DBA	1	0.4722	0.4137	0.4429	0.0397	0.0199	8.97	0.4193	0.0987	0.0570	23.54
	2	0.5279	0.4802	0.5040	0.0276	0.0138	5.48				
	3	0.3150	0.3068	0.3109	0.0256	0.0128	8.22				
FRM	1	0.4504	0.3550	0.4027	0.0593	0.0296	14.72	0.5069	0.1277	0.0737	25.20
	2	0.4991	0.4379	0.4685	0.0488	0.0244	10.42				
	3	0.6965	0.6022	0.6493	0.0559	0.0279	8.60				
ECZ	2	0.2843	0.2694	0.2769	0.0123	0.0061	4.43	0.3611	0.0733	0.0423	20.30
	3	0.4170	0.3751	0.3961	0.0468	0.0234	11.81				
	4	0.4129	0.4079	0.4104	0.0383	0.0191	9.33				
CYN	2	0.2843	0.2694	0.2769	0.0123	0.0061	4.43	0.3611	0.0733	0.0423	20.30
	3	0.4170	0.3751	0.3961	0.0468	0.0234	11.81				
	4	0.4129	0.4079	0.4104	0.0383	0.0191	9.33				
DCF	1	0.2695	0.2664	0.2680	0.0151	0.0075	5.62	0.2995	0.0417	0.0241	13.94
	2	0.3626	0.3311	0.3469	0.0373	0.0186	10.74				
	3	0.2892	0.2783	0.2838	0.0137	0.0068	4.81				
ATZ	1	0.2695	0.2664	0.2680	0.0151	0.0075	5.62	0.2995	0.0417	0.0241	13.94
	2	0.3626	0.3311	0.3469	0.0373	0.0186	10.74				
	3	0.2892	0.2783	0.2838	0.0137	0.0068	4.81				
Overall								0.3822	0.1043	0.0190	27.29

[a]Units are nmol/mg protein/min

The aromatase activities for the controls were divided by the average full enzyme activity control activities for each chemical and replicate to calculate percent of control values. Plots of the percent control activities for each chemical and replicate were prepared and examined visually for trends (see Appendix E). The full activity control and negative control percent of control values both ranged from about 80-120%, while the positive control percent of control values generally ranged between 45 and 70%. 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). This difference in beginning and end control activities is presented graphically in the statisticians report in Appendix E. The graphs show decreases between beginning and end percent of control activities generally on the order of 5-20%. These decreases are notable since only about 25 minutes elapsed between the beginning and end of the assay set. The results of the analysis of the control data using a mixed effects analysis of variance (ANOVA) model show that portion was a significant contributor to the variance in percent of control for each control type (except background). The coefficient of variance for the controls across all chemicals and replicates was approximately 27%. Most of this variance may be explained as the effect of the variance in measured protein content.

4.4.2 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 15. There was little tube to tube variance (%CV generally less than 10%) within a replicate, except at some high RC concentrations where the percent of control values were near zero.

Table 15. Percent of Control Values

RC	Replicate	Log[RC]	Percent of Control			Mean	±SD	SEM	%CV
			Tube 1	Tube 2	Tube 3				
AG	1	-3.00	9.70	9.29	8.47	9.15	0.63	0.36	6.84
		-4.00	40.48	46.24	44.66	43.79	2.97	1.72	6.79
		-5.00	86.93	56.61[a]	86.85	86.89	0.05	0.04	0.06
		-6.00	93.50	89.90	92.73	92.04	1.90	1.10	2.06
		-7.00	93.98	94.41	97.12	95.17	1.70	0.98	1.79
		-8.00	97.60	96.16	103.29	99.02	3.77	2.17	3.80
		-9.00	94.37	93.58	96.56	94.84	1.54	0.89	1.63
		-10.00	95.19	94.53	96.33	95.35	0.91	0.53	0.96
AG	2	-3.00	6.84	6.99	7.53	7.12	0.36	0.21	5.08
		-3.30	14.78	14.98	14.86	14.87	0.10	0.06	0.70
		-3.60	28.02	28.20	27.72	27.98	0.25	0.14	0.88
		-4.00	48.14	46.43	48.69	47.75	1.18	0.68	2.47
		-4.30	62.64	60.45	65.49	62.86	2.53	1.46	4.02
		-5.00	87.04	90.37	86.37	87.93	2.14	1.24	2.44
		-6.00	95.74	90.80	100.77	95.77	4.98	2.88	5.20
		-7.00	95.57	100.80	99.38	98.58	2.70	1.56	2.74

[a]Excluded as an outlier.

Table 15. Percent of Control Values (continued)

RC	Replicate	Log[RC]	Percent of Control			Mean	±SD	SEM	%CV
			Tube 1	Tube 2	Tube 3				
AG	3	-3.00	8.41	8.12	7.86	8.13	0.28	0.16	3.42
		-3.30	13.35	15.38	14.23	14.32	1.02	0.59	7.13
		-3.60	27.70	26.64	26.02	26.79	0.85	0.49	3.18
		-4.00	45.46	47.23	42.54	45.08	2.37	1.37	5.25
		-4.30	58.82	58.77	58.76	58.78	0.03	0.02	0.05
		-5.00	89.42	83.29	82.34	85.02	3.84	2.22	4.52
		-6.00	89.03	85.24	90.65	88.31	2.78	1.60	3.15
		-7.00	92.68	92.58	101.56	95.61	5.15	2.98	5.39
KCZ	1	-4.00	0.54	0.26	0.39	0.40	0.14	0.08	36.22
		-5.00	4.44	4.24	4.40	4.36	0.11	0.06	2.43
		-6.00	30.07	32.30	31.23	31.20	1.11	0.64	3.57
		-6.30	45.32	46.67	45.79	45.93	0.68	0.39	1.49
		-7.00	82.62	80.09	62.98	75.23	10.68	6.17	14.20
		-8.00	88.64	92.10	86.65	89.13	2.76	1.59	3.09
		-9.00	98.02	92.79	92.82	94.54	3.01	1.74	3.18
		-10.00	90.53	97.43	102.88	96.95	6.19	3.57	6.39
KCZ	2	-4.00	0.07	0.14	-0.01	0.07	0.08	0.04	118.02
		-5.00	5.15	4.71	4.74	4.87	0.25	0.14	5.08
		-6.00	35.39	34.92	32.15	34.15	1.75	1.01	5.13
		-6.30	44.23	48.17	48.64	47.02	2.43	1.40	5.16
		-7.00	81.13	75.46	74.54	77.05	3.57	2.06	4.63
		-8.00	89.37	80.80	90.40	86.85	5.27	3.04	6.07
		-9.00	84.03	83.52	91.53	86.36	4.48	2.59	5.19
		-10.00	89.82	85.48	91.92	89.07	3.28	1.90	3.69
KCZ	3	-4.00	0.42	0.45	0.46	0.44	0.02	0.01	4.88
		-5.00	4.06	4.01	4.48	4.18	0.26	0.15	6.12
		-6.00	31.26	29.93	32.49	31.23	1.28	0.74	4.10
		-6.30	48.68	48.45	47.46	48.20	0.65	0.37	1.34
		-7.00	77.16	84.01	82.17	81.11	3.54	2.05	4.37
		-8.00	99.09	92.41	100.35	97.28	4.27	2.46	4.38
		-9.00	95.85	98.96	98.08	97.63	1.60	0.92	1.64
		-10.00	90.45	89.13	103.35	94.31	7.85	4.53	8.33
PCZ	1	-3.00	-0.09	0.00	-0.06	-0.05	0.05	0.03	-87.29
		-4.00	0.01	-0.05	-0.01	-0.01	0.03	0.02	-215.65
		-5.00	0.32	0.21	0.33	0.29	0.07	0.04	23.14
		-6.00	3.20	3.23	3.80	3.41	0.34	0.20	9.94
		-7.00	28.62	25.05	26.05	26.57	1.84	1.06	6.94
		-8.00	64.91	70.46	76.88	70.75	5.99	3.46	8.47
		-9.00	101.83	90.36	92.06	94.75	6.19	3.57	6.53
		-10.00	91.59	89.20	95.16	91.98	3.00	1.73	3.27

(continued)

Table 15. Percent of Control Values (continued)

RC	Replicate	Log[RC]	Percent of Control			Mean	±SD	SEM	%CV
			Tube 1	Tube 2	Tube 3				
PCZ	2	-5.00	0.52	0.54	0.59	0.55	0.03	0.02	6.37
		-6.00	3.56	3.48	3.53	3.53	0.04	0.02	1.15
		-7.00	24.73	23.15	24.96	24.28	0.99	0.57	4.07
		-7.60	55.37	53.07	55.78	54.74	1.46	0.84	2.66
		-8.00	75.65	70.46	72.33	72.81	2.63	1.52	3.61
		-8.30	83.33	84.68	86.96	84.99	1.83	1.06	2.16
		-9.00	88.38	92.66	96.42	92.49	4.02	2.32	4.35
		-10.00	92.93	97.92	99.70	96.85	3.51	2.03	3.63
PCZ	3	-5.00	0.56	0.45	0.41	0.47	0.08	0.05	16.60
		-6.00	2.55	3.26	3.55	3.12	0.51	0.30	16.49
		-7.00	24.80	21.93	24.96	23.90	1.70	0.98	7.13
		-7.60	57.29	49.54	51.70	52.84	4.00	2.31	7.57
		-8.00	74.18	71.26	74.87	73.44	1.92	1.11	2.61
		-8.30	77.98	79.67	85.77	81.14	4.10	2.37	5.05
		-9.00	93.47	97.20	84.58	91.75	6.48	3.74	7.07
		-10.00	94.37	100.05	87.25	93.89	6.41	3.70	6.83
NYP	1	-3.00	0.14	0.05	0.07	0.09	0.05	0.03	56.99
		-4.00	1.57	1.93	1.94	1.81	0.21	0.12	11.76
		-5.00	84.06	87.35	84.74	85.38	1.74	1.00	2.03
		-6.00	104.41	108.77	101.35	104.84	3.73	2.15	3.55
		-7.00	104.53	101.83	100.72	102.36	1.96	1.13	1.91
		-8.00	97.26	81.69	102.00	93.65	10.62	6.13	11.34
		-9.00	99.64	100.17	95.91	98.57	2.32	1.34	2.35
		-10.00	102.55	102.91	102.43	102.63	0.25	0.14	0.24
NYP	2	-3.00	0.10	0.16	0.09	0.11	0.04	0.02	31.91
		-4.00	4.05	4.61	5.94	4.87	0.97	0.56	19.95
		-4.30	21.09	21.40	21.45	21.31	0.20	0.11	0.92
		-4.60	39.77	39.97	41.87	40.53	1.16	0.67	2.86
		-5.00	92.74	74.94	84.04	83.90	8.90	5.14	10.61
		-5.30	101.49	88.07	99.96	96.51	7.35	4.24	7.61
		-6.00	94.18	100.76	98.05	97.66	3.30	1.91	3.38
		-7.00	91.84	101.35	98.03	97.07	4.83	2.79	4.97
NYP	3	-3.00	0.05	0.14	0.12	0.11	0.05	0.03	44.21
		-4.00	5.49	2.82	3.77	4.03	1.35	0.78	33.57
		-4.30	20.11	21.77	18.23	20.04	1.77	1.02	8.84
		-4.60	39.44	43.14	39.53	40.70	2.11	1.22	5.18
		-5.00	90.90	85.03	85.45	87.13	3.27	1.89	3.76
		-5.30	107.47	102.55	108.95	106.32	3.35	1.93	3.15
		-6.00	97.09	98.68	106.97	100.92	5.30	3.06	5.26
		-7.00	102.23	98.77	105.48	102.16	3.36	1.94	3.29

(continued)

Table 15. Percent of Control Values (continued)

RC	Replicate	Log[RC]	Percent of Control			Mean	±SD	SEM	%CV
			Tube 1	Tube 1	Tube 1				
DBA	1	-4.00	92.13	96.40	100.34	96.29	4.11	2.37	4.27
		-5.00	106.57	96.58	93.73	98.96	6.74	3.89	6.82
		-6.00	91.83	90.55	95.87	92.75	2.78	1.60	2.99
		-6.30	93.13	99.34	98.47	96.98	3.37	1.94	3.47
		-7.00	91.81	92.91	91.00	91.90	0.95	0.55	1.04
		-8.00	98.65	102.97	99.21	100.27	2.35	1.36	2.34
		-9.00	99.39	92.00	93.13	94.84	3.98	2.30	4.20
		-10.00	95.79	93.58	94.32	94.56	1.13	0.65	1.19
DBA	2	-4.00	83.89	90.63	90.95	88.49	3.99	2.30	4.51
		-5.00	87.58	91.50	93.70	90.92	3.10	1.79	3.41
		-6.00	97.08	89.84	88.61	91.84	4.58	2.64	4.98
		-6.30	91.61	92.36	92.25	92.07	0.41	0.24	0.44
		-7.00	94.92	91.26	95.06	93.75	2.15	1.24	2.30
		-8.00	92.16	90.84	77.64	86.88	8.03	4.63	9.24
		-9.00	93.63	99.49	92.80	95.31	3.65	2.11	3.83
		-10.00	97.27	95.39	89.12	93.93	4.26	2.46	4.54
DBA	3	-4.00	93.28	93.61	100.18	95.69	3.89	2.25	4.07
		-5.00	100.29	98.39	97.82	98.83	1.30	0.75	1.31
		-6.00	97.47	98.43	100.49	98.79	1.54	0.89	1.56
		-6.30	96.56	94.69	91.96	94.40	2.31	1.33	2.45
		-7.00	100.10	105.76	98.69	101.52	3.74	2.16	3.68
		-8.00	99.59	96.97	94.81	97.12	2.39	1.38	2.46
		-9.00	97.13	102.71	96.39	98.74	3.45	1.99	3.50
		-10.00	91.49	96.57	98.24	95.43	3.52	2.03	3.69
FRM	1	-3.00	0.92	0.90	0.79	0.87	0.07	0.04	8.36
		-4.00	6.49	6.23	6.08	6.27	0.21	0.12	3.34
		-5.00	36.75	37.24	38.03	37.34	0.65	0.38	1.74
		-6.00	77.80	80.97	81.59	80.12	2.03	1.17	2.53
		-7.00	89.23	89.89	90.86	89.99	0.82	0.48	0.92
		-8.00	92.94	92.10	84.22	89.75	4.81	2.78	5.36
		-9.00	90.02	91.02	92.74	91.26	1.38	0.79	1.51
		-10.00	88.85	89.24	91.44	89.84	1.39	0.80	1.55
FRM	2	-3.00	1.04	1.00	1.04	1.03	0.02	0.01	2.26
		-4.00	6.44	6.45	6.39	6.43	0.03	0.02	0.49
		-4.60	19.59	20.97	19.39	19.98	0.86	0.50	4.30
		-5.00	37.47	37.07	33.09	35.88	2.42	1.40	6.75
		-5.30	50.17	47.69	48.91	48.92	1.24	0.72	2.54
		-6.00	77.04	86.72	85.97	83.24	5.38	3.11	6.47
		-7.00	93.34	94.44	88.66	92.15	3.07	1.77	3.33
		-8.00	90.07	92.57	89.35	90.66	1.69	0.98	1.87

(continued)

Table 15. Percent of Control Values (continued)

RC	Replicate	Log[RC]	Percent of Control			Mean	±SD	SEM	%CV
			Tube 1	Tube 2	Tube 3				
FRM	3	-3.00	0.93	0.99	0.96	0.96	0.03	0.02	3.32
		-4.00	6.14	6.10	6.32	6.18	0.11	0.07	1.86
		-4.60	19.51	18.85	19.55	19.30	0.39	0.23	2.04
		-5.00	35.04	33.36	32.12	33.51	1.47	0.85	4.39
		-5.30	49.70	50.32	49.72	49.91	0.35	0.20	0.70
		-6.00	76.60	76.29	81.21	78.03	2.76	1.59	3.53
		-7.00	93.45	84.74	87.92	88.70	4.40	2.54	4.97
		-8.00	87.92	88.51	93.62	90.02	3.14	1.81	3.48
ECZ	2	-6.00	0.27	0.22	0.28	0.25	0.03	0.02	11.50
		-7.00	2.34	1.91	1.97	2.07	0.23	0.13	11.23
		-8.00	15.70	16.58	17.84	16.71	1.08	0.62	6.45
		-8.30	30.12	30.26	27.35	29.25	1.64	0.95	5.61
		-9.00	77.24	75.89	71.69	74.94	2.90	1.67	3.86
		-9.30	90.46	88.90	91.19	90.18	1.17	0.68	1.30
		-9.60	91.93	91.32	91.23	91.49	0.38	0.22	0.42
		-10.00	94.75	104.01	100.60	99.78	4.69	2.70	4.70
ECZ	3	-6.00	0.30	0.25	0.33	0.29	0.04	0.02	12.82
		-7.00	1.93	2.11	1.99	2.01	0.09	0.05	4.70
		-8.00	15.30	18.01	17.75	17.02	1.49	0.86	8.78
		-8.30	30.18	31.25	31.09	30.84	0.58	0.33	1.87
		-9.00	77.02	70.66	76.43	74.70	3.51	2.03	4.70
		-9.30	82.15	81.32	85.15	82.87	2.01	1.16	2.43
		-9.60	95.62	95.12	94.18	94.98	0.73	0.42	0.77
		-10.00	94.88	90.51	98.34	94.58	3.92	2.27	4.15
ECZ	4	-6.00	0.19	0.25	0.24	0.22	0.03	0.02	15.22
		-7.00	2.12	2.21	2.47	2.27	0.18	0.11	8.10
		-8.00	17.74	19.92	19.63	19.10	1.18	0.68	6.20
		-8.30	32.01	37.55	32.83	34.13	2.99	1.73	8.76
		-9.00	71.02	76.36	66.03	71.14	5.17	2.98	7.26
		-9.30	89.72	85.38	96.35	90.48	5.53	3.19	6.11
		-9.60	99.17	93.98	91.43	94.86	3.94	2.28	4.15
		-10.00	98.37	88.70	109.79	98.95	10.55	6.09	10.67
CYN	2	-4.00	25.25	21.00	23.26	23.17	2.13	1.23	9.18
		-5.00	28.52	26.67	25.22	26.80	1.66	0.96	6.18
		-5.30	41.21	37.82	41.56	40.20	2.07	1.19	5.14
		-5.60	52.33	57.15	58.85	56.11	3.38	1.95	6.03
		-6.00	63.49	72.68	73.38	69.85	5.52	3.19	7.90
		-6.30	84.26	86.26	78.47	83.00	4.05	2.34	4.88
		-7.00	92.50	89.05	84.06	88.54	4.25	2.45	4.80
		-8.00	89.14	99.17	86.04	91.45	6.86	3.96	7.50

(continued)

Table 15. Percent of Control Values (continued)

RC	Replicate	Log[RC]	Percent of Control			Mean	±SD	SEM	%CV
			Tube 1	Tube 2	Tube 3				
CYN	3	-4.00	24.98	24.33	22.57	23.96	1.25	0.72	5.21
		-5.00	23.72	26.00	24.99	24.90	1.15	0.66	4.60
		-5.30	35.69	37.80	36.48	36.66	1.06	0.61	2.90
		-5.60	49.65	56.29	52.73	52.89	3.33	1.92	6.29
		-6.00	70.55	74.68	66.35	70.53	4.16	2.40	5.91
		-6.30	86.59	73.87	76.60	79.02	6.69	3.87	8.47
		-7.00	99.75	92.56	94.15	95.49	3.78	2.18	3.96
		-8.00	90.82	87.26	92.56	90.21	2.70	1.56	3.00
CYN	4	-4.00	27.67	27.66	26.76	27.37	0.52	0.30	1.91
		-5.00	29.15	27.21	30.49	28.95	1.65	0.95	5.69
		-5.30	42.71	44.95	47.85	45.17	2.58	1.49	5.70
		-5.60	64.22	59.47	58.66	60.78	3.00	1.73	4.94
		-6.00	73.08	70.30	76.34	73.24	3.02	1.75	4.13
		-6.30	82.37	85.22	85.93	84.51	1.88	1.09	2.23
		-7.00	93.48	86.39	93.66	91.18	4.15	2.40	4.55
		-8.00	97.54	94.12	89.28	93.65	4.15	2.39	4.43
DCF	1	-3.00	2.46	2.12	2.22	2.26	0.18	0.10	7.86
		-4.00	24.61	22.16	23.17	23.32	1.23	0.71	5.28
		-5.00	82.57	79.74	74.33	78.88	4.19	2.42	5.31
		-6.00	90.51	92.45	100.68	94.55	5.40	3.12	5.71
		-7.00	92.08	97.27	92.95	94.10	2.78	1.61	2.95
		-8.00	100.66	95.93	97.84	98.14	2.38	1.37	2.42
		-9.00	106.94	92.17	95.48	98.19	7.75	4.47	7.89
		-10.00	91.17	93.55	94.89	93.21	1.89	1.09	2.02
DCF	2	-3.00	2.77	1.84	2.10	2.23	0.48	0.28	21.36
		-3.30	4.75	6.68	5.30	5.57	0.99	0.57	17.83
		-3.60	11.06	11.48	14.40	12.31	1.82	1.05	14.78
		-4.00	23.76	29.31	34.20	29.09	5.22	3.02	17.95
		-5.00	74.13	77.04	79.60	76.92	2.74	1.58	3.56
		-6.00	113.81	106.65	89.72	103.39	12.37	7.14	11.97
		-7.00	100.71	102.22	100.46	101.13	0.95	0.55	0.94
		-8.00	115.12	99.72	113.15	109.33	8.38	4.84	7.67
DCF	3	-3.00	2.64	2.65	2.06	2.45	0.34	0.19	13.68
		-3.30	7.08	7.45	6.27	6.93	0.60	0.35	8.70
		-3.60	11.75	11.66	14.17	12.52	1.42	0.82	11.37
		-4.00	22.96	22.82	28.21	24.66	3.07	1.77	12.46
		-5.00	75.73	70.79	71.15	72.56	2.76	1.59	3.80
		-6.00	92.34	96.56	88.27	92.39	4.14	2.39	4.49
		-7.00	94.18	92.66	88.28	91.71	3.06	1.77	3.34
		-8.00	96.31	94.79	102.66	97.92	4.17	2.41	4.26

(continued)

Table 15. Percent of Control Values (continued)

RC	Replicate	Log[RC]	Percent of Control			Mean	±SD	SEM	%CV
			Tube 1	Tube 2	Tube 3				
ATZ	1	-3.00	93.24	94.01	85.30	90.85	4.82	2.78	5.31
		-4.00	97.36	93.55	98.65	96.52	2.65	1.53	2.74
		-5.00	99.88	95.07	92.70	95.88	3.66	2.11	3.82
		-6.00	97.93	88.44	95.88	94.08	5.00	2.89	5.31
		-7.00	99.24	101.24	93.64	98.04	3.94	2.27	4.01
		-8.00	92.22	106.39	89.47	96.03	9.08	5.24	9.45
		-9.00	91.55	96.66	98.15	95.46	3.46	2.00	3.63
		-10.00	94.14	90.61	85.69	90.15	4.24	2.45	4.71
ATZ	2	-3.00	92.75	78.70	80.48	83.97	7.65	4.42	9.11
		-3.30	91.24	91.96	88.44	90.54	1.86	1.07	2.05
		-3.60	88.83	94.47	91.82	91.71	2.82	1.63	3.08
		-4.00	95.35	101.64	96.02	97.67	3.45	1.99	3.54
		-4.30	102.60	99.15	97.52	99.75	2.59	1.50	2.60
		-5.00	100.39	101.94	93.76	98.70	4.35	2.51	4.40
		-6.00	100.67	94.91	106.59	100.72	5.84	3.37	5.80
		-7.00	93.58	111.96	114.40	106.65	11.38	6.57	10.67
ATZ	3	-3.00	79.82	82.96	84.37	82.39	2.33	1.34	2.82
		-3.30	85.18	85.51	83.21	84.63	1.25	0.72	1.47
		-3.60	90.55	87.36	89.53	89.15	1.63	0.94	1.82
		-4.00	95.93	93.35	94.18	94.49	1.32	0.76	1.39
		-4.30	87.56	95.25	93.58	92.13	4.05	2.34	4.39
		-5.00	94.97	97.73	93.97	95.56	1.95	1.12	2.04
		-6.00	96.93	93.12	93.53	94.53	2.09	1.21	2.21
		-7.00	98.09	88.64	89.53	92.08	5.22	3.01	5.66

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 16. Replicate to replicate reproducibility was good, with most %CV values less than 10%, except at some high RC concentrations were the percent of control value was near zero.

Table 16. Mean Percent of Control Per Replicate and Percent of Control Across Replicates

RC	Log[RC]	Mean Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	±SD	SEM	%CV
AG	-3.00	9.15	7.12	8.13	8.13	1.01	0.59	12.47
	-3.30	NA	14.87	14.32	14.60	0.39	0.28	2.67
	-3.60	NA	27.98	26.79	27.38	0.84	0.60	3.08
	-4.00	43.79	47.75	45.08	45.54	2.02	1.17	4.44
	-4.30	NA	62.86	58.78	60.82	2.88	2.04	4.74
	-5.00	86.89	87.93	85.02	86.61	1.48	0.85	1.70
	-6.00	92.04	95.77	88.31	92.04	3.73	2.15	4.05
	-7.00	95.17	98.58	95.61	96.45	1.86	1.07	1.93

(continued)

Table 16. 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
	-8.00	99.02	NA	NA	99.02	NC	NC	NC
	-9.00	94.84	NA	NA	94.84	NC	NC	NC
	-10.00	95.35	NA	NA	95.35	NC	NC	NC
KCZ	-4.00	0.40	0.07	0.44	0.30	0.21	0.12	68.23
	-5.00	4.36	4.87	4.18	4.47	0.36	0.21	7.98
	-6.00	31.20	34.15	31.23	32.20	1.70	0.98	5.27
	-6.30	45.93	47.02	48.20	47.05	1.14	0.66	2.42
	-7.00	75.23	77.05	81.11	77.80	3.01	1.74	3.87
	-8.00	89.13	86.85	97.28	91.09	5.48	3.17	6.02
	-9.00	94.54	86.36	97.63	92.84	5.82	3.36	6.27
	-10.00	96.95	89.07	94.31	93.44	4.01	2.31	4.29
PCZ	-3.00	-0.05	NA	NA	-0.05	NC	NC	NC
	-4.00	-0.01	NA	NA	-0.01	NC	NC	NC
	-5.00	0.29	0.55	0.47	0.43	0.14	0.08	31.04
	-6.00	3.41	3.53	3.12	3.35	0.21	0.12	6.26
	-7.00	26.57	24.28	23.90	24.92	1.45	0.83	5.80
	-7.60	NA	54.74	52.84	53.79	1.34	0.95	2.49
	-8.00	70.75	72.81	73.44	72.33	1.41	0.81	1.94
	-8.30	NA	84.99	81.14	83.06	2.72	1.92	3.28
	-9.00	94.75	92.49	91.75	92.99	1.56	0.90	1.68
	-10.00	91.98	96.85	93.89	94.24	2.45	1.42	2.60
NYP	-3.00	0.09	0.11	0.11	0.10	0.01	0.01	13.72
	-4.00	1.81	4.87	4.03	3.57	1.58	0.91	44.22
	-4.30	NA	21.31	20.04	20.67	0.90	0.64	4.37
	-4.60	NA	40.53	40.70	40.62	0.12	0.09	0.30
	-5.00	85.38	83.90	87.13	85.47	1.61	0.93	1.89
	-5.30	NA	96.51	106.32	101.41	6.94	4.91	6.85
	-6.00	104.84	97.66	100.92	101.14	3.60	2.08	3.55
	-7.00	102.36	97.07	102.16	100.53	3.00	1.73	2.98
	-8.00	93.65	NA	NA	93.65	NC	NC	NC
	-9.00	98.57	NA	NA	98.57	NC	NC	NC
	-10.00	102.63	NA	NA	102.63	NC	NC	NC
DBA	-4.00	96.29	88.49	95.69	93.49	4.34	2.51	4.64
	-5.00	98.96	90.92	98.83	96.24	4.60	2.66	4.78
	-6.00	92.75	91.84	98.79	94.46	3.78	2.18	4.00
	-6.30	96.98	92.07	94.40	94.48	2.45	1.42	2.60
	-7.00	91.90	93.75	101.52	95.72	5.10	2.95	5.33
	-8.00	100.27	86.88	97.12	94.76	7.00	4.04	7.39
	-9.00	94.84	95.31	98.74	96.30	2.13	1.23	2.22
	-10.00	94.56	93.93	95.43	94.64	0.76	0.44	0.80

(continued)

Table 16. 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	0.87	1.03	0.96	0.95	0.08	0.05	8.38
	-4.00	6.27	6.43	6.18	6.29	0.12	0.07	1.96
	-4.60	NA	19.98	19.30	19.64	0.48	0.34	2.44
	-5.00	37.34	35.88	33.51	35.58	1.94	1.12	5.44
	-5.30	NA	48.92	49.91	49.42	0.70	0.49	1.41
	-6.00	80.12	83.24	78.03	80.47	2.62	1.51	3.26
	-7.00	89.99	92.15	88.70	90.28	1.74	1.00	1.93
	-8.00	89.75	90.66	90.02	90.15	0.47	0.27	0.52
	-9.00	91.26	NA	NA	91.26	NC	NC	NC
	-10.00	89.84	NA	NA	89.84	NC	NC	NC
ECZ	-6.00	0.25	0.29	0.22	0.26	0.04	0.02	13.59
	-7.00	2.07	2.01	2.27	2.12	0.13	0.08	6.34
	-8.00	16.71	17.02	19.10	17.61	1.30	0.75	7.38
	-8.30	29.25	30.84	34.13	31.41	2.49	1.44	7.94
	-9.00	74.94	74.70	71.14	73.59	2.13	1.23	2.89
	-9.30	90.18	82.87	90.48	87.85	4.31	2.49	4.91
	-9.60	91.49	94.98	94.86	93.78	1.98	1.14	2.11
	-10.00	99.78	94.58	98.95	97.77	2.80	1.62	2.86
CYN	-4.00	23.17	23.96	27.37	24.83	2.23	1.29	8.98
	-5.00	26.80	24.90	28.95	26.89	2.03	1.17	7.53
	-5.30	40.20	36.66	45.17	40.68	4.28	2.47	10.52
	-5.60	56.11	52.89	60.78	56.60	3.97	2.29	7.01
	-6.00	69.85	70.53	73.24	71.20	1.79	1.04	2.52
	-6.30	83.00	79.02	84.51	82.17	2.83	1.64	3.45
	-7.00	88.54	95.49	91.18	91.73	3.51	2.03	3.82
	-8.00	91.45	90.21	93.65	91.77	1.74	1.00	1.90
DCF	-3.00	2.26	2.23	2.45	2.32	0.12	0.07	5.09
	-3.30	NA	5.57	6.93	6.25	0.96	0.68	15.37
	-3.60	NA	12.31	12.52	12.42	0.15	0.10	1.19
	-4.00	23.32	29.09	24.66	25.69	3.02	1.75	11.77
	-5.00	78.88	76.92	72.56	76.12	3.24	1.87	4.25
	-6.00	94.55	103.39	92.39	96.78	5.83	3.37	6.02
	-7.00	94.10	101.13	91.71	95.64	4.90	2.83	5.12
	-8.00	98.14	109.33	97.92	101.80	6.53	3.77	6.41
	-9.00	98.19	NA	NA	98.19	NC	NC	NC
	-10.00	93.21	NA	NA	93.21	NC	NC	NC

(continued)

Table 16. 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	90.85	83.97	82.39	85.74	4.50	2.60	5.25
	-3.30	NA	90.54	84.63	87.59	4.18	2.95	4.77
	-3.60	NA	91.71	89.15	90.43	1.81	1.28	2.00
	-4.00	96.52	97.67	94.49	96.23	1.61	0.93	1.68
	-4.30	NA	99.75	92.13	95.94	5.39	3.81	5.62
	-5.00	95.88	98.70	95.56	96.71	1.73	1.00	1.79
	-6.00	94.08	100.72	94.53	96.44	3.71	2.14	3.85
	-7.00	98.04	106.65	92.08	98.92	7.32	4.23	7.40
	-8.00	96.03	NA	NA	96.03	NC	NC	NC
	-9.00	95.46	NA	NA	95.46	NC	NC	NC
	-10.00	90.15	NA	NA	90.15	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 or CV calculated.

4.4.3 IC_{50} Values

For each reference chemical 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 concentration response curves for each replicate of each RC are presented in Appendix F.

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 25-92% 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 -5.526. This value is not encompassed by the 95% confidence interval (-5.74 to -5.59) for LogIC50 (see Table 19). 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.

Figure 1 shows the concentration response curve fits of each replicate per reference chemical superimposed on one plot. It is clear that the replicate to replicate variation in curve fit is low.

Figure 2 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 16) were fit to the model.

The Top, Bottom, IC_{50} and slope data are summarized in Table 17 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 (overall means, SE and 95% confidence intervals) for the response model parameters for each chemical are presented in Table 18. Graphs of the top, bottom, IC_{50} and slope means, standard errors and confidence intervals are included in statistician's report in Appendix E.

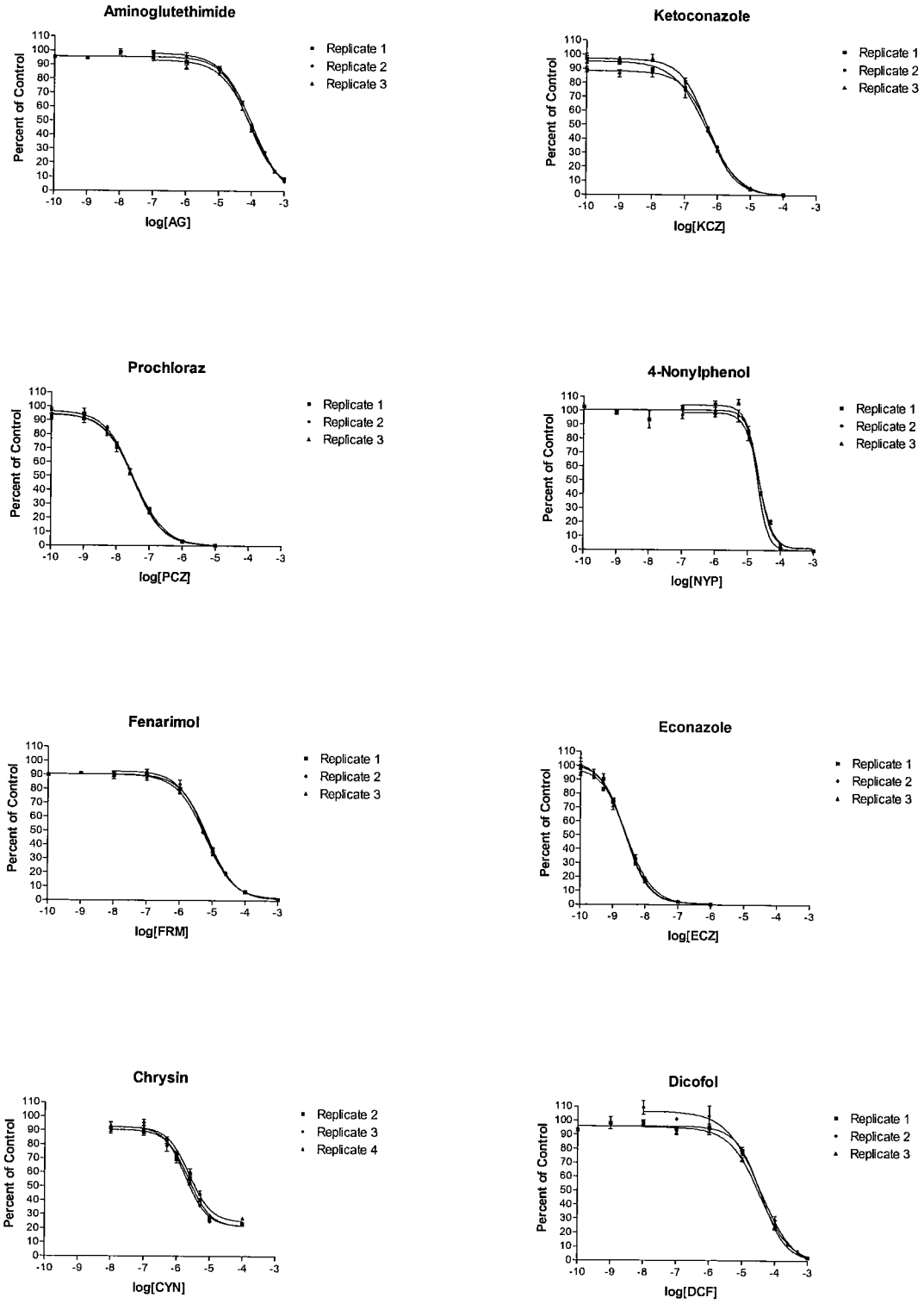

Figure 1. Concentration-Response Curve Fits – Overlay of Individual Replicate Results

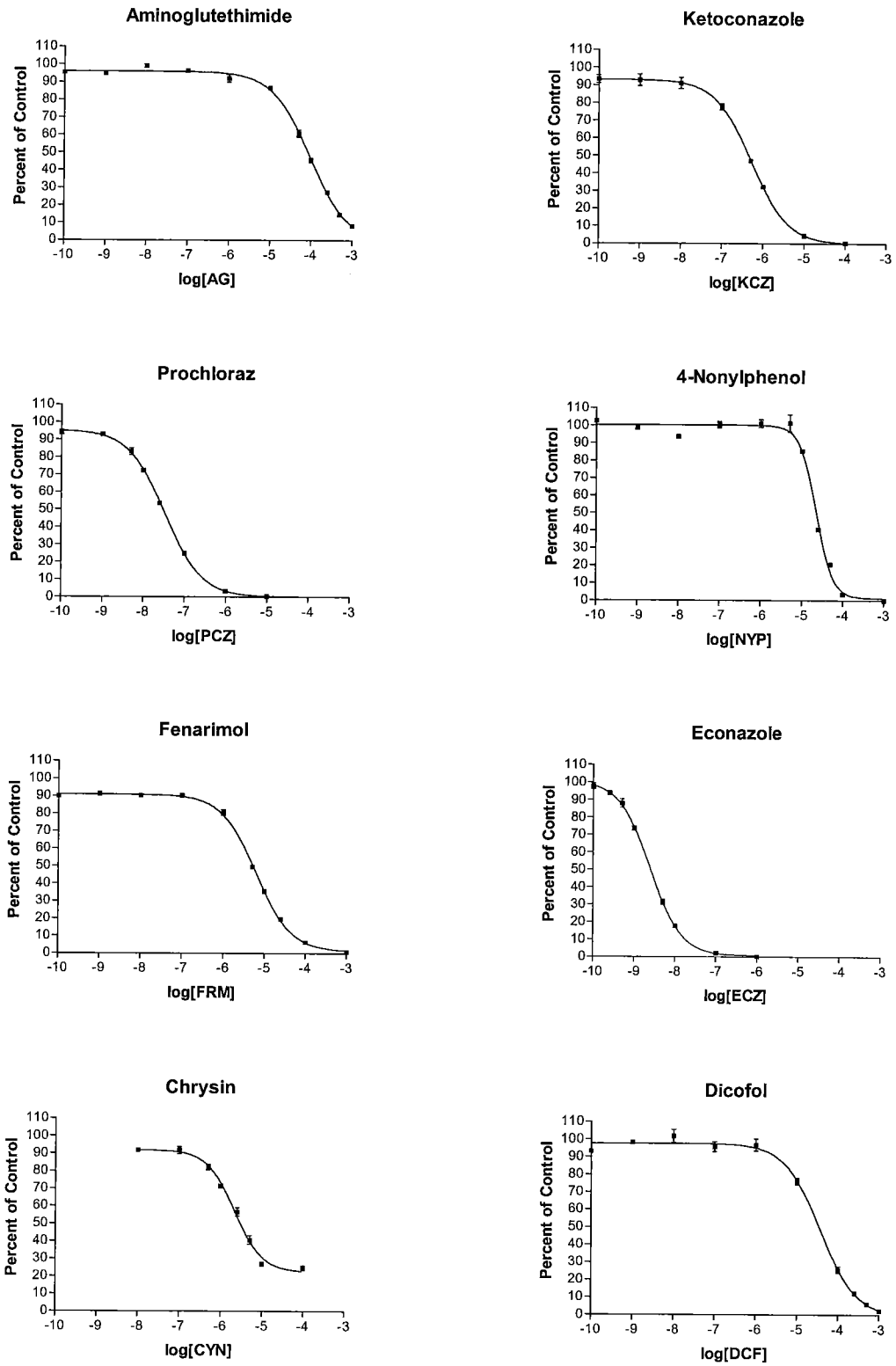

Figure 2. Average Concentration-Response Curve Fits

Table 17. 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	95.60	0.6988	2.781	2.934	-4.096	0.04096	-1.036	0.1105	Inhibitor
		2	98.23	1.009	-4.887	3.433	-3.978	0.03964	-0.9035	0.06290	Inhibitor
		3	93.28	1.397	-1.499	4.268	-4.018	0.05313	-0.9384	0.09583	Inhibitor
KCZ	2	1	95.08	1.68	-1.319	2.433	-6.332	0.04977	-0.8562	0.09097	Inhibitor
		2	88.06	1.08	-0.1461	1.587	-6.213	0.03158	-1.02	0.08696	Inhibitor
		3	96.83	1.211	0.2179	1.656	-6.308	0.03105	-1.056	0.08612	Inhibitor
PCZ	3	1	94.89	1.711	-0.1794	1.098	-7.459	0.04605	-0.9214	0.07189	Inhibitor
		2	96.65	1.060	0.2822	1.067	-7.487	0.02328	-0.9868	0.04696	Inhibitor
		3	94.31	1.692	0.1699	1.709	-7.482	0.03800	-0.9968	0.07788	Inhibitor
NYP	4	1	100.4	1.308	0.2218	2.936	-4.709	0.1469	-2.588	1.248	Inhibitor
		2	98.75	1.85	1.255	2.345	-4.654	0.02659	-2.018	0.2053	Inhibitor
		3	104.2	1.705	1.698	2.084	-4.673	0.02229	-2.186	0.2014	Inhibitor
DBA	5	1									no inhibition
		2									no inhibition
		3									no inhibition
FRM	6	1	90.51	0.5684	0.6013	0.9991	-5.152	0.02339	-1.041	0.05676	Inhibitor
		2	92.68	1.252	1.631	1.477	-5.219	0.02851	-1.049	0.07255	Inhibitor
		3	90.33	0.9310	0.8316	1.111	-5.218	0.02225	-0.9949	0.05099	Inhibitor
ECZ	7	2	100.6	1.571	0.6946	1.089	-8.614	0.02592	-1.216	0.06375	Inhibitor
		3	97.88	1.591	0.4549	1.094	-8.589	0.02698	-1.176	0.06406	Inhibitor
		4	103.7	3.627	0.1701	2.199	-8.617	0.05578	-1.043	0.1087	Inhibitor
CYN	8	2	90.54	1.870	21.16	2.513	-5.645	0.04807	-1.295	0.1526	Inhibitor
		3	92.6	1.927	21.55	2.429	-5.730	0.04671	-1.321	0.1533	Inhibitor
		4	92.48	1.645	24.61	2.254	-5.617	0.04349	-1.298	0.1393	Inhibitor
DCF	9	1	95.95	1.061	0.1887	2.846	-4.428	0.05282	-1.159	0.1085	Inhibitor
		2	106.5	2.287	-4.776	5.315	-4.453	0.08875	-0.8375	0.1125	Inhibitor
		3	95.29	1.157	-0.9486	2.385	-4.459	0.04765	-0.9354	0.07271	Inhibitor
ATZ	10	1									no inhibition
		2									no inhibition
		3									no inhibition

Status = codes as described in the statistical analysis section that describe the curve fit that lead to the IC_{50} calculation.

Table 18. Summary Statistics for Response Model Parameters by Chemical

Chemical	Dependent	Mean	Standard Error	Lower 95% CI	Upper 95% CI
AG	Top	95.82	1.35	92.96	98.68
AG	Bottom	-0.90	2.51	-6.22	4.42
AG	Log10(IC50)	-4.03	0.04	-4.11	-3.95
AG	Slope	-0.94	0.05	-1.04	-0.84
KCZ	Top	93.26	2.73	87.47	99.06
KCZ	Bottom	-0.22	1.04	-2.41	1.98
KCZ	Log10(IC50)	-6.28	0.04	-6.36	-6.20
KCZ	Slope	-0.98	0.06	-1.11	-0.85
PCZ	Top	95.73	0.82	94.00	97.46
PCZ	Bottom	0.08	0.70	-1.40	1.56
PCZ	Log10(IC50)	-7.48	0.02	-7.52	-7.44
PCZ	Slope	-0.97	0.04	-1.05	-0.90
NYP	Top	101.1	1.53	97.89	104.4
NYP	Bottom	1.22	1.38	-1.70	4.14
NYP	Log10(IC50)	-4.67	0.02	-4.70	-4.63
NYP	Slope	-2.26	0.17	-2.62	-1.91
FRM	Top	90.75	0.45	89.79	91.71
FRM	Bottom	0.89	0.66	-0.52	2.30
FRM	Log10(IC50)	-5.20	0.02	-5.24	-5.15
FRM	Slope	-1.02	0.03	-1.09	-0.95
ECZ	Top	99.78	1.32	96.98	102.6
ECZ	Bottom	0.53	0.73	-1.01	2.07
ECZ	Log10(IC50)	-8.60	0.02	-8.64	-8.57
ECZ	Slope	-1.17	0.04	-1.26	-1.09
CYN	Top	91.92	1.04	89.71	94.12
CYN	Bottom	22.58	1.38	19.65	25.51
CYN	Log10(IC50)	-5.66	0.03	-5.74	-5.59
CYN	Slope	-1.30	0.09	-1.49	-1.12
DCF	Top	98.99	3.50	91.58	106.4
DCF	Bottom	-0.93	1.73	-4.60	2.73
DCF	Log10(IC50)	-4.45	0.03	-4.52	-4.38
DCF	Slope	-0.97	0.09	-1.16	-0.79

5.0 DISCUSSION

This WA 4-17, Task 4 involved testing the response of human recombinant microsome aromatase activity to inhibition by ten reference chemicals. 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 recombinant microsomes used in this task had a reported protein concentration of 4.9 mg/mL. The average measured protein content of the microsome stock was 4.7 ± 1.1 mg/mL (24.2% CV).

QC standards of 12 and 110 μg/mL were included with each of the sixteen protein assays. Overall, the data show acceptable correlation (4.1% relative error) of calculated and known amounts for the high QC standard, while that found for the low QC standard was -17.7%.

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 recombinant microsomes was 0.3849 ± 0.1043 nmol/mg/min (27.1 % CV), the positive control activity averaged 0.2193 ± 0.0591 nmol/mg/min (26.9 %CV) and the negative control activity averaged 0.3822 ± 0.1043 nmol/mg/min (27.3 %CV). The data show decreases between beginning and end percent of control activities for each control type (except background) generally on the order of 5-20%. 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 positive and negative control percent of

control activities generally ranged from 45-70% and 80-120%, respectively, across all runs of the assay.

Each of the ten reference chemicals was added to the aromatase assay at eight predefined concentrations (usually 10^{-3} to 10^{-10} M) 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 a given technician.

The response curve was fitted by nonweighted least squares nonlinear regression analysis. The four-parameter model used in this work assignment led to satisfactory curve fits for the data sets where inhibitions of at least 80% were found. Because of the way Prism (Version 3.02) calculates estimates for $LogIC_{50}$, 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, as for chrysin in the current study and as discussed in Section 4.4.3 above.

IC_{50}s, slope, and top and bottom plateaus were estimated for each reference chemical using a four-parameter equation. There was little variance (3-15%) 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 4-17 Task 4, the performance of the aromatase assay was assessed using a recombinant microsome preparation and ten reference chemicals. The data reported here will be compared with data obtained from other participating laboratories using the same microsomal preparation and reference chemicals.

Aromatase activities for each type of control had coefficients of variance on the order of 27%. The coefficient of variance for the protein concentration of the microsomes was 24.2%, 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.

Because the curve parameters (top, bottom, IC50 and slope) are estimated based on percent of control values, the high CVs found for the control activities did not lead to high variances in the curve parameter estimates. For example, the variance in IC_{50} estimates was less than 15% 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 no 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.: 4-17
Task Number: 4
RTI Study Code: An05-947

TITLE: WA 4-17 Task 4: Conduct Multiple Chemical Studies with Recombinant Microsomes

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

AMENDMENTS:

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

Approved By:

James M. Mathews, Ph.D., DABT — Date 7-6-05
RTI Study Director

Jerry Johnson, Ph.D. DABT — Date 7-7-05
Work Assignment Leader, Battelle

Linda J. Phillips, Ph.D. — Date 7-8-05
EPA Project Officer

David P. Houchens, Ph.D. — Date 7/7/05
EDSP Program Manager, Battelle

Gary Timm, M.S., M.A. — Date 7/7/05
EPA Work Assignment Manager

Reviewed By:

Kim Collier, B.A. — Date 7/6/05
RTI, Quality Assurance Specialist

Terri L. Pollock, B.A. — Date 7-7-05
EDSP Quality Assurance Manager, Battelle

J. Thomas McClintock, Ph.D. — Date 7/7/05
EPA Quality Assurance Manager

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1.0 OBJECTIVES

TASK 4: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES

The objectives of this task are to test 10 reference chemicals using recombinant microsomes.

1.1 Justification for Test System

The test system for this study is recombinant microsomes. This test system was selected because it provides a commercially available source of the aromatase enzyme and since the assay is being evaluated for its potential to serve as a screening assay, the use of recombinant human enzyme 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 recombinant 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 4, 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
4-nonylphenol	104-40-5	$C_{15}H_{24}O$	220.4	Affects AR/ER; no aromatase inhibition

Reference chemical	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Basis for Selection
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

Recombinant microsomes will be supplied to each laboratory by RTI. 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 recombinant 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 0.008 mg/mL. The addition of 1 mL of that microsome dilution will result in a final approximate protein concentration of 0.004 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 to 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)	Recombinant
Microsomal Protein (mg/mL)[a]	0.004
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

Ten reference chemicals will be tested. 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. Each reference chemical will be tested at eight concentrations and there will be three (triplicate) repetitions for each concentration of a given replicate. 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. 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. A single replicate study of a given reference chemical is described in Table 4.

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 4. 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.: 4-17
Task Number: 4
RTI Study Code: An05-947

AMENDMENT 1

TITLE: WA 4-17 Task 4: Conduct Multiple Chemical Studies with Recombinant Microsomes

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.: 4-17
Task Number: 4
RTI Study Code: An05-947

AMENDMENT 2

TITLE: WA 4-17 Task 4: Conduct Multiple Chemical Studies with Recombinant Microsomes

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 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 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:

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

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.

WA 4-17, Task 4 Protocol Deviations

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, 3 and 4 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 J Black 9/28/05

Sherry Black Date
Research Chemist

Approved:

J Matt 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).

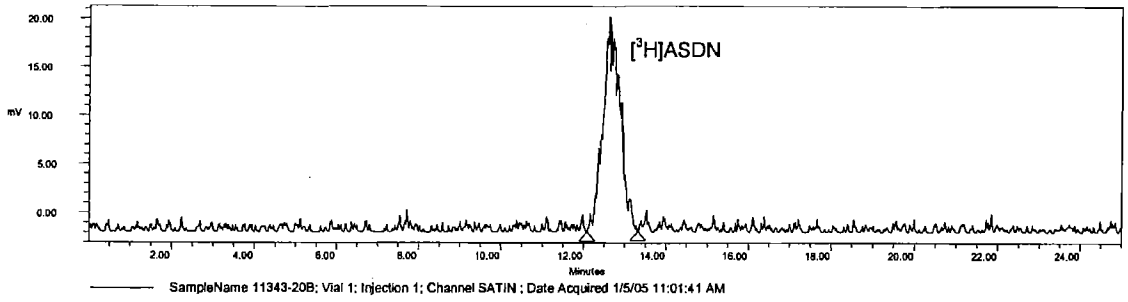
The radiochemical purity of the [^{3}H]ASDN (1:100 dilution in ethanol) was determined using high performance liquid chromatography (HPLC) and liquid scintillation counting. The HPLC system consists of a Waters 2690 Separations Module, a Waters 2487 Dual λ Absorbance Detector and a β-RAM Model 3 flow-through 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	2	Replicate #	1	Microsome type	Recombina Microsome ID 11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0194	31538	1625670	
2	0.0199	34396	1728442	
3	0.0196	36136	1843673	
4	0.0192	37813	1969427	
5	0.0191	35862	1877592	
			Average DPM/g soln	1808961
			SD	133925
			CV	7.40
			µCi/g soln	0.815

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	8.1158 g
Mass of dilution B used in substrate prep	4.5766 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563912 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00922 µg/g soln.
µg/g soln.

a. µCi/g soln 0.815
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.

= 0.563912 + 0.00922
= 0.573137 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.422 µCi/µg ASDN

903949 dpm/nmol

Assay Date 7/14/2005 | Test Chemical ID Ref Chem 1 | # Concentrations tested 8

Technician ID 2 | Replicate # 1 | Microsome type Recombinant | Microsome ID 11345-10

								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	0.0483
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

Blank 0.114

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

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

	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	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10		

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	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			

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
		Blank		0.114				

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

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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	1

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.9
Protein Concentration (dilution added to assay, mg/mL):	0.008164

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	9959	19916	19720	39440	0.1	180896	21.80	39294	0.0435	1	0.004	15	0.3550
			0.5	2	9762	19524			0.1					1		15	
	2	2	0.5	1	9578	19156	18922	37844	0.1	180896	20.92	37698	0.0417	1	0.004	15	0.3405
			0.5	2	9344	18688			0.1					1		15	
	3	2	0.5	1	9278	18556	17827	35654	0.1	180896	19.71	35508	0.0393	1	0.004	15	0.3208
			0.5	2	8549	17098			0.1					1		15	
	4	2	0.5	1	8230	16460	16436	32872	0.1	180896	18.17	32726	0.0362	1	0.004	15	0.2956
			0.5	2	8206	16412			0.1					1		15	
Background control	1	2	0.5	1	35	70	74	148	0.1	180896	0.08	2	0.0000	1	0.004	15	0.0000
			0.5	2	39	78			0.1					1		15	
	2	2	0.5	1	35	70	58	116	0.1	180896	0.06	-30	0.0000	1	0.004	15	-0.0003
			0.5	2	23	46			0.1					1		15	
	3	2	0.5	1	46	92	91	182	0.1	180896	0.10	36	0.0000	1	0.004	15	0.0003
			0.5	2	45	90			0.1					1		15	
	4	2	0.5	1	34	68	69	138	0.1	180896	0.08	-8	0.0000	1	0.004	15	-0.0001
			0.5	2	35	70			0.1					1		15	
Positive control	1	2	0.5	1	5229	10458	10622	21244	0.1	180896	11.74	21098	0.0233	1	0.004	15	0.1906
			0.5	2	5393	10786			0.1					1		15	
	2	2	0.5	1	4974	9948	10034	20068	0.1	180896	11.09	19922	0.0220	1	0.004	15	0.1800
			0.5	2	5060	10120			0.1					1		15	
	3	2	0.5	1	4511	9022	9054	18108	0.1	180896	10.01	17962	0.0199	1	0.004	15	0.1623
			0.5	2	4543	9086			0.1					1		15	
	4	2	0.5	1	4433	8866	8826	17652	0.1	180896	9.76	17506	0.0194	1	0.004	15	0.1581
			0.5	2	4393	8786			0.1					1		15	
Negative Control	1	2	0.5	1	9266	18532	18211	36422	0.1	180896	20.13	36276	0.0401	1	0.004	15	0.3277
			0.5	2	8945	17890			0.1					1		15	
	2	2	0.5	1	9125	18250	18153	36306	0.1	180896	20.07	36160	0.0400	1	0.004	15	0.3267
			0.5	2	9028	18056			0.1					1		15	
	3	2	0.5	1	8069	16138	16033	32066	0.1	180896	17.73	31920	0.0353	1	0.004	15	0.2884
			0.5	2	7964	15928			0.1					1		15	
	4	2	0.5	1	7328	14656	14595	29190	0.1	180896	16.14	29044	0.0321	1	0.004	15	0.2624
			0.5	2	7267	14534			0.1					1		15	
Ref Chem 1	1-1	2	0.5	1	887	1774	1833	3666	0.1	180896	2.03	3520	0.0039	1	0.004	15	0.0318
			0.5	2	946	1892			0.1					1		15	
	1-2	2	0.5	1	900	1800	1759	3518	0.1	180896	1.94	3372	0.0037	1	0.004	15	0.0305
			0.5	2	859	1718			0.1					1		15	
	1-3	2	0.5	1	836	1672	1610	3220	0.1	180896	1.78	3074	0.0034	1	0.004	15	0.0278
			0.5	2	774	1548			0.1					1		15	
	2-1	2	0.5	1	3690	7380	7422	14844	0.1	180896	8.21	14698	0.0163	1	0.004	15	0.1328
			0.5	2	3732	7464			0.1					1		15	
	2-2	2	0.5	1	4200	8400	8467	16934	0.1	180896	9.36	16788	0.0186	1	0.004	15	0.1517
			0.5	2	4267	8534			0.1					1		15	
	2-3	2	0.5	1	4117	8234	8180	16360	0.1	180896	9.04	16214	0.0179	1	0.004	15	0.1465
			0.5	2	4063	8126			0.1					1		15	
	3-1	2	0.5	1	7955	15910	15854	31708	0.1	180896	17.53	31562	0.0349	1	0.004	15	0.2851
			0.5	2	7899	15798			0.1					1		15	
	3-2	2	0.5	1	5085	10170	10349	20698	0.1	180896	11.44	20552	0.0227	1	0.004	15	0.1857
			0.5	2	5264	10528			0.1					1		15	
	3-3	2	0.5	1	8058	16116	15840	31680	0.1	180896	17.51	31534	0.0349	1	0.004	15	0.2849
			0.5	2	7782	15564			0.1					1		15	
	4-1	2	0.5	1	8557	17114	17046	34092	0.1	180896	18.85	33946	0.0376	1	0.004	15	0.3067
			0.5	2	8489	16978			0.1					1		15	
	4-2	2	0.5	1	8135	16270	16392	32784	0.1	180896	18.12	32638	0.0361	1	0.004	15	0.2948
			0.5	2	8257	16514			0.1					1		15	
	4-3	2	0.5	1	8463	16926	16906	33812	0.1	180896	18.69	33666	0.0372	1	0.004	15	0.3041
			0.5	2	8443	16886			0.1					1		15	
	5-1	2	0.5	1	8598	17196	17133	34266	0.1	180896	18.94	34120	0.0377	1	0.004	15	0.3082
			0.5	2	8535	17070			0.1					1		15	
	5-2	2	0.5	1	8542	17084	17212	34424	0.1	180896	19.03	34278	0.0379	1	0.004	15	0.3097
			0.5	2	8670	17340			0.1					1		15	
	5-3	2	0.5	1	8896	17792	17703	35406	0.1	180896	19.57	35260	0.0390	1	0.004	15	0.3185
			0.5	2	8807	17614			0.1					1		15	
	6-1	2	0.5	1	8974	17948	17791	35582	0.1	180896	19.67	35436	0.0392	1	0.004	15	0.3201
			0.5	2	8817	17634			0.1					1		15	
	6-2	2	0.5	1	8735	17470	17530	35060	0.1	180896	19.38	34914	0.0386	1	0.004	15	0.3154
			0.5	2	8795	17590			0.1					1		15	
	6-3	2	0.5	1	9411	18822	18823	37646	0.1	180896	20.81	37500	0.0415	1	0.004	15	0.3388
			0.5	2	9412	18824			0.1					1		15	
	7-1	2	0.5	1	8745	17490	17205	34410	0.1	180896	19.02	34264	0.0379	1	0.004	15	0.3095
			0.5	2	8460	16920			0.1					1		15	
	7-2	2	0.5	1	8471	16942	17060	34120	0.1	180896	18.86	33974	0.0376	1	0.004	15	0.3069
			0.5	2	8589	17178			0.1					1		15	
	7-3	2	0.5	1	8808	17616	17601	35202	0.1	180896	19.46	35056	0.0388	1	0.004	15	0.3167
			0.5	2	8793	17586			0.1					1		15	
	8-1	2	0.5	1	8781	17562	17353	34706	0.1	180896	19.19	34560	0.0382	1	0.004	15	0.3122
			0.5	2	8572	17144			0.1					1		15	
	8-2	2	0.5	1	8578	17156	17233	34466	0.1	180896	19.05	34320	0.0380	1	0.004	15	0.3100
			0.5	2	8655	17310			0.1					1		15	
	8-3	2	0.5	1	8723	17446	17560	35120	0.1	180896	19.41	34974	0.0367	1	0.004	15	0.3159
			0.5	2	8837	17674			0.1					1		15	

Assay Date	7/14/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	Replicate # 2 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.3478	0.0102
Full activity	End	0.3082	0.0178
Full activity	Overall	0.3280	0.0257
Background	Beginning	-0.0001	0.000204406
Background	End	0.0001	0.000281058
Background	Overall	0.0000	0.000248162
Positive	Beginning	0.1853	0.0075
Positive	End	0.1602	0.0029
Positive	Overall	0.1727	0.0152
Negative	Beginning	0.3272	0.0007
Negative	End	0.2754	0.0184
Negative	Overall	0.3013	0.0317

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 1	1	1	1.00E-03	-3.00	0.0318
Ref Chem 1	1	2	1.00E-03	-3.00	0.0305
Ref Chem 1	1	3	1.00E-03	-3.00	0.0278
Ref Chem 1	2	1	1.00E-04	-4.00	0.1328
Ref Chem 1	2	2	1.00E-04	-4.00	0.1517
Ref Chem 1	2	3	1.00E-04	-4.00	0.1465
Ref Chem 1	3	1	1.00E-05	-5.00	0.2851
Ref Chem 1	3	2	1.00E-05	-5.00	0.1857
Ref Chem 1	3	3	1.00E-05	-5.00	0.2849
Ref Chem 1	4	1	1.00E-06	-6.00	0.3067
Ref Chem 1	4	2	1.00E-06	-6.00	0.2948
Ref Chem 1	4	3	1.00E-06	-6.00	0.3041
Ref Chem 1	5	1	1.00E-07	-7.00	0.3082
Ref Chem 1	5	2	1.00E-07	-7.00	0.3097
Ref Chem 1	5	3	1.00E-07	-7.00	0.3185
Ref Chem 1	6	1	1.00E-08	-8.00	0.3201
Ref Chem 1	6	2	1.00E-08	-8.00	0.3154
Ref Chem 1	6	3	1.00E-08	-8.00	0.3388
Ref Chem 1	7	1	1.00E-09	-9.00	0.3095
Ref Chem 1	7	2	1.00E-09	-9.00	0.3069
Ref Chem 1	7	3	1.00E-09	-9.00	0.3167
Ref Chem 1	8	1	1.00E-10	-10.00	0.3122
Ref Chem 1	8	2	1.00E-10	-10.00	0.3100
Ref Chem 1	8	3	1.00E-10	-10.00	0.3159

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	9.70	9.29	8.47
2	-4.00	40.48	46.24	44.66
3	-5.00	86.93	56.61	86.85
4	-6.00	93.50	89.90	92.73
5	-7.00	93.98	94.41	97.12
6	-8.00	97.60	96.16	103.29
7	-9.00	94.37	93.58	96.56
8	-10.00	95.19	94.53	96.33

Aromatase Assay Spreadsheet

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8
Technician ID	2	Replicate #	2	Microsome type	Recombina Microsome ID 11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0206	41914	2034660	
2	0.0208	38305	1841587	
3	0.0196	41548	2119796	
4	0.0202	38762	1918911	
5	0.0199	41628	2091859	
			Average DPM/g soln	2001363
			SD	117953
			CV	5.89
			µCi/g soln	0.902

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	8.1332 g
Mass of dilution B used in substrate prep	4.5844 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563665 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01021 µg/g soln.
µg/g soln.

a. µCi/g soln 0.902
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.

= 0.563665 + 0.01021
= 0.573870 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.571 µCi/µg ASDN

998815 dpm/nmol

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8						
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10				

								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	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10				

								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	0.0402	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_{regr}, 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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.6
Protein Concentration (dilution added to assay, mg/mL):	0.00768

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	8479	16958	16703	33406	0.1	200136	16.69	33273	0.0333	1	0.004	15	0.2892
			0.5	2	8224	16448											
	2	2	0.5	1	8616	17232	17210	34420	0.1	200136	17.20	34287	0.0343	1	0.004	15	0.2980
			0.5	2	8594	17188											
	3	2	0.5	1	7436	14872	15075	30150	0.1	200136	15.06	30017	0.0301	1	0.004	15	0.2609
			0.5	2	7639	15278											
	4	2	0.5	1	7759	15518	15459	30918	0.1	200136	15.45	30785	0.0308	1	0.004	15	0.2675
			0.5	2	7700	15400											
Background control	1	2	0.5	1	34	68	64	128	0.1	200136	0.06	-8	0.0000	1	0.004	15	0.0000
			0.5	2	30	60											
	2	2	0.5	1	31	62	47	94	0.1	200136	0.05	-40	0.0000	1	0.004	15	-0.0003
			0.5	2	16	32											
	3	2	0.5	1	35	70	70	140	0.1	200136	0.07	7	0.0000	1	0.004	15	0.0001
			0.5	2	35	70											
	4	2	0.5	1	48	96	86	172	0.1	200136	0.09	39	0.0000	1	0.004	15	0.0003
			0.5	2	38	76											
Postive control	1	2	0.5	1	5137	10274	10217	20434	0.1	200136	10.21	20301	0.0203	1	0.004	15	0.1764
			0.5	2	5080	10160											
	2	2	0.5	1	5095	10190	10201	20402	0.1	200136	10.19	20269	0.0203	1	0.004	15	0.1761
			0.5	2	5106	10212											
	3	2	0.5	1	4772	9544	9465	18930	0.1	200136	9.46	18797	0.0188	1	0.004	15	0.1634
			0.5	2	4693	9386											
	4	2	0.5	1	4826	9652	9641	19282	0.1	200136	9.63	19149	0.0192	1	0.004	15	0.1664
			0.5	2	4815	9630											
Negative Control	1	2	0.5	1	7831	15662	15693	31386	0.1	200136	15.68	31253	0.0313	1	0.004	15	0.2716
			0.5	2	7862	15724											
	2	2	0.5	1	8074	16148	16137	32274	0.1	200136	16.13	32141	0.0322	1	0.004	15	0.2793
			0.5	2	8063	16126											
	3	2	0.5	1	7324	14648	14765	29530	0.1	200136	14.75	29397	0.0294	1	0.004	15	0.2555
			0.5	2	7441	14882											
	4	2	0.5	1	7396	14792	14966	29932	0.1	200136	14.96	29799	0.0298	1	0.004	15	0.2590
			0.5	2	7570	15140											
Ref Chem 1	1-1	2	0.5	1	573	1146	1165	2330	0.1	200136	1.16	2197	0.0022	1	0.004	15	0.0191
			0.5	2	592	1184											
	1-2	2	0.5	1	589	1178	1188	2376	0.1	200136	1.19	2243	0.0022	1	0.004	15	0.0195
			0.5	2	599	1198											
	1-3	2	0.5	1	648	1296	1275	2550	0.1	200136	1.27	2417	0.0024	1	0.004	15	0.0210
			0.5	2	627	1254											
	2-1	2	0.5	1	1177	2354	2438	4876	0.1	200136	2.44	4743	0.0047	1	0.004	15	0.0412
			0.5	2	1261	2522											
	2-2	2	0.5	1	1263	2526	2471	4942	0.1	200136	2.47	4809	0.0048	1	0.004	15	0.0418
			0.5	2	1208	2416											
	2-3	2	0.5	1	1235	2470	2451	4902	0.1	200136	2.45	4769	0.0048	1	0.004	15	0.0414
			0.5	2	1216	2432											
	3-1	2	0.5	1	2276	4552	4563	9126	0.1	200136	4.56	8993	0.0090	1	0.004	15	0.0781
			0.5	2	2287	4574											
	3-2	2	0.5	1	2267	4534	4592	9184	0.1	200136	4.59	9051	0.0091	1	0.004	15	0.0787
			0.5	2	2325	4650											
	3-3	2	0.5	1	2328	4656	4514	9028	0.1	200136	4.51	8895	0.0089	1	0.004	15	0.0773
			0.5	2	2186	4372											
	4-1	2	0.5	1	3925	7850	7791	15582	0.1	200136	7.79	15449	0.0155	1	0.004	15	0.1343
			0.5	2	3866	7732											
	4-2	2	0.5	1	3724	7448	7516	15032	0.1	200136	7.51	14899	0.0149	1	0.004	15	0.1295
			0.5	2	3792	7584											
	4-3	2	0.5	1	3953	7906	7879	15758	0.1	200136	7.87	15625	0.0156	1	0.004	15	0.1358
			0.5	2	3926	7852											
	5-1	2	0.5	1	5076	10152	10117	20234	0.1	200136	10.11	20101	0.0201	1	0.004	15	0.1747
			0.5	2	5041	10082											
	5-2	2	0.5	1	4853	9706	9766	19532	0.1	200136	9.76	19399	0.0194	1	0.004	15	0.1686
			0.5	2	4913	9826											
	5-3	2	0.5	1	5346	10692	10574	21148	0.1	200136	10.57	21015	0.0210	1	0.004	15	0.1826
			0.5	2	5228	10456											
	6-1	2	0.5	1	6975	13950	14032	28064	0.1	200136	14.02	27931	0.0280	1	0.004	15	0.2427
			0.5	2	7057	14114											
	6-2	2	0.5	1	7183	14366	14567	29134	0.1	200136	14.56	29001	0.0290	1	0.004	15	0.2520
			0.5	2	7384	14768											
	6-3	2	0.5	1	6908	13816	13925	27850	0.1	200136	13.92	27717	0.0277	1	0.004	15	0.2409
			0.5	2	7017	14034											
	7-1	2	0.5	1	7799	15598	15428	30856	0.1	200136	15.42	30723	0.0308	1	0.004	15	0.2670
			0.5	2	7629	15258											
	7-2	2	0.5	1	7335	14670	14636	29272	0.1	200136	14.63	29139	0.0292	1	0.004	15	0.2532
			0.5	2	7301	14602											
	7-3	2	0.5	1	8102	16204	16235	32470	0.1	200136	16.22	32337	0.0324	1	0.004	15	0.2810
			0.5	2	8133	16266											
	8-1	2	0.5	1	7666	15332	15401	30802	0.1	200136	15.39	30669	0.0307	1	0.004	15	0.2665
			0.5	2	7735	15470											
	8-2	2	0.5	1	8186	16372	16240	32480	0.1	200136	16.23	32347	0.0324	1	0.004	15	0.2811
			0.5	2	8054	16108											
	8-3	2	0.5	1	8012	16024	16012	32024	0.1	200136	16.00	31891	0.0319	1	0.004	15	0.2771
			0.5	2	8000	16000											

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.2936	0.0062
Full activity	End	0.2642	0.0047
Full activity	Overall	0.2789	0.0175
Background	Beginning	-0.0002	0.000208933
Background	End	0.0002	0.000196643
Background	Overall	0.0000	0.000280035
Positive	Beginning	0.1763	0.0002
Positive	End	0.1649	0.0022
Positive	Overall	0.1706	0.0067
Negative	Beginning	0.2755	0.0055
Negative	End	0.2572	0.0025
Negative	Overall	0.2663	0.0111

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 1	1	1	1.00E-03	-3.00	0.0191
Ref Chem 1	1	2	1.00E-03	-3.00	0.0195
Ref Chem 1	1	3	1.00E-03	-3.00	0.0210
Ref Chem 1	2	1	5.00E-04	-3.30	0.0412
Ref Chem 1	2	2	5.00E-04	-3.30	0.0418
Ref Chem 1	2	3	5.00E-04	-3.30	0.0414
Ref Chem 1	3	1	2.50E-04	-3.60	0.0781
Ref Chem 1	3	2	2.50E-04	-3.60	0.0787
Ref Chem 1	3	3	2.50E-04	-3.60	0.0773
Ref Chem 1	4	1	1.00E-04	-4.00	0.1343
Ref Chem 1	4	2	1.00E-04	-4.00	0.1295
Ref Chem 1	4	3	1.00E-04	-4.00	0.1358
Ref Chem 1	5	1	5.00E-05	-4.30	0.1747
Ref Chem 1	5	2	5.00E-05	-4.30	0.1686
Ref Chem 1	5	3	5.00E-05	-4.30	0.1826
Ref Chem 1	6	1	1.00E-05	-5.00	0.2427
Ref Chem 1	6	2	1.00E-05	-5.00	0.2520
Ref Chem 1	6	3	1.00E-05	-5.00	0.2409
Ref Chem 1	7	1	1.00E-06	-6.00	0.2670
Ref Chem 1	7	2	1.00E-06	-6.00	0.2532
Ref Chem 1	7	3	1.00E-06	-6.00	0.2810
Ref Chem 1	8	1	1.00E-07	-7.00	0.2665
Ref Chem 1	8	2	1.00E-07	-7.00	0.2811
Ref Chem 1	8	3	1.00E-07	-7.00	0.2771

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	6.84	6.99	7.53
2	-3.30	14.78	14.98	14.86
3	-3.60	28.02	28.20	27.72
4	-4.00	48.14	46.43	48.69
5	-4.30	62.64	60.45	65.49
6	-5.00	87.04	90.37	86.37
7	-6.00	95.74	90.80	100.77
8	-7.00	95.57	100.80	99.38

Aromatase Assay Spreadsheet

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8
Technician ID	2	Replicate #	3	Microsome type	Recombina Microsome ID 11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0192	33955	1768490	
2	0.0198	35898	1813030	
3	0.0203	39473	1944483	
4	0.0201	33650	1674129	
5	0.0201	37886	1884876	
			Average DPM/g soln	1817002
			SD	104451
			CV	5.75
			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	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	8.1129 g
Mass of dilution B used in substrate prep	4.562 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562314 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00927 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.

= 0.562314 + 0.00927
= 0.571580 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.432 µCi/µg ASDN

910441 dpm/nmol

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8					
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10			

								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	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			
		Blank	0.112	r^2=	0.990						
				m=	0.109						
				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	0.254	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	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10		

								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	0.0430
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
			Blank	0.112				

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

r^2= 0.989
m= 0.094
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	0.254	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	Recombina	Microsome ID	11345-10	Technician ID		Replicate #	3

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.5
Protein Concentration (dilution added to assay, mg/mL):	0.0075

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	11580	23160	22972	45944	0.1	181700	25.29	45666	0.0502	1	0.004	15	0.4459
			0.5	2	11392	22784											
	2	2	0.5	1	11959	23918	23670	47340	0.1	181700	26.05	47062	0.0517	1	0.004	15	0.4595
			0.5	2	11711	23422											
	3	2	0.5	1	9813	19626	19666	39332	0.1	181700	21.65	39054	0.0429	1	0.004	15	0.3813
			0.5	2	9653	19706											
	4	2	0.5	1	10009	20018	19840	39680	0.1	181700	21.84	39402	0.0433	1	0.004	15	0.3847
			0.5	2	9831	19662											
Background control	1	2	0.5	1	74	148	128	256	0.1	181700	0.14	-22	0.0000	1	0.004	15	-0.0002
			0.5	2	54	108											
	2	2	0.5	1	77	154	162	324	0.1	181700	0.18	46	0.0001	1	0.004	15	0.0004
			0.5	2	85	170											
	3	2	0.5	1	68	136	145	290	0.1	181700	0.16	12	0.0000	1	0.004	15	0.0001
			0.5	2	77	154											
	4	2	0.5	1	75	150	121	242	0.1	181700	0.13	-36	0.0000	1	0.004	15	-0.0004
			0.5	2	46	92											
Positive control	1	2	0.5	1	6049	12098	12018	24036	0.1	181700	13.23	23758	0.0261	1	0.004	15	0.2320
			0.5	2	5969	11938											
	2	2	0.5	1	6075	12150	12158	24316	0.1	181700	13.38	24038	0.0264	1	0.004	15	0.2347
			0.5	2	6083	12166											
	3	2	0.5	1	6214	12428	12337	24674	0.1	181700	13.58	24396	0.0268	1	0.004	15	0.2382
			0.5	2	6123	12246											
	4	2	0.5	1	5617	11234	11204	22408	0.1	181700	12.33	22130	0.0243	1	0.004	15	0.2161
			0.5	2	5587	11174											
Negative Control	1	2	0.5	1	10316	20632	20677	41354	0.1	181700	22.76	41076	0.0451	1	0.004	15	0.4010
			0.5	2	10361	20722											
	2	2	0.5	1	9704	19408	19440	38880	0.1	181700	21.40	38602	0.0424	1	0.004	15	0.3769
			0.5	2	9736	19472											
	3	2	0.5	1	9977	19954	19925	39850	0.1	181700	21.93	39572	0.0435	1	0.004	15	0.3864
			0.5	2	9948	19896											
	4	2	0.5	1	10728	21456	21460	42920	0.1	181700	23.62	42642	0.0468	1	0.004	15	0.4163
			0.5	2	10732	21464											
Ref Chem 1	1-1	2	0.5	1	960	1920	1939	3878	0.1	181700	2.13	3600	0.0040	1	0.004	15	0.0351
			0.5	2	979	1958											
	1-2	2	0.5	1	942	1884	1877	3754	0.1	181700	2.07	3476	0.0038	1	0.004	15	0.0339
			0.5	2	935	1870											
	1-3	2	0.5	1	948	1896	1820	3640	0.1	181700	2.00	3362	0.0037	1	0.004	15	0.0328
			0.5	2	872	1744											
	2-1	2	0.5	1	1514	3028	2995	5990	0.1	181700	3.30	5712	0.0063	1	0.004	15	0.0558
			0.5	2	1481	2962											
	2-2	2	0.5	1	1755	3510	3431	6862	0.1	181700	3.78	6584	0.0072	1	0.004	15	0.0643
			0.5	2	1676	3352											
	2-3	2	0.5	1	1583	3126	3185	6370	0.1	181700	3.51	6092	0.0067	1	0.004	15	0.0595
			0.5	2	1622	3244											
	3-1	2	0.5	1	3034	6068	6067	12134	0.1	181700	6.68	11856	0.0130	1	0.004	15	0.1158
			0.5	2	3033	6066											
	3-2	2	0.5	1	2992	5984	5839	11678	0.1	181700	6.43	11400	0.0125	1	0.004	15	0.1113
			0.5	2	2847	5694											
	3-3	2	0.5	1	2827	5654	5707	11414	0.1	181700	6.28	11136	0.0122	1	0.004	15	0.1087
			0.5	2	2880	5760											
	4-1	2	0.5	1	4985	9970	9867	19734	0.1	181700	10.86	19456	0.0214	1	0.004	15	0.1900
			0.5	2	4882	9764											
	4-2	2	0.5	1	5089	10178	10245	20490	0.1	181700	11.28	20212	0.0222	1	0.004	15	0.1973
			0.5	2	5156	10312											
	4-3	2	0.5	1	4653	9306	9242	18484	0.1	181700	10.17	18206	0.0200	1	0.004	15	0.1778
			0.5	2	4589	9178											
	5-1	2	0.5	1	6438	12876	12725	25450	0.1	181700	14.01	25172	0.0276	1	0.004	15	0.2458
			0.5	2	6287	12574											
	5-2	2	0.5	1	6250	12500	12715	25430	0.1	181700	14.00	25152	0.0276	1	0.004	15	0.2456
			0.5	2	6465	12930											
	5-3	2	0.5	1	6344	12688	12713	25426	0.1	181700	13.99	25148	0.0276	1	0.004	15	0.2455
			0.5	2	6369	12738											
	6-1	2	0.5	1	9617	19234	19273	38546	0.1	181700	21.21	38268	0.0420	1	0.004	15	0.3736
			0.5	2	9656	19312											
	6-2	2	0.5	1	8925	17850	17961	35922	0.1	181700	19.77	35644	0.0392	1	0.004	15	0.3480
			0.5	2	9036	18072											
	6-3	2	0.5	1	8914	17828	17758	35516	0.1	181700	19.55	35238	0.0387	1	0.004	15	0.3440
			0.5	2	8844	17688											
	7-1	2	0.5	1	9686	19372	19190	38380	0.1	181700	21.12	38102	0.0419	1	0.004	15	0.3720
			0.5	2	9504	19008											
	7-2	2	0.5	1	9144	18288	18378	36756	0.1	181700	20.23	36478	0.0401	1	0.004	15	0.3561
			0.5	2	9234	18468											
	7-3	2	0.5	1	9903	19806	19537	39074	0.1	181700	21.50	38796	0.0426	1	0.004	15	0.3788
			0.5	2	9634	19268											
	8-1	2	0.5	1	10034	20068	19970	39940	0.1	181700	21.98	39662	0.0436	1	0.004	15	0.3872
			0.5	2	9936	19872											
	8-2	2	0.5	1	9967	19934	19950	39900	0.1	181700	21.96	39622	0.0435	1	0.004	15	0.3866
			0.5	2	9983	19966											
	8-3	2	0.5	1	11023	22046	21870	43740	0.1	181700	24.07	43462	0.0477	1	0.004	15	0.4243
			0.5	2	10847	21694											

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 1	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.4527	0.0096
Full activity	End	0.3830	0.0024
Full activity	Overall	0.4178	0.0406
Background	Beginning	0.0001	0.00046945
Background	End	-0.0001	0.000331377
Background	Overall	0.0000	0.000358283
Positive	Beginning	0.2333	0.0019
Positive	End	0.2271	0.0156
Positive	Overall	0.2302	0.0098
Negative	Beginning	0.3890	0.0171
Negative	End	0.4013	0.0212
Negative	Overall	0.3951	0.0173

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 1	1	1	1.00E-03	-3.00	0.0351
Ref Chem 1	1	2	1.00E-03	-3.00	0.0339
Ref Chem 1	1	3	1.00E-03	-3.00	0.0328
Ref Chem 1	2	1	5.00E-04	-3.30	0.0558
Ref Chem 1	2	2	5.00E-04	-3.30	0.0643
Ref Chem 1	2	3	5.00E-04	-3.30	0.0595
Ref Chem 1	3	1	2.50E-04	-3.60	0.1158
Ref Chem 1	3	2	2.50E-04	-3.60	0.1113
Ref Chem 1	3	3	2.50E-04	-3.60	0.1087
Ref Chem 1	4	1	1.00E-04	-4.00	0.1900
Ref Chem 1	4	2	1.00E-04	-4.00	0.1973
Ref Chem 1	4	3	1.00E-04	-4.00	0.1778
Ref Chem 1	5	1	5.00E-05	-4.30	0.2458
Ref Chem 1	5	2	5.00E-05	-4.30	0.2456
Ref Chem 1	5	3	5.00E-05	-4.30	0.2455
Ref Chem 1	6	1	1.00E-05	-5.00	0.3736
Ref Chem 1	6	2	1.00E-05	-5.00	0.3480
Ref Chem 1	6	3	1.00E-05	-5.00	0.3440
Ref Chem 1	7	1	1.00E-06	-6.00	0.3720
Ref Chem 1	7	2	1.00E-06	-6.00	0.3561
Ref Chem 1	7	3	1.00E-06	-6.00	0.3788
Ref Chem 1	8	1	1.00E-07	-7.00	0.3872
Ref Chem 1	8	2	1.00E-07	-7.00	0.3868
Ref Chem 1	8	3	1.00E-07	-7.00	0.4243

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	8.41	8.12	7.86
2	-3.30	13.35	15.38	14.23
3	-3.60	27.70	26.64	26.02
4	-4.00	45.46	47.23	42.54
5	-4.30	58.82	58.77	58.76
6	-5.00	89.42	83.29	82.34
7	-6.00	89.03	85.24	90.65
8	-7.00	92.68	92.58	101.56

Aromatase Assay Spreadsheet

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8		
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0206	41914	2034660	
2	0.0208	38305	1841587	
3	0.0196	41548	2119796	
4	0.0202	38762	1918911	
5	0.0199	41628	2091859	
			Average DPM/g soln	2001363
			SD	117953
			CV	5.89
			µCi/g soln	0.902

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	8.1332 g
Mass of dilution B used in substrate prep	4.5844 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563665 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01021 µg/g soln.
µg/g soln.

a. µCi/g soln 0.902
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.

= 0.563665 + 0.01021
= 0.573870 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.571 µCi/µg ASDN

998815 dpm/nmol

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8				
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10		

								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
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_{reg}, 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	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10				

								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	0.0402	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 2	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	11345-10	Technician ID		Replicate #	1

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.6
Protein Concentration (dilution added to assay, mg/mL):	0.00768

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	8479	16958	16703	33406	0.1	200136	16.69	33273	0.0333	1	0.004	15	0.2892
			0.5	2	8224	16448											
	2	2	0.5	1	8616	17232	17210	34420	0.1	200136	17.20	34287	0.0343	1	0.004	15	0.2980
			0.5	2	8594	17188											
	3	2	0.5	1	7436	14872	15075	30150	0.1	200136	15.06	30017	0.0301	1	0.004	15	0.2609
			0.5	2	7639	15278											
	4	2	0.5	1	7759	15518	15459	30918	0.1	200136	15.45	30785	0.0308	1	0.004	15	0.2675
			0.5	2	7700	15400											
Background control	1	2	0.5	1	34	68	64	128	0.1	200136	0.06	-6	0.0000	1	0.004	15	0.0000
			0.5	2	30	60											
	2	2	0.5	1	31	62	47	94	0.1	200136	0.05	-40	0.0000	1	0.004	15	-0.0003
			0.5	2	16	32											
	3	2	0.5	1	35	70	70	140	0.1	200136	0.07	7	0.0000	1	0.004	15	0.0001
			0.5	2	35	70											
	4	2	0.5	1	48	96	86	172	0.1	200136	0.09	39	0.0000	1	0.004	15	0.0003
			0.5	2	38	76											
Positive control	1	2	0.5	1	5137	10274	10217	20434	0.1	200136	10.21	20301	0.0203	1	0.004	15	0.1764
			0.5	2	5080	10160											
	2	2	0.5	1	5095	10190	10201	20402	0.1	200136	10.19	20269	0.0203	1	0.004	15	0.1761
			0.5	2	5106	10212											
	3	2	0.5	1	4772	9544	9465	18930	0.1	200136	9.46	18797	0.0188	1	0.004	15	0.1634
			0.5	2	4693	9386											
	4	2	0.5	1	4826	9652	9641	19282	0.1	200136	9.63	19149	0.0192	1	0.004	15	0.1664
			0.5	2	4815	9630											
Negative Control	1	2	0.5	1	7831	15662	15693	31386	0.1	200136	15.68	31253	0.0313	1	0.004	15	0.2716
			0.5	2	7862	15724											
	2	2	0.5	1	8074	16148	16137	32274	0.1	200136	16.13	32141	0.0322	1	0.004	15	0.2793
			0.5	2	8063	16126											
	3	2	0.5	1	7324	14648	14765	29530	0.1	200136	14.75	29397	0.0294	1	0.004	15	0.2555
			0.5	2	7441	14882											
	4	2	0.5	1	7396	14792	14966	29932	0.1	200136	14.96	29799	0.0298	1	0.004	15	0.2590
			0.5	2	7570	15140											
Ref Chem 2	1-1	2	0.5	1	80	160	154	308	0.1	200136	0.15	175	0.0002	1	0.004	15	0.0015
			0.5	2	74	148											
	1-2	2	0.5	1	56	112	108	216	0.1	200136	0.11	83	0.0001	1	0.004	15	0.0007
			0.5	2	52	104											
	1-3	2	0.5	1	63	126	129	258	0.1	200136	0.13	125	0.0001	1	0.004	15	0.0011
			0.5	2	66	132											
	2-1	2	0.5	1	407	814	779	1558	0.1	200136	0.78	1425	0.0014	1	0.004	15	0.0124
			0.5	2	372	744											
	2-2	2	0.5	1	390	780	747	1494	0.1	200136	0.75	1361	0.0014	1	0.004	15	0.0118
			0.5	2	357	714											
	2-3	2	0.5	1	382	764	773	1546	0.1	200136	0.77	1413	0.0014	1	0.004	15	0.0123
			0.5	2	391	782											
	3-1	2	0.5	1	2496	4992	4892	9784	0.1	200136	4.89	9651	0.0097	1	0.004	15	0.0839
			0.5	2	2396	4792											
	3-2	2	0.5	1	2653	5306	5249	10498	0.1	200136	5.25	10365	0.0104	1	0.004	15	0.0901
			0.5	2	2596	5192											
	3-3	2	0.5	1	2549	5098	5078	10156	0.1	200136	5.07	10023	0.0100	1	0.004	15	0.0871
			0.5	2	2529	5058											
	4-1	2	0.5	1	3719	7438	7339	14678	0.1	200136	7.33	14545	0.0146	1	0.004	15	0.1264
			0.5	2	3620	7240											
	4-2	2	0.5	1	3724	7448	7555	15110	0.1	200136	7.55	14977	0.0150	1	0.004	15	0.1302
			0.5	2	3831	7662											
	4-3	2	0.5	1	3683	7366	7413	14826	0.1	200136	7.41	14693	0.0147	1	0.004	15	0.1277
			0.5	2	3730	7460											
	5-1	2	0.5	1	6727	13454	13323	26646	0.1	200136	13.31	26513	0.0265	1	0.004	15	0.2304
			0.5	2	6596	13192											
	5-2	2	0.5	1	6451	12902	12917	25834	0.1	200136	12.91	25701	0.0257	1	0.004	15	0.2233
			0.5	2	6466	12932											
	5-3	2	0.5	1	5066	10132	10172	20344	0.1	200136	10.17	20211	0.0202	1	0.004	15	0.1756
			0.5	2	5106	10212											
	6-1	2	0.5	1	7249	14498	14289	28578	0.1	200136	14.28	28445	0.0285	1	0.004	15	0.2472
			0.5	2	7040	14080											
	6-2	2	0.5	1	7429	14858	14844	29688	0.1	200136	14.83	29555	0.0296	1	0.004	15	0.2568
			0.5	2	7415	14830											
	6-3	2	0.5	1	7001	14002	13970	27940	0.1	200136	13.96	27807	0.0278	1	0.004	15	0.2417
			0.5	2	6969	13938											
	7-1	2	0.5	1	7955	15910	15794	31588	0.1	200136	15.78	31455	0.0315	1	0.004	15	0.2734
			0.5	2	7839	15678											
	7-2	2	0.5	1	7488	14976	14955	29910	0.1	200136	14.94	29777	0.0298	1	0.004	15	0.2588
			0.5	2	7467	14934											
	7-3	2	0.5	1	7483	14966	14960	29920	0.1	200136	14.95	29787	0.0298	1	0.004	15	0.2589
			0.5	2	7477	14954											
	8-1	2	0.5	1	7395	14790	14592	29184	0.1	200136	14.58	29051	0.0291	1	0.004	15	0.2525
			0.5	2	7197	14394											
	8-2	2	0.5	1	7854	15708	15699	31398	0.1	200136	15.69	31265	0.0313	1	0.004	15	0.2717
			0.5	2	7845	15690											
	8-3	2	0.5	1	8229	16458	16574	33148	0.1	200136	16.56	33015	0.0331	1	0.004	15	0.2869
			0.5	2	8345	16690											

Assay Date	7/18/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.2936	0.0062
Full activity	End	0.2642	0.0047
Full activity	Overall	0.2789	0.0175
Background	Beginning	-0.0002	0.000208933
Background	End	0.0002	0.000196643
Background	Overall	0.0000	0.000280035
Positive	Beginning	0.1763	0.0002
Positive	End	0.1649	0.0022
Positive	Overall	0.1706	0.0067
Negative	Beginning	0.2755	0.0055
Negative	End	0.2572	0.0025
Negative	Overall	0.2663	0.0111

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 2	1	1	1.00E-04	-4.00	0.0015
Ref Chem 2	1	2	1.00E-04	-4.00	0.0007
Ref Chem 2	1	3	1.00E-04	-4.00	0.0011
Ref Chem 2	2	1	1.00E-05	-5.00	0.0124
Ref Chem 2	2	2	1.00E-05	-5.00	0.0118
Ref Chem 2	2	3	1.00E-05	-5.00	0.0123
Ref Chem 2	3	1	1.00E-06	-6.00	0.0839
Ref Chem 2	3	2	1.00E-06	-6.00	0.0901
Ref Chem 2	3	3	1.00E-06	-6.00	0.0871
Ref Chem 2	4	1	5.00E-07	-6.30	0.1264
Ref Chem 2	4	2	5.00E-07	-6.30	0.1302
Ref Chem 2	4	3	5.00E-07	-6.30	0.1277
Ref Chem 2	5	1	1.00E-07	-7.00	0.2304
Ref Chem 2	5	2	1.00E-07	-7.00	0.2233
Ref Chem 2	5	3	1.00E-07	-7.00	0.1756
Ref Chem 2	6	1	1.00E-08	-8.00	0.2472
Ref Chem 2	6	2	1.00E-08	-8.00	0.2568
Ref Chem 2	6	3	1.00E-08	-8.00	0.2417
Ref Chem 2	7	1	1.00E-09	-9.00	0.2734
Ref Chem 2	7	2	1.00E-09	-9.00	0.2588
Ref Chem 2	7	3	1.00E-09	-9.00	0.2589
Ref Chem 2	8	1	1.00E-10	-10.00	0.2525
Ref Chem 2	8	2	1.00E-10	-10.00	0.2717
Ref Chem 2	8	3	1.00E-10	-10.00	0.2869

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.54	0.26	0.39
2	-5.00	4.44	4.24	4.40
3	-6.00	30.07	32.30	31.23
4	-6.30	45.32	46.67	45.79
5	-7.00	82.62	80.09	62.98
6	-8.00	88.64	92.10	86.65
7	-9.00	98.02	92.79	92.82
8	-10.00	90.53	97.43	102.88

Aromatase Assay Spreadsheet

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8		
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0192	33955	1768490	
2	0.0198	35898	1813030	
3	0.0203	39473	1944483	
4	0.0201	33650	1674129	
5	0.0201	37886	1884876	
			Average DPM/g soln	1817002
			SD	104451
			CV	5.75
			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	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	8.1129 g
Mass of dilution B used in substrate prep	4.562 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562314 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00927 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.

= 0.562314 + 0.00927
= 0.571580 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.432 µCi/µg ASDN

910441 dpm/nmol

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8						
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10				

								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		

Blank 0.112

r^2= 0.990
m= 0.109
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	0.254	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	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10		

								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	0.0430
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
			Blank	0.112				

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

r^2= 0.989
m= 0.094
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	0.254	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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.5
Protein Concentration (dilution added to assay, mg/mL):	0.007441

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	11580	23160	22972	45944	0.1	181700	25.29	45666	0.0902	1	0.004	15	0.4494
			0.5	2	11392	22784											
	2	2	0.5	1	11959	23918	23670	47340	0.1	181700	26.05	47062	0.0517	1	0.004	15	0.4631
			0.5	2	11711	23422											
	3	2	0.5	1	9813	19626	19666	39332	0.1	181700	21.65	39054	0.0429	1	0.004	15	0.3843
			0.5	2	9853	19706											
	4	2	0.5	1	10009	20018	19840	39680	0.1	181700	21.84	39402	0.0433	1	0.004	15	0.3877
			0.5	2	9831	19662											
Background control	1	2	0.5	1	74	148	128	256	0.1	181700	0.14	-22	0.0000	1	0.004	15	-0.0002
			0.5	2	54	108											
	2	2	0.5	1	77	154	162	324	0.1	181700	0.18	46	0.0001	1	0.004	15	0.0005
			0.5	2	85	170											
	3	2	0.5	1	68	136	145	290	0.1	181700	0.16	12	0.0000	1	0.004	15	0.0001
			0.5	2	77	154											
	4	2	0.5	1	75	150	121	242	0.1	181700	0.13	-36	0.0000	1	0.004	15	-0.0004
			0.5	2	46	92											
Positive control	1	2	0.5	1	6049	12098	12018	24036	0.1	181700	13.23	23758	0.0261	1	0.004	15	0.2338
			0.5	2	5969	11938											
	2	2	0.5	1	6075	12150	12158	24316	0.1	181700	13.38	24038	0.0264	1	0.004	15	0.2365
			0.5	2	6083	12166											
	3	2	0.5	1	6214	12428	12337	24674	0.1	181700	13.58	24396	0.0268	1	0.004	15	0.2401
			0.5	2	6123	12246											
	4	2	0.5	1	5617	11234	11204	22408	0.1	181700	12.33	22130	0.0243	1	0.004	15	0.2178
			0.5	2	5587	11174											
Negative Control	1	2	0.5	1	10316	20632	20677	41354	0.1	181700	22.76	41076	0.0451	1	0.004	15	0.4042
			0.5	2	10361	20722											
	2	2	0.5	1	9704	19408	19440	38880	0.1	181700	21.40	38602	0.0424	1	0.004	15	0.3799
			0.5	2	9736	19472											
	3	2	0.5	1	9977	19954	19925	39850	0.1	181700	21.93	39572	0.0435	1	0.004	15	0.3894
			0.5	2	9948	19896											
	4	2	0.5	1	10728	21456	21460	42920	0.1	181700	23.62	42642	0.0468	1	0.004	15	0.4196
			0.5	2	10732	21464											
Ref Chem 2	1-1	2	0.5	1	71	142	154	308	0.1	181700	0.17	30	0.0000	1	0.004	15	0.0003
			0.5	2	83	166											
	1-2	2	0.5	1	87	174	169	338	0.1	181700	0.19	60	0.0001	1	0.004	15	0.0006
			0.5	2	82	164											
	1-3	2	0.5	1	85	170	136	272	0.1	181700	0.15	-6	0.0000	1	0.004	15	-0.0001
			0.5	2	51	102											
	2-1	2	0.5	1	619	1238	1242	2484	0.1	181700	1.37	2206	0.0024	1	0.004	15	0.0217
			0.5	2	623	1246											
	2-2	2	0.5	1	574	1148	1147	2294	0.1	181700	1.26	2016	0.0022	1	0.004	15	0.0198
			0.5	2	573	1146											
	2-3	2	0.5	1	588	1176	1154	2308	0.1	181700	1.27	2030	0.0022	1	0.004	15	0.0200
			0.5	2	566	1132											
	3-1	2	0.25	1	1917	7668	7712	15424	0.1	181700	8.49	15146	0.0166	1	0.004	15	0.1490
			0.25	2	1939	7756											
	3-2	2	0.25	1	1892	7568	7612	15224	0.1	181700	8.38	14946	0.0164	1	0.004	15	0.1471
			0.25	2	1914	7656											
	3-3	2	0.5	1	3479	6958	7018	14036	0.1	181700	7.72	13758	0.0151	1	0.004	15	0.1354
			0.5	2	3539	7078											
	4-1	2	0.5	1	4840	9680	9603	19206	0.1	181700	10.57	18928	0.0208	1	0.004	15	0.1863
			0.5	2	4763	9526											
	4-2	2	0.5	1	5214	10428	10447	20894	0.1	181700	11.50	20616	0.0226	1	0.004	15	0.2029
			0.5	2	5233	10466											
	4-3	2	0.5	1	5275	10550	10548	21096	0.1	181700	11.61	20818	0.0229	1	0.004	15	0.2049
			0.5	2	5273	10546											
	5-1	2	0.5	1	8480	16960	17499	34998	0.1	181700	19.26	34720	0.0381	1	0.004	15	0.3417
			0.5	2	9019	18038											
	5-2	2	0.5	1	8221	16442	16287	32574	0.1	181700	17.93	32296	0.0355	1	0.004	15	0.3178
			0.5	2	8066	16132											
	5-3	2	0.5	1	8109	16218	16090	32180	0.1	181700	17.71	31902	0.0350	1	0.004	15	0.3139
			0.5	2	7981	15962											
	6-1	2	0.5	1	9752	19504	19262	38524	0.1	181700	21.20	38246	0.0420	1	0.004	15	0.3764
			0.5	2	9510	19020											
	6-2	2	0.5	1	8850	17700	17428	34856	0.1	181700	19.18	34578	0.0380	1	0.004	15	0.3403
			0.5	2	8578	17156											
	6-3	2	0.5	1	9630	19260	19482	38964	0.1	181700	21.44	38686	0.0425	1	0.004	15	0.3807
			0.5	2	9652	19704											
	7-1	2	0.5	1	8961	17922	18120	36240	0.1	181700	19.94	35962	0.0395	1	0.004	15	0.3539
			0.5	2	9159	18318											
	7-2	2	0.5	1	9013	18026	18010	36020	0.1	181700	19.82	35742	0.0393	1	0.004	15	0.3517
			0.5	2	8997	17994											
	7-3	2	0.5	1	9942	19884	19724	39448	0.1	181700	21.71	39170	0.0430	1	0.004	15	0.3855
			0.5	2	9782	19564											
	8-1	2	0.5	1	9812	19624	19358	38716	0.1	181700	21.31	38438	0.0422	1	0.004	15	0.3783
			0.5	2	9546	19092											
	8-2	2	0.5	1	9237	18474	18429	36858	0.1	181700	20.29	36580	0.0402	1	0.004	15	0.3600
			0.5	2	9192	18384											
	8-3	2	0.5	1	9876	19752	19807	39614	0.1	181700	21.80	39336	0.0432	1	0.004	15	0.3871
			0.5	2	9931	19862											

Assay Date	7/20/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.4563	0.0097
Full activity	End	0.3860	0.0024
Full activity	Overall	0.4211	0.0410
Background	Beginning	0.0001	0.000473169
Background	End	-0.0001	0.000334002
Background	Overall	0.0000	0.000361121
Positive	Beginning	0.2352	0.0019
Positive	End	0.2289	0.0158
Positive	Overall	0.2320	0.0099
Negative	Beginning	0.3920	0.0172
Negative	End	0.4045	0.0214
Negative	Overall	0.3983	0.0174

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 2	1	1	1.00E-04	-4.00	0.0003
Ref Chem 2	1	2	1.00E-04	-4.00	0.0006
Ref Chem 2	1	3	1.00E-04	-4.00	-0.0001
Ref Chem 2	2	1	1.00E-05	-5.00	0.0217
Ref Chem 2	2	2	1.00E-05	-5.00	0.0198
Ref Chem 2	2	3	1.00E-05	-5.00	0.0200
Ref Chem 2	3	1	1.00E-06	-6.00	0.1490
Ref Chem 2	3	2	1.00E-06	-6.00	0.1471
Ref Chem 2	3	3	1.00E-06	-6.00	0.1354
Ref Chem 2	4	1	5.00E-07	-6.30	0.1863
Ref Chem 2	4	2	5.00E-07	-6.30	0.2029
Ref Chem 2	4	3	5.00E-07	-6.30	0.2049
Ref Chem 2	5	1	1.00E-07	-7.00	0.3417
Ref Chem 2	5	2	1.00E-07	-7.00	0.3178
Ref Chem 2	5	3	1.00E-07	-7.00	0.3139
Ref Chem 2	6	1	1.00E-08	-8.00	0.3764
Ref Chem 2	6	2	1.00E-08	-8.00	0.3403
Ref Chem 2	6	3	1.00E-08	-8.00	0.3807
Ref Chem 2	7	1	1.00E-09	-9.00	0.3539
Ref Chem 2	7	2	1.00E-09	-9.00	0.3517
Ref Chem 2	7	3	1.00E-09	-9.00	0.3855
Ref Chem 2	8	1	1.00E-10	-10.00	0.3783
Ref Chem 2	8	2	1.00E-10	-10.00	0.3600
Ref Chem 2	8	3	1.00E-10	-10.00	0.3871

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.07	0.14	-0.01
2	-5.00	5.15	4.71	4.74
3	-6.00	35.39	34.92	32.15
4	-6.30	44.23	48.17	48.64
5	-7.00	81.13	75.46	74.54
6	-8.00	89.37	80.80	90.40
7	-9.00	84.03	83.52	91.53
8	-10.00	89.82	85.48	91.92

Aromatase Assay Spreadsheet

Assay Date	7/22/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8		
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11434-14

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	34553	1719055	
2	0.0199	35195	1768593	
3	0.0199	36159	1817035	
4	0.0204	36976	1812549	
5	0.0199	36195	1818844	
			Average DPM/g soln	1787215
			SD	43371
			CV	2.43
			µCi/g soln	0.805

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	8.1508 g
Mass of dilution B used in substrate prep	4.588 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.574147 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00911 µg/g soln.
µg/g soln.

a. µCi/g soln 0.805
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.

= 0.574147 + 0.00911
= 0.583261 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.380 µCi/µg ASDN

877581 dpm/nmol

Assay Date	7/22/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8						
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11434-14				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	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	Variables	Regression results	
250	200	200	0.00027	200	0.0532	0.570	0.461	0.0517	m, b	0.118	-0.003
125	100	200	0.00013	200	0.0266	0.368	0.258	0.0278	se_m, se_b	0.006	0.001
50	200	1000	0.00005	200	0.0106	0.247	0.138	0.0137	r^2, se_y	0.991	0.002
25	100	1000	0.00003	200	0.0053	0.179	0.069	0.0056	F, df	439	4
10	40	1000	0.00001	200	0.0021	0.141	0.031	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0011	0.122	0.013	-0.0010			

Regression results are calculated using the function LINEST

Blank	0.110	r^2=	0.991	
		m=	0.118	
		b=	-0.003	

	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	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11434-14

								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

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		

Regression results are calculated using the function LINEST

Blank	0.110	r^2=	0.981	
		m=	0.104	
		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	0.332	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	Recombina	Microsome ID	11434-14	Technician ID	2	Replicate #	3

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.4
Protein Concentration (dilution added to assay, mg/mL):	0.009033

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	Recombinan	Microsome ID	11434-14	Technician ID	2	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 dilutec 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	10186	20332	20143	40286	0.1	178722	22.54	40167	0.0458	1	0.005	15	0.3378
			0.5	2	9977	19954											
	2	2	0.5	1	10224	20448	20534	41068	0.1	178722	22.98	40949	0.0467	1	0.005	15	0.3444
			0.5	2	10310	20620											
	3	2	0.5	1	10192	20384	20446	40892	0.1	178722	22.88	40773	0.0465	1	0.005	15	0.3429
			0.5	2	10254	20508											
	4	2	0.5	1	9927	19854	19829	39658	0.1	178722	22.19	39539	0.0451	1	0.005	15	0.3325
			0.5	2	9902	19804											
Background control	1	2	0.5	1	36	72	62	124	0.1	178722	0.07	5	0.0000	1	0.005	15	0.0000
			0.5	2	26	52											
	2	2	0.5	1	29	58	66	132	0.1	178722	0.07	13	0.0000	1	0.005	15	0.0001
			0.5	2	37	74											
	3	2	0.5	1	27	54	60	120	0.1	178722	0.07	1	0.0000	1	0.005	15	0.0000
			0.5	2	33	66											
	4	2	0.5	1	23	46	51	102	0.1	178722	0.06	-18	0.0000	1	0.005	15	-0.0001
			0.5	2	28	56											
Positive control	1	2	0.5	1	5811	11622	11416	22832	0.1	178722	12.78	22713	0.0259	1	0.005	15	0.1910
			0.5	2	5605	11210											
	2	2	0.5	1	6356	12712	12741	25482	0.1	178722	14.26	25363	0.0289	1	0.005	15	0.2133
			0.5	2	6385	12770											
	3	2	0.5	1	6060	12120	12168	24336	0.1	178722	13.62	24217	0.0276	1	0.005	15	0.2036
			0.5	2	6108	12216											
	4	2	0.5	1	6475	12950	12749	25498	0.1	178722	14.27	25379	0.0289	1	0.005	15	0.2134
			0.5	2	6274	12548											
Negative Control	1	2	0.5	1	9915	19830	19649	39298	0.1	178722	21.99	39179	0.0446	1	0.005	15	0.3295
			0.5	2	9734	19468											
	2	2	0.5	1	9439	18878	18634	37268	0.1	178722	20.85	37149	0.0423	1	0.005	15	0.3124
			0.5	2	9195	18390											
	3	2	0.5	1	9503	19006	19035	38070	0.1	178722	21.30	37951	0.0432	1	0.005	15	0.3191
			0.5	2	9532	19064											
	4	2	0.5	1	9278	18556	18857	37714	0.1	178722	21.10	37595	0.0428	1	0.005	15	0.3161
			0.5	2	9579	19158											
Ref Chem 2	1-1	2	0.5	1	68	136	144	288	0.1	178722	0.16	169	0.0002	1	0.005	15	0.0014
			0.5	2	76	152											
	1-2	2	0.5	1	75	150	151	302	0.1	178722	0.17	183	0.0002	1	0.005	15	0.0015
			0.5	2	76	152											
	1-3	2	0.5	1	73	146	152	304	0.1	178722	0.17	185	0.0002	1	0.005	15	0.0016
			0.5	2	79	158											
	2-1	2	0.5	1	443	886	879	1758	0.1	178722	0.98	1639	0.0019	1	0.005	15	0.0138
			0.5	2	436	872											
	2-2	2	0.5	1	421	842	869	1738	0.1	178722	0.97	1619	0.0018	1	0.005	15	0.0136
			0.5	2	448	896											
	2-3	2	0.5	1	494	988	963	1926	0.1	178722	1.08	1807	0.0021	1	0.005	15	0.0152
			0.5	2	469	938											
	3-1	2	0.5	1	3203	6406	6368	12736	0.1	178722	7.13	12617	0.0144	1	0.005	15	0.1061
			0.5	2	3165	6330											
	3-2	2	0.5	1	3054	6108	6100	12200	0.1	178722	6.83	12081	0.0138	1	0.005	15	0.1016
			0.5	2	3046	6092											
	3-3	2	0.5	1	3339	6678	6616	13232	0.1	178722	7.40	13113	0.0149	1	0.005	15	0.1103
			0.5	2	3277	6554											
	4-1	2	0.5	1	4894	9788	9883	19766	0.1	178722	11.06	19647	0.0224	1	0.005	15	0.1652
			0.5	2	4989	9978											
	4-2	2	0.5	1	4893	9786	9836	19672	0.1	178722	11.01	19553	0.0223	1	0.005	15	0.1644
			0.5	2	4943	9886											
	4-3	2	0.5	1	4825	9650	9637	19274	0.1	178722	10.78	19155	0.0218	1	0.005	15	0.1611
			0.5	2	4812	9624											
	5-1	2	0.5	1	7824	15648	15630	31260	0.1	178722	17.49	31141	0.0355	1	0.005	15	0.2619
			0.5	2	7806	15612											
	5-2	2	0.5	1	8591	17182	17012	34024	0.1	178722	19.04	33905	0.0386	1	0.005	15	0.2851
			0.5	2	8421	16842											
	5-3	2	0.5	1	8317	16634	16640	33280	0.1	178722	18.62	33161	0.0378	1	0.005	15	0.2789
			0.5	2	8323	16646											
	6-1	2	0.5	1	10078	20156	20055	40110	0.1	178722	22.44	39991	0.0456	1	0.005	15	0.3363
			0.5	2	9977	19954											
	6-2	2	0.5	1	9352	18704	18707	37414	0.1	178722	20.93	37295	0.0425	1	0.005	15	0.3136
			0.5	2	9355	18710											
	6-3	2	0.5	1	10219	20438	20308	40616	0.1	178722	22.73	40497	0.0461	1	0.005	15	0.3406
			0.5	2	10089	20178											
	7-1	2	0.5	1	9705	19410	19401	38802	0.1	178722	21.71	38683	0.0441	1	0.005	15	0.3253
			0.5	2	9696	19392											
	7-2	2	0.5	1	9938	19876	20028	40056	0.1	178722	22.41	39937	0.0455	1	0.005	15	0.3358
			0.5	2	10090	20180											
	7-3	2	0.5	1	9970	19940	19650	39700	0.1	178722	22.21	39581	0.0451	1	0.005	15	0.3329
			0.5	2	9680	19760											
	8-1	2	0.5	1	9093	18186	18310	36620	0.1	178722	20.49	36501	0.0416	1	0.005	15	0.3069
			0.5	2	9217	18434											
	8-2	2	0.5	1	8957	17914	18045	36090	0.1	178722	20.19	35971	0.0410	1	0.005	15	0.3025
			0.5	2	9088	18176											
	8-3	2	0.5	1	10424	20848	20913	41826	0.1	178722	23.40	41707	0.0475	1	0.005	15	0.3507
			0.5	2	10489	20978											

Assay Date	7/22/2005	Test Chemical ID	Ref Chem 2	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11434-14	Technician ID	2	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3411	0.0047
Full activity	End	0.3377	0.0073
Full activity	Overall	0.3394	0.0054
Background	Beginning	0.0001	4.7571E-05
Background	End	-0.0001	0.000107035
Background	Overall	0.0000	0.000106704
Positive	Beginning	0.2021	0.0158
Positive	End	0.2085	0.0069
Positive	Overall	0.2053	0.0106
Negative	Beginning	0.3209	0.0121
Negative	End	0.3176	0.0021
Negative	Overall	0.3193	0.0073

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 2	1	1	1.00E-04	-4.00	0.0014
Ref Chem 2	1	2	1.00E-04	-4.00	0.0015
Ref Chem 2	1	3	1.00E-04	-4.00	0.0016
Ref Chem 2	2	1	1.00E-05	-5.00	0.0138
Ref Chem 2	2	2	1.00E-05	-5.00	0.0136
Ref Chem 2	2	3	1.00E-05	-5.00	0.0152
Ref Chem 2	3	1	1.00E-06	-6.00	0.1061
Ref Chem 2	3	2	1.00E-06	-6.00	0.1016
Ref Chem 2	3	3	1.00E-06	-6.00	0.1103
Ref Chem 2	4	1	5.00E-07	-6.30	0.1652
Ref Chem 2	4	2	5.00E-07	-6.30	0.1644
Ref Chem 2	4	3	5.00E-07	-6.30	0.1611
Ref Chem 2	5	1	1.00E-07	-7.00	0.2619
Ref Chem 2	5	2	1.00E-07	-7.00	0.2851
Ref Chem 2	5	3	1.00E-07	-7.00	0.2789
Ref Chem 2	6	1	1.00E-08	-8.00	0.3363
Ref Chem 2	6	2	1.00E-08	-8.00	0.3136
Ref Chem 2	6	3	1.00E-08	-8.00	0.3406
Ref Chem 2	7	1	1.00E-09	-9.00	0.3253
Ref Chem 2	7	2	1.00E-09	-9.00	0.3358
Ref Chem 2	7	3	1.00E-09	-9.00	0.3329
Ref Chem 2	8	1	1.00E-10	-10.00	0.3069
Ref Chem 2	8	2	1.00E-10	-10.00	0.3025
Ref Chem 2	8	3	1.00E-10	-10.00	0.3507

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.42	0.45	0.46
2	-5.00	4.06	4.01	4.48
3	-6.00	31.26	29.93	32.49
4	-6.30	48.68	48.45	47.46
5	-7.00	77.16	84.01	82.17
6	-8.00	99.09	92.41	100.35
7	-9.00	95.85	98.96	98.08
8	-10.00	90.45	89.13	103.35

Aromatase Assay Spreadsheet

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8		
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	33526	1684724	
2	0.0202	35479	1756386	
3	0.0199	34896	1753568	
4	0.0197	35356	1794721	
5	0.0200	35175	1758750	
			Average DPM/g soln	1749630
			SD	39970
			CV	2.28
			µCi/g soln	0.788

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	8.1124 g
Mass of dilution B used in substrate prep	4.5703 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563372 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00892 µg/g soln.
µg/g soln.

a. µCi/g soln	0.788
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.

= 0.563372 + 0.00892
= 0.572294 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.377 µCi/µg ASDN

875589 dpm/nmol

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8					
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10			

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

Regression results are calculated using the function LINEST

Blank	0.111

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	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	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	Variables	Regression results	
250	200	200	0.00025	200	0.0508	0.595	0.483	0.0426	m, b	0.090	-0.001
125	100	200	0.00013	200	0.0254	0.396	0.284	0.0247	se_m, se_b	0.004	0.001
50	200	1000	0.00005	200	0.0102	0.249	0.137	0.0115	r^2, se_y	0.993	0.001
25	100	1000	0.00003	200	0.0051	0.180	0.068	0.0053	F, df	400	3
10	40	1000	0.00001	200	0.0020	0.140	0.029	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.124	0.012	0.0003			

Regression results are calculated using the function LINEST

Blank	0.111	r^2=	0.993		
		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	Recombine	Microsome ID	11345-10	Technician ID	2	Replicate #	1

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.5
Protein Concentration (dilution added to assay, mg/mL):	0.007534

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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 dilutec 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	1317	2634	2447	4894	0.1	174963	2.80	4705	0.0054	1	0.004	15	0.0475
			0.5	2	1130	2260											
	2	2	0.5	1	11026	22052	22101	44202	0.1	174963	25.26	44013	0.0503	1	0.004	15	0.4448
			0.5	2	11075	22150											
	3	2	0.5	1	9954	19908	19848	39696	0.1	174963	22.69	39507	0.0451	1	0.004	15	0.3992
			0.5	2	9894	19788											
	4	2	0.5	1	8931	17862	17693	35386	0.1	174963	20.22	35197	0.0402	1	0.004	15	0.3557
			0.5	2	8762	17524											
Background control	1	2	0.5	1	41	82	87	174	0.1	174963	0.10	-15	0.0000	1	0.004	15	-0.0002
			0.5	2	46	92											
	2	2	0.5	1	39	78	87	174	0.1	174963	0.10	-15	0.0000	1	0.004	15	-0.0002
			0.5	2	48	96											
	3	2	0.5	1	56	112	119	238	0.1	174963	0.14	49	0.0001	1	0.004	15	0.0005
			0.5	2	63	126											
	4	2	0.5	1	45	90	85	170	0.1	174963	0.10	-19	0.0000	1	0.004	15	-0.0002
			0.5	2	40	80											
Positive control	1	2	0.5	1	6243	12486	12364	24728	0.1	174963	14.13	24539	0.0280	1	0.004	15	0.2480
			0.5	2	6121	12242											
	2	2	0.5	1	6738	13476	13297	26594	0.1	174963	15.20	26405	0.0302	1	0.004	15	0.2668
			0.5	2	6559	13118											
	3	2	0.5	1	4798	9596	9602	19204	0.1	174963	10.98	19015	0.0217	1	0.004	15	0.1922
			0.5	2	4804	9608											
	4	2	0.5	1	5305	10610	10568	21136	0.1	174963	12.08	20947	0.0239	1	0.004	15	0.2117
			0.5	2	5263	10526											
Negative Control	1	2	0.5	1	11118	22236	22057	44114	0.1	174963	25.21	43925	0.0502	1	0.004	15	0.4439
			0.5	2	10939	21878											
	2	2	0.5	1	11316	22632	22702	45404	0.1	174963	25.95	45215	0.0516	1	0.004	15	0.4569
			0.5	2	11386	22772											
	3	2	0.5	1	8249	16498	16373	32746	0.1	174963	18.72	32557	0.0372	1	0.004	15	0.3290
			0.5	2	8124	16248											
	4	2	0.5	1	9480	18960	18947	37894	0.1	174963	21.66	37705	0.0431	1	0.004	15	0.3810
			0.5	2	9467	18934											
Ref Chem 3	1-1	2	0.5	1	41	82	76	152	0.1	174963	0.09	-37	0.0000	1	0.004	15	-0.0004
			0.5	2	35	70											
	1-2	2	0.5	1	51	102	94	188	0.1	174963	0.11	-1	0.0000	1	0.004	15	0.0000
			0.5	2	43	86											
	1-3	2	0.5	1	38	76	82	164	0.1	174963	0.09	-25	0.0000	1	0.004	15	-0.0003
			0.5	2	44	88											
	2-1	2	0.5	1	58	116	97	194	0.1	174963	0.11	5	0.0000	1	0.004	15	0.0001
			0.5	2	39	78											
	2-2	2	0.5	1	43	86	85	170	0.1	174963	0.10	-19	0.0000	1	0.004	15	-0.0002
			0.5	2	42	84											
	2-3	2	0.5	1	45	90	93	186	0.1	174963	0.11	-3	0.0000	1	0.004	15	0.0000
			0.5	2	48	96											
	3-1	2	0.5	1	80	160	157	314	0.1	174963	0.18	125	0.0001	1	0.004	15	0.0013
			0.5	2	77	154											
	3-2	2	0.5	1	69	138	136	272	0.1	174963	0.16	83	0.0001	1	0.004	15	0.0008
			0.5	2	67	134											
	3-3	2	0.5	1	73	146	160	320	0.1	174963	0.18	131	0.0001	1	0.004	15	0.0013
			0.5	2	87	174											
	4-1	2	0.5	1	365	730	727	1454	0.1	174963	0.83	1265	0.0014	1	0.004	15	0.0128
			0.5	2	362	724											
	4-2	2	0.5	1	371	742	733	1466	0.1	174963	0.84	1277	0.0015	1	0.004	15	0.0129
			0.5	2	362	724											
	4-3	2	0.5	1	430	860	846	1692	0.1	174963	0.97	1503	0.0017	1	0.004	15	0.0152
			0.5	2	416	832											
	5-1	2	0.5	1	2948	5896	5757	11514	0.1	174963	6.58	11325	0.0129	1	0.004	15	0.1144
			0.5	2	2809	5618											
	5-2	2	0.5	1	2571	5142	5050	10100	0.1	174963	5.77	9911	0.0113	1	0.004	15	0.1002
			0.5	2	2479	4958											
	5-3	2	0.5	1	2561	5122	5248	10496	0.1	174963	6.00	10307	0.0118	1	0.004	15	0.1042
			0.5	2	2687	5374											
	6-1	2	0.5	1	6491	12982	12938	25876	0.1	174963	14.79	25687	0.0293	1	0.004	15	0.2596
			0.5	2	6447	12894											
	6-2	2	0.5	1	6925	13850	14035	28070	0.1	174963	16.04	27881	0.0318	1	0.004	15	0.2818
			0.5	2	7110	14220											
	6-3	2	0.5	1	7604	15208	15307	30614	0.1	174963	17.50	30425	0.0347	1	0.004	15	0.3075
			0.5	2	7703	15406											
	7-1	2	0.5	1	10073	20146	20242	40484	0.1	174963	23.14	40295	0.0460	1	0.004	15	0.4072
			0.5	2	10169	20338											
	7-2	2	0.5	1	9009	18018	17973	35946	0.1	174963	20.54	35757	0.0408	1	0.004	15	0.3613
			0.5	2	8964	17928											
	7-3	2	0.5	1	9171	18342	18310	36620	0.1	174963	20.93	36431	0.0416	1	0.004	15	0.3682
			0.5	2	9139	18278											
	8-1	2	0.5	1	9057	18114	18216	36432	0.1	174963	20.82	36243	0.0414	1	0.004	15	0.3663
			0.5	2	9159	18318											
	8-2	2	0.5	1	8845	17690	17743	35486	0.1	174963	20.28	35297	0.0403	1	0.004	15	0.3567
			0.5	2	8898	17796											
	8-3	2	0.5	1	9371	18742	18924	37848	0.1	174963	21.63	37659	0.0430	1	0.004	15	0.3806
			0.5	2	9553	19106											

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested		Microsome 8 type	Recombinant	Microsome ID 11345-10	Technician ID	Replicate 2 #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4448	
Full activity	End	0.3775	0.0308
Full activity	Overall	0.3999	0.0445
Background	Beginning	-0.0002	0
Background	End	0.0002	0.000485905
Background	Overall	0.0000	0.000330662
Positive	Beginning	0.2574	0.0133
Positive	End	0.2019	0.0138
Positive	Overall	0.2297	0.0339
Negative	Beginning	0.4504	0.0092
Negative	End	0.3550	0.0368
Negative	Overall	0.4027	0.0593

Note: Altered FEAC Beg and Overall averages to exclude Tube 1 - SLB 8/3/2005

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 3	1	1	1.00E-03	-3.00	-0.0004
Ref Chem 3	1	2	1.00E-03	-3.00	0.0000
Ref Chem 3	1	3	1.00E-03	-3.00	-0.0003
Ref Chem 3	2	1	1.00E-04	-4.00	0.0001
Ref Chem 3	2	2	1.00E-04	-4.00	-0.0002
Ref Chem 3	2	3	1.00E-04	-4.00	0.0000
Ref Chem 3	3	1	1.00E-05	-5.00	0.0013
Ref Chem 3	3	2	1.00E-05	-5.00	0.0008
Ref Chem 3	3	3	1.00E-05	-5.00	0.0013
Ref Chem 3	4	1	1.00E-06	-6.00	0.0128
Ref Chem 3	4	2	1.00E-06	-6.00	0.0129
Ref Chem 3	4	3	1.00E-06	-6.00	0.0152
Ref Chem 3	5	1	1.00E-07	-7.00	0.1144
Ref Chem 3	5	2	1.00E-07	-7.00	0.1002
Ref Chem 3	5	3	1.00E-07	-7.00	0.1042
Ref Chem 3	6	1	1.00E-08	-8.00	0.2596
Ref Chem 3	6	2	1.00E-08	-8.00	0.2818
Ref Chem 3	6	3	1.00E-08	-8.00	0.3075
Ref Chem 3	7	1	1.00E-09	-9.00	0.4072
Ref Chem 3	7	2	1.00E-09	-9.00	0.3613
Ref Chem 3	7	3	1.00E-09	-9.00	0.3682
Ref Chem 3	8	1	1.00E-10	-10.00	0.3663
Ref Chem 3	8	2	1.00E-10	-10.00	0.3567
Ref Chem 3	8	3	1.00E-10	-10.00	0.3806

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	-0.09	0.00	-0.06
2	-4.00	0.01	-0.05	-0.01
3	-5.00	0.32	0.21	0.33
4	-6.00	3.20	3.23	3.80
5	-7.00	28.62	25.05	26.05
6	-8.00	64.91	70.46	76.88
7	-9.00	101.83	90.36	92.06
8	-10.00	91.59	89.20	95.16

Aromatase Assay Spreadsheet

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8		
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	35354	1758905	
2	0.0199	36190	1818593	
3	0.0196	35884	1830816	
4	0.0199	39605	1990201	
5	0.0199	37187	1868693	
			Average DPM/g soln	1853442
			SD	86022
			CV	4.64
			µCi/g soln	0.835

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	8.1217 g
Mass of dilution B used in substrate prep	4.5722 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.568591 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00945 µg/g soln.
µg/g soln.

a. µCi/g soln 0.835
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.

= 0.568591 + 0.00945
= 0.578042 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.444 µCi/µg ASDN

918318 dpm/nmol

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID 11345-10

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	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10				

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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.7
Protein Concentration (dilution added to assay, mg/mL):	0.006136

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	10587	21174	21014	42028	0.1	185344	22.68	41914	0.0456	1	0.003	15	0.4959
			0.5	2	10427	20854											
	2	2	0.5	1	10527	21054	20936	41872	0.1	185344	22.59	41758	0.0455	1	0.003	15	0.4941
			0.5	2	10409	20818											
	3	2	0.5	1	8273	16546	16330	32660	0.1	185344	17.62	32546	0.0354	1	0.003	15	0.3851
			0.5	2	8057	16114											
	4	2	0.5	1	8503	17006	17103	34206	0.1	185344	18.46	34092	0.0371	1	0.003	15	0.4034
			0.5	2	8600	17200											
Background control	1	2	0.5	1	22	44	52	104	0.1	185344	0.06	-10	0.0000	1	0.003	15	-0.0001
			0.5	2	30	60											
	2	2	0.5	1	33	66	75	150	0.1	185344	0.08	36	0.0000	1	0.003	15	0.0004
			0.5	2	42	84											
	3	2	0.5	1	23	46	36	72	0.1	185344	0.04	-42	0.0000	1	0.003	15	-0.0005
			0.5	2	13	26											
	4	2	0.5	1	18	36	65	130	0.1	185344	0.07	16	0.0000	1	0.003	15	0.0002
			0.5	2	47	94											
Positive control	1	2	0.5	1	6269	12538	12323	24646	0.1	185344	13.30	24532	0.0267	1	0.003	15	0.2903
			0.5	2	6054	12108											
	2	2	0.5	1	6616	13232	12988	25976	0.1	185344	14.02	25862	0.0282	1	0.003	15	0.3060
			0.5	2	6372	12744											
	3	2	0.5	1	5094	10188	10273	20546	0.1	185344	11.09	20432	0.0222	1	0.003	15	0.2418
			0.5	2	5179	10358											
	4	2	0.5	1	5803	11606	11625	23250	0.1	185344	12.54	23136	0.0252	1	0.003	15	0.2737
			0.5	2	5822	11644											
Negative Control	1	2	0.5	1	11170	22340	21767	43534	0.1	185344	23.49	43420	0.0473	1	0.003	15	0.5137
			0.5	2	10597	21194											
	2	2	0.5	1	10302	20604	20532	41064	0.1	185344	22.16	40950	0.0446	1	0.003	15	0.4845
			0.5	2	10230	20460											
	3	2	0.5	1	8479	16958	16932	33864	0.1	185344	18.27	33750	0.0368	1	0.003	15	0.3993
			0.5	2	8453	16906											
	4	2	0.5	1	10120	20240	20196	40392	0.1	185344	21.79	40278	0.0439	1	0.003	15	0.4766
			0.5	2	10076	20152											
Ref Chem 3	1-1	2	0.5	1	88	176	154	308	0.1	185344	0.17	194	0.0002	1	0.003	15	0.0023
			0.5	2	66	132											
	1-2	2	0.5	1	70	140	159	318	0.1	185344	0.17	204	0.0002	1	0.003	15	0.0024
			0.5	2	89	178											
	1-3	2	0.5	1	81	162	167	334	0.1	185344	0.18	220	0.0002	1	0.003	15	0.0026
			0.5	2	86	172											
	2-1	2	0.5	1	345	690	726	1452	0.1	185344	0.78	1338	0.0015	1	0.003	15	0.0158
			0.5	2	381	762											
	2-2	2	0.5	1	349	698	711	1422	0.1	185344	0.77	1308	0.0014	1	0.003	15	0.0155
			0.5	2	362	724											
	2-3	2	0.5	1	364	728	721	1442	0.1	185344	0.78	1328	0.0014	1	0.003	15	0.0157
			0.5	2	357	714											
	3-1	2	0.5	1	2362	4724	4703	9406	0.1	185344	5.07	9292	0.0101	1	0.003	15	0.1099
			0.5	2	2341	4682											
	3-2	2	0.5	1	2194	4388	4406	8812	0.1	185344	4.75	8698	0.0095	1	0.003	15	0.1029
			0.5	2	2212	4424											
	3-3	2	0.5	1	2381	4762	4747	9494	0.1	185344	5.12	9380	0.0102	1	0.003	15	0.1110
			0.5	2	2366	4732											
	4-1	2	0.5	1	5300	10600	10461	20922	0.1	185344	11.29	20808	0.0227	1	0.003	15	0.2462
			0.5	2	5161	10322											
	4-2	2	0.5	1	5003	10006	10029	20058	0.1	185344	10.82	19944	0.0217	1	0.003	15	0.2360
			0.5	2	5026	10052											
	4-3	2	0.5	1	5276	10552	10537	21074	0.1	185344	11.37	20960	0.0228	1	0.003	15	0.2480
			0.5	2	5261	10522											
	5-1	2	0.5	1	7084	14168	14270	28540	0.1	185344	15.40	28426	0.0310	1	0.003	15	0.3363
			0.5	2	7186	14372											
	5-2	2	0.5	1	6673	13346	13295	26590	0.1	185344	14.35	26476	0.0288	1	0.003	15	0.3133
			0.5	2	6622	13244											
	5-3	2	0.5	1	6721	13442	13647	27294	0.1	185344	14.73	27180	0.0296	1	0.003	15	0.3216
			0.5	2	6926	13852											
	6-1	2	0.5	1	7913	15826	15714	31428	0.1	185344	16.96	31314	0.0341	1	0.003	15	0.3705
			0.5	2	7801	15602											
	6-2	2	0.5	1	7975	15950	15967	31934	0.1	185344	17.23	31820	0.0347	1	0.003	15	0.3765
			0.5	2	7992	15984											
	6-3	2	0.5	1	8199	16398	16395	32790	0.1	185344	17.69	32676	0.0356	1	0.003	15	0.3866
			0.5	2	8196	16392											
	7-1	2	0.5	1	8279	16558	16663	33326	0.1	185344	17.98	33212	0.0362	1	0.003	15	0.3930
			0.5	2	8384	16768											
	7-2	2	0.5	1	8716	17432	17466	34932	0.1	185344	18.85	34818	0.0379	1	0.003	15	0.4120
			0.5	2	8750	17500											
	7-3	2	0.5	1	9016	18032	18173	36346	0.1	185344	19.61	36232	0.0396	1	0.003	15	0.4287
			0.5	2	9157	18314											
	8-1	2	0.5	1	8730	17460	17517	35034	0.1	185344	18.90	34920	0.0380	1	0.003	15	0.4132
			0.5	2	8787	17574											
	8-2	2	0.5	1	9218	18436	18455	36910	0.1	185344	19.91	36796	0.0401	1	0.003	15	0.4354
			0.5	2	9237	18474											
	8-3	2	0.5	1	9392	18784	18790	37580	0.1	185344	20.28	37466	0.0408	1	0.003	15	0.4433
			0.5	2	9398	18796											

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.4950	0.0013
Full activity	End	0.3942	0.0129
Full activity	Overall	0.4446	0.0587
Background	Beginning	0.0002	0.000384856
Background	End	-0.0002	0.000485253
Background	Overall	0.0000	0.000399258
Positive	Beginning	0.2981	0.0111
Positive	End	0.2577	0.0226
Positive	Overall	0.2779	0.0275
Negative	Beginning	0.4991	0.0207
Negative	End	0.4379	0.0546
Negative	Overall	0.4685	0.0488

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 3	1	1	1.00E-05	-5.00	0.0023
Ref Chem 3	1	2	1.00E-05	-5.00	0.0024
Ref Chem 3	1	3	1.00E-05	-5.00	0.0026
Ref Chem 3	2	1	1.00E-06	-6.00	0.0158
Ref Chem 3	2	2	1.00E-06	-6.00	0.0155
Ref Chem 3	2	3	1.00E-06	-6.00	0.0157
Ref Chem 3	3	1	1.00E-07	-7.00	0.1099
Ref Chem 3	3	2	1.00E-07	-7.00	0.1029
Ref Chem 3	3	3	1.00E-07	-7.00	0.1110
Ref Chem 3	4	1	2.50E-08	-7.60	0.2462
Ref Chem 3	4	2	2.50E-08	-7.60	0.2360
Ref Chem 3	4	3	2.50E-08	-7.60	0.2480
Ref Chem 3	5	1	1.00E-08	-8.00	0.3363
Ref Chem 3	5	2	1.00E-08	-8.00	0.3133
Ref Chem 3	5	3	1.00E-08	-8.00	0.3216
Ref Chem 3	6	1	5.00E-09	-8.30	0.3705
Ref Chem 3	6	2	5.00E-09	-8.30	0.3765
Ref Chem 3	6	3	5.00E-09	-8.30	0.3866
Ref Chem 3	7	1	1.00E-09	-9.00	0.3930
Ref Chem 3	7	2	1.00E-09	-9.00	0.4120
Ref Chem 3	7	3	1.00E-09	-9.00	0.4287
Ref Chem 3	8	1	1.00E-10	-10.00	0.4132
Ref Chem 3	8	2	1.00E-10	-10.00	0.4354
Ref Chem 3	8	3	1.00E-10	-10.00	0.4433

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	0.52	0.54	0.59
2	-6.00	3.56	3.48	3.53
3	-7.00	24.73	23.15	24.96
4	-7.60	55.37	53.07	55.78
5	-8.00	75.65	70.46	72.33
6	-8.30	83.33	84.68	86.96
7	-9.00	88.38	92.66	96.42
8	-10.00	92.93	97.92	99.70

Aromatase Assay Spreadsheet

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8		
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0196	34190	1744388	
2	0.0197	34551	1753858	
3	0.0200	33954	1697700	
4	0.0194	33733	1738814	
5	0.0197	34085	1730203	
			Average DPM/g soln	1732993
			SD	21520
			CV	1.24
			µCi/g soln	0.781

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	8.0688 g
Mass of dilution B used in substrate prep	4.5295 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56136 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00884 µg/g soln.
µg/g soln.

a. µCi/g soln	0.781
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.

= 0.561360 + 0.00884
= 0.570197 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.369 µCi/µg ASDN

870453 dpm/nmol

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8		
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10

								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	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10				

Standards:	250	125	50	25	10	5	0
	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

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
25.1	100	11540-43

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}
250	200	200	0.00025	200	0.0502	0.583	0.462
125	100	200	0.00013	200	0.0251	0.390	0.269
50	200	1000	0.00005	200	0.0100	0.240	0.120
25	100	1000	0.00003	200	0.0050	0.185	0.064
10	40	1000	0.00001	200	0.0020	0.140	0.020
5	20	1000	0.00001	200	0.0010	0.128	0.007
			Blank	0.121			

Curve Output	Variables	Regression results	
0.0424	m, b	0.092	0.000
0.0246	se_m, se_b	0.004	0.000
0.0108	r^2, se_y	0.995	0.001
0.0057	F, df	650	3
0.0016	ss_{reg}, ss_{resid}	0.000	0.000
0.0004			

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 3	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	2.9
Protein Concentration (dilution added to assay, mg/mL):	0.004806

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	8	Microsome type	Recombinan	Microsome ID	11345-10	Technician ID	2	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	10651	21302	21112	42224	0.1	173299	24.36	42056	0.0483	1	0.002	15	0.6702
			0.5	2	10461	20922											
	2	2	0.5	1	11400	22800	22374	44748	0.1	173299	25.82	44580	0.0512	1	0.002	15	0.7104
			0.5	2	10974	21948											
	3	2	0.5	1	9469	18938	19084	38168	0.1	173299	22.02	38000	0.0437	1	0.002	15	0.6055
			0.5	2	9615	19230											
	4	2	0.5	1	9710	19420	19331	38662	0.1	173299	22.31	38494	0.0442	1	0.002	15	0.6134
			0.5	2	9621	19242											
Background control	1	2	0.5	1	36	72	78	156	0.1	173299	0.09	-13	0.0000	1	0.002	15	-0.0002
			0.5	2	42	84											
	2	2	0.5	1	37	74	83	166	0.1	173299	0.10	-3	0.0000	1	0.002	15	0.0000
			0.5	2	46	92											
	3	2	0.5	1	36	72	77	154	0.1	173299	0.09	-15	0.0000	1	0.002	15	-0.0002
			0.5	2	41	82											
	4	2	0.5	1	49	98	99	198	0.1	173299	0.11	30	0.0000	1	0.002	15	0.0005
			0.5	2	50	100											
Positive control	1	2	0.5	1	6019	12038	12033	24066	0.1	173299	13.89	23898	0.0275	1	0.002	15	0.3808
			0.5	2	6014	12028											
	2	2	0.5	1	6955	13910	13859	27718	0.1	173299	15.99	27550	0.0316	1	0.002	15	0.4390
			0.5	2	6904	13808											
	3	2	0.5	1	5559	11118	11107	22214	0.1	173299	12.82	22046	0.0253	1	0.002	15	0.3513
			0.5	2	5548	11096											
	4	2	0.5	1	5407	10814	10904	21808	0.1	173299	12.58	21640	0.0249	1	0.002	15	0.3448
			0.5	2	5497	10994											
Negative Control	1	2	0.5	1	10931	21862	22054	44108	0.1	173299	25.45	43940	0.0505	1	0.002	15	0.7002
			0.5	2	11123	22246											
	2	2	0.5	1	10958	21916	21823	43646	0.1	173299	25.19	43478	0.0499	1	0.002	15	0.6928
			0.5	2	10865	21730											
	3	2	0.5	1	9285	18570	18515	37030	0.1	173299	21.37	36862	0.0423	1	0.002	15	0.5874
			0.5	2	9230	18460											
	4	2	0.5	1	9810	19620	19443	38886	0.1	173299	22.44	38718	0.0445	1	0.002	15	0.6170
			0.5	2	9633	19266											
Ref Chem 3	1-1	2	0.5	1	99	198	198	396	0.1	173299	0.23	228	0.0003	1	0.002	15	0.0036
			0.5	2	99	198											
	1-2	2	0.5	1	80	160	176	352	0.1	173299	0.20	184	0.0002	1	0.002	15	0.0029
			0.5	2	96	192											
	1-3	2	0.5	1	76	152	167	334	0.1	173299	0.19	166	0.0002	1	0.002	15	0.0026
			0.5	2	91	182											
	2-1	2	0.5	1	299	598	604	1208	0.1	173299	0.70	1040	0.0012	1	0.002	15	0.0166
			0.5	2	305	610											
	2-2	2	0.5	1	361	722	748	1496	0.1	173299	0.86	1328	0.0015	1	0.002	15	0.0212
			0.5	2	387	774											
	2-3	2	0.5	1	395	790	808	1616	0.1	173299	0.93	1448	0.0017	1	0.002	15	0.0231
			0.5	2	413	826											
	3-1	2	0.5	1	2559	5118	5142	10284	0.1	173299	5.93	10116	0.0116	1	0.002	15	0.1612
			0.5	2	2583	5166											
	3-2	2	0.5	1	2265	4530	4556	9112	0.1	173299	5.26	8944	0.0103	1	0.002	15	0.1425
			0.5	2	2291	4582											
	3-3	2	0.5	1	2571	5142	5173	10346	0.1	173299	5.97	10178	0.0117	1	0.002	15	0.1622
			0.5	2	2602	5204											
	4-1	2	0.5	1	5871	11742	11767	23534	0.1	173299	13.58	23366	0.0268	1	0.002	15	0.3723
			0.5	2	5896	11792											
	4-2	2	0.5	1	5058	10116	10186	20372	0.1	173299	11.76	20204	0.0232	1	0.002	15	0.3219
			0.5	2	5128	10256											
	4-3	2	0.5	1	5220	10440	10626	21252	0.1	173299	12.26	21084	0.0242	1	0.002	15	0.3360
			0.5	2	5406	10812											
	5-1	2	0.5	1	7549	15098	15210	30420	0.1	173299	17.55	30252	0.0348	1	0.002	15	0.4821
			0.5	2	7661	15322											
	5-2	2	0.5	1	7343	14686	14615	29230	0.1	173299	16.87	29062	0.0334	1	0.002	15	0.4631
			0.5	2	7272	14544											
	5-3	2	0.5	1	7612	15224	15351	30702	0.1	173299	17.72	30534	0.0351	1	0.002	15	0.4866
			0.5	2	7739	15478											
	6-1	2	0.5	1	8084	16168	15985	31970	0.1	173299	18.45	31802	0.0365	1	0.002	15	0.5068
			0.5	2	7901	15802											
	6-2	2	0.5	1	8152	16304	16330	32660	0.1	173299	18.85	32492	0.0373	1	0.002	15	0.5178
			0.5	2	8178	16356											
	6-3	2	0.5	1	8753	17506	17574	35148	0.1	173299	20.28	34980	0.0402	1	0.002	15	0.5574
			0.5	2	8821	17642											
	7-1	2	0.5	1	9538	19076	19143	38286	0.1	173299	22.09	38118	0.0438	1	0.002	15	0.6074
			0.5	2	9605	19210											
	7-2	2	0.5	1	9916	19832	19904	39808	0.1	173299	22.97	39640	0.0455	1	0.002	15	0.6317
			0.5	2	9988	19976											
	7-3	2	0.5	1	8702	17404	17331	34662	0.1	173299	20.00	34494	0.0396	1	0.002	15	0.5497
			0.5	2	8629	17258											
	8-1	2	0.5	1	9776	19552	19327	38654	0.1	173299	22.30	38486	0.0442	1	0.002	15	0.6133
			0.5	2	9551	19102											
	8-2	2	0.5	1	10160	20320	20485	40970	0.1	173299	23.64	40802	0.0469	1	0.002	15	0.6502
			0.5	2	10325	20650											
	8-3	2	0.5	1	8921	17842	17875	35750	0.1	173299	20.63	35582	0.0409	1	0.002	15	0.5670
			0.5	2	8954	17908											

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 3	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.6903	0.0284
Full activity	End	0.6095	0.0056
Full activity	Overall	0.6499	0.0496
Background	Beginning	-0.0001	0.00011268
Background	End	0.0001	0.00049579
Background	Overall	0.0000	0.000324366
Positive	Beginning	0.4099	0.0412
Positive	End	0.3481	0.0046
Positive	Overall	0.3790	0.0430
Negative	Beginning	0.6965	0.0052
Negative	End	0.6022	0.0209
Negative	Overall	0.6493	0.0559

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 3	1	1	1.00E-05	-5.00	0.0036
Ref Chem 3	1	2	1.00E-05	-5.00	0.0029
Ref Chem 3	1	3	1.00E-05	-5.00	0.0026
Ref Chem 3	2	1	1.00E-06	-6.00	0.0166
Ref Chem 3	2	2	1.00E-06	-6.00	0.0212
Ref Chem 3	2	3	1.00E-06	-6.00	0.0231
Ref Chem 3	3	1	1.00E-07	-7.00	0.1612
Ref Chem 3	3	2	1.00E-07	-7.00	0.1425
Ref Chem 3	3	3	1.00E-07	-7.00	0.1622
Ref Chem 3	4	1	2.50E-08	-7.60	0.3723
Ref Chem 3	4	2	2.50E-08	-7.60	0.3219
Ref Chem 3	4	3	2.50E-08	-7.60	0.3360
Ref Chem 3	5	1	1.00E-08	-8.00	0.4821
Ref Chem 3	5	2	1.00E-08	-8.00	0.4631
Ref Chem 3	5	3	1.00E-08	-8.00	0.4866
Ref Chem 3	6	1	5.00E-09	-8.30	0.5068
Ref Chem 3	6	2	5.00E-09	-8.30	0.5178
Ref Chem 3	6	3	5.00E-09	-8.30	0.5574
Ref Chem 3	7	1	1.00E-09	-9.00	0.6074
Ref Chem 3	7	2	1.00E-09	-9.00	0.6317
Ref Chem 3	7	3	1.00E-09	-9.00	0.5497
Ref Chem 3	8	1	1.00E-10	-10.00	0.6133
Ref Chem 3	8	2	1.00E-10	-10.00	0.6502
Ref Chem 3	8	3	1.00E-10	-10.00	0.5670

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	0.56	0.45	0.41
2	-6.00	2.55	3.26	3.55
3	-7.00	24.80	21.93	24.96
4	-7.60	57.29	49.54	51.70
5	-8.00	74.18	71.26	74.87
6	-8.30	77.98	79.67	85.77
7	-9.00	93.47	97.20	84.58
8	-10.00	94.37	100.05	87.25

Aromatase Assay Spreadsheet

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8		
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	35636	1781800	
2	0.0196	34244	1747143	
3	0.0198	35536	1794747	
4	0.0203	35001	1724187	
5	0.0199	35444	1781106	
			Average DPM/g soln	1765797
			SD	29189
			CV	1.65
			µCi/g soln	0.795

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	8.1468 g
Mass of dilution B used in substrate prep	4.5797 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.567769 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00900 µg/g soln.
µg/g soln.

a. µCi/g soln	0.795
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.

= 0.567769 + 0.00900
= 0.576773 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.379 µCi/µg ASDN

876817 dpm/nmol

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8				
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10		

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
		Blank		0.119				

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

r^2= 0.985
m= 0.132
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	0.339	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		

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8						
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10				

								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
			Blank	0.119				

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

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		

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	1

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.2
Protein Concentration (dilution added to assay, mg/mL):	0.006974

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	9974	19948	19961	39922	0.1	176580	22.61	39789	0.0454	1	0.003	15	0.4338
			0.5	2	9987	19974											
	2	2	0.5	1	9838	19676	19600	39200	0.1	176580	22.20	39067	0.0446	1	0.003	15	0.4259
			0.5	2	9762	19524											
	3	2	0.5	1	9248	18496	18463	36926	0.1	176580	20.91	36793	0.0420	1	0.003	15	0.4011
			0.5	2	9215	18430											
	4	2	0.5	1	9430	18860	18823	37246	0.1	176580	21.09	37113	0.0423	1	0.003	15	0.4046
			0.5	2	9193	18386											
Background control	1	2	0.5	1	38	76	85	170	0.1	176580	0.10	37	0.0000	1	0.003	15	0.0004
			0.5	2	47	94											
	2	2	0.5	1	31	62	65	130	0.1	176580	0.07	-4	0.0000	1	0.003	15	0.0000
			0.5	2	34	68											
	3	2	0.5	1	24	48	51	102	0.1	176580	0.06	-32	0.0000	1	0.003	15	-0.0003
			0.5	2	27	54											
	4	2	0.5	1	37	74	66	132	0.1	176580	0.07	-2	0.0000	1	0.003	15	0.0000
			0.5	2	29	58											
Positive control	1	2	0.5	1	5591	11182	11141	22282	0.1	176580	12.62	22149	0.0253	1	0.003	15	0.2415
			0.5	2	5550	11100											
	2	2	0.5	1	5239	10478	10444	20888	0.1	176580	11.83	20755	0.0237	1	0.003	15	0.2263
			0.5	2	5205	10410											
	3	2	0.5	1	5362	10724	10786	21572	0.1	176580	12.22	21439	0.0245	1	0.003	15	0.2337
			0.5	2	5424	10848											
	4	2	0.5	1	5435	10870	10705	21410	0.1	176580	12.12	21277	0.0243	1	0.003	15	0.2320
			0.5	2	5270	10540											
Negative Control	1	2	0.5	1	11416	22832	22872	45744	0.1	176580	25.91	45611	0.0520	1	0.003	15	0.4973
			0.5	2	11456	22912											
	2	2	0.5	1	10313	20626	20569	41138	0.1	176580	23.30	41005	0.0468	1	0.003	15	0.4471
			0.5	2	10256	20512											
	3	2	0.5	1	9310	18620	18804	37608	0.1	176580	21.30	37475	0.0427	1	0.003	15	0.4086
			0.5	2	9494	18988											
	4	2	0.5	1	9517	19034	19272	38544	0.1	176580	21.83	38411	0.0438	1	0.003	15	0.4188
			0.5	2	9755	19510											
Ref Chem 4	1-1	2	0.5	1	42	84	94	188	0.1	176580	0.11	55	0.0001	1	0.003	15	0.0006
			0.5	2	52	104											
	1-2	2	0.5	1	32	64	76	152	0.1	176580	0.09	19	0.0000	1	0.003	15	0.0002
			0.5	2	44	88											
	1-3	2	0.5	1	41	82	80	160	0.1	176580	0.09	27	0.0000	1	0.003	15	0.0003
			0.5	2	39	78											
	2-1	2	0.5	1	175	350	366	732	0.1	176580	0.41	599	0.0007	1	0.003	15	0.0065
			0.5	2	191	382											
	2-2	2	0.5	1	236	472	435	870	0.1	176580	0.49	737	0.0008	1	0.003	15	0.0080
			0.5	2	199	398											
	2-3	2	0.5	1	216	432	438	876	0.1	176580	0.50	743	0.0008	1	0.003	15	0.0081
			0.5	2	222	444											
	3-1	2	0.5	1	8128	16256	16118	32236	0.1	176580	18.26	32103	0.0366	1	0.003	15	0.3500
			0.5	2	7990	15980											
	3-2	2	0.5	1	8333	16666	16746	33492	0.1	176580	18.97	33359	0.0380	1	0.003	15	0.3637
			0.5	2	8413	16826											
	3-3	2	0.5	1	8132	16264	16247	32494	0.1	176580	18.40	32361	0.0369	1	0.003	15	0.3528
			0.5	2	8115	16230											
	4-1	2	0.5	1	9995	19990	20004	40008	0.1	176580	22.66	39875	0.0455	1	0.003	15	0.4348
			0.5	2	10009	20018											
	4-2	2	0.5	1	10378	20756	20836	41672	0.1	176580	23.60	41539	0.0474	1	0.003	15	0.4529
			0.5	2	10458	20916											
	4-3	2	0.5	1	9690	19380	19420	38840	0.1	176580	22.00	38707	0.0441	1	0.003	15	0.4220
			0.5	2	9730	19460											
	5-1	2	0.5	1	10035	20070	20026	40052	0.1	176580	22.68	39919	0.0455	1	0.003	15	0.4352
			0.5	2	9991	19982											
	5-2	2	0.5	1	9733	19466	19512	39024	0.1	176580	22.10	38891	0.0444	1	0.003	15	0.4240
			0.5	2	9779	19558											
	5-3	2	0.5	1	9706	19412	19299	38598	0.1	176580	21.86	38465	0.0439	1	0.003	15	0.4194
			0.5	2	9593	19186											
	6-1	2	0.5	1	9326	18652	18639	37278	0.1	176580	21.11	37145	0.0424	1	0.003	15	0.4050
			0.5	2	9313	18626											
	6-2	2	0.5	1	7905	15810	15666	31332	0.1	176580	17.74	31199	0.0356	1	0.003	15	0.3402
			0.5	2	7761	15522											
	6-3	2	0.5	1	9823	19646	19543	39086	0.1	176580	22.14	38953	0.0444	1	0.003	15	0.4247
			0.5	2	9720	19440											
	7-1	2	0.5	1	9527	19054	19093	38186	0.1	176580	21.63	38053	0.0434	1	0.003	15	0.4149
			0.5	2	9566	19132											
	7-2	2	0.5	1	9593	19186	19194	38388	0.1	176580	21.74	38255	0.0436	1	0.003	15	0.4171
			0.5	2	9601	19202											
	7-3	2	0.5	1	9252	18504	18381	36762	0.1	176580	20.82	36629	0.0418	1	0.003	15	0.3994
			0.5	2	9129	18258											
	8-1	2	0.5	1	9888	19776	19648	39296	0.1	176580	22.25	39163	0.0447	1	0.003	15	0.4270
			0.5	2	9759	19518											
	8-2	2	0.5	1	9845	19690	19717	39434	0.1	176580	22.33	39301	0.0448	1	0.003	15	0.4285
			0.5	2	9872	19744											
	8-3	2	0.5	1	9814	19628	19626	39252	0.1	176580	22.23	39119	0.0446	1	0.003	15	0.4265
			0.5	2	9812	19624											

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4299	0.0056
Full activity	End	0.4029	0.0025
Full activity	Overall	0.4164	0.0160
Background	Beginning	0.0002	0.000308383
Background	End	-0.0002	0.000231287
Background	Overall	0.0000	0.000304439
Positive	Beginning	0.2339	0.0107
Positive	End	0.2329	0.0012
Positive	Overall	0.2334	0.0063
Negative	Beginning	0.4722	0.0355
Negative	End	0.4137	0.0072
Negative	Overall	0.4429	0.0397

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 4	1	1	1.00E-03	-3.00	0.0006
Ref Chem 4	1	2	1.00E-03	-3.00	0.0002
Ref Chem 4	1	3	1.00E-03	-3.00	0.0003
Ref Chem 4	2	1	1.00E-04	-4.00	0.0065
Ref Chem 4	2	2	1.00E-04	-4.00	0.0080
Ref Chem 4	2	3	1.00E-04	-4.00	0.0081
Ref Chem 4	3	1	1.00E-05	-5.00	0.3500
Ref Chem 4	3	2	1.00E-05	-5.00	0.3637
Ref Chem 4	3	3	1.00E-05	-5.00	0.3528
Ref Chem 4	4	1	1.00E-06	-6.00	0.4348
Ref Chem 4	4	2	1.00E-06	-6.00	0.4529
Ref Chem 4	4	3	1.00E-06	-6.00	0.4220
Ref Chem 4	5	1	1.00E-07	-7.00	0.4352
Ref Chem 4	5	2	1.00E-07	-7.00	0.4240
Ref Chem 4	5	3	1.00E-07	-7.00	0.4194
Ref Chem 4	6	1	1.00E-08	-8.00	0.4050
Ref Chem 4	6	2	1.00E-08	-8.00	0.3402
Ref Chem 4	6	3	1.00E-08	-8.00	0.4247
Ref Chem 4	7	1	1.00E-09	-9.00	0.4149
Ref Chem 4	7	2	1.00E-09	-9.00	0.4171
Ref Chem 4	7	3	1.00E-09	-9.00	0.3994
Ref Chem 4	8	1	1.00E-10	-10.00	0.4270
Ref Chem 4	8	2	1.00E-10	-10.00	0.4285
Ref Chem 4	8	3	1.00E-10	-10.00	0.4265

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.14	0.05	0.07
2	-4.00	1.57	1.93	1.94
3	-5.00	84.06	87.35	84.74
4	-6.00	104.41	108.77	101.35
5	-7.00	104.53	101.83	100.72
6	-8.00	97.26	81.69	102.00
7	-9.00	99.64	100.17	95.91
8	-10.00	102.55	102.91	102.43

Aromatase Assay Spreadsheet

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8		
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	30739	1544673	
2	0.0195	29040	1489231	
3	0.0205	32831	1601512	
4	0.0198	33856	1709899	
5	0.0202	35697	1767178	
			Average DPM/g soln	1622499
			SD	114922
			CV	7.08
			µCi/g soln	0.731

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	8.1548 g
Mass of dilution B used in substrate prep	4.5863 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.579277 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00827 µg/g soln.
µg/g soln.

a. µCi/g soln	0.731
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.

= 0.579277 + 0.00827
= 0.587551 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.244 µCi/µg ASDN

790883 dpm/nmol

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID 11345-10

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

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

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
26.3	100	11540-62

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 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	0.025	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	2	Replicate #	2	Microsome type	Recombinant	Microsome ID 11345-10

								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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.5
Protein Concentration (dilution added to assay, mg/mL):	0.005767

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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 dilutec 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	10357	20714	20603	41206	0.1	162250	25.40	41110	0.0520	1	0.003	15	0.6008
			0.5	2	10246	20492											
	2	2	0.5	1	9202	18404	18492	36984	0.1	162250	22.79	36888	0.0466	1	0.003	15	0.5391
			0.5	2	9290	18580											
	3	2	0.5	1	8879	17758	17822	35644	0.1	162250	21.97	35548	0.0449	1	0.003	15	0.5195
			0.5	2	8943	17886											
	4	2	0.5	1	8935	17870	17908	35816	0.1	162250	22.07	35720	0.0452	1	0.003	15	0.5221
			0.5	2	8973	17946											
Background control	1	2	0.5	1	27	54	55	110	0.1	162250	0.07	14	0.0000	1	0.003	15	0.0002
			0.5	2	28	56											
	2	2	0.5	1	42	84	73	146	0.1	162250	0.09	50	0.0001	1	0.003	15	0.0007
			0.5	2	31	62											
	3	2	0.5	1	12	24	37	74	0.1	162250	0.05	-22	0.0000	1	0.003	15	-0.0003
			0.5	2	25	50											
	4	2	0.5	1	11	22	27	54	0.1	162250	0.03	-42	-0.0001	1	0.003	15	-0.0006
			0.5	2	16	32											
Positive control	1	2	0.5	1	4680	9360	9424	18848	0.1	162250	11.62	18752	0.0237	1	0.003	15	0.2741
			0.5	2	4744	9488											
	2	2	0.5	1	4881	9762	9726	19452	0.1	162250	11.99	19356	0.0245	1	0.003	15	0.2829
			0.5	2	4845	9690											
	3	2	0.5	1	4550	9100	9041	18082	0.1	162250	11.14	17986	0.0227	1	0.003	15	0.2629
			0.5	2	4491	8982											
	4	2	0.5	1	4275	8550	8506	17012	0.1	162250	10.49	16916	0.0214	1	0.003	15	0.2472
			0.5	2	4231	8462											
Negative Control	1	2	0.5	1	9001	18002	18082	36164	0.1	162250	22.29	36068	0.0456	1	0.003	15	0.5271
			0.5	2	9081	18162											
	2	2	0.5	1	9062	18124	18134	36268	0.1	162250	22.35	36172	0.0457	1	0.003	15	0.5287
			0.5	2	9072	18144											
	3	2	0.5	1	8267	16534	16406	32812	0.1	162250	20.22	32716	0.0414	1	0.003	15	0.4782
			0.5	2	8139	16278											
	4	2	0.5	1	8226	16452	16543	33086	0.1	162250	20.39	32990	0.0417	1	0.003	15	0.4822
			0.5	2	8317	16634											
Ref Chem 4	1-1	2	0.5	1	32	64	67	134	0.1	162250	0.08	38	0.0000	1	0.003	15	0.0006
			0.5	2	35	70											
	1-2	2	0.5	1	40	80	77	154	0.1	162250	0.09	58	0.0001	1	0.003	15	0.0008
			0.5	2	37	74											
	1-3	2	0.5	1	33	66	64	128	0.1	162250	0.08	32	0.0000	1	0.003	15	0.0005
			0.5	2	31	62											
	2-1	2	0.5	1	396	792	804	1608	0.1	162250	0.99	1512	0.0019	1	0.003	15	0.0221
			0.5	2	408	816											
	2-2	2	0.5	1	451	902	909	1818	0.1	162250	1.12	1722	0.0022	1	0.003	15	0.0252
			0.5	2	458	916											
	2-3	2	0.5	1	577	1154	1157	2314	0.1	162250	1.43	2218	0.0028	1	0.003	15	0.0324
			0.5	2	580	1160											
	3-1	2	0.5	1	2015	4030	3983	7966	0.1	162250	4.91	7870	0.0100	1	0.003	15	0.1150
			0.5	2	1968	3936											
	3-2	2	0.5	1	2044	4088	4040	8080	0.1	162250	4.98	7984	0.0101	1	0.003	15	0.1167
			0.5	2	1996	3992											
	3-3	2	0.5	1	2005	4010	4051	8102	0.1	162250	4.99	8006	0.0101	1	0.003	15	0.1170
			0.5	2	2046	4092											
	4-1	2	0.5	1	3755	7510	7468	14936	0.1	162250	9.21	14840	0.0188	1	0.003	15	0.2169
			0.5	2	3713	7426											
	4-2	2	0.5	1	3771	7542	7505	15010	0.1	162250	9.25	14914	0.0189	1	0.003	15	0.2180
			0.5	2	3734	7468											
	4-3	2	0.5	1	3931	7862	7860	15720	0.1	162250	9.69	15624	0.0198	1	0.003	15	0.2284
			0.5	2	3929	7858											
	5-1	2	0.5	1	8753	17506	17351	34702	0.1	162250	21.39	34606	0.0438	1	0.003	15	0.5056
			0.5	2	8598	17196											
	5-2	2	0.5	1	7077	14154	14030	28060	0.1	162250	17.29	27964	0.0354	1	0.003	15	0.4087
			0.5	2	6953	13906											
	5-3	2	0.5	1	7851	15702	15728	31456	0.1	162250	19.39	31360	0.0397	1	0.003	15	0.4583
			0.5	2	7877	15754											
	6-1	2	0.5	1	9416	18832	18985	37970	0.1	162250	23.40	37874	0.0479	1	0.003	15	0.5535
			0.5	2	9569	19138											
	6-2	2	0.5	1	8174	16348	16480	32960	0.1	162250	20.31	32864	0.0416	1	0.003	15	0.4803
			0.5	2	8306	16612											
	6-3	2	0.5	1	9394	18788	18698	37396	0.1	162250	23.05	37300	0.0472	1	0.003	15	0.5452
			0.5	2	9304	18608											
	7-1	2	0.5	1	8799	17598	17621	35242	0.1	162250	21.72	35146	0.0444	1	0.003	15	0.5137
			0.5	2	8822	17644											
	7-2	2	0.5	1	9475	18950	18848	37696	0.1	162250	23.23	37600	0.0475	1	0.003	15	0.5495
			0.5	2	9373	18746											
	7-3	2	0.5	1	9132	18264	18342	36684	0.1	162250	22.61	36588	0.0463	1	0.003	15	0.5347
			0.5	2	9210	18420											
	8-1	2	0.5	1	8643	17286	17183	34366	0.1	162250	21.18	34270	0.0433	1	0.003	15	0.5009
			0.5	2	8540	17080											
	8-2	2	0.5	1	9428	18856	18958	37916	0.1	162250	23.37	37820	0.0478	1	0.003	15	0.5528
			0.5	2	9530	19060											
	8-3	2	0.5	1	9054	18108	18338	36676	0.1	162250	22.60	36580	0.0463	1	0.003	15	0.5346
			0.5	2	9284	18568											

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.5700	0.0436
Full activity	End	0.5208	0.0018
Full activity	Overall	0.5454	0.0380
Background	Beginning	0.0005	0.000372049
Background	End	-0.0005	0.000206694
Background	Overall	0.0000	0.000593322
Positive	Beginning	0.2785	0.0062
Positive	End	0.2551	0.0111
Positive	Overall	0.2668	0.0154
Negative	Beginning	0.5279	0.0011
Negative	End	0.4802	0.0028
Negative	Overall	0.5040	0.0276

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 4	1	1	1.00E-03	-3.00	0.0006
Ref Chem 4	1	2	1.00E-03	-3.00	0.0008
Ref Chem 4	1	3	1.00E-03	-3.00	0.0005
Ref Chem 4	2	1	1.00E-04	-4.00	0.0221
Ref Chem 4	2	2	1.00E-04	-4.00	0.0252
Ref Chem 4	2	3	1.00E-04	-4.00	0.0324
Ref Chem 4	3	1	5.00E-05	-4.30	0.1150
Ref Chem 4	3	2	5.00E-05	-4.30	0.1167
Ref Chem 4	3	3	5.00E-05	-4.30	0.1170
Ref Chem 4	4	1	2.50E-05	-4.60	0.2169
Ref Chem 4	4	2	2.50E-05	-4.60	0.2180
Ref Chem 4	4	3	2.50E-05	-4.60	0.2284
Ref Chem 4	5	1	1.00E-05	-5.00	0.5058
Ref Chem 4	5	2	1.00E-05	-5.00	0.4087
Ref Chem 4	5	3	1.00E-05	-5.00	0.4583
Ref Chem 4	6	1	5.00E-06	-5.30	0.5535
Ref Chem 4	6	2	5.00E-06	-5.30	0.4803
Ref Chem 4	6	3	5.00E-06	-5.30	0.5452
Ref Chem 4	7	1	1.00E-06	-6.00	0.5137
Ref Chem 4	7	2	1.00E-06	-6.00	0.5495
Ref Chem 4	7	3	1.00E-06	-6.00	0.5347
Ref Chem 4	8	1	1.00E-07	-7.00	0.5009
Ref Chem 4	8	2	1.00E-07	-7.00	0.5528
Ref Chem 4	8	3	1.00E-07	-7.00	0.5346

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.10	0.16	0.09
2	-4.00	4.05	4.61	5.94
3	-4.30	21.09	21.40	21.45
4	-4.60	39.77	39.97	41.87
5	-5.00	92.74	74.94	84.04
6	-5.30	101.49	88.07	99.96
7	-6.00	94.18	100.76	98.05
8	-7.00	91.84	101.35	98.03

Aromatase Assay Spreadsheet

Assay Date	9/29/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8		
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	29420	1485859	
2	0.0197	29306	1487614	
3	0.0196	31970	1631122	
4	0.0194	30889	1592216	
5	0.0198	33285	1681061	
			Average DPM/g soln	1575574
			SD	87000
			CV	5.52
			µCi/g soln	0.710

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	8.0062 g
Mass of dilution B used in substrate prep	4.5114 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.574758 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00803 µg/g soln.
µg/g soln.

a. µCi/g soln 0.710
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.

= 0.574758 + 0.00803
= 0.582792 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.218 µCi/µg ASDN

774280 dpm/nmol

Assay Date	9/29/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8				
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.1	100	11345-27
	0.515	0.364	0.234	0.186	0.147	0.126	0.125			
	0.518	0.351	0.235	0.185	0.140	0.136	0.119			
	0.503	0.326	0.246	0.176	0.158	0.134	0.123			

Samples:	11540-6A	11540-6B	11540-27H	11345-27I	11540-60D	11540-60C
	0.314	0.142	0.360	0.135	0.196	0.194
	0.358	0.136	0.350	0.142	0.198	0.186
			0.354	0.136	0.185	0.184

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	0.512	0.390	0.0484
125	100	200	0.00013	200	0.0251	0.347	0.225	0.0269
50	200	1000	0.00005	200	0.0100	0.238	0.116	0.0127
25	100	1000	0.00003	200	0.0050	0.182	0.060	0.0054
10	40	1000	0.00001	200	0.0020	0.148	0.026	0.0010
5	20	1000	0.00001	200	0.0010	0.132	0.010	-0.0011
			Blank	0.122				

Variables	Regression results	
m, b	0.130	-0.002
se_m, se_b	0.007	0.001
r^2, se_y	0.989	0.002
F, df	372	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.989
m= 0.130
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	0.314	0.192	0.023	200	1	1	0.113	0.127	0.11
11540-6A	0.358	0.236	0.028	200	1	1	0.142		
11540-6A									
11540-6B	0.142	0.020	0.000	200	1	1	0.001	-0.001	0.012
11540-6B	0.136	0.014	-0.001	200	1	1	-0.003		
11540-6B									
11540-27H	0.360	0.238	0.029	200	1	50	7.1	7.0	
11540-27H	0.350	0.228	0.027	200	1	50	6.8		
11540-27H	0.354	0.232	0.028	200	1	50	7.0		
11345-27I	0.135	0.013	-0.001	200	1	600	-2.2	-1.2	
11345-27I	0.142	0.020	0.000	200	1	600	0.5		
11345-27I	0.136	0.014	-0.001	200	1	600	-1.8		
11540-60D	0.196	0.074	0.007	200	1	320	11.6	10.9	
11540-60D	0.198	0.076	0.007	200	1	320	12.0		
11540-60D	0.185	0.063	0.006	200	1	320	9.3		
11540-60C	0.194	0.072	0.007	200	1	800	27.8	24.7	
11540-60C	0.186	0.064	0.006	200	1	800	23.7		
11540-60C	0.184	0.062	0.006	200	1	800	22.6		

Assay Date	9/29/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8		
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.1	100	11345-27
	0.515	0.364	0.234	0.186	0.147	0.126	0.125			
	0.518	0.351	0.235	0.185	0.140	0.136	0.119			
	0.503	0.326	0.246	0.176	0.158	0.134	0.123			

Samples:	11540-6A	11540-6B	11540-27H	11345-27I	11540-60D	11540-60C
	0.314	0.142	0.360	0.135	0.196	0.194
	0.358	0.136	0.350	0.142	0.198	0.186
			0.354	0.136	0.185	0.184

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	0.512	0.390	0.0426
125	100	200	0.00013	200	0.0251	0.347	0.225	0.0241
50	200	1000	0.00005	200	0.0100	0.238	0.116	0.0119
25	100	1000	0.00003	200	0.0050	0.182	0.060	0.0056
10	40	1000	0.00001	200	0.0020	0.148	0.026	0.0017
5	20	1000	0.00001	200	0.0010	0.132	0.010	-0.0001

Variables	Regression results	
m, b	0.112	-0.001
se_m, se_b	0.008	0.001
r^2, se_y	0.985	0.001
F, df	193	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

Blank	0.122	r^2=	0.985
		m=	0.112
		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	0.314	0.192	0.020	200	1	1	0.102	0.114	0.11
11540-6A	0.358	0.236	0.025	200	1	1	0.127		
11540-6A									
11540-6B	0.142	0.020	0.001	200	1	1	0.005	0.003	0.012
11540-6B	0.136	0.014	0.000	200	1	1	0.002		
11540-6B									
11540-27H	0.360	0.238	0.026	200	1	50	6.4	6.2	
11540-27H	0.350	0.228	0.024	200	1	50	6.1		
11540-27H	0.354	0.232	0.025	200	1	50	6.2		
11345-27I	0.135	0.013	0.000	200	1	600	0.7	1.6	
11345-27I	0.142	0.020	0.001	200	1	600	3.1		
11345-27I	0.136	0.014	0.000	200	1	600	1.1		
11540-60D	0.196	0.074	0.007	200	1	320	11.4	10.8	
11540-60D	0.198	0.076	0.007	200	1	320	11.7		
11540-60D	0.185	0.063	0.006	200	1	320	9.4		
11540-60C	0.194	0.072	0.007	200	1	800	27.5	24.8	
11540-60C	0.186	0.064	0.006	200	1	800	23.9		
11540-60C	0.184	0.062	0.006	200	1	800	23.0		

Assay Date	9/29/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.2
Protein Concentration (dilution added to assay, mg/mL):	0.010396

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	9/29/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	11345-10	Technician ID	2	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	9124	18248	18218	36436	0.1	157557	23.13	36303	0.0469	1	0.005	15	0.3007
			0.5	2	9094	18188											
	2	2	0.5	1	9504	19008	18807	37614	0.1	157557	23.87	37481	0.0484	1	0.005	15	0.3104
			0.5	2	9303	18606											
	3	2	0.5	1	8737	17474	17376	34752	0.1	157557	22.06	34619	0.0447	1	0.005	15	0.2867
			0.5	2	8639	17278											
	4	2	0.5	1	8747	17494	17393	34786	0.1	157557	22.08	34653	0.0448	1	0.005	15	0.2870
			0.5	2	8646	17292											
Background control	1	2	0.5	1	36	72	79	158	0.1	157557	0.10	25	0.0000	1	0.005	15	0.0002
			0.5	2	43	86											
	2	2	0.5	1	28	56	61	122	0.1	157557	0.08	-11	0.0000	1	0.005	15	-0.0001
			0.5	2	33	66											
	3	2	0.5	1	22	44	56	112	0.1	157557	0.07	-21	0.0000	1	0.005	15	-0.0002
			0.5	2	34	68											
	4	2	0.5	1	32	64	70	140	0.1	157557	0.09	7	0.0000	1	0.005	15	0.0001
			0.5	2	38	76											
Positive control	1	2	0.5	1	4990	9980	9916	19832	0.1	157557	12.59	19699	0.0254	1	0.005	15	0.1631
			0.5	2	4926	9852											
	2	2	0.5	1	5594	11188	11042	22084	0.1	157557	14.02	21951	0.0284	1	0.005	15	0.1818
			0.5	2	5448	10896											
	3	2	0.5	1	5110	10220	10180	20360	0.1	157557	12.92	20227	0.0261	1	0.005	15	0.1675
			0.5	2	5070	10140											
	4	2	0.5	1	4566	9132	9139	18278	0.1	157557	11.60	18145	0.0234	1	0.005	15	0.1503
			0.5	2	4573	9146											
Negative Control	1	2	0.5	1	11004	22008	20683	41366	0.1	157557	26.25	41233	0.0533	1	0.005	15	0.3415
			0.5	2	9679	19358											
	2	2	0.5	1	8799	17598	17483	34966	0.1	157557	22.19	34833	0.0450	1	0.005	15	0.2885
			0.5	2	8684	17368											
	3	2	0.5	1	8880	17760	17647	35294	0.1	157557	22.40	35161	0.0454	1	0.005	15	0.2912
			0.5	2	8767	17534											
	4	2	0.5	1	9763	19526	19533	39066	0.1	157557	24.79	38933	0.0503	1	0.005	15	0.3224
			0.5	2	9770	19540											
Ref Chem 4	1-1	2	0.5	1	41	82	76	152	0.1	157557	0.10	19	0.0000	1	0.005	15	0.0002
			0.5	2	35	70											
	1-2	2	0.5	1	48	96	92	184	0.1	157557	0.12	51	0.0001	1	0.005	15	0.0004
			0.5	2	44	88											
	1-3	2	0.5	1	43	86	88	176	0.1	157557	0.11	43	0.0001	1	0.005	15	0.0004
			0.5	2	45	90											
	2-1	2	0.5	1	517	1034	1048	2096	0.1	157557	1.33	1963	0.0025	1	0.005	15	0.0163
			0.5	2	531	1062											
	2-2	2	0.5	1	280	560	571	1142	0.1	157557	0.72	1009	0.0013	1	0.005	15	0.0084
			0.5	2	291	582											
	2-3	2	0.5	1	372	744	741	1482	0.1	157557	0.94	1349	0.0017	1	0.005	15	0.0112
			0.5	2	369	738											
	3-1	2	0.5	1	1833	3666	3663	7326	0.1	157557	4.65	7193	0.0093	1	0.005	15	0.0596
			0.5	2	1830	3660											
	3-2	2	0.5	1	2035	4070	3959	7918	0.1	157557	5.03	7785	0.0101	1	0.005	15	0.0645
			0.5	2	1924	3848											
	3-3	2	0.5	1	1694	3388	3326	6652	0.1	157557	4.22	6519	0.0084	1	0.005	15	0.0540
			0.5	2	1632	3264											
	4-1	2	0.5	1	3543	7086	7119	14238	0.1	157557	9.04	14105	0.0182	1	0.005	15	0.1168
			0.5	2	3576	7152											
	4-2	2	0.5	1	3893	7786	7781	15562	0.1	157557	9.88	15429	0.0199	1	0.005	15	0.1278
			0.5	2	3888	7776											
	4-3	2	0.5	1	3596	7192	7136	14272	0.1	157557	9.06	14139	0.0183	1	0.005	15	0.1171
			0.5	2	3540	7080											
	5-1	2	0.5	1	8152	16304	16321	32642	0.1	157557	20.72	32509	0.0420	1	0.005	15	0.2692
			0.5	2	8169	16338											
	5-2	2	0.5	1	7562	15124	15272	30544	0.1	157557	19.39	30411	0.0393	1	0.005	15	0.2519
			0.5	2	7710	15420											
	5-3	2	0.5	1	7626	15252	15346	30692	0.1	157557	19.48	30559	0.0395	1	0.005	15	0.2531
			0.5	2	7720	15440											
	6-1	2	0.5	1	9670	19340	19284	38568	0.1	157557	24.48	38435	0.0496	1	0.005	15	0.3183
			0.5	2	9614	19228											
	6-2	2	0.5	1	9192	18384	18405	36810	0.1	157557	23.36	36677	0.0474	1	0.005	15	0.3038
			0.5	2	9213	18426											
	6-3	2	0.5	1	9803	19606	19549	39098	0.1	157557	24.82	38965	0.0503	1	0.005	15	0.3227
			0.5	2	9746	19492											
	7-1	2	0.5	1	8723	17446	17429	34858	0.1	157557	22.12	34725	0.0448	1	0.005	15	0.2876
			0.5	2	8706	17412											
	7-2	2	0.5	1	8886	17772	17713	35426	0.1	157557	22.48	35293	0.0456	1	0.005	15	0.2923
			0.5	2	8827	17654											
	7-3	2	0.5	1	9542	19084	19195	38390	0.1	157557	24.37	38257	0.0494	1	0.005	15	0.3168
			0.5	2	9653	19306											
	8-1	2	0.5	1	9220	18440	18348	36696	0.1	157557	23.29	36563	0.0472	1	0.005	15	0.3028
			0.5	2	9128	18256											
	8-2	2	0.5	1	9123	18246	17728	35456	0.1	157557	22.50	35323	0.0456	1	0.005	15	0.2925
			0.5	2	8605	17210											
	8-3	2	0.5	1	9411	18822	18929	37858	0.1	157557	24.03	37725	0.0487	1	0.005	15	0.3124
			0.5	2	9518	19036											

Assay Date	9/29/2005	Test Chemical ID	Ref Chem 4	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	Replicate # 2 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3055	0.0069
Full activity	End	0.2869	0.0002
Full activity	Overall	0.2962	0.0115
Background	Beginning	0.0001	0.000210824
Background	End	-0.0001	0.000163974
Background	Overall	0.0000	0.000168105
Positive	Beginning	0.1725	0.0132
Positive	End	0.1589	0.0122
Positive	Overall	0.1657	0.0130
Negative	Beginning	0.3150	0.0375
Negative	End	0.3068	0.0221
Negative	Overall	0.3109	0.0256

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 4	1	1	1.00E-03	-3.00	0.0002
Ref Chem 4	1	2	1.00E-03	-3.00	0.0004
Ref Chem 4	1	3	1.00E-03	-3.00	0.0004
Ref Chem 4	2	1	1.00E-04	-4.00	0.0163
Ref Chem 4	2	2	1.00E-04	-4.00	0.0084
Ref Chem 4	2	3	1.00E-04	-4.00	0.0112
Ref Chem 4	3	1	5.00E-05	-4.30	0.0596
Ref Chem 4	3	2	5.00E-05	-4.30	0.0645
Ref Chem 4	3	3	5.00E-05	-4.30	0.0540
Ref Chem 4	4	1	2.50E-05	-4.60	0.1168
Ref Chem 4	4	2	2.50E-05	-4.60	0.1278
Ref Chem 4	4	3	2.50E-05	-4.60	0.1171
Ref Chem 4	5	1	1.00E-05	-5.00	0.2692
Ref Chem 4	5	2	1.00E-05	-5.00	0.2519
Ref Chem 4	5	3	1.00E-05	-5.00	0.2531
Ref Chem 4	6	1	5.00E-06	-5.30	0.3183
Ref Chem 4	6	2	5.00E-06	-5.30	0.3038
Ref Chem 4	6	3	5.00E-06	-5.30	0.3227
Ref Chem 4	7	1	1.00E-06	-6.00	0.2876
Ref Chem 4	7	2	1.00E-06	-6.00	0.2923
Ref Chem 4	7	3	1.00E-06	-6.00	0.3168
Ref Chem 4	8	1	1.00E-07	-7.00	0.3028
Ref Chem 4	8	2	1.00E-07	-7.00	0.2925
Ref Chem 4	8	3	1.00E-07	-7.00	0.3124

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.05	0.14	0.12
2	-4.00	5.49	2.82	3.77
3	-4.30	20.11	21.77	18.23
4	-4.60	39.44	43.14	39.53
5	-5.00	90.90	85.03	85.45
6	-5.30	107.47	102.55	108.95
7	-6.00	97.09	98.68	106.97
8	-7.00	102.23	98.77	105.48

Aromatase Assay Spreadsheet

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8		
Technician ID	2	Replicate #	1	Microsome type	Recombina	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	35636	1781800	
2	0.0196	34244	1747143	
3	0.0198	35536	1794747	
4	0.0203	35001	1724187	
5	0.0199	35444	1781106	
			Average DPM/g soln	1765797
			SD	29189
			CV	1.65
			µCi/g soln	0.795

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	8.1468 g
Mass of dilution B used in substrate prep	4.5797 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.567769 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00900 µg/g soln.
µg/g soln.

a. µCi/g soln 0.795
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.

= 0.567769 + 0.00900
= 0.576773 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.379 µCi/µg ASDN

876817 dpm/nmol

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8		
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10

								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	0.0481
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
			Blank	0.119				

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

r^2= 0.985
m= 0.132
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	0.339	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		

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID 11345-10

								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	

Regression results are calculated using the function LINEST

Blank	0.119	r^2=	0.969
		m=	0.112
		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	0.220	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		

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	1

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.2
Protein Concentration (dilution added to assay, mg/mL):	0.006974

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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 dilutec 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	9974	19948	19961	39922	0.1	176580	22.61	39789	0.0454	1	0.003	15	0.4338
			0.5	2	9987	19974											
	2	2	0.5	1	9838	19676	19600	39200	0.1	176580	22.20	39067	0.0446	1	0.003	15	0.4259
			0.5	2	9762	19524											
	3	2	0.5	1	9248	18496	18463	36926	0.1	176580	20.91	36793	0.0420	1	0.003	15	0.4011
			0.5	2	9215	18430											
	4	2	0.5	1	9430	18860	18623	37246	0.1	176580	21.09	37113	0.0423	1	0.003	15	0.4046
			0.5	2	9193	18386											
Background control	1	2	0.5	1	38	76	85	170	0.1	176580	0.10	37	0.0000	1	0.003	15	0.0004
			0.5	2	47	94											
	2	2	0.5	1	31	62	65	130	0.1	176580	0.07	-4	0.0000	1	0.003	15	0.0000
			0.5	2	34	68											
	3	2	0.5	1	24	48	51	102	0.1	176580	0.06	-32	0.0000	1	0.003	15	-0.0003
			0.5	2	27	54											
	4	2	0.5	1	37	74	66	132	0.1	176580	0.07	-2	0.0000	1	0.003	15	0.0000
			0.5	2	29	58											
Positive control	1	2	0.5	1	5591	11182	11141	22282	0.1	176580	12.62	22149	0.0253	1	0.003	15	0.2415
			0.5	2	5550	11100											
	2	2	0.5	1	5239	10478	10444	20888	0.1	176580	11.83	20755	0.0237	1	0.003	15	0.2263
			0.5	2	5205	10410											
	3	2	0.5	1	5362	10724	10786	21572	0.1	176580	12.22	21439	0.0245	1	0.003	15	0.2337
			0.5	2	5424	10848											
	4	2	0.5	1	5435	10870	10705	21410	0.1	176580	12.12	21277	0.0243	1	0.003	15	0.2320
			0.5	2	5270	10540											
Negative Control	1	2	0.5	1	11416	22832	22872	45744	0.1	176580	25.91	45611	0.0520	1	0.003	15	0.4973
			0.5	2	11456	22912											
	2	2	0.5	1	10313	20626	20569	41138	0.1	176580	23.30	41005	0.0468	1	0.003	15	0.4471
			0.5	2	10256	20512											
	3	2	0.5	1	9310	18620	18804	37608	0.1	176580	21.30	37475	0.0427	1	0.003	15	0.4086
			0.5	2	9494	18988											
	4	2	0.5	1	9517	19034	19272	38544	0.1	176580	21.83	38411	0.0438	1	0.003	15	0.4188
			0.5	2	9755	19510											
Ref Chem 5	1-1	2	0.5	1	8939	17878	17659	35318	0.1	176580	20.00	35185	0.0401	1	0.003	15	0.3836
			0.5	2	8720	17440											
	1-2	2	0.5	1	9229	18458	18475	36950	0.1	176580	20.93	36817	0.0420	1	0.003	15	0.4014
			0.5	2	9246	18492											
	1-3	2	0.5	1	9700	19400	19227	38454	0.1	176580	21.78	38321	0.0437	1	0.003	15	0.4178
			0.5	2	9527	19054											
	2-1	2	0.5	1	10187	20374	20417	40834	0.1	176580	23.12	40701	0.0464	1	0.003	15	0.4438
			0.5	2	10230	20460											
	2-2	2	0.5	1	9310	18620	18508	37016	0.1	176580	20.96	36883	0.0421	1	0.003	15	0.4021
			0.5	2	9198	18396											
	2-3	2	0.5	1	8941	17882	17965	35930	0.1	176580	20.35	35797	0.0408	1	0.003	15	0.3903
			0.5	2	9024	18048											
	3-1	2	0.5	1	8903	17806	17601	35202	0.1	176580	19.94	35069	0.0400	1	0.003	15	0.3824
			0.5	2	8698	17396											
	3-2	2	0.5	1	8697	17394	17357	34714	0.1	176580	19.66	34581	0.0394	1	0.003	15	0.3770
			0.5	2	8660	17320											
	3-3	2	0.5	1	9310	18620	18373	36746	0.1	176580	20.81	36613	0.0418	1	0.003	15	0.3992
			0.5	2	9063	18126											
	4-1	2	0.5	1	8837	17674	17849	35698	0.1	176580	20.22	35565	0.0406	1	0.003	15	0.3878
			0.5	2	9012	18024											
	4-2	2	0.5	1	9507	19014	19036	38072	0.1	176580	21.56	37939	0.0433	1	0.003	15	0.4136
			0.5	2	9529	19058											
	4-3	2	0.5	1	9446	18892	18869	37738	0.1	176580	21.37	37605	0.0429	1	0.003	15	0.4100
			0.5	2	9423	18846											
	5-1	2	0.5	1	8864	17728	17597	35194	0.1	176580	19.93	35061	0.0400	1	0.003	15	0.3823
			0.5	2	8733	17466											
	5-2	2	0.5	1	9016	18032	17807	35614	0.1	176580	20.17	35481	0.0405	1	0.003	15	0.3868
			0.5	2	8791	17582											
	5-3	2	0.5	1	8744	17488	17444	34888	0.1	176580	19.76	34755	0.0396	1	0.003	15	0.3789
			0.5	2	8700	17400											
	6-1	2	0.5	1	9434	18868	18904	37808	0.1	176580	21.41	37675	0.0430	1	0.003	15	0.4108
			0.5	2	9470	18940											
	6-2	2	0.5	1	9946	19892	19728	39456	0.1	176580	22.34	39323	0.0448	1	0.003	15	0.4287
			0.5	2	9782	19564											
	6-3	2	0.5	1	9586	19172	19010	38020	0.1	176580	21.53	37887	0.0432	1	0.003	15	0.4131
			0.5	2	9424	18848											
	7-1	2	0.5	1	9628	19256	19045	38090	0.1	176580	21.57	37957	0.0433	1	0.003	15	0.4138
			0.5	2	9417	18834											
	7-2	2	0.5	1	8885	17770	17634	35268	0.1	176580	19.97	35135	0.0401	1	0.003	15	0.3831
			0.5	2	8749	17498											
	7-3	2	0.5	1	9028	18056	17849	35698	0.1	176580	20.22	35565	0.0406	1	0.003	15	0.3878
			0.5	2	8821	17642											
	8-1	2	0.5	1	9194	18388	18358	36716	0.1	176580	20.79	36583	0.0417	1	0.003	15	0.3989
			0.5	2	9164	18328											
	8-2	2	0.5	1	8993	17986	17936	35872	0.1	176580	20.31	35739	0.0408	1	0.003	15	0.3897
			0.5	2	8943	17886											
	8-3	2	0.5	1	8952	17904	18077	36154	0.1	176580	20.47	36021	0.0411	1	0.003	15	0.3927
			0.5	2	9125	18250											

Assay Date	9/21/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4299	0.0056
Full activity	End	0.4029	0.0025
Full activity	Overall	0.4164	0.0160
Background	Beginning	0.0002	0.000308383
Background	End	-0.0002	0.000231287
Background	Overall	0.0000	0.000304439
Positive	Beginning	0.2339	0.0107
Positive	End	0.2329	0.0012
Positive	Overall	0.2334	0.0063
Negative	Beginning	0.4722	0.0355
Negative	End	0.4137	0.0072
Negative	Overall	0.4429	0.0397

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 5	1	1	1.00E-04	-4.00	0.3836
Ref Chem 5	1	2	1.00E-04	-4.00	0.4014
Ref Chem 5	1	3	1.00E-04	-4.00	0.4178
Ref Chem 5	2	1	1.00E-05	-5.00	0.4438
Ref Chem 5	2	2	1.00E-05	-5.00	0.4021
Ref Chem 5	2	3	1.00E-05	-5.00	0.3903
Ref Chem 5	3	1	1.00E-06	-6.00	0.3824
Ref Chem 5	3	2	1.00E-06	-6.00	0.3770
Ref Chem 5	3	3	1.00E-06	-6.00	0.3992
Ref Chem 5	4	1	5.00E-07	-6.30	0.3878
Ref Chem 5	4	2	5.00E-07	-6.30	0.4136
Ref Chem 5	4	3	5.00E-07	-6.30	0.4100
Ref Chem 5	5	1	1.00E-07	-7.00	0.3823
Ref Chem 5	5	2	1.00E-07	-7.00	0.3868
Ref Chem 5	5	3	1.00E-07	-7.00	0.3789
Ref Chem 5	6	1	1.00E-08	-8.00	0.4108
Ref Chem 5	6	2	1.00E-08	-8.00	0.4287
Ref Chem 5	6	3	1.00E-08	-8.00	0.4131
Ref Chem 5	7	1	1.00E-09	-9.00	0.4138
Ref Chem 5	7	2	1.00E-09	-9.00	0.3831
Ref Chem 5	7	3	1.00E-09	-9.00	0.3878
Ref Chem 5	8	1	1.00E-10	-10.00	0.3989
Ref Chem 5	8	2	1.00E-10	-10.00	0.3897
Ref Chem 5	8	3	1.00E-10	-10.00	0.3927

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	92.13	96.40	100.34
2	-5.00	106.57	96.58	93.73
3	-6.00	91.83	90.55	95.87
4	-6.30	93.13	99.34	98.47
5	-7.00	91.81	92.91	91.00
6	-8.00	98.65	102.97	99.21
7	-9.00	99.39	92.00	93.13
8	-10.00	95.79	93.58	94.32

Aromatase Assay Spreadsheet

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8
Technician ID	2	Replicate #	2	Microsome type	Recombina Microsome ID 11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	30739	1544673	
2	0.0195	29040	1489231	
3	0.0205	32831	1601512	
4	0.0198	33856	1709899	
5	0.0202	35697	1767178	
			Average DPM/g soln	1622499
			SD	114922
			CV	7.08
			µCi/g soln	0.731

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	8.1548 g
Mass of dilution B used in substrate prep	4.5863 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.579277 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00827 µg/g soln.
µg/g soln.

a. µCi/g soln	0.731
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.

= 0.579277 + 0.00827
= 0.587551 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.244 µCi/µg ASDN

790883 dpm/nmol

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8						
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.510	0.355	0.244	0.178	0.148	0.122	0.128	26.3	100	11540-62
	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

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		

Blank 0.127

r^2= 0.992
m= 0.125
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	0.212	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	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10				

								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 5	# Concentrations tested	8	Microsome type	Recombinε Microsome ID	11345-10	Technician ID	2	Replicate #	2

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.5
Protein Concentration (dilution added to assay, mg/mL):	0.005767

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	Recombinan	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol^{3}H$_2$O 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^{3}H$_2$O 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	10357	20714	20603	41206	0.1	162250	25.40	41110	0.0520	1	0.003	15	0.6006
			0.5	2	10246	20492											
	2	2	0.5	1	9202	18404	18492	36984	0.1	162250	22.79	36888	0.0466	1	0.003	15	0.5391
			0.5	2	9290	18580											
	3	2	0.5	1	8879	17758	17822	35644	0.1	162250	21.97	35548	0.0449	1	0.003	15	0.5195
			0.5	2	8943	17886											
	4	2	0.5	1	8935	17870	17908	35816	0.1	162250	22.07	35720	0.0452	1	0.003	15	0.5221
			0.5	2	8973	17946											
Background control	1	2	0.5	1	27	54	55	110	0.1	162250	0.07	14	0.0000	1	0.003	15	0.0002
			0.5	2	28	56											
	2	2	0.5	1	42	84	73	146	0.1	162250	0.09	50	0.0001	1	0.003	15	0.0007
			0.5	2	31	62											
	3	2	0.5	1	12	24	37	74	0.1	162250	0.05	-22	0.0000	1	0.003	15	-0.0003
			0.5	2	25	50											
	4	2	0.5	1	11	22	27	54	0.1	162250	0.03	-42	-0.0001	1	0.003	15	-0.0006
			0.5	2	16	32											
Positive control	1	2	0.5	1	4680	9360	9424	18848	0.1	162250	11.62	18752	0.0237	1	0.003	15	0.2741
			0.5	2	4744	9488											
	2	2	0.5	1	4881	9762	9726	19452	0.1	162250	11.99	19356	0.0245	1	0.003	15	0.2829
			0.5	2	4845	9690											
	3	2	0.5	1	4550	9100	9041	18082	0.1	162250	11.14	17986	0.0227	1	0.003	15	0.2629
			0.5	2	4491	8982											
	4	2	0.5	1	4275	8550	8506	17012	0.1	162250	10.49	16916	0.0214	1	0.003	15	0.2472
			0.5	2	4231	8462											
Negative Control	1	2	0.5	1	9001	18002	18082	36164	0.1	162250	22.29	36068	0.0456	1	0.003	15	0.5271
			0.5	2	9081	18162											
	2	2	0.5	1	9062	18124	18134	36268	0.1	162250	22.35	36172	0.0457	1	0.003	15	0.5287
			0.5	2	9072	18144											
	3	2	0.5	1	8267	16534	16406	32812	0.1	162250	20.22	32716	0.0414	1	0.003	15	0.4782
			0.5	2	8139	16278											
	4	2	0.5	1	8226	16452	16543	33086	0.1	162250	20.39	32990	0.0417	1	0.003	15	0.4822
			0.5	2	8317	16634											
Ref Chem 5	1-1	2	0.5	1	7963	15926	15700	31400	0.1	162250	19.35	31304	0.0396	1	0.003	15	0.4575
			0.5	2	7737	15474											
	1-2	2	0.5	1	8507	17014	16958	33916	0.1	162250	20.90	33820	0.0428	1	0.003	15	0.4943
			0.5	2	8451	16902											
	1-3	2	0.5	1	8548	17096	17017	34034	0.1	162250	20.98	33938	0.0429	1	0.003	15	0.4960
			0.5	2	8469	16938											
	2-1	2	0.5	1	8266	16532	16388	32776	0.1	162250	20.20	32680	0.0413	1	0.003	15	0.4776
			0.5	2	8122	16244											
	2-2	2	0.5	1	8483	16966	17120	34240	0.1	162250	21.10	34144	0.0432	1	0.003	15	0.4990
			0.5	2	8637	17274											
	2-3	2	0.5	1	8821	17642	17531	35062	0.1	162250	21.61	34966	0.0442	1	0.003	15	0.5110
			0.5	2	8710	17420											
	3-1	2	0.5	1	9206	18412	18162	36324	0.1	162250	22.39	36228	0.0458	1	0.003	15	0.5295
			0.5	2	8956	17912											
	3-2	2	0.5	1	8317	16634	16811	33622	0.1	162250	20.72	33526	0.0424	1	0.003	15	0.4900
			0.5	2	8494	16988											
	3-3	2	0.5	1	8375	16750	16581	33162	0.1	162250	20.44	33066	0.0418	1	0.003	15	0.4833
			0.5	2	8206	16412											
	4-1	2	0.5	1	8600	17200	17140	34280	0.1	162250	21.13	34184	0.0432	1	0.003	15	0.4996
			0.5	2	8540	17080											
	4-2	2	0.5	1	8666	17332	17281	34562	0.1	162250	21.30	34466	0.0436	1	0.003	15	0.5037
			0.5	2	8615	17230											
	4-3	2	0.5	1	8734	17468	17260	34520	0.1	162250	21.28	34424	0.0435	1	0.003	15	0.5031
			0.5	2	8526	17052											
	5-1	2	0.5	1	8957	17914	17759	35518	0.1	162250	21.89	35422	0.0448	1	0.003	15	0.5177
			0.5	2	8802	17604											
	5-2	2	0.5	1	8471	16942	17076	34152	0.1	162250	21.05	34056	0.0431	1	0.003	15	0.4977
			0.5	2	8605	17210											
	5-3	2	0.5	1	8963	17926	17784	35568	0.1	162250	21.92	35472	0.0449	1	0.003	15	0.5184
			0.5	2	8821	17642											
	6-1	2	0.5	1	8498	16996	17244	34488	0.1	162250	21.26	34392	0.0435	1	0.003	15	0.5027
			0.5	2	8746	17492											
	6-2	2	0.5	1	8561	17122	16997	33994	0.1	162250	20.95	33898	0.0429	1	0.003	15	0.4954
			0.5	2	8436	16872											
	6-3	2	0.5	1	7325	14650	14535	29070	0.1	162250	17.92	28974	0.0366	1	0.003	15	0.4235
			0.5	2	7210	14420											
	7-1	2	0.5	1	8781	17562	17518	35036	0.1	162250	21.59	34940	0.0442	1	0.003	15	0.5107
			0.5	2	8737	17474											
	7-2	2	0.5	1	9232	18464	18612	37224	0.1	162250	22.94	37128	0.0469	1	0.003	15	0.5426
			0.5	2	9380	18760											
	7-3	2	0.5	1	8708	17416	17362	34724	0.1	162250	21.40	34628	0.0438	1	0.003	15	0.5061
			0.5	2	8654	17308											
	8-1	2	0.5	1	9151	18302	18196	36392	0.1	162250	22.43	36296	0.0459	1	0.003	15	0.5305
			0.5	2	9045	18090											
	8-2	2	0.5	1	8935	17870	17847	35694	0.1	162250	22.00	35598	0.0450	1	0.003	15	0.5203
			0.5	2	8912	17824											
	8-3	2	0.5	1	8398	16796	16677	33354	0.1	162250	20.56	33258	0.0421	1	0.003	15	0.4861
			0.5	2	8279	16558											

Assay Date	9/23/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.5700	0.0436
Full activity	End	0.5208	0.0018
Full activity	Overall	0.5454	0.0380
Background	Beginning	0.0005	0.000372049
Background	End	-0.0005	0.000206694
Background	Overall	0.0000	0.000593322
Positive	Beginning	0.2785	0.0062
Positive	End	0.2551	0.0111
Positive	Overall	0.2668	0.0154
Negative	Beginning	0.5279	0.0011
Negative	End	0.4802	0.0028
Negative	Overall	0.5040	0.0276

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 5	1	1	1.00E-04	-4.00	0.4575
Ref Chem 5	1	2	1.00E-04	-4.00	0.4943
Ref Chem 5	1	3	1.00E-04	-4.00	0.4960
Ref Chem 5	2	1	1.00E-05	-5.00	0.4776
Ref Chem 5	2	2	1.00E-05	-5.00	0.4990
Ref Chem 5	2	3	1.00E-05	-5.00	0.5110
Ref Chem 5	3	1	1.00E-06	-6.00	0.5295
Ref Chem 5	3	2	1.00E-06	-6.00	0.4900
Ref Chem 5	3	3	1.00E-06	-6.00	0.4833
Ref Chem 5	4	1	5.00E-07	-6.30	0.4996
Ref Chem 5	4	2	5.00E-07	-6.30	0.5037
Ref Chem 5	4	3	5.00E-07	-6.30	0.5031
Ref Chem 5	5	1	1.00E-07	-7.00	0.5177
Ref Chem 5	5	2	1.00E-07	-7.00	0.4977
Ref Chem 5	5	3	1.00E-07	-7.00	0.5184
Ref Chem 5	6	1	1.00E-08	-8.00	0.5027
Ref Chem 5	6	2	1.00E-08	-8.00	0.4954
Ref Chem 5	6	3	1.00E-08	-8.00	0.4235
Ref Chem 5	7	1	1.00E-09	-9.00	0.5107
Ref Chem 5	7	2	1.00E-09	-9.00	0.5426
Ref Chem 5	7	3	1.00E-09	-9.00	0.5061
Ref Chem 5	8	1	1.00E-10	-10.00	0.5305
Ref Chem 5	8	2	1.00E-10	-10.00	0.5203
Ref Chem 5	8	3	1.00E-10	-10.00	0.4861

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	83.89	90.63	90.95
2	-5.00	87.58	91.50	93.70
3	-6.00	97.08	89.84	88.61
4	-6.30	91.61	92.36	92.25
5	-7.00	94.92	91.26	95.06
6	-8.00	92.16	90.84	77.64
7	-9.00	93.63	99.49	92.80
8	-10.00	97.27	95.39	89.12

Aromatase Assay Spreadsheet

Assay Date	9/29/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8		
Technician ID	2	Replicate #	3	Microsome type	Recombina	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	29420	1485859	
2	0.0197	29306	1487614	
3	0.0196	31970	1631122	
4	0.0194	30889	1592216	
5	0.0198	33285	1681061	
			Average DPM/g soln	1575574
			SD	87000
			CV	5.52
			µCi/g soln	0.710

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	8.0062 g
Mass of dilution B used in substrate prep	4.5114 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.574758 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00803 µg/g soln.
µg/g soln.

a. µCi/g soln 0.710
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.

= 0.574758 + 0.00803
= 0.582792 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.218 µCi/µg ASDN

774280 dpm/nmol

Assay Date	9/29/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8					
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10			

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.515	0.364	0.234	0.186	0.147	0.126	0.125	25.1	100	11345-27
	0.518	0.351	0.235	0.185	0.140	0.136	0.119			
	0.503	0.326	0.246	0.176	0.158	0.134	0.123			

Samples:	11540-6A	11540-6B	11540-27H	11345-27I	11540-60D	11540-60C
	0.314	0.142	0.360	0.135	0.196	0.194
	0.358	0.136	0.350	0.142	0.198	0.186
			0.354	0.136	0.185	0.184

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	0.512	0.390	0.0484
125	100	200	0.00013	200	0.0251	0.347	0.225	0.0269
50	200	1000	0.00005	200	0.0100	0.238	0.116	0.0127
25	100	1000	0.00003	200	0.0050	0.182	0.060	0.0054
10	40	1000	0.00001	200	0.0020	0.148	0.026	0.0010
5	20	1000	0.00001	200	0.0010	0.132	0.010	-0.0011
			Blank	0.122				

Variables	Regression results	
m, b	0.130	-0.002
se_m, se_b	0.007	0.001
r^2, se_y	0.989	0.002
F, df	372	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.989
m= 0.130
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	0.314	0.192	0.023	200	1	1	0.113	0.127	0.11
11540-6A	0.358	0.236	0.028	200	1	1	0.142		
11540-6A									
11540-6B	0.142	0.020	0.000	200	1	1	0.001	-0.001	0.012
11540-6B	0.136	0.014	-0.001	200	1	1	-0.003		
11540-6B									
11540-27H	0.360	0.238	0.029	200	1	50	7.1	7.0	
11540-27H	0.350	0.228	0.027	200	1	50	6.8		
11540-27H	0.354	0.232	0.028	200	1	50	7.0		
11345-27I	0.135	0.013	-0.001	200	1	600	-2.2	-1.2	
11345-27I	0.142	0.020	0.000	200	1	600	0.5		
11345-27I	0.136	0.014	-0.001	200	1	600	-1.8		
11540-60D	0.196	0.074	0.007	200	1	320	11.6	10.9	
11540-60D	0.198	0.076	0.007	200	1	320	12.0		
11540-60D	0.185	0.063	0.006	200	1	320	9.3		
11540-60C	0.194	0.072	0.007	200	1	800	27.8	24.7	
11540-60C	0.186	0.064	0.006	200	1	800	23.7		
11540-60C	0.184	0.062	0.006	200	1	800	22.6		

Assay Date	9/29/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8				
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10		

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.515	0.364	0.234	0.186	0.147	0.126	0.125	25.1	100	11345-27
	0.518	0.351	0.235	0.185	0.140	0.136	0.119			
	0.503	0.326	0.246	0.176	0.158	0.134	0.123			

Samples:	11540-6A	11540-6B	11540-27H	11345-27I	11540-60D	11540-60C
	0.314	0.142	0.360	0.135	0.196	0.194
	0.358	0.136	0.350	0.142	0.198	0.186
			0.354	0.136	0.185	0.184

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	0.512	0.390	0.0426
125	100	200	0.00013	200	0.0251	0.347	0.225	0.0241
50	200	1000	0.00005	200	0.0100	0.238	0.116	0.0119
25	100	1000	0.00003	200	0.0050	0.182	0.060	0.0056
10	40	1000	0.00001	200	0.0020	0.148	0.026	0.0017
5	20	1000	0.00001	200	0.0010	0.132	0.010	-0.0001
			Blank	0.122				

Variables	Regression results	
m, b	0.112	-0.001
se_m, se_b	0.008	0.001
r^2, se_y	0.985	0.001
F, df	193	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.985
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	0.314	0.192	0.020	200	1	1	0.102	0.114	0.11
11540-6A	0.358	0.236	0.025	200	1	1	0.127		
11540-6A									
11540-6B	0.142	0.020	0.001	200	1	1	0.005	0.003	0.012
11540-6B	0.136	0.014	0.000	200	1	1	0.002		
11540-6B									
11540-27H	0.360	0.238	0.026	200	1	50	6.4	6.2	
11540-27H	0.350	0.228	0.024	200	1	50	6.1		
11540-27H	0.354	0.232	0.025	200	1	50	6.2		
11345-27I	0.135	0.013	0.000	200	1	600	0.7	1.6	
11345-27I	0.142	0.020	0.001	200	1	600	3.1		
11345-27I	0.136	0.014	0.000	200	1	600	1.1		
11540-60D	0.196	0.074	0.007	200	1	320	11.4	10.8	
11540-60D	0.198	0.076	0.007	200	1	320	11.7		
11540-60D	0.185	0.063	0.006	200	1	320	9.4		
11540-60C	0.194	0.072	0.007	200	1	800	27.5	24.8	
11540-60C	0.186	0.064	0.006	200	1	800	23.9		
11540-60C	0.184	0.062	0.006	200	1	800	23.0		

Assay Date	9/29/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.2
Protein Concentration (dilution added to assay, mg/mL):	0.010396

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	9/29/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	11345-10	Technician ID	2	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	9124	18248	18218	36436	0.1	157557	23.13	36303	0.0469	1	0.005	15	0.3007
			0.5	2	9094	18188											
	2	2	0.5	1	9504	19008	18807	37614	0.1	157557	23.87	37481	0.0484	1	0.005	15	0.3104
			0.5	2	9303	18606											
	3	2	0.5	1	8737	17474	17376	34752	0.1	157557	22.06	34619	0.0447	1	0.005	15	0.2867
			0.5	2	8639	17278											
	4	2	0.5	1	8747	17494	17393	34786	0.1	157557	22.08	34653	0.0448	1	0.005	15	0.2870
			0.5	2	8646	17292											
Background control	1	2	0.5	1	36	72	79	158	0.1	157557	0.10	25	0.0000	1	0.005	15	0.0002
			0.5	2	43	86											
	2	2	0.5	1	28	56	61	122	0.1	157557	0.08	-11	0.0000	1	0.005	15	-0.0001
			0.5	2	33	66											
	3	2	0.5	1	22	44	56	112	0.1	157557	0.07	-21	0.0000	1	0.005	15	-0.0002
			0.5	2	34	68											
	4	2	0.5	1	32	64	70	140	0.1	157557	0.09	7	0.0000	1	0.005	15	0.0001
			0.5	2	38	76											
Positive control	1	2	0.5	1	4990	9980	9916	19832	0.1	157557	12.59	19699	0.0254	1	0.005	15	0.1631
			0.5	2	4926	9852											
	2	2	0.5	1	5594	11188	11042	22084	0.1	157557	14.02	21951	0.0284	1	0.005	15	0.1818
			0.5	2	5448	10896											
	3	2	0.5	1	5110	10220	10180	20360	0.1	157557	12.92	20227	0.0261	1	0.005	15	0.1675
			0.5	2	5070	10140											
	4	2	0.5	1	4566	9132	9139	18278	0.1	157557	11.60	18145	0.0234	1	0.005	15	0.1503
			0.5	2	4573	9146											
Negative Control	1	2	0.5	1	11004	22008	20683	41366	0.1	157557	26.25	41233	0.0533	1	0.005	15	0.3415
			0.5	2	9679	19358											
	2	2	0.5	1	8799	17598	17483	34966	0.1	157557	22.19	34833	0.0450	1	0.005	15	0.2885
			0.5	2	8684	17368											
	3	2	0.5	1	8880	17760	17647	35294	0.1	157557	22.40	35161	0.0454	1	0.005	15	0.2912
			0.5	2	8767	17534											
	4	2	0.5	1	9763	19526	19533	39066	0.1	157557	24.79	38933	0.0503	1	0.005	15	0.3224
			0.5	2	9770	19540											
Ref Chem 5	1-1	2	0.5	1	8295	16590	16747	33494	0.1	157557	21.26	33361	0.0431	1	0.005	15	0.2763
			0.5	2	8452	16904											
	1-2	2	0.5	1	8488	16976	16806	33612	0.1	157557	21.33	33479	0.0432	1	0.005	15	0.2773
			0.5	2	8318	16636											
	1-3	2	0.5	1	8919	17838	17981	35962	0.1	157557	22.82	35829	0.0463	1	0.005	15	0.2967
			0.5	2	9062	18124											
	2-1	2	0.5	1	9096	18192	18001	36002	0.1	157557	22.85	35869	0.0463	1	0.005	15	0.2971
			0.5	2	8905	17810											
	2-2	2	0.5	1	8849	17698	17660	35320	0.1	157557	22.42	35187	0.0454	1	0.005	15	0.2914
			0.5	2	8811	17622											
	2-3	2	0.5	1	8799	17598	17559	35118	0.1	157557	22.29	34985	0.0452	1	0.005	15	0.2897
			0.5	2	8760	17520											
	3-1	2	0.5	1	8872	17744	17496	34992	0.1	157557	22.21	34859	0.0450	1	0.005	15	0.2887
			0.5	2	8624	17248											
	3-2	2	0.5	1	8850	17700	17667	35334	0.1	157557	22.43	35201	0.0455	1	0.005	15	0.2915
			0.5	2	8817	17634											
	3-3	2	0.5	1	9123	18246	18036	36072	0.1	157557	22.89	35939	0.0464	1	0.005	15	0.2976
			0.5	2	8913	17826											
	4-1	2	0.5	1	8648	17296	17333	34666	0.1	157557	22.00	34533	0.0446	1	0.005	15	0.2860
			0.5	2	8685	17370											
	4-2	2	0.5	1	8577	17154	16999	33998	0.1	157557	21.58	33865	0.0437	1	0.005	15	0.2805
			0.5	2	8422	16844											
	4-3	2	0.5	1	8292	16584	16511	33022	0.1	157557	20.96	32889	0.0425	1	0.005	15	0.2724
			0.5	2	8219	16438											
	5-1	2	0.5	1	8935	17870	17967	35934	0.1	157557	22.81	35801	0.0462	1	0.005	15	0.2965
			0.5	2	9032	18064											
	5-2	2	0.5	1	9550	19100	18978	37956	0.1	157557	24.09	37823	0.0488	1	0.005	15	0.3132
			0.5	2	9428	18856											
	5-3	2	0.5	1	8812	17624	17714	35428	0.1	157557	22.49	35295	0.0456	1	0.005	15	0.2923
			0.5	2	8902	17804											
	6-1	2	0.5	1	8990	17980	17875	35750	0.1	157557	22.69	35617	0.0460	1	0.005	15	0.2950
			0.5	2	8885	17770											
	6-2	2	0.5	1	8678	17356	17407	34814	0.1	157557	22.10	34681	0.0448	1	0.005	15	0.2872
			0.5	2	8729	17458											
	6-3	2	0.5	1	8439	16878	17021	34042	0.1	157557	21.61	33909	0.0438	1	0.005	15	0.2808
			0.5	2	8582	17164											
	7-1	2	0.5	1	8681	17362	17436	34872	0.1	157557	22.13	34739	0.0449	1	0.005	15	0.2877
			0.5	2	8755	17510											
	7-2	2	0.5	1	9264	18528	18433	36866	0.1	157557	23.40	36733	0.0474	1	0.005	15	0.3042
			0.5	2	9169	18338											
	7-3	2	0.5	1	8690	17380	17303	34606	0.1	157557	21.96	34473	0.0445	1	0.005	15	0.2855
			0.5	2	8613	17226											
	8-1	2	0.5	1	8131	16262	16426	32852	0.1	157557	20.85	32719	0.0423	1	0.005	15	0.2710
			0.5	2	8295	16590											
	8-2	2	0.5	1	8697	17394	17335	34670	0.1	157557	22.00	34537	0.0446	1	0.005	15	0.2860
			0.5	2	8638	17276											
	8-3	2	0.5	1	8904	17808	17634	35268	0.1	157557	22.38	35135	0.0454	1	0.005	15	0.2910
			0.5	2	8730	17460											

Assay Date	9/29/2005	Test Chemical ID	Ref Chem 5	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID 11345-10	Technician ID	Replicate 2 # 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3055	0.0069
Full activity	End	0.2869	0.0002
Full activity	Overall	0.2962	0.0115
Background	Beginning	0.0001	0.000210824
Background	End	-0.0001	0.000163974
Background	Overall	0.0000	0.000168105
Positive	Beginning	0.1725	0.0132
Positive	End	0.1589	0.0122
Positive	Overall	0.1657	0.0130
Negative	Beginning	0.3150	0.0375
Negative	End	0.3068	0.0221
Negative	Overall	0.3109	0.0256

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 5	1	1	1.00E-04	-4.00	0.2763
Ref Chem 5	1	2	1.00E-04	-4.00	0.2773
Ref Chem 5	1	3	1.00E-04	-4.00	0.2967
Ref Chem 5	2	1	1.00E-05	-5.00	0.2971
Ref Chem 5	2	2	1.00E-05	-5.00	0.2914
Ref Chem 5	2	3	1.00E-05	-5.00	0.2897
Ref Chem 5	3	1	1.00E-06	-6.00	0.2887
Ref Chem 5	3	2	1.00E-06	-6.00	0.2915
Ref Chem 5	3	3	1.00E-06	-6.00	0.2976
Ref Chem 5	4	1	5.00E-07	-6.30	0.2860
Ref Chem 5	4	2	5.00E-07	-6.30	0.2805
Ref Chem 5	4	3	5.00E-07	-6.30	0.2724
Ref Chem 5	5	1	1.00E-07	-7.00	0.2965
Ref Chem 5	5	2	1.00E-07	-7.00	0.3132
Ref Chem 5	5	3	1.00E-07	-7.00	0.2923
Ref Chem 5	6	1	1.00E-08	-8.00	0.2950
Ref Chem 5	6	2	1.00E-08	-8.00	0.2872
Ref Chem 5	6	3	1.00E-08	-8.00	0.2808
Ref Chem 5	7	1	1.00E-09	-9.00	0.2877
Ref Chem 5	7	2	1.00E-09	-9.00	0.3042
Ref Chem 5	7	3	1.00E-09	-9.00	0.2855
Ref Chem 5	8	1	1.00E-10	-10.00	0.2710
Ref Chem 5	8	2	1.00E-10	-10.00	0.2860
Ref Chem 5	8	3	1.00E-10	-10.00	0.2910

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	93.28	93.61	100.18
2	-5.00	100.29	98.39	97.82
3	-6.00	97.47	98.43	100.49
4	-6.30	96.56	94.69	91.96
5	-7.00	100.10	105.76	98.69
6	-8.00	99.59	96.97	94.81
7	-9.00	97.13	102.71	96.39
8	-10.00	91.49	96.57	98.24

Aromatase Assay Spreadsheet

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8		
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	33526	1684724	
2	0.0202	35479	1756386	
3	0.0199	34896	1753568	
4	0.0197	35356	1794721	
5	0.0200	35175	1758750	
			Average DPM/g soln	1749630
			SD	39970
			CV	2.28
			µCi/g soln	0.788

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	8.1124 g
Mass of dilution B used in substrate prep	4.5703 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563372 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00892 µg/g soln.
µg/g soln.

a. µCi/g soln 0.788
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.

= 0.563372 + 0.00892
= 0.572294 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.377 µCi/µg ASDN

875589 dpm/nmol

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8						
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10				

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	Variables	Regression results	
250	200	200	0.00025	200	0.0508	0.595	0.483	0.0488	m, b	0.105	-0.002
125	100	200	0.00013	200	0.0254	0.396	0.284	0.0279	se_m, se_b	0.005	0.001
50	200	1000	0.00005	200	0.0102	0.249	0.137	0.0124	r^2, se_y	0.990	0.002
25	100	1000	0.00003	200	0.0051	0.180	0.068	0.0052	F, df	383	4
10	40	1000	0.00001	200	0.0020	0.140	0.029	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.124	0.012	-0.0007			

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	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.4	100	
	0.592	0.398	0.252	0.176	0.138	0.122	0.101			
	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	Variables	Regression results	
250	200	200	0.00025	200	0.0508	0.595	0.483	0.0426	m, b	0.090	-0.001
125	100	200	0.00013	200	0.0254	0.396	0.284	0.0247	se_m, se_b	0.004	0.001
50	200	1000	0.00005	200	0.0102	0.249	0.137	0.0115	r^2, se_y	0.993	0.001
25	100	1000	0.00003	200	0.0051	0.180	0.068	0.0053	F, df	400	3
10	40	1000	0.00001	200	0.0020	0.140	0.029	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.124	0.012	0.0003			

Regression results are calculated using the function LINEST

Blank	0.111	r^2=	0.993
		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 6	# Concentrations tested	8	Microsome type	Recombinz Microsome ID	11345-10	Technician ID	2	Replicate #	1

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.5
Protein Concentration (dilution added to assay, mg/mL):	0.007534

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	1317	2634	2447	4894	0.1	174963	2.80	4705	0.0054	1	0.004	15	0.0475
			0.5	2	1130	2260											
	2	2	0.5	1	11026	22052	22101	44202	0.1	174963	25.26	44013	0.0503	1	0.004	15	0.4448
			0.5	2	11075	22150											
	3	2	0.5	1	9954	19908	19848	39696	0.1	174963	22.69	39507	0.0451	1	0.004	15	0.3992
			0.5	2	9894	19788											
	4	2	0.5	1	8931	17862	17693	35386	0.1	174963	20.22	35197	0.0402	1	0.004	15	0.3557
			0.5	2	8762	17524											
Background control	1	2	0.5	1	41	82	87	174	0.1	174963	0.10	-15	0.0000	1	0.004	15	-0.0002
			0.5	2	46	92											
	2	2	0.5	1	39	78	87	174	0.1	174963	0.10	-15	0.0000	1	0.004	15	-0.0002
			0.5	2	48	96											
	3	2	0.5	1	56	112	119	238	0.1	174963	0.14	49	0.0001	1	0.004	15	0.0005
			0.5	2	63	126											
	4	2	0.5	1	45	90	85	170	0.1	174963	0.10	-19	0.0000	1	0.004	15	-0.0002
			0.5	2	40	80											
Positive control	1	2	0.5	1	6243	12486	12364	24728	0.1	174963	14.13	24539	0.0280	1	0.004	15	0.2480
			0.5	2	6121	12242											
	2	2	0.5	1	6738	13476	13297	26594	0.1	174963	15.20	26405	0.0302	1	0.004	15	0.2668
			0.5	2	6559	13118											
	3	2	0.5	1	4798	9596	9602	19204	0.1	174963	10.98	19015	0.0217	1	0.004	15	0.1922
			0.5	2	4804	9608											
	4	2	0.5	1	5305	10610	10568	21136	0.1	174963	12.08	20947	0.0239	1	0.004	15	0.2117
			0.5	2	5263	10526											
Negative Control	1	2	0.5	1	11118	22236	22057	44114	0.1	174963	25.21	43925	0.0502	1	0.004	15	0.4439
			0.5	2	10939	21878											
	2	2	0.5	1	11316	22632	22702	45404	0.1	174963	25.95	45215	0.0516	1	0.004	15	0.4569
			0.5	2	11386	22772											
	3	2	0.5	1	8249	16498	16373	32746	0.1	174963	18.72	32557	0.0372	1	0.004	15	0.3290
			0.5	2	8124	16248											
	4	2	0.5	1	9480	18960	18947	37894	0.1	174963	21.66	37705	0.0431	1	0.004	15	0.3810
			0.5	2	9467	18934											
Ref Chem 6	1-1	2	0.5	1	147	294	277	554	0.1	174963	0.32	365	0.0004	1	0.004	15	0.0037
			0.5	2	130	260											
	1-2	2	0.5	1	138	276	272	544	0.1	174963	0.31	355	0.0004	1	0.004	15	0.0036
			0.5	2	134	268											
	1-3	2	0.5	1	125	250	250	500	0.1	174963	0.29	311	0.0004	1	0.004	15	0.0031
			0.5	2	125	250											
	2-1	2	0.5	1	699	1398	1379	2758	0.1	174963	1.58	2569	0.0029	1	0.004	15	0.0260
			0.5	2	680	1360											
	2-2	2	0.5	1	681	1362	1328	2656	0.1	174963	1.52	2467	0.0028	1	0.004	15	0.0249
			0.5	2	647	1294											
	2-3	2	0.5	1	682	1364	1297	2594	0.1	174963	1.48	2405	0.0027	1	0.004	15	0.0243
			0.5	2	615	1230											
	3-1	2	0.5	1	3676	7352	7365	14730	0.1	174963	8.42	14541	0.0166	1	0.004	15	0.1469
			0.5	2	3689	7378											
	3-2	2	0.5	1	3683	7366	7463	14926	0.1	174963	8.53	14737	0.0168	1	0.004	15	0.1489
			0.5	2	3780	7560											
	3-3	2	0.5	1	3811	7622	7620	15240	0.1	174963	8.71	15051	0.0172	1	0.004	15	0.1521
			0.5	2	3809	7618											
	4-1	2	0.5	1	7880	15760	15489	30978	0.1	174963	17.71	30789	0.0352	1	0.004	15	0.3111
			0.5	2	7609	15218											
	4-2	2	0.5	1	8048	16096	16115	32230	0.1	174963	18.42	32041	0.0366	1	0.004	15	0.3238
			0.5	2	8067	16134											
	4-3	2	0.5	1	8103	16206	16238	32476	0.1	174963	18.56	32287	0.0369	1	0.004	15	0.3263
			0.5	2	8135	16270											
	5-1	2	0.5	1	8839	17678	17749	35498	0.1	174963	20.29	35309	0.0403	1	0.004	15	0.3568
			0.5	2	8910	17820											
	5-2	2	0.5	1	9029	18058	17880	35760	0.1	174963	20.44	35571	0.0406	1	0.004	15	0.3595
			0.5	2	8851	17702											
	5-3	2	0.5	1	9121	18242	18073	36146	0.1	174963	20.66	35957	0.0411	1	0.004	15	0.3634
			0.5	2	8952	17904											
	6-1	2	0.5	1	9183	18366	18484	36968	0.1	174963	21.13	36779	0.0420	1	0.004	15	0.3717
			0.5	2	9301	18602											
	6-2	2	0.5	1	9116	18232	18317	36634	0.1	174963	20.94	36445	0.0416	1	0.004	15	0.3683
			0.5	2	9201	18402											
	6-3	2	0.5	1	8410	16820	16759	33518	0.1	174963	19.16	33329	0.0381	1	0.004	15	0.3368
			0.5	2	8349	16698											
	7-1	2	0.5	1	8990	17980	17906	35812	0.1	174963	20.47	35623	0.0407	1	0.004	15	0.3600
			0.5	2	8916	17832											
	7-2	2	0.5	1	8929	17858	18104	36208	0.1	174963	20.69	36019	0.0411	1	0.004	15	0.3640
			0.5	2	9175	18350											
	7-3	2	0.5	1	9307	18614	18444	36888	0.1	174963	21.08	36699	0.0419	1	0.004	15	0.3709
			0.5	2	9137	18274											
	8-1	2	0.5	1	8838	17676	17675	35350	0.1	174963	20.20	35161	0.0402	1	0.004	15	0.3563
			0.5	2	8837	17674											
	8-2	2	0.5	1	8850	17700	17752	35504	0.1	174963	20.29	35315	0.0403	1	0.004	15	0.3569
			0.5	2	8902	17804											
	8-3	2	0.5	1	9156	18312	18186	36372	0.1	174963	20.79	36183	0.0413	1	0.004	15	0.3656
			0.5	2	9030	18060											

Assay Date	8/2/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	Replicate # 2 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4448	
Full activity	End	0.3775	0.0308
Full activity	Overall	0.3999	0.0445
Background	Beginning	-0.0002	0
Background	End	0.0002	0.000485905
Background	Overall	0.0000	0.000330662
Positive	Beginning	0.2574	0.0133
Positive	End	0.2019	0.0138
Positive	Overall	0.2297	0.0339
Negative	Beginning	0.4504	0.0092
Negative	End	0.3550	0.0368
Negative	Overall	0.4027	0.0593

Note: Altered FEAC Beg and Overall averages to exclude Tube 1 - SLB 8/3/2005

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 6	1	1	1.00E-03	-3.00	0.0037
Ref Chem 6	1	2	1.00E-03	-3.00	0.0036
Ref Chem 6	1	3	1.00E-03	-3.00	0.0031
Ref Chem 6	2	1	1.00E-04	-4.00	0.0260
Ref Chem 6	2	2	1.00E-04	-4.00	0.0249
Ref Chem 6	2	3	1.00E-04	-4.00	0.0243
Ref Chem 6	3	1	1.00E-05	-5.00	0.1469
Ref Chem 6	3	2	1.00E-05	-5.00	0.1489
Ref Chem 6	3	3	1.00E-05	-5.00	0.1521
Ref Chem 6	4	1	1.00E-06	-6.00	0.3111
Ref Chem 6	4	2	1.00E-06	-6.00	0.3238
Ref Chem 6	4	3	1.00E-06	-6.00	0.3263
Ref Chem 6	5	1	1.00E-07	-7.00	0.3568
Ref Chem 6	5	2	1.00E-07	-7.00	0.3595
Ref Chem 6	5	3	1.00E-07	-7.00	0.3634
Ref Chem 6	6	1	1.00E-08	-8.00	0.3717
Ref Chem 6	6	2	1.00E-08	-8.00	0.3683
Ref Chem 6	6	3	1.00E-08	-8.00	0.3368
Ref Chem 6	7	1	1.00E-09	-9.00	0.3600
Ref Chem 6	7	2	1.00E-09	-9.00	0.3640
Ref Chem 6	7	3	1.00E-09	-9.00	0.3709
Ref Chem 6	8	1	1.00E-10	-10.00	0.3553
Ref Chem 6	8	2	1.00E-10	-10.00	0.3569
Ref Chem 6	8	3	1.00E-10	-10.00	0.3656

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.92	0.90	0.79
2	-4.00	6.49	6.23	6.08
3	-5.00	36.75	37.24	38.03
4	-6.00	77.80	80.97	81.59
5	-7.00	89.23	89.89	90.86
6	-8.00	92.94	92.10	84.22
7	-9.00	90.02	91.02	92.74
8	-10.00	88.85	89.24	91.44

Aromatase Assay Spreadsheet

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8		
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	35354	1758905	
2	0.0199	36190	1818593	
3	0.0196	35884	1830816	
4	0.0199	39605	1990201	
5	0.0199	37187	1868693	
			Average DPM/g soln	1853442
			SD	86022
			CV	4.64
			µCi/g soln	0.835

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	8.1217 g
Mass of dilution B used in substrate prep	4.5722 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.568591 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00945 µg/g soln.
µg/g soln.

a. µCi/g soln 0.835
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.

= 0.568591 + 0.00945
= 0.578042 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.444 µCi/µg ASDN

918318 dpm/nmol

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8					
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.4	100	11540-42
	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			

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
			Blank	0.113				

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

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	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10

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	0.0431
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

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

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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.7
Protein Concentration (dilution added to assay, mg/mL):	0.006136

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	Recombinan Microsome ID	11345-10	Technician ID	2	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol^{3}H$_2$O 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^{3}H$_2$O 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	10567	21174	21014	42028	0.1	185344	22.68	41914	0.0456	1	0.003	15	0.4959
			0.5	2	10427	20854											
	2	2	0.5	1	10527	21054	20936	41872	0.1	185344	22.59	41758	0.0455	1	0.003	15	0.4941
			0.5	2	10409	20818											
	3	2	0.5	1	8273	16546	16330	32660	0.1	185344	17.62	32546	0.0354	1	0.003	15	0.3851
			0.5	2	8057	16114											
	4	2	0.5	1	8503	17006	17103	34206	0.1	185344	18.46	34092	0.0371	1	0.003	15	0.4034
			0.5	2	8600	17200											
Background control	1	2	0.5	1	22	44	52	104	0.1	185344	0.06	-10	0.0000	1	0.003	15	-0.0001
			0.5	2	30	60											
	2	2	0.5	1	33	66	75	150	0.1	185344	0.08	36	0.0000	1	0.003	15	0.0004
			0.5	2	42	84											
	3	2	0.5	1	23	46	36	72	0.1	185344	0.04	-42	0.0000	1	0.003	15	-0.0005
			0.5	2	13	26											
	4	2	0.5	1	18	36	65	130	0.1	185344	0.07	16	0.0000	1	0.003	15	0.0002
			0.5	2	47	94											
Positive control	1	2	0.5	1	6269	12538	12323	24646	0.1	185344	13.30	24532	0.0267	1	0.003	15	0.2903
			0.5	2	6054	12108											
	2	2	0.5	1	6616	13232	12988	25976	0.1	185344	14.02	25862	0.0282	1	0.003	15	0.3060
			0.5	2	6372	12744											
	3	2	0.5	1	5094	10188	10273	20546	0.1	185344	11.09	20432	0.0222	1	0.003	15	0.2418
			0.5	2	5179	10358											
	4	2	0.5	1	5803	11606	11625	23250	0.1	185344	12.54	23136	0.0252	1	0.003	15	0.2737
			0.5	2	5822	11644											
Negative Control	1	2	0.5	1	11170	22340	21767	43534	0.1	185344	23.49	43420	0.0473	1	0.003	15	0.5137
			0.5	2	10597	21194											
	2	2	0.5	1	10302	20604	20532	41064	0.1	185344	22.16	40950	0.0446	1	0.003	15	0.4845
			0.5	2	10230	20460											
	3	2	0.5	1	8479	16958	16932	33864	0.1	185344	18.27	33750	0.0368	1	0.003	15	0.3993
			0.5	2	8453	16906											
	4	2	0.5	1	10120	20240	20196	40392	0.1	185344	21.79	40278	0.0439	1	0.003	15	0.4766
			0.5	2	10076	20152											
Ref Chem 6	1-1	2	0.5	1	139	278	252	504	0.1	185344	0.27	390	0.0004	1	0.003	15	0.0046
			0.5	2	113	226											
	1-2	2	0.5	1	131	262	245	490	0.1	185344	0.26	376	0.0004	1	0.003	15	0.0044
			0.5	2	114	228											
	1-3	2	0.5	1	127	254	253	506	0.1	185344	0.27	392	0.0004	1	0.003	15	0.0046
			0.5	2	126	252											
	2-1	2	0.5	1	633	1266	1267	2534	0.1	185344	1.37	2420	0.0026	1	0.003	15	0.0286
			0.5	2	634	1268											
	2-2	2	0.5	1	640	1280	1269	2538	0.1	185344	1.37	2424	0.0026	1	0.003	15	0.0287
			0.5	2	629	1258											
	2-3	2	0.5	1	626	1252	1258	2516	0.1	185344	1.36	2402	0.0026	1	0.003	15	0.0284
			0.5	2	632	1264											
	3-1	2	0.5	1	1861	3722	3737	7474	0.1	185344	4.03	7360	0.0080	1	0.003	15	0.0871
			0.5	2	1876	3752											
	3-2	2	0.5	1	1997	3994	3997	7994	0.1	185344	4.31	7880	0.0086	1	0.003	15	0.0932
			0.5	2	2000	4000											
	3-3	2	0.5	1	1871	3742	3701	7402	0.1	185344	3.99	7288	0.0079	1	0.003	15	0.0862
			0.5	2	1830	3660											
	4-1	2	0.5	1	3561	7122	7098	14196	0.1	185344	7.66	14082	0.0153	1	0.003	15	0.1666
			0.5	2	3537	7074											
	4-2	2	0.5	1	3496	6992	7022	14044	0.1	185344	7.58	13930	0.0152	1	0.003	15	0.1648
			0.5	2	3526	7052											
	4-3	2	0.5	1	3167	6334	6275	12550	0.1	185344	6.77	12436	0.0135	1	0.003	15	0.1471
			0.5	2	3108	6216											
	5-1	2	0.5	1	4766	9532	9484	18968	0.1	185344	10.23	18854	0.0205	1	0.003	15	0.2231
			0.5	2	4718	9436											
	5-2	2	0.5	1	4510	9020	9017	18034	0.1	185344	9.73	17920	0.0195	1	0.003	15	0.2120
			0.5	2	4507	9014											
	5-3	2	0.5	1	4635	9270	9247	18494	0.1	185344	9.98	18380	0.0200	1	0.003	15	0.2175
			0.5	2	4612	9224											
	6-1	2	0.5	1	7317	14634	14532	29064	0.1	185344	15.68	28950	0.0315	1	0.003	15	0.3425
			0.5	2	7216	14430											
	6-2	2	0.5	1	8177	16354	16350	32700	0.1	185344	17.64	32586	0.0355	1	0.003	15	0.3856
			0.5	2	8173	16346											
	6-3	2	0.5	1	8146	16292	16210	32420	0.1	185344	17.49	32306	0.0352	1	0.003	15	0.3822
			0.5	2	8064	16128											
	7-1	2	0.5	1	8782	17564	17595	35190	0.1	185344	18.99	35078	0.0382	1	0.003	15	0.4150
			0.5	2	8813	17626											
	7-2	2	0.5	1	8981	17962	17802	35604	0.1	185344	19.21	35490	0.0386	1	0.003	15	0.4199
			0.5	2	8821	17642											
	7-3	2	0.5	1	8393	16786	16715	33430	0.1	185344	18.04	33316	0.0363	1	0.003	15	0.3942
			0.5	2	8322	16644											
	8-1	2	0.5	1	8505	17010	16980	33960	0.1	185344	18.32	33846	0.0369	1	0.003	15	0.4005
			0.5	2	8475	16950											
	8-2	2	0.5	1	8750	17500	17450	34900	0.1	185344	18.83	34786	0.0379	1	0.003	15	0.4116
			0.5	2	8700	17400											
	8-3	2	0.5	1	8534	17068	16844	33688	0.1	185344	18.18	33574	0.0366	1	0.003	15	0.3972
			0.5	2	8310	16620											

Assay Date	8/4/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested		Microsome 8 type	Recombinant	Microsome ID	11345-10	Technician ID	Replicate # 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.4950	0.0013
Full activity	End	0.3942	0.0129
Full activity	Overall	0.4446	0.0587
Background	Beginning	0.0002	0.000384856
Background	End	-0.0002	0.000485253
Background	Overall	0.0000	0.000399258
Positive	Beginning	0.2981	0.0111
Positive	End	0.2577	0.0226
Positive	Overall	0.2779	0.0275
Negative	Beginning	0.4991	0.0207
Negative	End	0.4379	0.0546
Negative	Overall	0.4685	0.0488

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 6	1	1	1.00E-03	-3.00	0.0046
Ref Chem 6	1	2	1.00E-03	-3.00	0.0044
Ref Chem 6	1	3	1.00E-03	-3.00	0.0046
Ref Chem 6	2	1	1.00E-04	-4.00	0.0286
Ref Chem 6	2	2	1.00E-04	-4.00	0.0287
Ref Chem 6	2	3	1.00E-04	-4.00	0.0284
Ref Chem 6	3	1	2.50E-05	-4.60	0.0871
Ref Chem 6	3	2	2.50E-05	-4.60	0.0932
Ref Chem 6	3	3	2.50E-05	-4.60	0.0862
Ref Chem 6	4	1	1.00E-05	-5.00	0.1666
Ref Chem 6	4	2	1.00E-05	-5.00	0.1648
Ref Chem 6	4	3	1.00E-05	-5.00	0.1471
Ref Chem 6	5	1	5.00E-06	-5.30	0.2231
Ref Chem 6	5	2	5.00E-06	-5.30	0.2120
Ref Chem 6	5	3	5.00E-06	-5.30	0.2175
Ref Chem 6	6	1	1.00E-06	-6.00	0.3425
Ref Chem 6	6	2	1.00E-06	-6.00	0.3856
Ref Chem 6	6	3	1.00E-06	-6.00	0.3822
Ref Chem 6	7	1	1.00E-07	-7.00	0.4150
Ref Chem 6	7	2	1.00E-07	-7.00	0.4199
Ref Chem 6	7	3	1.00E-07	-7.00	0.3942
Ref Chem 6	8	1	1.00E-08	-8.00	0.4005
Ref Chem 6	8	2	1.00E-08	-8.00	0.4116
Ref Chem 6	8	3	1.00E-08	-8.00	0.3972

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.04	1.00	1.04
2	-4.00	6.44	6.45	6.39
3	-4.60	19.59	20.97	19.39
4	-5.00	37.47	37.07	33.09
5	-5.30	50.17	47.69	48.91
6	-6.00	77.04	86.72	85.97
7	-7.00	93.34	94.44	88.66
8	-8.00	90.07	92.57	89.35

Aromatase Assay Spreadsheet

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8		
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0196	34190	1744388	
2	0.0197	34551	1753858	
3	0.0200	33954	1697700	
4	0.0194	33733	1738814	
5	0.0197	34085	1730203	
			Average DPM/g soln	1732993
			SD	21520
			CV	1.24
			µCi/g soln	0.781

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	8.0688 g
Mass of dilution B used in substrate prep	4.5295 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56136 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00884 µg/g soln.
µg/g soln.

a. µCi/g soln 0.781
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.

= 0.561360 + 0.00884
= 0.570197 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.369 µCi/µg ASDN

870453 dpm/nmol

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8						
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10				

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

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

Blank 0.121

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	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10				

								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	Variables	Regression results	
250	200	200	0.00025	200	0.0502	0.583	0.462	0.0424	m, b	0.092	0.000
125	100	200	0.00013	200	0.0251	0.390	0.269	0.0246	se_m, se_b	0.004	0.000
50	200	1000	0.00005	200	0.0100	0.240	0.120	0.0108	r^2, se_y	0.995	0.001
25	100	1000	0.00003	200	0.0050	0.185	0.064	0.0057	F, df	650	3
10	40	1000	0.00001	200	0.0020	0.140	0.020	0.0016	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.128	0.007	0.0004			

Regression results are calculated using the function LINEST

Blank	0.121	r^2=	0.995
		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 6	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	2.9
Protein Concentration (dilution added to assay, mg/mL):	0.004806

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	8/5/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	11345-10	Technician ID	2	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 dilutec 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	10661	21302	21112	42224	0.1	173299	24.36	42056	0.0483	1	0.002	15	0.6702
			0.5	2	10461	20922											
	2	2	0.5	1	11400	22800	22374	44748	0.1	173299	25.82	44580	0.0512	1	0.002	15	0.7104
			0.5	2	10974	21948											
	3	2	0.5	1	9469	18938	19084	38168	0.1	173299	22.02	38000	0.0437	1	0.002	15	0.6055
			0.5	2	9615	19230											
	4	2	0.5	1	9710	19420	19331	38662	0.1	173299	22.31	38494	0.0442	1	0.002	15	0.6134
			0.5	2	9621	19242											
Background control	1	2	0.5	1	36	72	78	156	0.1	173299	0.09	-13	0.0000	1	0.002	15	-0.0002
			0.5	2	42	84											
	2	2	0.5	1	37	74	83	166	0.1	173299	0.10	-3	0.0000	1	0.002	15	0.0000
			0.5	2	46	92											
	3	2	0.5	1	36	72	77	154	0.1	173299	0.09	-15	0.0000	1	0.002	15	-0.0002
			0.5	2	41	82											
	4	2	0.5	1	49	98	99	198	0.1	173299	0.11	30	0.0000	1	0.002	15	0.0005
			0.5	2	50	100											
Positive control	1	2	0.5	1	6019	12038	12033	24066	0.1	173299	13.89	23898	0.0275	1	0.002	15	0.3808
			0.5	2	6014	12028											
	2	2	0.5	1	6955	13910	13859	27718	0.1	173299	15.99	27550	0.0316	1	0.002	15	0.4390
			0.5	2	6904	13808											
	3	2	0.5	1	5559	11118	11107	22214	0.1	173299	12.82	22046	0.0253	1	0.002	15	0.3513
			0.5	2	5548	11096											
	4	2	0.5	1	5407	10814	10904	21808	0.1	173299	12.58	21640	0.0249	1	0.002	15	0.3448
			0.5	2	5497	10994											
Negative Control	1	2	0.5	1	10931	21862	22054	44108	0.1	173299	25.45	43940	0.0505	1	0.002	15	0.7002
			0.5	2	11123	22246											
	2	2	0.5	1	10958	21916	21823	43646	0.1	173299	25.19	43478	0.0499	1	0.002	15	0.6928
			0.5	2	10865	21730											
	3	2	0.5	1	9285	18570	18515	37030	0.1	173299	21.37	36862	0.0423	1	0.002	15	0.5874
			0.5	2	9230	18460											
	4	2	0.5	1	9810	19620	19443	38886	0.1	173299	22.44	38718	0.0445	1	0.002	15	0.6170
			0.5	2	9633	19266											
Ref Chem 6	1-1	2	0.5	1	148	296	274	548	0.1	173299	0.32	380	0.0004	1	0.002	15	0.0060
			0.5	2	126	252											
	1-2	2	0.5	1	141	282	287	574	0.1	173299	0.33	406	0.0005	1	0.002	15	0.0065
			0.5	2	146	292											
	1-3	2	0.5	1	151	302	280	560	0.1	173299	0.32	392	0.0004	1	0.002	15	0.0062
			0.5	2	129	258											
	2-1	2	0.5	1	670	1340	1336	2672	0.1	173299	1.54	2504	0.0029	1	0.002	15	0.0399
			0.5	2	666	1332											
	2-2	2	0.5	1	659	1318	1328	2656	0.1	173299	1.53	2488	0.0029	1	0.002	15	0.0396
			0.5	2	669	1338											
	2-3	2	0.5	1	657	1314	1372	2744	0.1	173299	1.58	2576	0.0030	1	0.002	15	0.0410
			0.5	2	715	1430											
	3-1	2	0.5	1	2057	4114	4063	8126	0.1	173299	4.69	7958	0.0091	1	0.002	15	0.1268
			0.5	2	2006	4012											
	3-2	2	0.5	1	1973	3946	3928	7856	0.1	173299	4.53	7688	0.0088	1	0.002	15	0.1225
			0.5	2	1955	3910											
	3-3	2	0.5	1	2006	4012	4071	8142	0.1	173299	4.70	7974	0.0092	1	0.002	15	0.1271
			0.5	2	2065	4130											
	4-1	2	0.5	1	3615	7230	7230	14460	0.1	173299	8.34	14292	0.0164	1	0.002	15	0.2277
			0.5	2	3615	7230											
	4-2	2	0.5	1	3477	6954	6886	13772	0.1	173299	7.95	13604	0.0156	1	0.002	15	0.2168
			0.5	2	3409	6818											
	4-3	2	0.5	1	3299	6598	6633	13266	0.1	173299	7.65	13098	0.0150	1	0.002	15	0.2087
			0.5	2	3334	6668											
	5-1	2	0.5	1	5113	10226	10218	20436	0.1	173299	11.79	20268	0.0233	1	0.002	15	0.3230
			0.5	2	5105	10210											
	5-2	2	0.5	1	5176	10352	10344	20688	0.1	173299	11.94	20520	0.0236	1	0.002	15	0.3270
			0.5	2	5168	10336											
	5-3	2	0.5	1	5154	10308	10223	20446	0.1	173299	11.80	20278	0.0233	1	0.002	15	0.3231
			0.5	2	5069	10138											
	6-1	2	0.5	1	7823	15646	15704	31408	0.1	173299	18.12	31240	0.0359	1	0.002	15	0.4978
			0.5	2	7881	15762											
	6-2	2	0.5	1	7679	15358	15641	31282	0.1	173299	18.05	31114	0.0357	1	0.002	15	0.4958
			0.5	2	7962	15924											
	6-3	2	0.5	1	8308	16616	16644	33288	0.1	173299	19.21	33120	0.0380	1	0.002	15	0.5278
			0.5	2	8336	16672											
	7-1	2	0.5	1	9513	19026	19139	38278	0.1	173299	22.09	38110	0.0438	1	0.002	15	0.6073
			0.5	2	9626	19252											
	7-2	2	0.5	1	8683	17366	17364	34728	0.1	173299	20.04	34560	0.0397	1	0.002	15	0.5507
			0.5	2	8681	17362											
	7-3	2	0.5	1	8975	17950	18013	36026	0.1	173299	20.79	35858	0.0412	1	0.002	15	0.5714
			0.5	2	9038	18076											
	8-1	2	0.5	1	9018	18036	18013	36026	0.1	173299	20.79	35858	0.0412	1	0.002	15	0.5714
			0.5	2	8995	17990											
	8-2	2	0.5	1	9068	18136	18132	36264	0.1	173299	20.93	36096	0.0415	1	0.002	15	0.5752
			0.5	2	9064	18128											
	8-3	2	0.5	1	9593	19186	19175	38350	0.1	173299	22.13	38182	0.0439	1	0.002	15	0.6084
			0.5	2	9582	19164											

Assay Date	8/5/2005	Test Chemical ID	Ref Chem 6	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.6903	0.0284
Full activity	End	0.6095	0.0056
Full activity	Overall	0.6499	0.0496
Background	Beginning	-0.0001	0.00011268
Background	End	0.0001	0.00049579
Background	Overall	0.0000	0.000324366
Positive	Beginning	0.4099	0.0412
Positive	End	0.3481	0.0046
Positive	Overall	0.3790	0.0430
Negative	Beginning	0.6965	0.0052
Negative	End	0.6022	0.0209
Negative	Overall	0.6493	0.0559

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 6	1	1	1.00E-03	-3.00	0.0060
Ref Chem 6	1	2	1.00E-03	-3.00	0.0065
Ref Chem 6	1	3	1.00E-03	-3.00	0.0062
Ref Chem 6	2	1	1.00E-04	-4.00	0.0399
Ref Chem 6	2	2	1.00E-04	-4.00	0.0396
Ref Chem 6	2	3	1.00E-04	-4.00	0.0410
Ref Chem 6	3	1	2.50E-05	-4.60	0.1268
Ref Chem 6	3	2	2.50E-05	-4.60	0.1225
Ref Chem 6	3	3	2.50E-05	-4.60	0.1271
Ref Chem 6	4	1	1.00E-05	-5.00	0.2277
Ref Chem 6	4	2	1.00E-05	-5.00	0.2168
Ref Chem 6	4	3	1.00E-05	-5.00	0.2087
Ref Chem 6	5	1	5.00E-06	-5.30	0.3230
Ref Chem 6	5	2	5.00E-06	-5.30	0.3270
Ref Chem 6	5	3	5.00E-06	-5.30	0.3231
Ref Chem 6	6	1	1.00E-06	-6.00	0.4978
Ref Chem 6	6	2	1.00E-06	-6.00	0.4958
Ref Chem 6	6	3	1.00E-06	-6.00	0.5278
Ref Chem 6	7	1	1.00E-07	-7.00	0.6073
Ref Chem 6	7	2	1.00E-07	-7.00	0.5507
Ref Chem 6	7	3	1.00E-07	-7.00	0.5714
Ref Chem 6	8	1	1.00E-08	-8.00	0.5714
Ref Chem 6	8	2	1.00E-08	-8.00	0.5752
Ref Chem 6	8	3	1.00E-08	-8.00	0.6084

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.93	0.99	0.96
2	-4.00	6.14	6.10	6.32
3	-4.60	19.51	18.85	19.55
4	-5.00	35.04	33.36	32.12
5	-5.30	49.70	50.32	49.72
6	-6.00	76.60	76.29	81.21
7	-7.00	93.45	84.74	87.92
8	-8.00	87.92	88.51	93.62

Aromatase Assay Spreadsheet

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8		
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	31847	1600352	
2	0.0197	30126	1529239	
3	0.0201	34596	1721194	
4	0.0199	33372	1676985	
5	0.0202	35146	1739901	
			Average DPM/g soln	1653534
			SD	87842
			CV	5.31
			µCi/g soln	0.745

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	8.0194 g
Mass of dilution B used in substrate prep	4.4702 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.574146 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00843 µg/g soln.
µg/g soln.

a. µCi/g soln 0.745
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.

= 0.574146 + 0.00843
= 0.582578 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.279 µCi/µg ASDN

812891 dpm/nmol

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8					
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10			

								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

Blank 0.115

r^2= 0.983
m= 0.111
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	0.364	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	2	Replicate #	2	Microsome type	Recombinant	Microsome ID 11345-10

								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	0.0396
125	100	200	0.00013	200	0.0251	0.394	0.279	0.0247
50	200	1000	0.00005	200	0.0100	0.239	0.124	0.0107
25	100	1000	0.00003	200	0.0050	0.179	0.064	0.0054
10	40	1000	0.00001	200	0.0020	0.139	0.024	0.0017
5	20	1000	0.00001	200	0.0010	0.126	0.011	0.0006

Variables	Regression results	
m, b	0.090	0.000
se_m, se_b	0.003	0.000
r^2, se_y	0.997	0.001
F, df	1143	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

			r^2=	0.997
Blank	0.115		m=	0.090
			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	0.364	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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Microsome Dilution Details

Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.3
Protein Concentration (dilution added to assay, mg/mL):	0.010495

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	8/24/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol^{3}H$_2$O 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^{3}H$_2$O 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	10058	20116	19661	39322	0.1	165353	23.78	39172	0.0482	1	0.005	15	0.3061
			0.5	2	9603	19206											
	2	2	0.5	1	10716	21432	21073	42146	0.1	165353	25.49	41996	0.0517	1	0.005	15	0.3282
			0.5	2	10357	20714											
	3	2	0.5	1	8315	16630	16533	33066	0.1	165353	20.00	32916	0.0405	1	0.005	15	0.2572
			0.5	2	8218	16436											
	4	2	0.5	1	7641	15282	15226	30452	0.1	165353	18.42	30302	0.0373	1	0.005	15	0.2368
			0.5	2	7585	15170											
Background control	1	2	0.5	1	38	76	70	140	0.1	165353	0.08	-10	0.0000	1	0.005	15	-0.0001
			0.5	2	32	64											
	2	2	0.5	1	37	74	69	138	0.1	165353	0.08	-12	0.0000	1	0.005	15	-0.0001
			0.5	2	32	64											
	3	2	0.5	1	38	76	77	154	0.1	165353	0.09	4	0.0000	1	0.005	15	0.0000
			0.5	2	39	78											
	4	2	0.5	1	40	80	84	168	0.1	165353	0.10	18	0.0000	1	0.005	15	0.0001
			0.5	2	44	88											
Positive control	1	2	0.5	1	5216	10432	10248	20496	0.1	165353	12.40	20346	0.0250	1	0.005	15	0.1590
			0.5	2	5032	10064											
	2	2	0.5	1	5153	10306	10276	20552	0.1	165353	12.43	20402	0.0251	1	0.005	15	0.1594
			0.5	2	5123	10246											
	3	2	0.5	1	4619	9238	9140	18280	0.1	165353	11.06	18130	0.0223	1	0.005	15	0.1417
			0.5	2	4521	9042											
	4	2	0.5	1	5023	10046	9938	19876	0.1	165353	12.02	19726	0.0243	1	0.005	15	0.1541
			0.5	2	4915	9830											
Negative Control	1	2	0.5	1	9240	18480	18342	36684	0.1	165353	22.19	36534	0.0449	1	0.005	15	0.2855
			0.5	2	9102	18204											
	2	2	0.5	1	9098	18196	18194	36388	0.1	165353	22.01	36238	0.0446	1	0.005	15	0.2832
			0.5	2	9096	18192											
	3	2	0.5	1	8275	16550	16634	33268	0.1	165353	20.12	33118	0.0407	1	0.005	15	0.2588
			0.5	2	8359	16718											
	4	2	0.5	1	8964	17928	17998	35996	0.1	165353	21.77	35846	0.0441	1	0.005	15	0.2801
			0.5	2	9034	18068											
Ref Chem 7	1-1	2	0.5	1	58	116	123	246	0.1	165353	0.15	96	0.0001	1	0.005	15	0.0008
			0.5	2	65	130											
	1-2	2	0.5	1	69	138	115	230	0.1	165353	0.14	80	0.0001	1	0.005	15	0.0006
			0.5	2	46	92											
	1-3	2	0.5	1	60	120	125	250	0.1	165353	0.15	100	0.0001	1	0.005	15	0.0008
			0.5	2	65	130											
	2-1	2	0.5	1	263	526	497	994	0.1	165353	0.60	844	0.0010	1	0.005	15	0.0066
			0.5	2	234	468											
	2-2	2	0.5	1	214	428	419	838	0.1	165353	0.51	688	0.0008	1	0.005	15	0.0054
			0.5	2	205	410											
	2-3	2	0.5	1	203	406	431	862	0.1	165353	0.52	712	0.0009	1	0.005	15	0.0056
			0.5	2	228	456											
	3-1	2	0.5	1	1443	2886	2908	5816	0.1	165353	3.52	5666	0.0070	1	0.005	15	0.0443
			0.5	2	1465	2930											
	3-2	2	0.5	1	1500	3000	3067	6134	0.1	165353	3.71	5984	0.0074	1	0.005	15	0.0468
			0.5	2	1567	3134											
	3-3	2	0.5	1	1653	3306	3295	6590	0.1	165353	3.99	6440	0.0079	1	0.005	15	0.0503
			0.5	2	1642	3284											
	4-1	2	0.5	1	2772	5544	5511	11022	0.1	165353	6.67	10872	0.0134	1	0.005	15	0.0850
			0.5	2	2739	5478											
	4-2	2	0.5	1	2777	5554	5537	11074	0.1	165353	6.70	10924	0.0134	1	0.005	15	0.0854
			0.5	2	2760	5520											
	4-3	2	0.5	1	2498	4996	5012	10024	0.1	165353	6.06	9874	0.0121	1	0.005	15	0.0772
			0.5	2	2514	5028											
	5-1	2	0.5	1	6983	13966	14015	28030	0.1	165353	16.95	27880	0.0343	1	0.005	15	0.2179
			0.5	2	7032	14064											
	5-2	2	0.5	1	6769	13538	13772	27544	0.1	165353	16.66	27394	0.0337	1	0.005	15	0.2141
			0.5	2	7003	14006											
	5-3	2	0.5	1	6511	13022	13013	26026	0.1	165353	15.74	25876	0.0318	1	0.005	15	0.2022
			0.5	2	6502	13004											
	6-1	2	0.5	1	8174	16348	16401	32802	0.1	165353	19.84	32652	0.0402	1	0.005	15	0.2551
			0.5	2	8227	16454											
	6-2	2	0.5	1	8097	16194	16119	32238	0.1	165353	19.50	32088	0.0395	1	0.005	15	0.2507
			0.5	2	8022	16044											
	6-3	2	0.5	1	8193	16386	16534	33068	0.1	165353	20.00	32918	0.0405	1	0.005	15	0.2572
			0.5	2	8341	16682											
	7-1	2	0.5	1	8317	16634	16666	33332	0.1	165353	20.16	33182	0.0408	1	0.005	15	0.2593
			0.5	2	8349	16698											
	7-2	2	0.5	1	8204	16408	16556	33112	0.1	165353	20.02	32962	0.0405	1	0.005	15	0.2576
			0.5	2	8352	16704											
	7-3	2	0.5	1	8277	16554	16540	33080	0.1	165353	20.01	32930	0.0405	1	0.005	15	0.2573
			0.5	2	8263	16526											
	8-1	2	0.5	1	8566	17132	17175	34350	0.1	165353	20.77	34200	0.0421	1	0.005	15	0.2672
			0.5	2	8609	17218											
	8-2	2	0.5	1	8847	17694	18847	37694	0.1	165353	22.80	37544	0.0462	1	0.005	15	0.2934
			0.5	2	10000	20000											
	8-3	2	0.5	1	9058	18116	18231	36462	0.1	165353	22.05	36312	0.0447	1	0.005	15	0.2837
			0.5	2	9173	18346											

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID 11345-10	Technician ID	Replicate # 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3171	0.0156
Full activity	End	0.2470	0.0144
Full activity	Overall	0.2821	0.0423
Background	Beginning	-0.0001	1.10508E-05
Background	End	0.0001	7.73556E-05
Background	Overall	0.0000	0.000109025
Positive	Beginning	0.1592	0.0003
Positive	End	0.1479	0.0088
Positive	Overall	0.1536	0.0083
Negative	Beginning	0.2843	0.0016
Negative	End	0.2694	0.0151
Negative	Overall	0.2769	0.0123

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 7	1	1	1.00E-06	-6.00	0.0008
Ref Chem 7	1	2	1.00E-06	-6.00	0.0006
Ref Chem 7	1	3	1.00E-06	-6.00	0.0008
Ref Chem 7	2	1	1.00E-07	-7.00	0.0066
Ref Chem 7	2	2	1.00E-07	-7.00	0.0054
Ref Chem 7	2	3	1.00E-07	-7.00	0.0056
Ref Chem 7	3	1	1.00E-08	-8.00	0.0443
Ref Chem 7	3	2	1.00E-08	-8.00	0.0468
Ref Chem 7	3	3	1.00E-08	-8.00	0.0503
Ref Chem 7	4	1	5.00E-09	-8.30	0.0850
Ref Chem 7	4	2	5.00E-09	-8.30	0.0854
Ref Chem 7	4	3	5.00E-09	-8.30	0.0772
Ref Chem 7	5	1	1.00E-09	-9.00	0.2179
Ref Chem 7	5	2	1.00E-09	-9.00	0.2141
Ref Chem 7	5	3	1.00E-09	-9.00	0.2022
Ref Chem 7	6	1	5.00E-10	-9.30	0.2551
Ref Chem 7	6	2	5.00E-10	-9.30	0.2507
Ref Chem 7	6	3	5.00E-10	-9.30	0.2572
Ref Chem 7	7	1	2.50E-10	-9.60	0.2593
Ref Chem 7	7	2	2.50E-10	-9.60	0.2576
Ref Chem 7	7	3	2.50E-10	-9.60	0.2573
Ref Chem 7	8	1	1.00E-10	-10.00	0.2672
Ref Chem 7	8	2	1.00E-10	-10.00	0.2934
Ref Chem 7	8	3	1.00E-10	-10.00	0.2837

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-6.00	0.27	0.22	0.28
2	-7.00	2.34	1.91	1.97
3	-8.00	15.70	16.58	17.84
4	-8.30	30.12	30.26	27.35
5	-9.00	77.24	75.89	71.69
6	-9.30	90.46	88.90	91.19
7	-9.60	91.93	91.32	91.23
8	-10.00	94.75	104.01	100.60

Aromatase Assay Spreadsheet

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8
Technician ID	2	Replicate #	3	Microsome type	Recombina Microsome ID 11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0192	32589	1697344	
2	0.0206	37715	1830825	
3	0.0199	37630	1890955	
4	0.0198	36206	1828586	
5	0.0199	38380	1928643	
			Average DPM/g soln	1835271
			SD	87903
			CV	4.79
			µCi/g soln	0.827

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	8.0232 g
Mass of dilution B used in substrate prep	4.4781 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.558144 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00936 µg/g soln.
µg/g soln.

a. µCi/g soln 0.827
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.

= 0.558144 + 0.00936
= 0.567502 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.457 µCi/µg ASDN

926202 dpm/nmol

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8				
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10		

								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	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10		

								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	0.0431
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

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

Regression results are calculated using the function LINEST

Blank	0.113	r^2=	0.981
		m=	0.115
		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	0.358	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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate # 3

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.4
Protein Concentration (dilution added to assay, mg/mL):	0.005609

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	9058	18116	17839	35678	0.1	183527	19.44	35512	0.0383	1	0.003	15	0.4557
			0.5	2	8781	17562											
	2	2	0.5	1	8469	16938	16902	33804	0.1	183527	18.42	33638	0.0363	1	0.003	15	0.4316
			0.5	2	8433	16866											
	3	2	0.5	1	8051	16102	15984	31968	0.1	183527	17.42	31802	0.0343	1	0.003	15	0.4081
			0.5	2	7933	15866											
	4	2	0.5	1	7201	14402	14290	28580	0.1	183527	15.57	28414	0.0307	1	0.003	15	0.3646
			0.5	2	7089	14178											
Background control	1	2	0.5	1	37	74	73	146	0.1	183527	0.08	-20	0.0000	1	0.003	15	-0.0003
			0.5	2	36	72											
	2	2	0.5	1	36	72	73	146	0.1	183527	0.08	-20	0.0000	1	0.003	15	-0.0003
			0.5	2	37	74											
	3	2	0.5	1	39	78	95	190	0.1	183527	0.10	24	0.0000	1	0.003	15	0.0003
			0.5	2	56	112											
	4	2	0.5	1	52	104	91	182	0.1	183527	0.10	16	0.0000	1	0.003	15	0.0002
			0.5	2	39	78											
Positive control	1	2	0.5	1	4945	9890	9929	19858	0.1	183527	10.82	19692	0.0213	1	0.003	15	0.2527
			0.5	2	4984	9968											
	2	2	0.5	1	5027	10054	10073	20146	0.1	183527	10.98	19980	0.0216	1	0.003	15	0.2564
			0.5	2	5046	10092											
	3	2	0.5	1	4455	8910	9080	18160	0.1	183527	9.89	17994	0.0194	1	0.003	15	0.2309
			0.5	2	4625	9250											
	4	2	0.5	1	4550	9100	9075	18150	0.1	183527	9.89	17984	0.0194	1	0.003	15	0.2308
			0.5	2	4525	9050											
Negative Control	1	2	0.5	1	8655	17310	17165	34330	0.1	183527	18.71	34164	0.0369	1	0.003	15	0.4384
			0.5	2	8510	17020											
	2	2	0.5	1	7732	15464	15499	30998	0.1	183527	16.89	30832	0.0333	1	0.003	15	0.3956
			0.5	2	7767	15534											
	3	2	0.5	1	6441	12882	12981	25962	0.1	183527	14.15	25796	0.0279	1	0.003	15	0.3310
			0.5	2	6540	13080											
	4	2	0.5	1	8385	16770	16421	32842	0.1	183527	17.89	32676	0.0353	1	0.003	15	0.4193
			0.5	2	8036	16072											
Ref Chem 7	1-1	2	0.5	1	56	112	132	264	0.1	183527	0.14	98	0.0001	1	0.003	15	0.0013
			0.5	2	76	152											
	1-2	2	0.5	1	59	118	124	248	0.1	183527	0.14	82	0.0001	1	0.003	15	0.0011
			0.5	2	65	130											
	1-3	2	0.5	1	64	128	136	272	0.1	183527	0.15	106	0.0001	1	0.003	15	0.0014
			0.5	2	72	144											
	2-1	2	0.5	1	181	362	395	790	0.1	183527	0.43	624	0.0007	1	0.003	15	0.0080
			0.5	2	214	428											
	2-2	2	0.5	1	215	430	425	850	0.1	183527	0.46	684	0.0007	1	0.003	15	0.0088
			0.5	2	210	420											
	2-3	2	0.5	1	207	414	405	810	0.1	183527	0.44	644	0.0007	1	0.003	15	0.0083
			0.5	2	198	396											
	3-1	2	0.5	1	1320	2640	2557	5114	0.1	183527	2.79	4948	0.0053	1	0.003	15	0.0635
			0.5	2	1237	2474											
	3-2	2	0.5	1	1502	3004	2995	5990	0.1	183527	3.26	5824	0.0063	1	0.003	15	0.0747
			0.5	2	1493	2986											
	3-3	2	0.5	1	1496	2992	2953	5906	0.1	183527	3.22	5740	0.0062	1	0.003	15	0.0737
			0.5	2	1457	2914											
	4-1	2	0.5	1	2485	4970	4963	9926	0.1	183527	5.41	9760	0.0105	1	0.003	15	0.1252
			0.5	2	2478	4956											
	4-2	2	0.5	1	2551	5102	5136	10272	0.1	183527	5.60	10106	0.0109	1	0.003	15	0.1297
			0.5	2	2585	5170											
	4-3	2	0.5	1	2534	5068	5110	10220	0.1	183527	5.57	10054	0.0109	1	0.003	15	0.1290
			0.5	2	2576	5152											
	5-1	2	0.5	1	6289	12578	12538	25076	0.1	183527	13.66	24910	0.0269	1	0.003	15	0.3196
			0.5	2	6249	12498											
	5-2	2	0.5	1	5761	11522	11510	23020	0.1	183527	12.54	22854	0.0247	1	0.003	15	0.2933
			0.5	2	5749	11498											
	5-3	2	0.5	1	6242	12484	12442	24884	0.1	183527	13.56	24718	0.0267	1	0.003	15	0.3172
			0.5	2	6200	12400											
	6-1	2	0.5	1	6664	13328	13368	26736	0.1	183527	14.57	26570	0.0287	1	0.003	15	0.3409
			0.5	2	6704	13408											
	6-2	2	0.5	1	6595	13190	13233	26466	0.1	183527	14.42	26300	0.0284	1	0.003	15	0.3375
			0.5	2	6638	13276											
	6-3	2	0.5	1	6949	13898	13852	27704	0.1	183527	15.10	27538	0.0297	1	0.003	15	0.3534
			0.5	2	6903	13806											
	7-1	2	0.5	1	7811	15622	15546	31092	0.1	183527	16.94	30926	0.0334	1	0.003	15	0.3968
			0.5	2	7735	15470											
	7-2	2	0.5	1	7812	15624	15485	30930	0.1	183527	16.85	30764	0.0332	1	0.003	15	0.3948
			0.5	2	7653	15306											
	7-3	2	0.5	1	7859	15718	15313	30626	0.1	183527	16.69	30460	0.0329	1	0.003	15	0.3909
			0.5	2	7454	14908											
	8-1	2	0.5	1	7609	15218	15426	30852	0.1	183527	16.81	30686	0.0331	1	0.003	15	0.3938
			0.5	2	7817	15634											
	8-2	2	0.5	1	7362	14724	14719	29438	0.1	183527	16.04	29272	0.0316	1	0.003	15	0.3756
			0.5	2	7357	14714											
	8-3	2	0.5	1	7983	15966	15985	31970	0.1	183527	17.42	31804	0.0343	1	0.003	15	0.4081
			0.5	2	8002	16004											

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.4437	0.0170
Full activity	End	0.3863	0.0307
Full activity	Overall	0.4150	0.0388
Background	Beginning	-0.0003	0
Background	End	0.0003	7.25871E-05
Background	Overall	0.0000	0.000299284
Positive	Beginning	0.2545	0.0026
Positive	End	0.2308	0.0001
Positive	Overall	0.2427	0.0138
Negative	Beginning	0.4170	0.0302
Negative	End	0.3751	0.0624
Negative	Overall	0.3961	0.0468

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 7	1	1	1.00E-06	-6.00	0.0013
Ref Chem 7	1	2	1.00E-06	-6.00	0.0011
Ref Chem 7	1	3	1.00E-06	-6.00	0.0014
Ref Chem 7	2	1	1.00E-07	-7.00	0.0080
Ref Chem 7	2	2	1.00E-07	-7.00	0.0088
Ref Chem 7	2	3	1.00E-07	-7.00	0.0083
Ref Chem 7	3	1	1.00E-08	-8.00	0.0635
Ref Chem 7	3	2	1.00E-08	-8.00	0.0747
Ref Chem 7	3	3	1.00E-08	-8.00	0.0737
Ref Chem 7	4	1	5.00E-09	-8.30	0.1252
Ref Chem 7	4	2	5.00E-09	-8.30	0.1297
Ref Chem 7	4	3	5.00E-09	-8.30	0.1290
Ref Chem 7	5	1	1.00E-09	-9.00	0.3196
Ref Chem 7	5	2	1.00E-09	-9.00	0.2933
Ref Chem 7	5	3	1.00E-09	-9.00	0.3172
Ref Chem 7	6	1	5.00E-10	-9.30	0.3409
Ref Chem 7	6	2	5.00E-10	-9.30	0.3375
Ref Chem 7	6	3	5.00E-10	-9.30	0.3534
Ref Chem 7	7	1	2.50E-10	-9.60	0.3968
Ref Chem 7	7	2	2.50E-10	-9.60	0.3948
Ref Chem 7	7	3	2.50E-10	-9.60	0.3909
Ref Chem 7	8	1	1.00E-10	-10.00	0.3938
Ref Chem 7	8	2	1.00E-10	-10.00	0.3756
Ref Chem 7	8	3	1.00E-10	-10.00	0.4081

Percent of control values				
		Replicate		
Level	Log[test substance]	1	2	3
1	-6.00	0.30	0.25	0.33
2	-7.00	1.93	2.11	1.99
3	-8.00	15.30	18.01	17.75
4	-8.30	30.18	31.25	31.09
5	-9.00	77.02	70.66	76.43
6	-9.30	82.15	81.32	85.15
7	-9.60	95.62	95.12	94.18
8	-10.00	94.88	90.51	98.34

Aromatase Assay Spreadsheet

Assay Date	8/31/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8		
Technician ID	2	Replicate #	4	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	32096	1596816	
2	0.0198	32619	1647424	
3	0.0193	33433	1732280	
4	0.0196	33331	1700561	
5	0.0195	31665	1623846	
			Average DPM/g soln	1660185
			SD	55513
			CV	3.34
			µCi/g soln	0.748

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	8.1336 g
Mass of dilution B used in substrate prep	4.5875 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.575299 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00847 µg/g soln.
µg/g soln.

a. µCi/g soln 0.748
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.

= 0.575299 + 0.00847
= 0.583764 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.281 µCi/µg ASDN

814502 dpm/nmol

Assay Date	8/31/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8						
Technician ID	2	Replicate #	4	Microsome type	Recombinant	Microsome ID	11345-10				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.485	0.312	0.226	0.173	0.141	0.126	0.106	25.3	100	11540-49
	0.483	0.328	0.229	0.182	0.131	0.124	0.108			
	0.491	0.323	0.219	0.172	0.137	0.120	0.112			

Samples:	11540-6A	11540-6B	11345-21I
	0.325	0.142	0.133
	0.326	0.136	0.135
			0.137

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	0.0506	0.486	0.378	0.0492
125	100	200	0.00013	200	0.0253	0.321	0.212	0.0263
50	200	1000	0.00005	200	0.0101	0.225	0.116	0.0129
25	100	1000	0.00003	200	0.0051	0.176	0.067	0.0061
10	40	1000	0.00001	200	0.0020	0.136	0.028	0.0007
5	20	1000	0.00001	200	0.0010	0.123	0.015	-0.0011
			Blank	0.109				

Variables	Regression results	
m, b	0.139	-0.003
se_m, se_b	0.007	0.001
r^2, se_y	0.990	0.002
F, df	402	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.990
m= 0.139
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	0.325	0.216	0.027	200	1	1	0.134	0.134	0.11
11540-6A	0.326	0.217	0.027	200	1	1	0.135		
11540-6A									
11540-6B	0.142	0.033	0.001	200	1	1	0.007	0.005	0.012
11540-6B	0.136	0.027	0.001	200	1	1	0.003		
11540-6B									
					1	800			
					1	800			
					1	800			
11345-21I	0.133	0.024	0.000	200	1	600	0.7	1.5	
11345-21I	0.135	0.026	0.001	200	1	600	1.5		
11345-21I	0.137	0.028	0.001	200	1	600	2.4		

Assay Date	8/31/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8						
Technician ID	2	Replicate #	4	Microsome type	Recombinant	Microsome ID	11345-10				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.3	100	11540-49
	0.485	0.312	0.226	0.173	0.141	0.126	0.106			
	0.483	0.328	0.229	0.182	0.131	0.124	0.108			
	0.491	0.323	0.219	0.172	0.137	0.120	0.112			

Samples:	11540-6A	11540-6B	11345-21I	
	0.325	0.142	0.133	
	0.326	0.136	0.135	
			0.137	

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	0.0506	0.486	0.378	0.0442
125	100	200	0.00013	200	0.0253	0.321	0.212	0.0240
50	200	1000	0.00005	200	0.0101	0.225	0.116	0.0122
25	100	1000	0.00003	200	0.0051	0.176	0.067	0.0062
10	40	1000	0.00001	200	0.0020	0.136	0.028	0.0014
5	20	1000	0.00001	200	0.0010	0.123	0.015	-0.0002
			Blank	0.109				

Variables	Regression results	
m, b	0.122	-0.002
se_m, se_b	0.011	0.001
r^2, se_y	0.977	0.002
F, df	125	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

			r^2=	0.977
			m=	0.122
			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	0.325	0.216	0.024	200	1	1	0.122	0.123	0.11
11540-6A	0.326	0.217	0.025	200	1	1	0.123		
11540-6A									
11540-6B	0.142	0.033	0.002	200	1	1	0.010	0.008	0.012
11540-6B	0.136	0.027	0.001	200	1	1	0.007		
11540-6B									
					1	800			
					1	800			
					1	800			
11345-21I	0.133	0.024	0.001	200	1	600	2.9	3.6	
11345-21I	0.135	0.026	0.001	200	1	600	3.6		
11345-21I	0.137	0.028	0.001	200	1	600	4.4		

Assay Date	8/31/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	4

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.6
Protein Concentration (dilution added to assay, mg/mL):	0.00605

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	8/31/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	11345-10	Technician ID	2	Replicate #	4

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 (Inibal)	% 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	7473	14946	14862	29724	0.1	166019	17.90	29632	0.0364	1	0.003	15	0.4009
			0.5	2	7389	14778											
	2	2	0.5	1	8772	17544	17584	35168	0.1	166019	21.18	35076	0.0431	1	0.003	15	0.4745
			0.5	2	8812	17624											
	3	2	0.5	1	6632	13264	13137	26274	0.1	166019	15.83	26182	0.0321	1	0.003	15	0.3542
			0.5	2	6505	13010											
	4	2	0.5	1	6199	12398	12277	24554	0.1	166019	14.79	24462	0.0300	1	0.003	15	0.3309
			0.5	2	6078	12156											
Background control	1	2	0.5	1	21	42	38	76	0.1	166019	0.05	-17	0.0000	1	0.003	15	-0.0002
			0.5	2	17	34											
	2	2	0.5	1	28	56	50	100	0.1	166019	0.06	8	0.0000	1	0.003	15	0.0001
			0.5	2	22	44											
	3	2	0.5	1	17	34	38	76	0.1	166019	0.05	-17	0.0000	1	0.003	15	-0.0002
			0.5	2	21	42											
	4	2	0.5	1	34	68	59	118	0.1	166019	0.07	26	0.0000	1	0.003	15	0.0003
			0.5	2	25	50											
Positive control	1	2	0.5	1	4882	9764	9686	19372	0.1	166019	11.67	19280	0.0237	1	0.003	15	0.2608
			0.5	2	4804	9608											
	2	2	0.5	1	4250	8500	8500	17000	0.1	166019	10.24	16908	0.0208	1	0.003	15	0.2287
			0.5	2	4250	8500											
	3	2	0.5	1	4232	8464	8547	17094	0.1	166019	10.30	17002	0.0209	1	0.003	15	0.2300
			0.5	2	4315	8630											
	4	2	0.5	1	4156	8312	8319	16638	0.1	166019	10.02	16546	0.0203	1	0.003	15	0.2238
			0.5	2	4163	8326											
Negative Control	1	2	0.5	1	7886	15772	15718	31436	0.1	166019	18.94	31344	0.0385	1	0.003	15	0.4240
			0.5	2	7832	15664											
	2	2	0.5	1	7438	14876	14894	29788	0.1	166019	17.94	29696	0.0365	1	0.003	15	0.4017
			0.5	2	7456	14912											
	3	2	0.5	1	6287	12574	13442	26884	0.1	166019	16.19	26792	0.0329	1	0.003	15	0.3624
			0.5	2	7155	14310											
	4	2	0.5	1	8441	16882	16798	33596	0.1	166019	20.24	33504	0.0411	1	0.003	15	0.4533
			0.5	2	8357	16714											
Ref Chem 7	1-1	2	0.5	1	36	72	73	146	0.1	166019	0.09	54	0.0001	1	0.003	15	0.0007
			0.5	2	37	74											
	1-2	2	0.5	1	35	70	82	164	0.1	166019	0.10	72	0.0001	1	0.003	15	0.0010
			0.5	2	47	94											
	1-3	2	0.5	1	39	78	81	162	0.1	166019	0.10	70	0.0001	1	0.003	15	0.0009
			0.5	2	42	84											
	2-1	2	0.5	1	185	370	352	704	0.1	166019	0.42	612	0.0008	1	0.003	15	0.0083
			0.5	2	167	334											
	2-2	2	0.5	1	177	354	365	730	0.1	166019	0.44	638	0.0008	1	0.003	15	0.0086
			0.5	2	188	376											
	2-3	2	0.5	1	208	416	403	806	0.1	166019	0.49	714	0.0009	1	0.003	15	0.0097
			0.5	2	195	390											
	3-1	2	0.5	1	1292	2584	2604	5208	0.1	166019	3.14	5116	0.0063	1	0.003	15	0.0692
			0.5	2	1312	2624											
	3-2	2	0.5	1	1456	2912	2919	5838	0.1	166019	3.52	5746	0.0071	1	0.003	15	0.0777
			0.5	2	1463	2926											
	3-3	2	0.5	1	1452	2904	2876	5752	0.1	166019	3.46	5660	0.0069	1	0.003	15	0.0766
			0.5	2	1424	2848											
	4-1	2	0.5	1	2361	4722	4662	9324	0.1	166019	5.62	9232	0.0113	1	0.003	15	0.1249
			0.5	2	2301	4602											
	4-2	2	0.5	1	2723	5446	5461	10922	0.1	166019	6.58	10830	0.0133	1	0.003	15	0.1465
			0.5	2	2738	5476											
	4-3	2	0.5	1	2362	4724	4780	9560	0.1	166019	5.76	9468	0.0116	1	0.003	15	0.1281
			0.5	2	2418	4836											
	5-1	2	0.5	1	5213	10426	10286	20572	0.1	166019	12.39	20480	0.0251	1	0.003	15	0.2771
			0.5	2	5073	10146											
	5-2	2	0.5	1	5563	11126	11057	22114	0.1	166019	13.32	22022	0.0270	1	0.003	15	0.2979
			0.5	2	5494	10988											
	5-3	2	0.5	1	4789	9578	9567	19134	0.1	166019	11.53	19042	0.0234	1	0.003	15	0.2576
			0.5	2	4778	9556											
	6-1	2	0.5	1	6625	13250	12983	25966	0.1	166019	15.64	25874	0.0318	1	0.003	15	0.3500
			0.5	2	6358	12716											
	6-2	2	0.5	1	6650	13300	12357	24714	0.1	166019	14.89	24622	0.0302	1	0.003	15	0.3331
			0.5	2	5707	11414											
	6-3	2	0.5	1	7233	14466	13939	27878	0.1	166019	16.79	27786	0.0341	1	0.003	15	0.3759
			0.5	2	6706	13412											
	7-1	2	0.5	1	7175	14350	14345	28690	0.1	166019	17.28	28598	0.0351	1	0.003	15	0.3869
			0.5	2	7170	14340											
	7-2	2	0.5	1	6682	13364	13597	27194	0.1	166019	16.38	27102	0.0333	1	0.003	15	0.3666
			0.5	2	6915	13830											
	7-3	2	0.5	1	6630	13260	13230	26460	0.1	166019	15.94	26368	0.0324	1	0.003	15	0.3567
			0.5	2	6600	13200											
	8-1	2	0.5	1	6949	13898	14230	28460	0.1	166019	17.14	28368	0.0348	1	0.003	15	0.3838
			0.5	2	7281	14562											
	8-2	2	0.5	1	6388	12776	12836	25672	0.1	166019	15.46	25580	0.0314	1	0.003	15	0.3461
			0.5	2	6448	12896											
	8-3	2	0.5	1	7985	15970	15876	31752	0.1	166019	19.13	31660	0.0389	1	0.003	15	0.4283
			0.5	2	7891	15782											

Assay Date	8/31/2005	Test Chemical ID	Ref Chem 7	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	4

Control Type	Portion	Average	SD
Full activity	Beginning	0.4377	0.0521
Full activity	End	0.3426	0.0165
Full activity	Overall	0.3901	0.0633
Background	Beginning	-0.0001	0.000229587
Background	End	0.0001	0.000401777
Background	Overall	0.0000	0.000276261
Positive	Beginning	0.2448	0.0227
Positive	End	0.2269	0.0044
Positive	Overall	0.2358	0.0169
Negative	Beginning	0.4129	0.0158
Negative	End	0.4079	0.0642
Negative	Overall	0.4104	0.0383

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 7	1	1	1.00E-06	-6.00	0.0007
Ref Chem 7	1	2	1.00E-06	-6.00	0.0010
Ref Chem 7	1	3	1.00E-06	-6.00	0.0009
Ref Chem 7	2	1	1.00E-07	-7.00	0.0083
Ref Chem 7	2	2	1.00E-07	-7.00	0.0086
Ref Chem 7	2	3	1.00E-07	-7.00	0.0097
Ref Chem 7	3	1	1.00E-08	-8.00	0.0692
Ref Chem 7	3	2	1.00E-08	-8.00	0.0777
Ref Chem 7	3	3	1.00E-08	-8.00	0.0766
Ref Chem 7	4	1	5.00E-09	-8.30	0.1249
Ref Chem 7	4	2	5.00E-09	-8.30	0.1465
Ref Chem 7	4	3	5.00E-09	-8.30	0.1281
Ref Chem 7	5	1	1.00E-09	-9.00	0.2771
Ref Chem 7	5	2	1.00E-09	-9.00	0.2979
Ref Chem 7	5	3	1.00E-09	-9.00	0.2576
Ref Chem 7	6	1	5.00E-10	-9.30	0.3500
Ref Chem 7	6	2	5.00E-10	-9.30	0.3331
Ref Chem 7	6	3	5.00E-10	-9.30	0.3759
Ref Chem 7	7	1	2.50E-10	-9.60	0.3869
Ref Chem 7	7	2	2.50E-10	-9.60	0.3666
Ref Chem 7	7	3	2.50E-10	-9.60	0.3567
Ref Chem 7	8	1	1.00E-10	-10.00	0.3838
Ref Chem 7	8	2	1.00E-10	-10.00	0.3461
Ref Chem 7	8	3	1.00E-10	-10.00	0.4283

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-6.00	0.19	0.25	0.24
2	-7.00	2.12	2.21	2.47
3	-8.00	17.74	19.92	19.63
4	-8.30	32.01	37.55	32.83
5	-9.00	71.02	76.36	66.03
6	-9.30	89.72	85.38	96.35
7	-9.60	99.17	93.98	91.43
8	-10.00	98.37	88.70	109.79

Aromatase Assay Spreadsheet

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8		
Technician ID	2	Replicate #	2	Microsome type	Recombina	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	31847	1600352	
2	0.0197	30126	1529239	
3	0.0201	34596	1721194	
4	0.0199	33372	1676985	
5	0.0202	35146	1739901	
			Average DPM/g soln	1653534
			SD	87842
			CV	5.31
			µCi/g soln	0.745

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	8.0194 g
Mass of dilution B used in substrate prep	4.4702 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.574146 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00843 µg/g soln.
µg/g soln.

a. µCi/g soln 0.745
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.

= 0.574146 + 0.00843
= 0.582578 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.279 µCi/µg ASDN

812891 dpm/nmol

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 8		# Concentrations tested	8					
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.559	0.399	0.239	0.176	0.130	0.127	0.108	25.1	100	11540-47
	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	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10			

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.559	0.399	0.239	0.176	0.130	0.127	0.108	25.1	100	11540-47
	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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.3
Protein Concentration (dilution added to assay, mg/mL):	0.010495

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	10058	20116	19661	39322	0.1	165353	23.78	39172	0.0482	1	0.005	15	0.3061
			0.5	2	9603	19206											
	2	2	0.5	1	10716	21432	21073	42146	0.1	165353	25.49	41996	0.0517	1	0.005	15	0.3282
			0.5	2	10357	20714											
	3	2	0.5	1	8315	16630	16533	33066	0.1	165353	20.00	32916	0.0405	1	0.005	15	0.2572
			0.5	2	8218	16436											
	4	2	0.5	1	7641	15282	15226	30452	0.1	165353	18.42	30302	0.0373	1	0.005	15	0.2368
			0.5	2	7585	15170											
Background control	1	2	0.5	1	38	76	70	140	0.1	165353	0.08	-10	0.0000	1	0.005	15	-0.0001
			0.5	2	32	64											
	2	2	0.5	1	37	74	69	138	0.1	165353	0.08	-12	0.0000	1	0.005	15	-0.0001
			0.5	2	32	64											
	3	2	0.5	1	38	76	77	154	0.1	165353	0.09	4	0.0000	1	0.005	15	0.0000
			0.5	2	39	78											
	4	2	0.5	1	40	80	84	168	0.1	165353	0.10	18	0.0000	1	0.005	15	0.0001
			0.5	2	44	88											
Positive control	1	2	0.5	1	5216	10432	10248	20496	0.1	165353	12.40	20346	0.0250	1	0.005	15	0.1590
			0.5	2	5032	10064											
	2	2	0.5	1	5153	10306	10276	20552	0.1	165353	12.43	20402	0.0251	1	0.005	15	0.1594
			0.5	2	5123	10246											
	3	2	0.5	1	4619	9238	9140	18280	0.1	165353	11.06	18130	0.0223	1	0.005	15	0.1417
			0.5	2	4521	9042											
	4	2	0.5	1	5023	10046	9938	19876	0.1	165353	12.02	19726	0.0243	1	0.005	15	0.1541
			0.5	2	4915	9830											
Negative Control	1	2	0.5	1	9240	18480	18342	36684	0.1	165353	22.19	36534	0.0449	1	0.005	15	0.2855
			0.5	2	9102	18204											
	2	2	0.5	1	9098	18196	18194	36388	0.1	165353	22.01	36238	0.0446	1	0.005	15	0.2832
			0.5	2	9096	18192											
	3	2	0.5	1	8275	16550	16634	33268	0.1	165353	20.12	33118	0.0407	1	0.005	15	0.2588
			0.5	2	8359	16718											
	4	2	0.5	1	8964	17928	17998	35996	0.1	165353	21.77	35846	0.0441	1	0.005	15	0.2801
			0.5	2	9034	18068											
Ref Chem 8	1-1	2	0.5	1	2326	4652	4632	9264	0.1	165353	5.60	9114	0.0112	1	0.005	15	0.0712
			0.5	2	2306	4612											
	1-2	2	0.5	1	1924	3848	3865	7730	0.1	165353	4.67	7580	0.0093	1	0.005	15	0.0592
			0.5	2	1941	3882											
	1-3	2	0.5	1	2095	4190	4273	8546	0.1	165353	5.17	8396	0.0103	1	0.005	15	0.0656
			0.5	2	2178	4356											
	2-1	2	0.5	1	2625	5250	5223	10446	0.1	165353	6.32	10296	0.0127	1	0.005	15	0.0805
			0.5	2	2598	5196											
	2-2	2	0.5	1	2411	4822	4888	9776	0.1	165353	5.91	9626	0.0118	1	0.005	15	0.0752
			0.5	2	2477	4954											
	2-3	2	0.5	1	2355	4710	4627	9254	0.1	165353	5.60	9104	0.0112	1	0.005	15	0.0711
			0.5	2	2272	4544											
	3-1	2	0.5	1	3761	7522	7513	15026	0.1	165353	9.09	14876	0.0183	1	0.005	15	0.1162
			0.5	2	3752	7504											
	3-2	2	0.5	1	3413	6826	6901	13802	0.1	165353	8.35	13652	0.0168	1	0.005	15	0.1067
			0.5	2	3488	6976											
	3-3	2	0.5	1	3821	7642	7576	15152	0.1	165353	9.16	15002	0.0185	1	0.005	15	0.1172
			0.5	2	3755	7510											
	4-1	2	0.5	1	4739	9478	9520	19040	0.1	165353	11.51	18890	0.0232	1	0.005	15	0.1476
			0.5	2	4781	9562											
	4-2	2	0.5	1	5293	10586	10389	20778	0.1	165353	12.57	20628	0.0254	1	0.005	15	0.1612
			0.5	2	5096	10192											
	4-3	2	0.5	1	5393	10786	10697	21394	0.1	165353	12.94	21244	0.0261	1	0.005	15	0.1660
			0.5	2	5304	10608											
	5-1	2	0.5	1	5725	11450	11533	23066	0.1	165353	13.95	22916	0.0282	1	0.005	15	0.1791
			0.5	2	5808	11616											
	5-2	2	0.5	1	6553	13106	13192	26384	0.1	165353	15.96	26234	0.0323	1	0.005	15	0.2050
			0.5	2	6638	13278											
	5-3	2	0.5	1	6681	13362	13319	26638	0.1	165353	16.11	26488	0.0326	1	0.005	15	0.2070
			0.5	2	6638	13276											
	6-1	2	0.5	1	7801	15602	15283	30566	0.1	165353	18.49	30416	0.0374	1	0.005	15	0.2377
			0.5	2	7482	14964											
	6-2	2	0.5	1	7905	15810	15643	31286	0.1	165353	18.92	31136	0.0383	1	0.005	15	0.2433
			0.5	2	7738	15476											
	6-3	2	0.5	1	7141	14282	14237	28474	0.1	165353	17.22	28324	0.0348	1	0.005	15	0.2213
			0.5	2	7096	14192											
	7-1	2	0.5	1	8359	16718	16770	33540	0.1	165353	20.28	33390	0.0411	1	0.005	15	0.2609
			0.5	2	8411	16822											
	7-2	2	0.5	1	8115	16230	16147	32294	0.1	165353	19.53	32144	0.0395	1	0.005	15	0.2512
			0.5	2	8032	16064											
	7-3	2	0.5	1	7674	15348	15246	30492	0.1	165353	18.44	30342	0.0373	1	0.005	15	0.2371
			0.5	2	7572	15144											
	8-1	2	0.5	1	8053	16106	16164	32328	0.1	165353	19.55	32178	0.0396	1	0.005	15	0.2514
			0.5	2	8111	16222											
	8-2	2	0.5	1	8962	17924	17973	35946	0.1	165353	21.74	35796	0.0440	1	0.005	15	0.2797
			0.5	2	9011	18022											
	8-3	2	0.5	1	7839	15678	15603	31206	0.1	165353	18.87	31056	0.0382	1	0.005	15	0.2427
			0.5	2	7764	15528											

Assay Date	8/24/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested		Microsome 8 type	Recombinant	Microsome ID	11345-10	Technician ID	Replicate # 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3171	0.0156
Full activity	End	0.2470	0.0144
Full activity	Overall	0.2821	0.0423
Background	Beginning	-0.0001	1.10508E-05
Background	End	0.0001	7.73556E-05
Background	Overall	0.0000	0.000109025
Positive	Beginning	0.1592	0.0003
Positive	End	0.1479	0.0088
Positive	Overall	0.1536	0.0083
Negative	Beginning	0.2843	0.0016
Negative	End	0.2694	0.0151
Negative	Overall	0.2769	0.0123

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 8	1	1	1.00E-04	-4.00	0.0712
Ref Chem 8	1	2	1.00E-04	-4.00	0.0592
Ref Chem 8	1	3	1.00E-04	-4.00	0.0656
Ref Chem 8	2	1	1.00E-05	-5.00	0.0805
Ref Chem 8	2	2	1.00E-05	-5.00	0.0752
Ref Chem 8	2	3	1.00E-05	-5.00	0.0711
Ref Chem 8	3	1	5.00E-06	-5.30	0.1162
Ref Chem 8	3	2	5.00E-06	-5.30	0.1067
Ref Chem 8	3	3	5.00E-06	-5.30	0.1172
Ref Chem 8	4	1	2.50E-06	-5.60	0.1476
Ref Chem 8	4	2	2.50E-06	-5.60	0.1612
Ref Chem 8	4	3	2.50E-06	-5.60	0.1660
Ref Chem 8	5	1	1.00E-06	-6.00	0.1791
Ref Chem 8	5	2	1.00E-06	-6.00	0.2050
Ref Chem 8	5	3	1.00E-06	-6.00	0.2070
Ref Chem 8	6	1	5.00E-07	-6.30	0.2377
Ref Chem 8	6	2	5.00E-07	-6.30	0.2433
Ref Chem 8	6	3	5.00E-07	-6.30	0.2213
Ref Chem 8	7	1	1.00E-07	-7.00	0.2609
Ref Chem 8	7	2	1.00E-07	-7.00	0.2512
Ref Chem 8	7	3	1.00E-07	-7.00	0.2371
Ref Chem 8	8	1	1.00E-08	-8.00	0.2514
Ref Chem 8	8	2	1.00E-08	-8.00	0.2797
Ref Chem 8	8	3	1.00E-08	-8.00	0.2427

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	25.25	21.00	23.26
2	-5.00	28.52	26.67	25.22
3	-5.30	41.21	37.82	41.56
4	-5.60	52.33	57.15	58.85
5	-6.00	63.49	72.68	73.38
6	-6.30	84.26	86.26	78.47
7	-7.00	92.50	89.05	84.06
8	-8.00	89.14	99.17	86.04

Aromatase Assay Spreadsheet

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8
Technician ID	2	Replicate #	3	Microsome type	Recombina Microsome ID 11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0192	32589	1697344	
2	0.0206	37715	1830825	
3	0.0199	37630	1890955	
4	0.0198	36206	1828586	
5	0.0199	38380	1928643	
			Average DPM/g soln	1835271
			SD	87903
			CV	4.79
			µCi/g soln	0.827

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	8.0232 g
Mass of dilution B used in substrate prep	4.4781 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.558144 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00936 µg/g soln.
µg/g soln.

a. µCi/g soln 0.827
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.

= 0.558144 + 0.00936
= 0.567502 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.457 µCi/µg ASDN

926202 dpm/nmol

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8	
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID 11345-10

								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 8	# Concentrations tested	8					
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10			

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 8	# Concentrations tested	8	Microsome type	Recombinz	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.4
Protein Concentration (dilution added to assay, mg/mL):	0.005609

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	9058	18116	17839	35678	0.1	183527	19.44	35512	0.0383	1	0.003	15	0.4557
			0.5	2	8781	17562											
	2	2	0.5	1	8469	16938	16902	33804	0.1	183527	18.42	33638	0.0363	1	0.003	15	0.4316
			0.5	2	8433	16866											
	3	2	0.5	1	8051	16102	15984	31968	0.1	183527	17.42	31802	0.0343	1	0.003	15	0.4081
			0.5	2	7933	15866											
	4	2	0.5	1	7201	14402	14290	28580	0.1	183527	15.57	28414	0.0307	1	0.003	15	0.3646
			0.5	2	7089	14178											
Background control	1	2	0.5	1	37	74	73	146	0.1	183527	0.08	-20	0.0000	1	0.003	15	-0.0003
			0.5	2	36	72											
	2	2	0.5	1	36	72	73	146	0.1	183527	0.08	-20	0.0000	1	0.003	15	-0.0003
			0.5	2	37	74											
	3	2	0.5	1	39	78	95	190	0.1	183527	0.10	24	0.0000	1	0.003	15	0.0003
			0.5	2	56	112											
	4	2	0.5	1	52	104	91	182	0.1	183527	0.10	16	0.0000	1	0.003	15	0.0002
			0.5	2	39	78											
Positive control	1	2	0.5	1	4945	9890	9929	19858	0.1	183527	10.82	19692	0.0213	1	0.003	15	0.2527
			0.5	2	4984	9968											
	2	2	0.5	1	5027	10054	10073	20146	0.1	183527	10.98	19980	0.0216	1	0.003	15	0.2564
			0.5	2	5046	10092											
	3	2	0.5	1	4455	8910	9080	18160	0.1	183527	9.89	17994	0.0194	1	0.003	15	0.2309
			0.5	2	4625	9250											
	4	2	0.5	1	4550	9100	9075	18150	0.1	183527	9.89	17984	0.0194	1	0.003	15	0.2308
			0.5	2	4525	9050											
Negative Control	1	2	0.5	1	8655	17310	17165	34330	0.1	183527	18.71	34164	0.0369	1	0.003	15	0.4384
			0.5	2	8510	17020											
	2	2	0.5	1	7732	15464	15499	30998	0.1	183527	16.89	30832	0.0333	1	0.003	15	0.3956
			0.5	2	7767	15534											
	3	2	0.5	1	6441	12882	12981	25962	0.1	183527	14.15	25796	0.0279	1	0.003	15	0.3310
			0.5	2	6540	13080											
	4	2	0.5	1	8385	16770	16421	32842	0.1	183527	17.89	32676	0.0353	1	0.003	15	0.4193
			0.5	2	8036	16072											
Ref Chem 8	1-1	2	0.5	1	2071	4142	4122	8244	0.1	183527	4.49	8078	0.0087	1	0.003	15	0.1037
			0.5	2	2051	4102											
	1-2	2	0.5	1	1995	3990	4017	8034	0.1	183527	4.38	7868	0.0085	1	0.003	15	0.1010
			0.5	2	2022	4044											
	1-3	2	0.5	1	1920	3840	3732	7464	0.1	183527	4.07	7298	0.0079	1	0.003	15	0.0936
			0.5	2	1812	3624											
	2-1	2	0.5	1	1934	3868	3918	7836	0.1	183527	4.27	7670	0.0083	1	0.003	15	0.0984
			0.5	2	1984	3968											
	2-2	2	0.5	1	2154	4308	4288	8576	0.1	183527	4.67	8410	0.0091	1	0.003	15	0.1079
			0.5	2	2134	4268											
	2-3	2	0.5	1	2026	4052	4124	8248	0.1	183527	4.49	8082	0.0087	1	0.003	15	0.1037
			0.5	2	2098	4196											
	3-1	2	0.5	1	3008	6016	5855	11710	0.1	183527	6.38	11544	0.0125	1	0.003	15	0.1481
			0.5	2	2847	5694											
	3-2	2	0.5	1	3153	6306	6195	12390	0.1	183527	6.75	12224	0.0132	1	0.003	15	0.1569
			0.5	2	3042	6084											
	3-3	2	0.5	1	2995	5990	5982	11964	0.1	183527	6.52	11798	0.0127	1	0.003	15	0.1514
			0.5	2	2987	5974											
	4-1	2	0.5	1	4035	8070	8111	16222	0.1	183527	8.84	16056	0.0173	1	0.003	15	0.2060
			0.5	2	4076	8152											
	4-2	2	0.5	1	4625	9250	9186	18372	0.1	183527	10.01	18206	0.0197	1	0.003	15	0.2336
			0.5	2	4561	9122											
	4-3	2	0.5	1	4282	8564	8610	17220	0.1	183527	9.38	17054	0.0184	1	0.003	15	0.2188
			0.5	2	4328	8656											
	5-1	2	0.5	1	5787	11574	11492	22984	0.1	183527	12.52	22818	0.0246	1	0.003	15	0.2928
			0.5	2	5705	11410											
	5-2	2	0.5	1	6072	12144	12159	24318	0.1	183527	13.25	24152	0.0261	1	0.003	15	0.3099
			0.5	2	6087	12174											
	5-3	2	0.5	1	5288	10576	10812	21624	0.1	183527	11.78	21458	0.0232	1	0.003	15	0.2753
			0.5	2	5524	11048											
	6-1	2	0.5	1	7010	14020	14085	28170	0.1	183527	15.35	28004	0.0302	1	0.003	15	0.3593
			0.5	2	7075	14150											
	6-2	2	0.5	1	6015	12030	12029	24058	0.1	183527	13.11	23892	0.0258	1	0.003	15	0.3066
			0.5	2	6014	12028											
	6-3	2	0.5	1	6265	12530	12469	24938	0.1	183527	13.59	24772	0.0267	1	0.003	15	0.3179
			0.5	2	6204	12408											
	7-1	2	0.5	1	8178	16356	16214	32428	0.1	183527	17.67	32262	0.0348	1	0.003	15	0.4140
			0.5	2	8036	16072											
	7-2	2	0.5	1	7532	15064	15051	30102	0.1	183527	16.40	29936	0.0323	1	0.003	15	0.3841
			0.5	2	7519	15038											
	7-3	2	0.5	1	7695	15390	15307	30614	0.1	183527	16.68	30448	0.0329	1	0.003	15	0.3907
			0.5	2	7612	15224											
	8-1	2	0.5	1	7429	14858	14770	29540	0.1	183527	16.10	29374	0.0317	1	0.003	15	0.3769
			0.5	2	7341	14682											
	8-2	2	0.5	1	7129	14258	14193	28386	0.1	183527	15.47	28220	0.0305	1	0.003	15	0.3621
			0.5	2	7064	14128											
	8-3	2	0.5	1	7692	15384	15050	30100	0.1	183527	16.40	29934	0.0323	1	0.003	15	0.3841
			0.5	2	7358	14716											

Assay Date	8/26/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested		Microsome 8 type	Recombinant	Microsome ID 11345-10	Technician ID	Replicate #	2	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.4437	0.0170
Full activity	End	0.3863	0.0307
Full activity	Overall	0.4150	0.0388
Background	Beginning	-0.0003	0
Background	End	0.0003	7.25871E-05
Background	Overall	0.0000	0.000299284
Positive	Beginning	0.2545	0.0026
Positive	End	0.2308	0.0001
Positive	Overall	0.2427	0.0138
Negative	Beginning	0.4170	0.0302
Negative	End	0.3751	0.0624
Negative	Overall	0.3961	0.0468

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 8	1	1	1.00E-04	-4.00	0.1037
Ref Chem 8	1	2	1.00E-04	-4.00	0.1010
Ref Chem 8	1	3	1.00E-04	-4.00	0.0936
Ref Chem 8	2	1	1.00E-05	-5.00	0.0984
Ref Chem 8	2	2	1.00E-05	-5.00	0.1079
Ref Chem 8	2	3	1.00E-05	-5.00	0.1037
Ref Chem 8	3	1	5.00E-06	-5.30	0.1481
Ref Chem 8	3	2	5.00E-06	-5.30	0.1569
Ref Chem 8	3	3	5.00E-06	-5.30	0.1514
Ref Chem 8	4	1	2.50E-06	-5.60	0.2060
Ref Chem 8	4	2	2.50E-06	-5.60	0.2336
Ref Chem 8	4	3	2.50E-06	-5.60	0.2188
Ref Chem 8	5	1	1.00E-06	-6.00	0.2928
Ref Chem 8	5	2	1.00E-06	-6.00	0.3099
Ref Chem 8	5	3	1.00E-06	-6.00	0.2753
Ref Chem 8	6	1	5.00E-07	-6.30	0.3593
Ref Chem 8	6	2	5.00E-07	-6.30	0.3066
Ref Chem 8	6	3	5.00E-07	-6.30	0.3179
Ref Chem 8	7	1	1.00E-07	-7.00	0.4140
Ref Chem 8	7	2	1.00E-07	-7.00	0.3841
Ref Chem 8	7	3	1.00E-07	-7.00	0.3907
Ref Chem 8	8	1	1.00E-08	-8.00	0.3769
Ref Chem 8	8	2	1.00E-08	-8.00	0.3621
Ref Chem 8	8	3	1.00E-08	-8.00	0.3841

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	24.98	24.33	22.57
2	-5.00	23.72	26.00	24.99
3	-5.30	35.69	37.80	36.48
4	-5.60	49.65	56.29	52.73
5	-6.00	70.55	74.68	66.35
6	-6.30	86.59	73.87	76.60
7	-7.00	99.75	92.56	94.15
8	-8.00	90.82	87.26	92.56

Aromatase Assay Spreadsheet

Assay Date	8/31/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8		
Technician ID	2	Replicate #	4	Microsome type	Recombina	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	32096	1596816	
2	0.0198	32619	1647424	
3	0.0193	33433	1732280	
4	0.0196	33331	1700561	
5	0.0195	31665	1623846	
			Average DPM/g soln	1660185
			SD	55513
			CV	3.34
			µCi/g soln	0.748

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	8.1336 g
Mass of dilution B used in substrate prep	4.5875 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.575299 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00847 µg/g soln.
µg/g soln.

a. µCi/g soln 0.748
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.

= 0.575299 + 0.00847
= 0.583764 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.281 µCi/µg ASDN

814502 dpm/nmol

Assay Date	8/31/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8						
Technician ID	2	Replicate #	4	Microsome type	Recombinant	Microsome ID	11345-10				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.3	100	11540-49
	0.485	0.312	0.226	0.173	0.141	0.126	0.106			
	0.483	0.328	0.229	0.182	0.131	0.124	0.108			
	0.491	0.323	0.219	0.172	0.137	0.120	0.112			

Samples:	11540-6A	11540-6B	11345-21I
	0.325	0.142	0.133
	0.326	0.136	0.135
			0.137

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	0.0506	0.486	0.378	0.0492
125	100	200	0.00013	200	0.0253	0.321	0.212	0.0263
50	200	1000	0.00005	200	0.0101	0.225	0.116	0.0129
25	100	1000	0.00003	200	0.0051	0.176	0.067	0.0061
10	40	1000	0.00001	200	0.0020	0.136	0.028	0.0007
5	20	1000	0.00001	200	0.0010	0.123	0.015	-0.0011

Variables	Regression results	
m, b	0.139	-0.003
se_m, se_b	0.007	0.001
r^2, se_y	0.990	0.002
F, df	402	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank	0.109	r^2=	0.990
		m=	0.139
		b=	-0.003

	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	0.325	0.216	0.027	200	1	1	0.134	0.134	0.11
11540-6A	0.326	0.217	0.027	200	1	1	0.135		
11540-6A									
11540-6B	0.142	0.033	0.001	200	1	1	0.007	0.005	0.012
11540-6B	0.136	0.027	0.001	200	1	1	0.003		
11540-6B									
					1	800			
					1	800			
					1	800			
11345-21I	0.133	0.024	0.000	200	1	600	0.7	1.5	
11345-21I	0.135	0.026	0.001	200	1	600	1.5		
11345-21I	0.137	0.028	0.001	200	1	600	2.4		

Assay Date	8/31/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8						
Technician ID	2	Replicate #	4	Microsome type	Recombinant	Microsome ID	11345-10				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.3	100	11540-49
	0.485	0.312	0.226	0.173	0.141	0.126	0.106			
	0.483	0.328	0.229	0.182	0.131	0.124	0.108			
	0.491	0.323	0.219	0.172	0.137	0.120	0.112			
Samples:	11540-6A	11540-6B		11345-21I						
	0.325	0.142		0.133						
	0.326	0.136		0.135						
				0.137						

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	0.0506	0.486	0.378	0.0442	m, b	0.122	-0.002
125	100	200	0.00013	200	0.0253	0.321	0.212	0.0240	se_m, se_b	0.011	0.001
50	200	1000	0.00005	200	0.0101	0.225	0.116	0.0122	r^2, se_y	0.977	0.002
25	100	1000	0.00003	200	0.0051	0.176	0.067	0.0062	F, df	125	3
10	40	1000	0.00001	200	0.0020	0.136	0.028	0.0014	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.123	0.015	-0.0002			

Regression results are calculated using the function LINEST

Blank	0.109	r^2=	0.977		
		m=	0.122		
		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	0.325	0.216	0.024	200	1	1	0.122	0.123	0.11
11540-6A	0.326	0.217	0.025	200	1	1	0.123		
11540-6A									
11540-6B	0.142	0.033	0.002	200	1	1	0.010	0.008	0.012
11540-6B	0.136	0.027	0.001	200	1	1	0.007		
11540-6B					1	800			
					1	800			
					1	800			
11345-21I	0.133	0.024	0.001	200	1	600	2.9	3.6	
11345-21I	0.135	0.026	0.001	200	1	600	3.6		
11345-21I	0.137	0.028	0.001	200	1	600	4.4		

Assay Date	8/31/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	4

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	1.7	mL total volume
	10	dilution factor
Dilution B	1.5	mL microsome Dilution A used
	90	mL total volume
	60	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.6
Protein Concentration (dilution added to assay, mg/mL):	0.00605

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	8/31/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	11345-10	Technician ID	2	Replicate #	4

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	7473	14946	14862	29724	0.1	166019	17.90	29632	0.0364	1	0.003	15	0.4009
			0.5	2	7389	14778											
	2	2	0.5	1	8772	17544	17584	35168	0.1	166019	21.18	35076	0.0431	1	0.003	15	0.4745
			0.5	2	8812	17624											
	3	2	0.5	1	8632	13264	13137	26274	0.1	166019	15.83	26182	0.0321	1	0.003	15	0.3542
			0.5	2	6505	13010											
	4	2	0.5	1	6199	12398	12277	24554	0.1	166019	14.79	24462	0.0300	1	0.003	15	0.3309
			0.5	2	6078	12156											
Background control	1	2	0.5	1	21	42	38	76	0.1	166019	0.05	-17	0.0000	1	0.003	15	-0.0002
			0.5	2	17	34											
	2	2	0.5	1	28	56	50	100	0.1	166019	0.06	8	0.0000	1	0.003	15	0.0001
			0.5	2	22	44											
	3	2	0.5	1	17	34	38	76	0.1	166019	0.05	-17	0.0000	1	0.003	15	-0.0002
			0.5	2	21	42											
	4	2	0.5	1	34	68	59	118	0.1	166019	0.07	26	0.0000	1	0.003	15	0.0003
			0.5	2	25	50											
Positive control	1	2	0.5	1	4882	9764	9686	19372	0.1	166019	11.67	19280	0.0237	1	0.003	15	0.2608
			0.5	2	4804	9608											
	2	2	0.5	1	4250	8500	8500	17000	0.1	166019	10.24	16908	0.0208	1	0.003	15	0.2287
			0.5	2	4250	8500											
	3	2	0.5	1	4232	8464	8547	17094	0.1	166019	10.30	17002	0.0209	1	0.003	15	0.2300
			0.5	2	4315	8630											
	4	2	0.5	1	4156	8312	8319	16638	0.1	166019	10.02	16546	0.0203	1	0.003	15	0.2238
			0.5	2	4163	8326											
Negative Control	1	2	0.5	1	7886	15772	15718	31436	0.1	166019	18.94	31344	0.0385	1	0.003	15	0.4240
			0.5	2	7832	15664											
	2	2	0.5	1	7438	14876	14894	29788	0.1	166019	17.94	29696	0.0365	1	0.003	15	0.4017
			0.5	2	7456	14912											
	3	2	0.5	1	6287	12574	13442	26884	0.1	166019	16.19	26792	0.0329	1	0.003	15	0.3624
			0.5	2	7155	14310											
	4	2	0.5	1	8441	16882	16798	33596	0.1	166019	20.24	33504	0.0411	1	0.003	15	0.4533
			0.5	2	8357	16714											
Ref Chem 8	1-1	2	0.5	1	2014	4028	4036	8072	0.1	166019	4.86	7980	0.0098	1	0.003	15	0.1080
			0.5	2	2022	4044											
	1-2	2	0.5	1	2037	4074	4035	8070	0.1	166019	4.86	7978	0.0098	1	0.003	15	0.1079
			0.5	2	1998	3996											
	1-3	2	0.5	1	1937	3874	3905	7810	0.1	166019	4.70	7718	0.0095	1	0.003	15	0.1044
			0.5	2	1968	3936											
	2-1	2	0.5	1	2116	4232	4250	8500	0.1	166019	5.12	8408	0.0103	1	0.003	15	0.1137
			0.5	2	2134	4268											
	2-2	2	0.5	1	1966	3932	3970	7940	0.1	166019	4.78	7848	0.0096	1	0.003	15	0.1062
			0.5	2	2004	4008											
	2-3	2	0.5	1	2221	4442	4442	8884	0.1	166019	5.35	8792	0.0108	1	0.003	15	0.1189
			0.5	2	2221	4442											
	3-1	2	0.5	1	3153	6306	6205	12410	0.1	166019	7.48	12318	0.0151	1	0.003	15	0.1666
			0.5	2	3052	6104											
	3-2	2	0.5	1	3292	6584	6528	13056	0.1	166019	7.86	12964	0.0159	1	0.003	15	0.1754
			0.5	2	3236	6472											
	3-3	2	0.5	1	3492	6984	6946	13892	0.1	166019	8.37	13800	0.0169	1	0.003	15	0.1867
			0.5	2	3454	6908											
	4-1	2	0.5	1	4573	9146	9306	18612	0.1	166019	11.21	18520	0.0227	1	0.003	15	0.2505
			0.5	2	4733	9466											
	4-2	2	0.5	1	4286	8572	8621	17242	0.1	166019	10.39	17150	0.0211	1	0.003	15	0.2320
			0.5	2	4335	8670											
	4-3	2	0.5	1	4247	8494	8505	17010	0.1	166019	10.25	16918	0.0208	1	0.003	15	0.2289
			0.5	2	4258	8516											
	5-1	2	0.5	1	5267	10534	10584	21168	0.1	166019	12.75	21076	0.0259	1	0.003	15	0.2851
			0.5	2	5317	10634											
	5-2	2	0.5	1	5121	10242	10182	20364	0.1	166019	12.27	20272	0.0249	1	0.003	15	0.2742
			0.5	2	5061	10122											
	5-3	2	0.5	1	5525	11050	11053	22106	0.1	166019	13.32	22014	0.0270	1	0.003	15	0.2978
			0.5	2	5528	11056											
	6-1	2	0.5	1	5947	11894	11923	23846	0.1	166019	14.36	23754	0.0292	1	0.003	15	0.3214
			0.5	2	5976	11952											
	6-2	2	0.5	1	6168	12336	12334	24668	0.1	166019	14.86	24576	0.0302	1	0.003	15	0.3325
			0.5	2	6166	12332											
	6-3	2	0.5	1	6199	12398	12436	24872	0.1	166019	14.98	24780	0.0304	1	0.003	15	0.3352
			0.5	2	6237	12474											
	7-1	2	0.5	1	6800	13600	13525	27050	0.1	166019	16.29	26958	0.0331	1	0.003	15	0.3647
			0.5	2	6725	13450											
	7-2	2	0.5	1	6240	12480	12502	25004	0.1	166019	15.06	24912	0.0306	1	0.003	15	0.3370
			0.5	2	6262	12524											
	7-3	2	0.5	1	6778	13556	13551	27102	0.1	166019	16.32	27010	0.0332	1	0.003	15	0.3654
			0.5	2	6773	13546											
	8-1	2	0.5	1	7082	14164	14110	28220	0.1	166019	17.00	28128	0.0345	1	0.003	15	0.3805
			0.5	2	7028	14056											
	8-2	2	0.5	1	6957	13914	13617	27234	0.1	166019	16.40	27142	0.0333	1	0.003	15	0.3672
			0.5	2	6660	13320											
	8-3	2	0.5	1	6485	12970	12920	25840	0.1	166019	15.56	25748	0.0316	1	0.003	15	0.3483
			0.5	2	6435	12870											

Assay Date	8/31/2005	Test Chemical ID	Ref Chem 8	# Concentrations tested		Microsome 8 type	Recombinant	Microsome ID 11345-10	Technician ID	2	Replicate #	4

Control Type	Portion	Average	SD
Full activity	Beginning	0.4377	0.0521
Full activity	End	0.3426	0.0165
Full activity	Overall	0.3901	0.0633
Background	Beginning	-0.0001	0.000229587
Background	End	0.0001	0.000401777
Background	Overall	0.0000	0.000276261
Positive	Beginning	0.2448	0.0227
Positive	End	0.2269	0.0044
Positive	Overall	0.2358	0.0169
Negative	Beginning	0.4129	0.0158
Negative	End	0.4079	0.0642
Negative	Overall	0.4104	0.0383

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 8	1	1	1.00E-04	-4.00	0.1080
Ref Chem 8	1	2	1.00E-04	-4.00	0.1079
Ref Chem 8	1	3	1.00E-04	-4.00	0.1044
Ref Chem 8	2	1	1.00E-05	-5.00	0.1137
Ref Chem 8	2	2	1.00E-05	-5.00	0.1062
Ref Chem 8	2	3	1.00E-05	-5.00	0.1189
Ref Chem 8	3	1	5.00E-06	-5.30	0.1666
Ref Chem 8	3	2	5.00E-06	-5.30	0.1754
Ref Chem 8	3	3	5.00E-06	-5.30	0.1867
Ref Chem 8	4	1	2.50E-06	-5.60	0.2505
Ref Chem 8	4	2	2.50E-06	-5.60	0.2320
Ref Chem 8	4	3	2.50E-06	-5.60	0.2289
Ref Chem 8	5	1	1.00E-06	-6.00	0.2851
Ref Chem 8	5	2	1.00E-06	-6.00	0.2742
Ref Chem 8	5	3	1.00E-06	-6.00	0.2978
Ref Chem 8	6	1	5.00E-07	-6.30	0.3214
Ref Chem 8	6	2	5.00E-07	-6.30	0.3325
Ref Chem 8	6	3	5.00E-07	-6.30	0.3352
Ref Chem 8	7	1	1.00E-07	-7.00	0.3647
Ref Chem 8	7	2	1.00E-07	-7.00	0.3370
Ref Chem 8	7	3	1.00E-07	-7.00	0.3654
Ref Chem 8	8	1	1.00E-08	-8.00	0.3805
Ref Chem 8	8	2	1.00E-08	-8.00	0.3672
Ref Chem 8	8	3	1.00E-08	-8.00	0.3483

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	27.67	27.66	26.76
2	-5.00	29.15	27.21	30.49
3	-5.30	42.71	44.95	47.85
4	-5.60	64.22	59.47	58.66
5	-6.00	73.08	70.30	76.34
6	-6.30	82.37	85.22	85.93
7	-7.00	93.48	86.39	93.66
8	-8.00	97.54	94.12	89.28

Aromatase Assay Spreadsheet

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8		
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0194	31398	1618454	
2	0.0195	31799	1630718	
3	0.0194	33072	1704742	
4	0.0199	30897	1552613	
5	0.0205	33802	1648878	
			Average DPM/g soln	1631081
			SD	54908
			CV	3.37
			µCi/g soln	0.735

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	8.1188 g
Mass of dilution B used in substrate prep	4.5659 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562386 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00832 µg/g soln.
µg/g soln.

a. µCi/g soln	0.735
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.

= 0.562386 + 0.00832
= 0.570703 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.287 µCi/µg ASDN

818537 dpm/nmol

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID 11345-10

								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

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

Blank	0.117	r^2=	0.989	
		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	2	Replicate #	1	Microsome type	Recombinant	Microsome ID 11345-10

								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

		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_{regr}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

Blank	0.117	r^2=	0.992	
		m=	0.096	
		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	0.369	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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	1

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.3
Protein Concentration (dilution added to assay, mg/mL):	0.010561

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	9/9/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	11345-10	Technician ID	2	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	9441	18882	18626	37252	0.1	163108	22.84	37176	0.0454	1	0.005	15	0.2867
			0.5	2	9185	18370											
	2	2	0.5	1	9896	19792	19512	39024	0.1	163108	23.93	38948	0.0476	1	0.005	15	0.3004
			0.5	2	9616	19232											
	3	2	0.5	1	8270	16540	16277	32554	0.1	163108	19.96	32478	0.0397	1	0.005	15	0.2505
			0.5	2	8007	16014											
	4	2	0.5	1	8605	17210	17387	34774	0.1	163108	21.32	34698	0.0424	1	0.005	15	0.2676
			0.5	2	8782	17564											
Background control	1	2	0.5	1	17	34	36	72	0.1	163108	0.04	-4	0.0000	1	0.005	15	0.0000
			0.5	2	19	38											
	2	2	0.5	1	25	50	47	94	0.1	163108	0.06	18	0.0000	1	0.005	15	0.0001
			0.5	2	22	44											
	3	2	0.5	1	13	26	26	52	0.1	163108	0.03	-24	0.0000	1	0.005	15	-0.0002
			0.5	2	13	26											
	4	2	0.5	1	17	34	43	86	0.1	163108	0.05	10	0.0000	1	0.005	15	0.0001
			0.5	2	26	52											
Positive control	1	2	0.5	1	5376	10752	10712	21424	0.1	163108	13.13	21348	0.0261	1	0.005	15	0.1646
			0.5	2	5336	10672											
	2	2	0.5	1	4999	9998	10046	20092	0.1	163108	12.32	20016	0.0245	1	0.005	15	0.1544
			0.5	2	5047	10094											
	3	2	0.5	1	5548	11096	11027	22054	0.1	163108	13.52	21978	0.0269	1	0.005	15	0.1695
			0.5	2	5479	10958											
	4	2	0.5	1	5309	10618	10668	21336	0.1	163108	13.08	21260	0.0260	1	0.005	15	0.1640
			0.5	2	5359	10718											
Negative Control	1	2	0.5	1	8688	17376	17431	34862	0.1	163108	21.37	34786	0.0425	1	0.005	15	0.2683
			0.5	2	8743	17486											
	2	2	0.5	1	8691	17382	17592	35184	0.1	163108	21.57	35108	0.0429	1	0.005	15	0.2708
			0.5	2	8901	17802											
	3	2	0.5	1	7984	15968	16127	32254	0.1	163108	19.77	32178	0.0393	1	0.005	15	0.2482
			0.5	2	8143	16286											
	4	2	0.5	1	9212	18424	18495	36990	0.1	163108	22.68	36914	0.0451	1	0.005	15	0.2847
			0.5	2	9283	18566											
Ref Chem 9	1-1	2	0.5	1	230	460	479	958	0.1	163108	0.59	882	0.0011	1	0.005	15	0.0068
			0.5	2	249	498											
	1-2	2	0.5	1	198	396	417	834	0.1	163108	0.51	758	0.0009	1	0.005	15	0.0058
			0.5	2	219	438											
	1-3	2	0.5	1	214	428	435	870	0.1	163108	0.53	794	0.0010	1	0.005	15	0.0061
			0.5	2	221	442											
	2-1	2	0.5	1	2232	4464	4447	8894	0.1	163108	5.45	8818	0.0108	1	0.005	15	0.0680
			0.5	2	2215	4430											
	2-2	2	0.5	1	1991	3982	4008	8016	0.1	163108	4.91	7940	0.0097	1	0.005	15	0.0612
			0.5	2	2017	4034											
	2-3	2	0.5	1	2109	4218	4188	8376	0.1	163108	5.14	8300	0.0101	1	0.005	15	0.0640
			0.5	2	2079	4158											
	3-1	2	0.5	1	7495	14990	14829	29658	0.1	163108	18.18	29582	0.0361	1	0.005	15	0.2281
			0.5	2	7334	14668											
	3-2	2	0.5	1	7205	14410	14321	28642	0.1	163108	17.56	28566	0.0349	1	0.005	15	0.2203
			0.5	2	7116	14232											
	3-3	2	0.5	1	6609	13218	13352	26704	0.1	163108	16.37	26628	0.0325	1	0.005	15	0.2054
			0.5	2	6743	13486											
	4-1	2	0.5	1	8050	16100	16251	32502	0.1	163108	19.93	32426	0.0396	1	0.005	15	0.2501
			0.5	2	8201	16402											
	4-2	2	0.5	1	8272	16544	16599	33198	0.1	163108	20.35	33122	0.0405	1	0.005	15	0.2554
			0.5	2	8327	16654											
	4-3	2	0.5	1	9024	18048	18073	36146	0.1	163108	22.16	36070	0.0441	1	0.005	15	0.2782
			0.5	2	9049	18098											
	5-1	2	0.5	1	8265	16530	16531	33062	0.1	163108	20.27	32986	0.0403	1	0.005	15	0.2544
			0.5	2	8266	16532											
	5-2	2	0.5	1	8707	17414	17461	34922	0.1	163108	21.41	34846	0.0426	1	0.005	15	0.2687
			0.5	2	8754	17508											
	5-3	2	0.5	1	8240	16480	16687	33374	0.1	163108	20.46	33298	0.0407	1	0.005	15	0.2568
			0.5	2	8447	16894											
	6-1	2	0.5	1	9076	18152	18068	36136	0.1	163108	22.15	36060	0.0441	1	0.005	15	0.2781
			0.5	2	8992	17984											
	6-2	2	0.5	1	8561	17122	17221	34442	0.1	163108	21.12	34366	0.0420	1	0.005	15	0.2650
			0.5	2	8660	17320											
	6-3	2	0.5	1	8799	17598	17563	35126	0.1	163108	21.54	35050	0.0428	1	0.005	15	0.2703
			0.5	2	8764	17528											
	7-1	2	0.5	1	9503	19006	19193	38386	0.1	163108	23.53	38310	0.0468	1	0.005	15	0.2955
			0.5	2	9690	19380											
	7-2	2	0.5	1	8437	16874	16548	33096	0.1	163108	20.29	33020	0.0403	1	0.005	15	0.2547
			0.5	2	8111	16222											
	7-3	2	0.5	1	8585	17170	17140	34280	0.1	163108	21.02	34204	0.0418	1	0.005	15	0.2638
			0.5	2	8556	17110											
	8-1	2	0.5	1	8126	16252	16369	32738	0.1	163108	20.07	32662	0.0399	1	0.005	15	0.2519
			0.5	2	8243	16486											
	8-2	2	0.5	1	8450	16900	16796	33592	0.1	163108	20.59	33516	0.0409	1	0.005	15	0.2585
			0.5	2	8346	16692											
	8-3	2	0.5	1	8506	17012	17036	34072	0.1	163108	20.89	33996	0.0415	1	0.005	15	0.2622
			0.5	2	8530	17060											

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.2935	0.0097
Full activity	End	0.2590	0.0121
Full activity	Overall	0.2763	0.0218
Background	Beginning	0.0001	0.000119975
Background	End	-0.0001	0.000185416
Background	Overall	0.0000	0.000141928
Positive	Beginning	0.1595	0.0073
Positive	End	0.1667	0.0039
Positive	Overall	0.1631	0.0063
Negative	Beginning	0.2695	0.0018
Negative	End	0.2664	0.0258
Negative	Overall	0.2680	0.0151

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 9	1	1	1.00E-03	-3.00	0.0068
Ref Chem 9	1	2	1.00E-03	-3.00	0.0058
Ref Chem 9	1	3	1.00E-03	-3.00	0.0061
Ref Chem 9	2	1	1.00E-04	-4.00	0.0680
Ref Chem 9	2	2	1.00E-04	-4.00	0.0612
Ref Chem 9	2	3	1.00E-04	-4.00	0.0640
Ref Chem 9	3	1	1.00E-05	-5.00	0.2281
Ref Chem 9	3	2	1.00E-05	-5.00	0.2203
Ref Chem 9	3	3	1.00E-05	-5.00	0.2054
Ref Chem 9	4	1	1.00E-06	-6.00	0.2501
Ref Chem 9	4	2	1.00E-06	-6.00	0.2554
Ref Chem 9	4	3	1.00E-06	-6.00	0.2782
Ref Chem 9	5	1	1.00E-07	-7.00	0.2544
Ref Chem 9	5	2	1.00E-07	-7.00	0.2687
Ref Chem 9	5	3	1.00E-07	-7.00	0.2568
Ref Chem 9	6	1	1.00E-08	-8.00	0.2781
Ref Chem 9	6	2	1.00E-08	-8.00	0.2650
Ref Chem 9	6	3	1.00E-08	-8.00	0.2703
Ref Chem 9	7	1	1.00E-09	-9.00	0.2955
Ref Chem 9	7	2	1.00E-09	-9.00	0.2547
Ref Chem 9	7	3	1.00E-09	-9.00	0.2638
Ref Chem 9	8	1	1.00E-10	-10.00	0.2519
Ref Chem 9	8	2	1.00E-10	-10.00	0.2585
Ref Chem 9	8	3	1.00E-10	-10.00	0.2622

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.46	2.12	2.22
2	-4.00	24.61	22.16	23.17
3	-5.00	82.57	79.74	74.33
4	-6.00	90.51	92.45	100.68
5	-7.00	92.08	97.27	92.95
6	-8.00	100.66	95.93	97.84
7	-9.00	106.94	92.17	95.48
8	-10.00	91.17	93.55	94.89

Aromatase Assay Spreadsheet

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8		
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0196	31383	1601173	
2	0.0199	31776	1596784	
3	0.0198	33854	1709798	
4	0.0200	33828	1691400	
5	0.0205	34970	1705854	
			Average DPM/g soln	1661002
			SD	57053
			CV	3.43
			µCi/g soln	0.748

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	8.1193 g
Mass of dilution B used in substrate prep	4.5679 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.568224 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00847 µg/g soln.
µg/g soln.

a. µCi/g soln 0.748
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.

= 0.568224 + 0.00847
= 0.576693 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.297 µCi/µg ASDN

824894 dpm/nmol

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8						
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10				

								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

Regression results are calculated using the function LINEST

r^2= 0.982
m= 0.120
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	0.354	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	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10				

								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	Variables	Regression results	
250	200	200	0.00026	200	0.0518	0.544	0.426	0.0409	m, b	0.097	0.000
125	100	200	0.00013	200	0.0259	0.384	0.266	0.0253	se_m, se_b	0.004	0.001
50	200	1000	0.00005	200	0.0104	0.242	0.123	0.0115	r^2, se_y	0.995	0.001
25	100	1000	0.00003	200	0.0052	0.181	0.062	0.0056	F, df	548	3
10	40	1000	0.00001	200	0.0021	0.140	0.021	0.0016	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.129	0.011	0.0006			

Regression results are calculated using the function LINEST

Blank	0.119	r^2=	0.995	
		m=	0.097	
		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	0.354	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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.6
Protein Concentration (dilution added to assay, mg/mL):	0.009302

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	10765	21530	21548	43096	0.1	166100	25.95	42977	0.0521	1	0.005	15	0.3734
			0.5	2	10783	21566											
	2	2	0.5	1	10105	20210	20249	40498	0.1	166100	24.38	40379	0.0490	1	0.005	15	0.3508
			0.5	2	10144	20288											
	3	2	0.5	1	7596	15192	15138	30276	0.1	166100	18.23	30157	0.0366	1	0.005	15	0.2620
			0.5	2	7542	15084											
	4	2	0.5	1	9027	18054	18166	36332	0.1	166100	21.87	36213	0.0439	1	0.005	15	0.3146
			0.5	2	9139	18278											
Background control	1	2	0.5	1	20	40	54	108	0.1	166100	0.07	-11	0.0000	1	0.005	15	-0.0001
			0.5	2	34	68											
	2	2	0.5	1	32	64	59	118	0.1	166100	0.07	-1	0.0000	1	0.005	15	0.0000
			0.5	2	27	54											
	3	2	0.5	1	35	70	68	136	0.1	166100	0.08	17	0.0000	1	0.005	15	0.0001
			0.5	2	33	66											
	4	2	0.5	1	29	58	57	114	0.1	166100	0.07	-5	0.0000	1	0.005	15	0.0000
			0.5	2	28	56											
Positive control	1	2	0.5	1	5552	11104	11328	22656	0.1	166100	13.64	22537	0.0273	1	0.005	15	0.1958
			0.5	2	5776	11552											
	2	2	0.5	1	5314	10628	10655	21310	0.1	166100	12.83	21191	0.0257	1	0.005	15	0.1841
			0.5	2	5341	10682											
	3	2	0.5	1	5132	10264	10314	20628	0.1	166100	12.42	20509	0.0249	1	0.005	15	0.1782
			0.5	2	5182	10364											
	4	2	0.5	1	5650	11300	11721	23442	0.1	166100	14.11	23323	0.0283	1	0.005	15	0.2026
			0.5	2	6071	12142											
Negative Control	1	2	0.5	1	10980	21960	21768	43536	0.1	166100	26.21	43417	0.0526	1	0.005	15	0.3772
			0.5	2	10788	21576											
	2	2	0.5	1	10007	20014	20090	40180	0.1	166100	24.19	40061	0.0486	1	0.005	15	0.3481
			0.5	2	10083	20166											
	3	2	0.5	1	8512	17024	16982	33964	0.1	166100	20.45	33845	0.0410	1	0.005	15	0.2941
			0.5	2	8470	16940											
	4	2	0.5	1	10547	21094	21244	42488	0.1	166100	25.58	42369	0.0514	1	0.005	15	0.3681
			0.5	2	10697	21394											
Ref Chem 9	1-1	2	0.5	1	298	596	577	1154	0.1	166100	0.69	1035	0.0013	1	0.005	15	0.0090
			0.5	2	279	558											
	1-2	2	0.5	1	199	398	404	808	0.1	166100	0.49	689	0.0008	1	0.005	15	0.0060
			0.5	2	205	410											
	1-3	2	0.5	1	208	416	452	904	0.1	166100	0.54	785	0.0010	1	0.005	15	0.0068
			0.5	2	244	488											
	2-1	2	0.5	1	483	966	948	1896	0.1	166100	1.14	1777	0.0022	1	0.005	15	0.0154
			0.5	2	465	930											
	2-2	2	0.5	1	662	1324	1309	2618	0.1	166100	1.58	2499	0.0030	1	0.005	15	0.0217
			0.5	2	647	1294											
	2-3	2	0.5	1	546	1092	1051	2102	0.1	166100	1.27	1983	0.0024	1	0.005	15	0.0172
			0.5	2	505	1010											
	3-1	2	0.5	1	1085	2170	2130	4260	0.1	166100	2.56	4141	0.0050	1	0.005	15	0.0360
			0.5	2	1045	2090											
	3-2	2	0.5	1	1117	2234	2208	4416	0.1	166100	2.66	4297	0.0052	1	0.005	15	0.0373
			0.5	2	1091	2182											
	3-3	2	0.5	1	1355	2710	2755	5510	0.1	166100	3.32	5391	0.0065	1	0.005	15	0.0468
			0.5	2	1400	2800											
	4-1	2	0.5	1	2177	4354	4507	9014	0.1	166100	5.43	8895	0.0108	1	0.005	15	0.0773
			0.5	2	2330	4660											
	4-2	2	0.5	1	2770	5540	5546	11092	0.1	166100	6.68	10973	0.0133	1	0.005	15	0.0953
			0.5	2	2776	5552											
	4-3	2	0.5	1	3288	6576	6461	12922	0.1	166100	7.78	12803	0.0155	1	0.005	15	0.1112
			0.5	2	3173	6346											
	5-1	2	0.5	1	6889	13778	13933	27866	0.1	166100	16.78	27747	0.0336	1	0.005	15	0.2411
			0.5	2	7044	14088											
	5-2	2	0.5	1	7200	14400	14479	28958	0.1	166100	17.43	28839	0.0350	1	0.005	15	0.2506
			0.5	2	7279	14558											
	5-3	2	0.5	1	7504	15008	14957	29914	0.1	166100	18.01	29795	0.0361	1	0.005	15	0.2589
			0.5	2	7453	14906											
	6-1	2	0.5	1	10761	21522	21360	42720	0.1	166100	25.72	42601	0.0516	1	0.005	15	0.3701
			0.5	2	10599	21198											
	6-2	2	0.5	1	10041	20082	20019	40038	0.1	166100	24.10	39919	0.0484	1	0.005	15	0.3468
			0.5	2	9978	19956											
	6-3	2	0.5	1	8348	16696	16851	33702	0.1	166100	20.29	33583	0.0407	1	0.005	15	0.2918
			0.5	2	8503	17006											
	7-1	2	0.5	1	9470	18940	18908	37816	0.1	166100	22.77	37697	0.0457	1	0.005	15	0.3275
			0.5	2	9438	18876											
	7-2	2	0.5	1	9601	19202	19190	38380	0.1	166100	23.11	38261	0.0464	1	0.005	15	0.3324
			0.5	2	9589	19178											
	7-3	2	0.5	1	9525	19050	18862	37724	0.1	166100	22.71	37605	0.0456	1	0.005	15	0.3267
			0.5	2	9337	18674											
	8-1	2	0.5	1	10709	21418	21606	43212	0.1	166100	26.02	43093	0.0522	1	0.005	15	0.3744
			0.5	2	10897	21794											
	8-2	2	0.5	1	9441	18882	18723	37446	0.1	166100	22.54	37327	0.0453	1	0.005	15	0.3243
			0.5	2	9282	18564											
	8-3	2	0.5	1	10493	20986	21236	42472	0.1	166100	25.57	42353	0.0513	1	0.005	15	0.3680
			0.5	2	10743	21486											

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3621	0.0160
Full activity	End	0.2883	0.0372
Full activity	Overall	0.3252	0.0486
Background	Beginning	-0.0001	6.14356E-05
Background	End	0.0001	0.000135158
Background	Overall	0.0000	0.000104741
Positive	Beginning	0.1900	0.0083
Positive	End	0.1904	0.0173
Positive	Overall	0.1902	0.0111
Negative	Beginning	0.3626	0.0206
Negative	End	0.3311	0.0524
Negative	Overall	0.3469	0.0373

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 9	1	1	1.00E-03	-3.00	0.0090
Ref Chem 9	1	2	1.00E-03	-3.00	0.0060
Ref Chem 9	1	3	1.00E-03	-3.00	0.0068
Ref Chem 9	2	1	5.00E-04	-3.30	0.0154
Ref Chem 9	2	2	5.00E-04	-3.30	0.0217
Ref Chem 9	2	3	5.00E-04	-3.30	0.0172
Ref Chem 9	3	1	2.50E-04	-3.60	0.0360
Ref Chem 9	3	2	2.50E-04	-3.60	0.0373
Ref Chem 9	3	3	2.50E-04	-3.60	0.0468
Ref Chem 9	4	1	1.00E-04	-4.00	0.0773
Ref Chem 9	4	2	1.00E-04	-4.00	0.0953
Ref Chem 9	4	3	1.00E-04	-4.00	0.1112
Ref Chem 9	5	1	1.00E-05	-5.00	0.2411
Ref Chem 9	5	2	1.00E-05	-5.00	0.2506
Ref Chem 9	5	3	1.00E-05	-5.00	0.2589
Ref Chem 9	6	1	1.00E-06	-6.00	0.3701
Ref Chem 9	6	2	1.00E-06	-6.00	0.3468
Ref Chem 9	6	3	1.00E-06	-6.00	0.2918
Ref Chem 9	7	1	1.00E-07	-7.00	0.3275
Ref Chem 9	7	2	1.00E-07	-7.00	0.3324
Ref Chem 9	7	3	1.00E-07	-7.00	0.3267
Ref Chem 9	8	1	1.00E-08	-8.00	0.3744
Ref Chem 9	8	2	1.00E-08	-8.00	0.3243
Ref Chem 9	8	3	1.00E-08	-8.00	0.3680

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.77	1.84	2.10
2	-3.30	4.75	6.68	5.30
3	-3.60	11.06	11.48	14.40
4	-4.00	23.76	29.31	34.20
5	-5.00	74.13	77.04	79.60
6	-6.00	113.81	106.65	89.72
7	-7.00	100.71	102.22	100.46
8	-8.00	115.12	99.72	113.15

Aromatase Assay Spreadsheet

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8		
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	29591	1494495	
2	0.0197	29345	1489594	
3	0.0199	32377	1626985	
4	0.0200	34301	1715050	
5	0.0198	32649	1648939	
			Average DPM/g soln	1595013
			SD	99444
			CV	6.23
			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	10.2	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	8.1078 g
Mass of dilution B used in substrate prep	4.5707 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.575016 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.

= 0.575016 + 0.00813
= 0.583149 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.232 µCi/µg ASDN

783353 dpm/nmol

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8						
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.5	100	11540-54
	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			

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	Variables	Regression results	
250	200	200	0.00026	200	0.0510	0.549	0.433	0.0486	m, b	0.118	-0.002
125	100	200	0.00013	200	0.0255	0.376	0.260	0.0282	se_m, se_b	0.007	0.002
50	200	1000	0.00005	200	0.0102	0.245	0.129	0.0127	r^2, se_y	0.985	0.003
25	100	1000	0.00003	200	0.0051	0.187	0.071	0.0059	F, df	268	4
10	40	1000	0.00001	200	0.0020	0.146	0.030	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.123	0.007	-0.0016			

Regression results are calculated using the function LINEST

Blank	0.116	r^2=	0.985	
		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	0.359	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	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10								

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.5	100	11540-54
	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			
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	Variables	Regression results	
250	200	200	0.00026	200	0.0510	0.549	0.433	0.0417	m, b	0.099	-0.001
125	100	200	0.00013	200	0.0255	0.376	0.260	0.0246	se_m, se_b	0.007	0.001
50	200	1000	0.00005	200	0.0102	0.245	0.129	0.0117	r^2, se_y	0.986	0.001
25	100	1000	0.00003	200	0.0051	0.187	0.071	0.0060	F, df	214	3
10	40	1000	0.00001	200	0.0020	0.146	0.030	0.0020	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.123	0.007	-0.0003			
			Blank	0.116	r^2=	0.986					
					m=	0.099					
					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	0.359	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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.9
Protein Concentration (dilution added to assay, mg/mL):	0.009859

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	Recombinan	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol^{2}H$_2$O 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^{2}H$_2$O 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	9357	18714	18654	37308	0.1	159501	23.39	37192	0.0475	1	0.005	15	0.3211
			0.5	2	9297	18594											
	2	2	0.5	1	8994	17988	17741	35482	0.1	159501	22.25	35366	0.0451	1	0.005	15	0.3053
			0.5	2	8747	17494											
	3	2	0.5	1	8510	17020	16862	33724	0.1	159501	21.14	33608	0.0429	1	0.005	15	0.2901
			0.5	2	8352	16704											
	4	2	0.5	1	8304	16608	16518	33036	0.1	159501	20.71	32920	0.0420	1	0.005	15	0.2842
			0.5	2	8214	16428											
Background control	1	2	0.5	1	16	32	36	72	0.1	159501	0.05	-44	-0.0001	1	0.005	15	-0.0004
			0.5	2	20	40											
	2	2	0.5	1	43	86	72	144	0.1	159501	0.09	28	0.0000	1	0.005	15	0.0002
			0.5	2	29	58											
	3	2	0.5	1	30	60	60	120	0.1	159501	0.08	4	0.0000	1	0.005	15	0.0000
			0.5	2	30	60											
	4	2	0.5	1	41	82	64	128	0.1	159501	0.08	12	0.0000	1	0.005	15	0.0001
			0.5	2	23	46											
Positive control	1	2	0.5	1	4854	9708	9527	19054	0.1	159501	11.95	18938	0.0242	1	0.005	15	0.1635
			0.5	2	4673	9346											
	2	2	0.5	1	4971	9942	10062	20124	0.1	159501	12.62	20008	0.0255	1	0.005	15	0.1727
			0.5	2	5091	10182											
	3	2	0.5	1	4420	8840	8728	17456	0.1	159501	10.94	17340	0.0221	1	0.005	15	0.1497
			0.5	2	4308	8616											
	4	2	0.5	1	4619	9238	9292	18584	0.1	159501	11.65	18468	0.0236	1	0.005	15	0.1594
			0.5	2	4673	9346											
Negative Control	1	2	0.5	1	8150	16300	16282	32564	0.1	159501	20.42	32448	0.0414	1	0.005	15	0.2801
			0.5	2	8132	16264											
	2	2	0.5	1	8746	17492	17339	34678	0.1	159501	21.74	34562	0.0441	1	0.005	15	0.2984
			0.5	2	8593	17186											
	3	2	0.5	1	7820	15640	15499	30998	0.1	159501	19.43	30882	0.0394	1	0.005	15	0.2666
			0.5	2	7679	15358											
	4	2	0.5	1	8440	16880	16854	33708	0.1	159501	21.13	33592	0.0429	1	0.005	15	0.2900
			0.5	2	8414	16828											
Ref Chem 9	1-1	2	0.5	1	264	528	517	1034	0.1	159501	0.65	918	0.0012	1	0.005	15	0.0079
			0.5	2	253	506											
	1-2	2	0.5	1	263	526	519	1038	0.1	159501	0.65	922	0.0012	1	0.005	15	0.0080
			0.5	2	256	512											
	1-3	2	0.5	1	212	424	417	834	0.1	159501	0.52	718	0.0009	1	0.005	15	0.0062
			0.5	2	205	410											
	2-1	2	0.5	1	636	1272	1289	2578	0.1	159501	1.62	2462	0.0031	1	0.005	15	0.0213
			0.5	2	653	1306											
	2-2	2	0.5	1	683	1366	1353	2706	0.1	159501	1.70	2590	0.0033	1	0.005	15	0.0224
			0.5	2	670	1340											
	2-3	2	0.5	1	590	1180	1148	2296	0.1	159501	1.44	2180	0.0028	1	0.005	15	0.0188
			0.5	2	558	1116											
	3-1	2	0.5	1	1089	2178	2100	4200	0.1	159501	2.63	4084	0.0052	1	0.005	15	0.0353
			0.5	2	1011	2022											
	3-2	2	0.5	1	1030	2060	2085	4170	0.1	159501	2.61	4054	0.0052	1	0.005	15	0.0350
			0.5	2	1055	2110											
	3-3	2	0.5	1	1256	2512	2521	5042	0.1	159501	3.16	4926	0.0063	1	0.005	15	0.0425
			0.5	2	1265	2530											
	4-1	2	0.5	1	2053	4126	4049	8098	0.1	159501	5.08	7982	0.0102	1	0.005	15	0.0689
			0.5	2	1986	3972											
	4-2	2	0.5	1	2035	4070	4026	8052	0.1	159501	5.05	7936	0.0101	1	0.005	15	0.0685
			0.5	2	1991	3982											
	4-3	2	0.5	1	2401	4802	4963	9926	0.1	159501	6.22	9810	0.0125	1	0.005	15	0.0847
			0.5	2	2562	5124											
	5-1	2	0.5	1	6682	13364	13225	26450	0.1	159501	16.58	26334	0.0336	1	0.005	15	0.2273
			0.5	2	6543	13086											
	5-2	2	0.5	1	6171	12342	12366	24732	0.1	159501	15.51	24616	0.0314	1	0.005	15	0.2125
			0.5	2	6195	12390											
	5-3	2	0.5	1	6216	12432	12428	24856	0.1	159501	15.58	24740	0.0316	1	0.005	15	0.2136
			0.5	2	6212	12424											
	6-1	2	0.5	1	7992	15984	16112	32224	0.1	159501	20.20	32108	0.0410	1	0.005	15	0.2772
			0.5	2	8120	16240											
	6-2	2	0.5	1	8443	16886	16845	33690	0.1	159501	21.12	33574	0.0429	1	0.005	15	0.2898
			0.5	2	8402	16804											
	6-3	2	0.5	1	7631	15262	15404	30808	0.1	159501	19.32	30692	0.0392	1	0.005	15	0.2649
			0.5	2	7773	15546											
	7-1	2	0.5	1	8145	16290	16432	32864	0.1	159501	20.60	32748	0.0418	1	0.005	15	0.2827
			0.5	2	8287	16574											
	7-2	2	0.5	1	8099	16198	16168	32336	0.1	159501	20.27	32220	0.0411	1	0.005	15	0.2781
			0.5	2	8069	16138											
	7-3	2	0.5	1	7719	15438	15406	30812	0.1	159501	19.32	30696	0.0392	1	0.005	15	0.2650
			0.5	2	7687	15374											
	8-1	2	0.5	1	8376	16752	16802	33604	0.1	159501	21.07	33488	0.0427	1	0.005	15	0.2891
			0.5	2	8426	16852											
	8-2	2	0.5	1	8256	16512	16538	33076	0.1	159501	20.74	32960	0.0421	1	0.005	15	0.2845
			0.5	2	8282	16564											
	8-3	2	0.5	1	8877	17754	17906	35812	0.1	159501	22.45	35696	0.0456	1	0.005	15	0.3081
			0.5	2	9029	18058											

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 9	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3132	0.0111
Full activity	End	0.2871	0.0042
Full activity	Overall	0.3002	0.0165
Background	Beginning	-0.0001	0.000439486
Background	End	0.0001	4.88318E-05
Background	Overall	0.0000	0.000267463
Positive	Beginning	0.1681	0.0065
Positive	End	0.1546	0.0069
Positive	Overall	0.1613	0.0095
Negative	Beginning	0.2892	0.0129
Negative	End	0.2783	0.0165
Negative	Overall	0.2838	0.0137

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 9	1	1	1.00E-03	-3.00	0.0079
Ref Chem 9	1	2	1.00E-03	-3.00	0.0080
Ref Chem 9	1	3	1.00E-03	-3.00	0.0062
Ref Chem 9	2	1	5.00E-04	-3.30	0.0213
Ref Chem 9	2	2	5.00E-04	-3.30	0.0224
Ref Chem 9	2	3	5.00E-04	-3.30	0.0188
Ref Chem 9	3	1	2.50E-04	-3.60	0.0353
Ref Chem 9	3	2	2.50E-04	-3.60	0.0350
Ref Chem 9	3	3	2.50E-04	-3.60	0.0425
Ref Chem 9	4	1	1.00E-04	-4.00	0.0689
Ref Chem 9	4	2	1.00E-04	-4.00	0.0685
Ref Chem 9	4	3	1.00E-04	-4.00	0.0847
Ref Chem 9	5	1	1.00E-05	-5.00	0.2273
Ref Chem 9	5	2	1.00E-05	-5.00	0.2125
Ref Chem 9	5	3	1.00E-05	-5.00	0.2136
Ref Chem 9	6	1	1.00E-06	-6.00	0.2772
Ref Chem 9	6	2	1.00E-06	-6.00	0.2898
Ref Chem 9	6	3	1.00E-06	-6.00	0.2649
Ref Chem 9	7	1	1.00E-07	-7.00	0.2827
Ref Chem 9	7	2	1.00E-07	-7.00	0.2781
Ref Chem 9	7	3	1.00E-07	-7.00	0.2650
Ref Chem 9	8	1	1.00E-08	-8.00	0.2891
Ref Chem 9	8	2	1.00E-08	-8.00	0.2845
Ref Chem 9	8	3	1.00E-08	-8.00	0.3081

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.64	2.65	2.06
2	-3.30	7.08	7.45	6.27
3	-3.60	11.75	11.66	14.17
4	-4.00	22.96	22.82	28.21
5	-5.00	75.73	70.79	71.15
6	-6.00	92.34	96.56	88.27
7	-7.00	94.18	92.66	88.28
8	-8.00	96.31	94.79	102.66

Aromatase Assay Spreadsheet

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8
Technician ID	2	Replicate #	1	Microsome type	Recombina Microsome ID 11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0194	31398	1618454	
2	0.0195	31799	1630718	
3	0.0194	33072	1704742	
4	0.0199	30897	1552613	
5	0.0205	33802	1648878	
			Average DPM/g soln	1631081
			SD	54908
			CV	3.37
			µCi/g soln	0.735

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	8.1188 g
Mass of dilution B used in substrate prep	4.5659 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562386 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00832 µg/g soln.
µg/g soln.

a. µCi/g soln	0.735
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.

= 0.562386 + 0.00832
= 0.570703 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.287 µCi/µg ASDN

818537 dpm/nmol

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8						
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10				

								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

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

Blank	0.117	r^2=	0.989	
		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 10	# Concentrations tested	8					
Technician ID	2	Replicate #	1	Microsome type	Recombinant	Microsome ID	11345-10			

								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 10	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	1

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.3
Protein Concentration (dilution added to assay, mg/mL):	0.010561

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	9441	18882	18626	37252	0.1	163108	22.84	37176	0.0454	1	0.005	15	0.2867
			0.5	2	9185	18370											
	2	2	0.5	1	9896	19792	19512	39024	0.1	163108	23.93	38948	0.0476	1	0.005	15	0.3004
			0.5	2	9616	19232											
	3	2	0.5	1	8270	16540	16277	32554	0.1	163108	19.96	32478	0.0397	1	0.005	15	0.2505
			0.5	2	8007	16014											
	4	2	0.5	1	8605	17210	17387	34774	0.1	163108	21.32	34698	0.0424	1	0.005	15	0.2676
			0.5	2	8782	17564											
Background control	1	2	0.5	1	17	34	36	72	0.1	163108	0.04	-4	0.0000	1	0.005	15	0.0000
			0.5	2	19	38											
	2	2	0.5	1	25	50	47	94	0.1	163108	0.06	18	0.0000	1	0.005	15	0.0001
			0.5	2	22	44											
	3	2	0.5	1	13	26	26	52	0.1	163108	0.03	-24	0.0000	1	0.005	15	-0.0002
			0.5	2	13	26											
	4	2	0.5	1	17	34	43	86	0.1	163108	0.05	10	0.0000	1	0.005	15	0.0001
			0.5	2	26	52											
Positive control	1	2	0.5	1	5376	10752	10712	21424	0.1	163108	13.13	21348	0.0261	1	0.005	15	0.1646
			0.5	2	5336	10672											
	2	2	0.5	1	4999	9998	10046	20092	0.1	163108	12.32	20016	0.0245	1	0.005	15	0.1544
			0.5	2	5047	10094											
	3	2	0.5	1	5548	11096	11027	22054	0.1	163108	13.52	21978	0.0269	1	0.005	15	0.1695
			0.5	2	5479	10958											
	4	2	0.5	1	5309	10618	10668	21336	0.1	163108	13.08	21260	0.0260	1	0.005	15	0.1640
			0.5	2	5359	10718											
Negative Control	1	2	0.5	1	8688	17376	17431	34862	0.1	163108	21.37	34786	0.0425	1	0.005	15	0.2683
			0.5	2	8743	17486											
	2	2	0.5	1	8691	17382	17592	35184	0.1	163108	21.57	35108	0.0429	1	0.005	15	0.2708
			0.5	2	8901	17802											
	3	2	0.5	1	7984	15968	16127	32254	0.1	163108	19.77	32178	0.0393	1	0.005	15	0.2482
			0.5	2	8143	16286											
	4	2	0.5	1	9212	18424	18495	36990	0.1	163108	22.68	36914	0.0451	1	0.005	15	0.2847
			0.5	2	9283	18566											
Ref Chem 10	1-1	2	0.5	1	8547	17094	16739	33478	0.1	163108	20.53	33402	0.0408	1	0.005	15	0.2576
			0.5	2	8192	16384											
	1-2	2	0.5	1	8391	16782	16878	33756	0.1	163108	20.70	33680	0.0411	1	0.005	15	0.2597
			0.5	2	8487	16974											
	1-3	2	0.5	1	7687	15374	15318	30636	0.1	163108	18.78	30560	0.0373	1	0.005	15	0.2357
			0.5	2	7631	15262											
	2-1	2	0.5	1	8761	17522	17478	34956	0.1	163108	21.43	34880	0.0426	1	0.005	15	0.2690
			0.5	2	8717	17434											
	2-2	2	0.5	1	8449	16898	16796	33592	0.1	163108	20.59	33516	0.0409	1	0.005	15	0.2585
			0.5	2	8347	16694											
	2-3	2	0.5	1	8968	17936	17708	35416	0.1	163108	21.71	35340	0.0432	1	0.005	15	0.2726
			0.5	2	8740	17480											
	3-1	2	0.5	1	8966	17932	17929	35858	0.1	163108	21.98	35782	0.0437	1	0.005	15	0.2760
			0.5	2	8963	17926											
	3-2	2	0.5	1	8495	16990	17068	34136	0.1	163108	20.93	34060	0.0416	1	0.005	15	0.2627
			0.5	2	8573	17146											
	3-3	2	0.5	1	8342	16684	16642	33284	0.1	163108	20.41	33208	0.0406	1	0.005	15	0.2561
			0.5	2	8300	16600											
	4-1	2	0.5	1	8828	17656	17580	35160	0.1	163108	21.56	35084	0.0429	1	0.005	15	0.2706
			0.5	2	8752	17504											
	4-2	2	0.5	1	7913	15826	15879	31758	0.1	163108	19.47	31682	0.0387	1	0.005	15	0.2443
			0.5	2	7966	15932											
	4-3	2	0.5	1	8558	17116	17213	34426	0.1	163108	21.11	34350	0.0420	1	0.005	15	0.2649
			0.5	2	8655	17310											
	5-1	2	0.5	1	8848	17696	17814	35628	0.1	163108	21.84	35552	0.0434	1	0.005	15	0.2742
			0.5	2	8966	17932											
	5-2	2	0.5	1	9107	18214	18172	36344	0.1	163108	22.28	36268	0.0443	1	0.005	15	0.2797
			0.5	2	9065	18130											
	5-3	2	0.5	1	8290	16580	16812	33624	0.1	163108	20.61	33548	0.0410	1	0.005	15	0.2587
			0.5	2	8522	17044											
	6-1	2	0.5	1	8258	16516	16557	33114	0.1	163108	20.30	33038	0.0404	1	0.005	15	0.2548
			0.5	2	8299	16598											
	6-2	2	0.5	1	9666	19332	19095	38190	0.1	163108	23.41	38114	0.0466	1	0.005	15	0.2939
			0.5	2	9429	18858											
	6-3	2	0.5	1	8057	16114	16065	32130	0.1	163108	19.70	32054	0.0392	1	0.005	15	0.2472
			0.5	2	8008	16016											
	7-1	2	0.5	1	8271	16542	16437	32874	0.1	163108	20.15	32798	0.0401	1	0.005	15	0.2529
			0.5	2	8166	16332											
	7-2	2	0.5	1	8649	17298	17353	34706	0.1	163108	21.28	34630	0.0423	1	0.005	15	0.2671
			0.5	2	8704	17408											
	7-3	2	0.5	1	8778	17556	17620	35240	0.1	163108	21.61	35164	0.0430	1	0.005	15	0.2712
			0.5	2	8842	17684											
	8-1	2	0.5	1	8432	16864	16901	33802	0.1	163108	20.72	33726	0.0412	1	0.005	15	0.2601
			0.5	2	8469	16938											
	8-2	2	0.5	1	8195	16390	16269	32538	0.1	163108	19.95	32462	0.0397	1	0.005	15	0.2504
			0.5	2	8074	16148											
	8-3	2	0.5	1	7991	15982	15388	30776	0.1	163108	18.87	30700	0.0375	1	0.005	15	0.2368
			0.5	2	7397	14794											

Assay Date	9/8/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	Replicate # 2 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.2935	0.0097
Full activity	End	0.2590	0.0121
Full activity	Overall	0.2763	0.0218
Background	Beginning	0.0001	0.000119975
Background	End	-0.0001	0.000185416
Background	Overall	0.0000	0.000141928
Positive	Beginning	0.1595	0.0073
Positive	End	0.1667	0.0039
Positive	Overall	0.1631	0.0063
Negative	Beginning	0.2695	0.0018
Negative	End	0.2664	0.0258
Negative	Overall	0.2680	0.0151

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 10	1	1	1.00E-03	-3.00	0.2576
Ref Chem 10	1	2	1.00E-03	-3.00	0.2597
Ref Chem 10	1	3	1.00E-03	-3.00	0.2357
Ref Chem 10	2	1	1.00E-04	-4.00	0.2690
Ref Chem 10	2	2	1.00E-04	-4.00	0.2585
Ref Chem 10	2	3	1.00E-04	-4.00	0.2726
Ref Chem 10	3	1	1.00E-05	-5.00	0.2760
Ref Chem 10	3	2	1.00E-05	-5.00	0.2627
Ref Chem 10	3	3	1.00E-05	-5.00	0.2561
Ref Chem 10	4	1	1.00E-06	-6.00	0.2706
Ref Chem 10	4	2	1.00E-06	-6.00	0.2443
Ref Chem 10	4	3	1.00E-06	-6.00	0.2649
Ref Chem 10	5	1	1.00E-07	-7.00	0.2742
Ref Chem 10	5	2	1.00E-07	-7.00	0.2797
Ref Chem 10	5	3	1.00E-07	-7.00	0.2587
Ref Chem 10	6	1	1.00E-08	-8.00	0.2548
Ref Chem 10	6	2	1.00E-08	-8.00	0.2939
Ref Chem 10	6	3	1.00E-08	-8.00	0.2472
Ref Chem 10	7	1	1.00E-09	-9.00	0.2529
Ref Chem 10	7	2	1.00E-09	-9.00	0.2671
Ref Chem 10	7	3	1.00E-09	-9.00	0.2712
Ref Chem 10	8	1	1.00E-10	-10.00	0.2601
Ref Chem 10	8	2	1.00E-10	-10.00	0.2504
Ref Chem 10	8	3	1.00E-10	-10.00	0.2368

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	93.24	94.01	85.30
2	-4.00	97.36	93.55	98.65
3	-5.00	99.88	95.07	92.70
4	-6.00	97.93	88.44	95.88
5	-7.00	99.24	101.24	93.64
6	-8.00	92.22	106.39	89.47
7	-9.00	91.55	96.66	98.15
8	-10.00	94.14	90.61	85.69

Aromatase Assay Spreadsheet

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8		
Technician ID	2	Replicate #	2	Microsome type	Recombina	Microsome ID	11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0196	31383	1601173	
2	0.0199	31776	1596784	
3	0.0198	33854	1709798	
4	0.0200	33828	1691400	
5	0.0205	34970	1705854	
			Average DPM/g soln	1661002
			SD	57053
			CV	3.43
			µCi/g soln	0.748

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	8.1193 g
Mass of dilution B used in substrate prep	4.5679 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.568224 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00847 µg/g soln.
µg/g soln.

a. µCi/g soln	0.748
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.

= 0.568224 + 0.00847
= 0.576693 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.297 µCi/µg ASDN

824894 dpm/nmol

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8				
Technician ID	2	Replicate #	2	Microsome type	Recombinant	Microsome ID	11345-10		

								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	
Blank	0.119		m, b	0.120	-0.002
			se_m, se_b	0.008	0.002
r^2=	0.982		r^2, se_y	0.982	0.003
m=	0.120		F, df	221	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	0.354	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	2	Replicate #	2	Microsome type	Recombinant	Microsome ID 11345-10

								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

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

Blank	0.119	r^2=	0.995
		m=	0.097
		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	0.354	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	Recombina	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.6
Protein Concentration (dilution added to assay, mg/mL):	0.009302

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	10765	21530	21548	43096	0.1	166100	25.95	42977	0.0521	1	0.005	15	0.3734
			0.5	2	10783	21566											
	2	2	0.5	1	10105	20210	20249	40498	0.1	166100	24.38	40379	0.0490	1	0.005	15	0.3508
			0.5	2	10144	20288											
	3	2	0.5	1	7596	15192	15138	30276	0.1	166100	18.23	30157	0.0366	1	0.005	15	0.2620
			0.5	2	7542	15084											
	4	2	0.5	1	9027	18054	18166	36332	0.1	166100	21.87	36213	0.0439	1	0.005	15	0.3146
			0.5	2	9139	18278											
Background control	1	2	0.5	1	20	40	54	108	0.1	166100	0.07	-11	0.0000	1	0.005	15	-0.0001
			0.5	2	34	68											
	2	2	0.5	1	32	64	59	118	0.1	166100	0.07	-1	0.0000	1	0.005	15	0.0000
			0.5	2	27	54											
	3	2	0.5	1	35	70	68	136	0.1	166100	0.08	17	0.0000	1	0.005	15	0.0001
			0.5	2	33	66											
	4	2	0.5	1	29	58	57	114	0.1	166100	0.07	-5	0.0000	1	0.005	15	0.0000
			0.5	2	28	56											
Positive control	1	2	0.5	1	5552	11104	11328	22656	0.1	166100	13.64	22537	0.0273	1	0.005	15	0.1958
			0.5	2	5776	11552											
	2	2	0.5	1	5314	10628	10655	21310	0.1	166100	12.83	21191	0.0257	1	0.005	15	0.1841
			0.5	2	5341	10682											
	3	2	0.5	1	5132	10264	10314	20628	0.1	166100	12.42	20509	0.0249	1	0.005	15	0.1782
			0.5	2	5182	10364											
	4	2	0.5	1	5650	11300	11721	23442	0.1	166100	14.11	23323	0.0283	1	0.005	15	0.2026
			0.5	2	6071	12142											
Negative Control	1	2	0.5	1	10980	21960	21768	43536	0.1	166100	26.21	43417	0.0526	1	0.005	15	0.3772
			0.5	2	10788	21576											
	2	2	0.5	1	10007	20014	20090	40180	0.1	166100	24.19	40061	0.0486	1	0.005	15	0.3481
			0.5	2	10083	20166											
	3	2	0.5	1	8512	17024	16982	33964	0.1	166100	20.45	33845	0.0410	1	0.005	15	0.2941
			0.5	2	8470	16940											
	4	2	0.5	1	10547	21094	21244	42488	0.1	166100	25.58	42369	0.0514	1	0.005	15	0.3681
			0.5	2	10697	21394											
Ref Chem 10	1-1	2	0.5	1	8737	17474	17418	34836	0.1	166100	20.97	34717	0.0421	1	0.005	15	0.3016
			0.5	2	8681	17362											
	1-2	2	0.5	1	7466	14932	14788	29576	0.1	166100	17.81	29457	0.0357	1	0.005	15	0.2559
			0.5	2	7322	14644											
	1-3	2	0.5	1	7633	15266	15122	30244	0.1	166100	18.21	30125	0.0365	1	0.005	15	0.2617
			0.5	2	7489	14978											
	2-1	2	0.5	1	8537	17074	17135	34270	0.1	166100	20.63	34151	0.0414	1	0.005	15	0.2967
			0.5	2	8598	17196											
	2-2	2	0.5	1	8745	17490	17270	34540	0.1	166100	20.79	34421	0.0417	1	0.005	15	0.2991
			0.5	2	8525	17050											
	2-3	2	0.5	1	8301	16602	16611	33222	0.1	166100	20.00	33103	0.0401	1	0.005	15	0.2876
			0.5	2	8310	16620											
	3-1	2	0.5	1	8613	17226	16685	33370	0.1	166100	20.09	33251	0.0403	1	0.005	15	0.2889
			0.5	2	8072	16144											
	3-2	2	0.5	1	8830	17660	17741	35482	0.1	166100	21.36	35363	0.0429	1	0.005	15	0.3072
			0.5	2	8911	17822											
	3-3	2	0.5	1	8634	17268	17244	34488	0.1	166100	20.76	34369	0.0417	1	0.005	15	0.2986
			0.5	2	8610	17220											
	4-1	2	0.5	1	9016	18032	17905	35810	0.1	166100	21.56	35691	0.0433	1	0.005	15	0.3101
			0.5	2	8889	17778											
	4-2	2	0.5	1	9545	19090	19082	38164	0.1	166100	22.98	38045	0.0461	1	0.005	15	0.3305
			0.5	2	9537	19074											
	4-3	2	0.5	1	9117	18234	18030	36060	0.1	166100	21.71	35941	0.0436	1	0.005	15	0.3123
			0.5	2	8913	17826											
	5-1	2	0.5	1	9653	19306	19261	38522	0.1	166100	23.19	38403	0.0466	1	0.005	15	0.3337
			0.5	2	9608	19216											
	5-2	2	0.5	1	9205	18410	18616	37232	0.1	166100	22.42	37113	0.0450	1	0.005	15	0.3224
			0.5	2	9411	18822											
	5-3	2	0.5	1	9128	18256	18311	36622	0.1	166100	22.05	36503	0.0443	1	0.005	15	0.3171
			0.5	2	9183	18366											
	6-1	2	0.5	1	9341	18682	18848	37696	0.1	166100	22.69	37577	0.0456	1	0.005	15	0.3265
			0.5	2	9507	19014											
	6-2	2	0.5	1	9597	19194	19139	38278	0.1	166100	23.05	38159	0.0463	1	0.005	15	0.3315
			0.5	2	9542	19084											
	6-3	2	0.5	1	8874	17748	17607	35214	0.1	166100	21.20	35095	0.0425	1	0.005	15	0.3049
			0.5	2	8733	17466											
	7-1	2	0.5	1	9437	18874	18901	37802	0.1	166100	22.76	37683	0.0457	1	0.005	15	0.3274
			0.5	2	9464	18928											
	7-2	2	0.5	1	8848	17696	17822	35644	0.1	166100	21.46	35525	0.0431	1	0.005	15	0.3087
			0.5	2	8974	17948											
	7-3	2	0.5	1	9967	19934	20009	40018	0.1	166100	24.09	39899	0.0484	1	0.005	15	0.3467
			0.5	2	10042	20084											
	8-1	2	0.5	1	8772	17544	17574	35148	0.1	166100	21.16	35029	0.0425	1	0.005	15	0.3043
			0.5	2	8802	17604											
	8-2	2	0.5	1	10911	21822	21013	42026	0.1	166100	25.30	41907	0.0508	1	0.005	15	0.3641
			0.5	2	10102	20204											
	8-3	2	0.5	1	10724	21448	21470	42940	0.1	166100	25.85	42821	0.0519	1	0.005	15	0.3720
			0.5	2	10746	21492											

Assay Date	9/13/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	2	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3621	0.0160
Full activity	End	0.2883	0.0372
Full activity	Overall	0.3252	0.0486
Background	Beginning	-0.0001	6.14356E-05
Background	End	0.0001	0.000135158
Background	Overall	0.0000	0.000104741
Positive	Beginning	0.1900	0.0083
Positive	End	0.1904	0.0173
Positive	Overall	0.1902	0.0111
Negative	Beginning	0.3626	0.0206
Negative	End	0.3311	0.0524
Negative	Overall	0.3469	0.0373

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 10	1	1	1.00E-03	-3.00	0.3016
Ref Chem 10	1	2	1.00E-03	-3.00	0.2559
Ref Chem 10	1	3	1.00E-03	-3.00	0.2617
Ref Chem 10	2	1	5.00E-04	-3.30	0.2967
Ref Chem 10	2	2	5.00E-04	-3.30	0.2991
Ref Chem 10	2	3	5.00E-04	-3.30	0.2876
Ref Chem 10	3	1	2.50E-04	-3.60	0.2889
Ref Chem 10	3	2	2.50E-04	-3.60	0.3072
Ref Chem 10	3	3	2.50E-04	-3.60	0.2986
Ref Chem 10	4	1	1.00E-04	-4.00	0.3101
Ref Chem 10	4	2	1.00E-04	-4.00	0.3305
Ref Chem 10	4	3	1.00E-04	-4.00	0.3123
Ref Chem 10	5	1	5.00E-05	-4.30	0.3337
Ref Chem 10	5	2	5.00E-05	-4.30	0.3224
Ref Chem 10	5	3	5.00E-05	-4.30	0.3171
Ref Chem 10	6	1	1.00E-05	-5.00	0.3265
Ref Chem 10	6	2	1.00E-05	-5.00	0.3315
Ref Chem 10	6	3	1.00E-05	-5.00	0.3049
Ref Chem 10	7	1	1.00E-06	-6.00	0.3274
Ref Chem 10	7	2	1.00E-06	-6.00	0.3087
Ref Chem 10	7	3	1.00E-06	-6.00	0.3467
Ref Chem 10	8	1	1.00E-07	-7.00	0.3043
Ref Chem 10	8	2	1.00E-07	-7.00	0.3641
Ref Chem 10	8	3	1.00E-07	-7.00	0.3720

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	92.75	78.70	80.48
2	-3.30	91.24	91.96	88.44
3	-3.60	88.83	94.47	91.82
4	-4.00	95.35	101.64	96.02
5	-4.30	102.60	99.15	97.52
6	-5.00	100.39	101.94	93.76
7	-6.00	100.67	94.91	106.59
8	-7.00	93.58	111.96	114.40

Aromatase Assay Spreadsheet

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8
Technician ID	2	Replicate #	3	Microsome type	Recombina Microsome ID 11345-10

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	29591	1494495	
2	0.0197	29345	1489594	
3	0.0199	32377	1626985	
4	0.0200	34301	1715050	
5	0.0198	32649	1648939	
			Average DPM/g soln	1595013
			SD	99444
			CV	6.23
			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	10.2	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	8.1078 g
Mass of dilution B used in substrate prep	4.5707 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.575016 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.

= 0.575016 + 0.00813
= 0.583149 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.232 µCi/µg ASDN

783353 dpm/nmol

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8						
Technician ID	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.5	100	11540-54
	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			

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	Variables	Regression results	
250	200	200	0.00026	200	0.0510	0.549	0.433	0.0486	m, b	0.118	-0.002
125	100	200	0.00013	200	0.0255	0.376	0.260	0.0282	se_m, se_b	0.007	0.002
50	200	1000	0.00005	200	0.0102	0.245	0.129	0.0127	r^2, se_y	0.985	0.003
25	100	1000	0.00003	200	0.0051	0.187	0.071	0.0059	F, df	268	4
10	40	1000	0.00001	200	0.0020	0.146	0.030	0.0011	ss_{regr}, ss_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.123	0.007	-0.0016			

Regression results are calculated using the function LINEST

Blank	0.116	r^2=	0.985	
		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	0.359	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	2	Replicate #	3	Microsome type	Recombinant	Microsome ID	11345-10		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25.5	100	11540-54
	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			

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

Blank 0.116

r^2= 0.986
m= 0.099
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	0.359	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	Recombina	Microsome ID	11345-10	Technician ID	Replicate #	2	3

Microsome Dilution Details		
Dilution A	0.17	mL microsome Stock used
	8.5	mL total volume
	50	dilution factor
Dilution B	7	mL microsome Dilution A used
	84	mL total volume
	12	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.9
Protein Concentration (dilution added to assay, mg/mL):	0.009859

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	Recombinan	Microsome ID	11345-10	Technician ID	2	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	9357	18714	18654	37308	0.1	159501	23.39	37192	0.0475	1	0.005	15	0.3211
			0.5	2	9297	18594											
	2	2	0.5	1	8994	17988	17741	35482	0.1	159501	22.25	35366	0.0451	1	0.005	15	0.3053
			0.5	2	8747	17494											
	3	2	0.5	1	8510	17020	16862	33724	0.1	159501	21.14	33608	0.0429	1	0.005	15	0.2901
			0.5	2	8352	16704											
	4	2	0.5	1	8304	16608	16518	33036	0.1	159501	20.71	32920	0.0420	1	0.005	15	0.2842
			0.5	2	8214	16428											
Background control	1	2	0.5	1	16	32	36	72	0.1	159501	0.05	-44	-0.0001	1	0.005	15	-0.0004
			0.5	2	20	40											
	2	2	0.5	1	43	86	72	144	0.1	159501	0.09	28	0.0000	1	0.005	15	0.0002
			0.5	2	29	58											
	3	2	0.5	1	30	60	60	120	0.1	159501	0.08	4	0.0000	1	0.005	15	0.0000
			0.5	2	30	60											
	4	2	0.5	1	41	82	64	128	0.1	159501	0.08	12	0.0000	1	0.005	15	0.0001
			0.5	2	23	46											
Positive control	1	2	0.5	1	4854	9708	9527	19054	0.1	159501	11.95	18938	0.0242	1	0.005	15	0.1635
			0.5	2	4673	9346											
	2	2	0.5	1	4971	9942	10062	20124	0.1	159501	12.62	20008	0.0255	1	0.005	15	0.1727
			0.5	2	5091	10182											
	3	2	0.5	1	4420	8840	8728	17456	0.1	159501	10.94	17340	0.0221	1	0.005	15	0.1497
			0.5	2	4308	8616											
	4	2	0.5	1	4619	9238	9292	18584	0.1	159501	11.65	18468	0.0236	1	0.005	15	0.1594
			0.5	2	4673	9346											
Negative Control	1	2	0.5	1	8150	16300	16262	32564	0.1	159501	20.42	32448	0.0414	1	0.005	15	0.2801
			0.5	2	8132	16264											
	2	2	0.5	1	8746	17492	17339	34678	0.1	159501	21.74	34562	0.0441	1	0.005	15	0.2984
			0.5	2	8593	17186											
	3	2	0.5	1	7820	15640	15499	30998	0.1	159501	19.43	30882	0.0394	1	0.005	15	0.2666
			0.5	2	7679	15358											
	4	2	0.5	1	8440	16880	16854	33708	0.1	159501	21.13	33592	0.0429	1	0.005	15	0.2900
			0.5	2	8414	16828											
Ref Chem 10	1-1	2	0.5	1	7054	14108	13936	27872	0.1	159501	17.47	27756	0.0354	1	0.005	15	0.2396
			0.5	2	6882	13764											
	1-2	2	0.5	1	7297	14594	14482	28964	0.1	159501	18.16	28848	0.0368	1	0.005	15	0.2490
			0.5	2	7185	14370											
	1-3	2	0.5	1	7368	14732	14726	29452	0.1	159501	18.47	29336	0.0374	1	0.005	15	0.2532
			0.5	2	7360	14720											
	2-1	2	0.5	1	7515	15030	14868	29736	0.1	159501	18.64	29620	0.0378	1	0.005	15	0.2557
			0.5	2	7353	14706											
	2-2	2	0.5	1	7597	15194	14925	29850	0.1	159501	18.71	29734	0.0380	1	0.005	15	0.2567
			0.5	2	7328	14656											
	2-3	2	0.5	1	7296	14592	14524	29048	0.1	159501	18.21	28932	0.0369	1	0.005	15	0.2498
			0.5	2	7228	14456											
	3-1	2	0.5	1	7977	15954	15800	31600	0.1	159501	19.81	31484	0.0402	1	0.005	15	0.2718
			0.5	2	7823	15646											
	3-2	2	0.5	1	7626	15252	15247	30494	0.1	159501	19.12	30378	0.0388	1	0.005	15	0.2622
			0.5	2	7621	15242											
	3-3	2	0.5	1	7561	15122	15624	31248	0.1	159501	19.59	31132	0.0397	1	0.005	15	0.2687
			0.5	2	8063	16126											
	4-1	2	0.5	1	8382	16764	16736	33472	0.1	159501	20.99	33356	0.0426	1	0.005	15	0.2879
			0.5	2	8354	16708											
	4-2	2	0.5	1	8211	16422	16287	32574	0.1	159501	20.42	32458	0.0414	1	0.005	15	0.2802
			0.5	2	8076	16152											
	4-3	2	0.5	1	8283	16566	16432	32864	0.1	159501	20.60	32748	0.0418	1	0.005	15	0.2827
			0.5	2	8149	16298											
	5-1	2	0.5	1	7782	15524	15281	30562	0.1	159501	19.16	30446	0.0389	1	0.005	15	0.2628
			0.5	2	7519	15038											
	5-2	2	0.5	1	8462	16924	16618	33236	0.1	159501	20.84	33120	0.0423	1	0.005	15	0.2859
			0.5	2	8156	16312											
	5-3	2	0.5	1	8329	16658	16328	32656	0.1	159501	20.47	32540	0.0415	1	0.005	15	0.2809
			0.5	2	7999	15998											
	6-1	2	0.5	1	8410	16820	16570	33140	0.1	159501	20.78	33024	0.0422	1	0.005	15	0.2851
			0.5	2	8160	16320											
	6-2	2	0.5	1	8554	17108	17049	34098	0.1	159501	21.38	33982	0.0434	1	0.005	15	0.2933
			0.5	2	8495	16990											
	6-3	2	0.5	1	8313	16626	16395	32790	0.1	159501	20.56	32674	0.0417	1	0.005	15	0.2821
			0.5	2	8082	16164											
	7-1	2	0.5	1	8564	17128	16910	33820	0.1	159501	21.20	33704	0.0430	1	0.005	15	0.2909
			0.5	2	8346	16692											
	7-2	2	0.5	1	8187	16374	16247	32494	0.1	159501	20.37	32378	0.0413	1	0.005	15	0.2795
			0.5	2	8060	16120											
	7-3	2	0.5	1	8211	16422	16319	32638	0.1	159501	20.46	32522	0.0415	1	0.005	15	0.2807
			0.5	2	8108	16216											
	8-1	2	0.5	1	8652	17304	17111	34222	0.1	159501	21.46	34106	0.0435	1	0.005	15	0.2944
			0.5	2	8459	16918											
	8-2	2	0.5	1	7767	15534	15469	30938	0.1	159501	19.40	30822	0.0393	1	0.005	15	0.2661
			0.5	2	7702	15404											
	8-3	2	0.5	1	7913	15826	15623	31246	0.1	159501	19.59	31130	0.0397	1	0.005	15	0.2687
			0.5	2	7710	15420											

Assay Date	9/15/2005	Test Chemical ID	Ref Chem 10	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	11345-10	Technician ID	Replicate # 2	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3132	0.0111
Full activity	End	0.2871	0.0042
Full activity	Overall	0.3002	0.0165
Background	Beginning	-0.0001	0.000439486
Background	End	0.0001	4.88318E-05
Background	Overall	0.0000	0.000267463
Positive	Beginning	0.1681	0.0065
Positive	End	0.1546	0.0069
Positive	Overall	0.1613	0.0095
Negative	Beginning	0.2892	0.0129
Negative	End	0.2783	0.0165
Negative	Overall	0.2838	0.0137

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Ref Chem 10	1	1	1.00E-03	-3.00	0.2396
Ref Chem 10	1	2	1.00E-03	-3.00	0.2490
Ref Chem 10	1	3	1.00E-03	-3.00	0.2532
Ref Chem 10	2	1	5.00E-04	-3.30	0.2557
Ref Chem 10	2	2	5.00E-04	-3.30	0.2567
Ref Chem 10	2	3	5.00E-04	-3.30	0.2498
Ref Chem 10	3	1	2.50E-04	-3.60	0.2718
Ref Chem 10	3	2	2.50E-04	-3.60	0.2622
Ref Chem 10	3	3	2.50E-04	-3.60	0.2687
Ref Chem 10	4	1	1.00E-04	-4.00	0.2879
Ref Chem 10	4	2	1.00E-04	-4.00	0.2802
Ref Chem 10	4	3	1.00E-04	-4.00	0.2827
Ref Chem 10	5	1	5.00E-05	-4.30	0.2628
Ref Chem 10	5	2	5.00E-05	-4.30	0.2859
Ref Chem 10	5	3	5.00E-05	-4.30	0.2809
Ref Chem 10	6	1	1.00E-05	-5.00	0.2851
Ref Chem 10	6	2	1.00E-05	-5.00	0.2933
Ref Chem 10	6	3	1.00E-05	-5.00	0.2821
Ref Chem 10	7	1	1.00E-06	-6.00	0.2909
Ref Chem 10	7	2	1.00E-06	-6.00	0.2795
Ref Chem 10	7	3	1.00E-06	-6.00	0.2807
Ref Chem 10	8	1	1.00E-07	-7.00	0.2944
Ref Chem 10	8	2	1.00E-07	-7.00	0.2661
Ref Chem 10	8	3	1.00E-07	-7.00	0.2687

Percent of control values				
		Replicate		
Level	Log[test substance]	1	2	3
1	-3.00	79.82	82.96	84.37
2	-3.30	85.18	85.51	83.21
3	-3.60	90.55	87.36	89.53
4	-4.00	95.93	93.35	94.18
5	-4.30	87.56	95.25	93.58
6	-5.00	94.97	97.73	93.97
7	-6.00	96.93	93.12	93.53
8	-7.00	98.09	88.64	89.53

Appendix E

Statistical Analysis Report

Report on the Statistical Analysis for WA 4-17, Task 4

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	95.82061	1.34722	92.96463	98.67660
AG	Bottom	-0.89731	2.50853	-6.21515	4.42053
AG	Log10(IC50)	-4.03116	0.03746	-4.11058	-3.95175
AG	Slope	-0.93653	0.04748	-1.03719	-0.83588
KCZ	Top	93.26440	2.73269	87.47135	99.05745
KCZ	Bottom	-0.21638	1.03660	-2.41387	1.98110
KCZ	Log10(IC50)	-6.27942	0.03689	-6.35763	-6.20121
KCZ	Slope	-0.98033	0.06016	-1.10786	-0.85281
PCZ	Top	95.73157	0.81576	94.00223	97.46092
PCZ	Bottom	0.07669	0.69840	-1.40384	1.55723
PCZ	Log10(IC50)	-7.48146	0.01823	-7.52011	-7.44282
PCZ	Slope	-0.97325	0.03510	-1.04765	-0.89884
NYP	Top	101.1217	1.52616	97.88635	104.3570
NYP	Bottom	1.22119	1.37606	-1.69593	4.13830
NYP	Log10(IC50)	-4.66574	0.01697	-4.70171	-4.62977
NYP	Slope	-2.26351	0.16816	-2.61998	-1.90704
FRM	Top	90.75079	0.45236	89.79183	91.70974
FRM	Bottom	0.89138	0.66367	-0.51554	2.29830
FRM	Log10(IC50)	-5.19541	0.02264	-5.24341	-5.14741
FRM	Slope	-1.02268	0.03361	-1.09394	-0.95142
ECZ	Top	99.78080	1.31892	96.98481	102.5768
ECZ	Bottom	0.53086	0.72825	-1.01296	2.07468
ECZ	Log10(IC50)	-8.60351	0.01772	-8.64109	-8.56594

Chemical	Dependent	Mean	Standard Error	Lower 95% CI	Upper 95% CI
ECZ	Slope	-1.17354	0.04173	-1.26199	-1.08508
CYN	Top	91.91506	1.03986	89.71067	94.11946
CYN	Bottom	22.58026	1.38057	19.65359	25.50693
CYN	Log10(IC50)	-5.66286	0.03428	-5.73553	-5.59018
CYN	Slope	-1.30421	0.08545	-1.48535	-1.12306
DCF	Top	98.99385	3.49811	91.57819	106.4095
DCF	Bottom	-0.93388	1.72861	-4.59837	2.73060
DCF	Log10(IC50)	-4.44618	0.03287	-4.51585	-4.37650
DCF	Slope	-0.97429	0.08657	-1.15781	-0.79078

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 between ±14%, and have no discernable pattern in the variation of the percent of control values above and below the 0% line or by portion, except that there appears to be less variation in the percent of control values for the last 4 chemicals (ECZ, CYN, DCF and ATZ). Exhibit 38 shows percent of control values for full activity control that, for the most part, vary between 80% and 120% 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 80% and 120% of control. The percent of control values for the first 2 chemicals (AG and KCZ) only have percent of control values at 100% of control and lower, whereas the rest of the chemicals have percent of control values somewhat evenly distributed above and below 100% of control. Exhibit 40 shows percent of control values for positive control that vary between 45% and 70% and also display the pattern of higher beginning portion values and lower end portion values for most of the chemicals and replicates. Exhibits 41 through 44 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. All of the percent of control differences for background control in Exhibit 41 appear along the zero percent reference line, however, the majority of the percent of control differences for full activity, negative and positive control in Exhibits 42 through 44 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 except background control. The reference chemical effect was not significant for any of the control types. The reference chemical by portion interaction term was significant for each control type except negative 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 and full activity control are essentially zero, along with the estimates for each of the portion by replicate within chemical interaction terms.

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	0.36	0.5545
Background control	Chemical*Portion	9	20	2.85	0.0245
Full activity control	Chemical	9	20	0.05	0.9999
Full activity control	Portion	1	20	276.99	<.0001
Full activity control	Chemical*Portion	9	20	3.53	0.0090
Negative Control	Chemical	9	20	0.88	0.5601
Negative Control	Portion	1	20	39.25	<.0001
Negative Control	Chemical*Portion	9	20	1.70	0.1542
Positive control	Chemical	9	20	1.16	0.3700
Positive control	Portion	1	20	67.29	<.0001
Positive control	Chemical*Portion	9	20	4.84	0.0016

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)	1E-30	.	.	.
Background control	Residual	0.003901	0.000552	7.07	<.0001
Full activity control	Replicate(Chemical)	1E-30	.	.	.
Full activity control	Portion* Replicate(Chemical)	1E-30	.	.	.
Full activity control	Residual	21.4916	3.0701	7.00	<.0001
Negative Control	Replicate(Chemical)	14.8540	8.8335	1.68	0.0927
Negative Control	Portion* Replicate(Chemical)	1E-30	.	.	.
Negative Control	Residual	49.5401	7.8330	6.32	<.0001
Positive control	Replicate(Chemical)	9.2443	3.6239	2.55	0.0107
Positive control	Portion* Replicate(Chemical)	1E-30	.	.	.
Positive control	Residual	8.6588	1.3690	6.33	<.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 reference chemicals NYP, DBA, ECZ and CYN for the variation in the background control percent of control values. The portion effects within reference chemical for the negative control are significant for reference chemicals PCZ, NYP, DBA and FRM while AG also displays some borderline significance. For the full activity control, every reference chemical contains significant portion effects. All of the reference chemicals except KCZ, DCF and ATZ have significant portion effects for the positive control values. The significant portion effects results within most of the reference chemicals for full activity and positive control 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	-0.02688	0.02550
Background control	Chemical*Portion	AG	End	0.02688	0.02550
Background control	Chemical*Portion	KCZ	Begin	-0.00700	0.02550
Background control	Chemical*Portion	KCZ	End	0.007004	0.02550
Background control	Chemical*Portion	PCZ	Begin	-0.00723	0.02550
Background control	Chemical*Portion	PCZ	End	0.007234	0.02550

Control	Effect	Chemical	Portion	Estimate	Standard Error
Background control	Chemical*Portion	NYP	Begin	0.04951	0.02550
Background control	Chemical*Portion	NYP	End	-0.04951	0.02550
Background control	Chemical*Portion	DBA	Begin	0.04951	0.02550
Background control	Chemical*Portion	DBA	End	-0.04951	0.02550
Background control	Chemical*Portion	FRM	Begin	-0.00723	0.02550
Background control	Chemical*Portion	FRM	End	0.007234	0.02550
Background control	Chemical*Portion	ECZ	Begin	-0.03597	0.02550
Background control	Chemical*Portion	ECZ	End	0.03597	0.02550
Background control	Chemical*Portion	CYN	Begin	-0.03597	0.02550
Background control	Chemical*Portion	CYN	End	0.03597	0.02550
Background control	Chemical*Portion	DCF	Begin	-0.00650	0.02550
Background control	Chemical*Portion	DCF	End	0.006499	0.02550
Background control	Chemical*Portion	ATZ	Begin	-0.00650	0.02550
Background control	Chemical*Portion	ATZ	End	0.006499	0.02550
Full activity control	Chemical*Portion	AG	Begin	106.54	1.8926
Full activity control	Chemical*Portion	AG	End	93.4558	1.8926
Full activity control	Chemical*Portion	KCZ	Begin	104.70	1.8926
Full activity control	Chemical*Portion	KCZ	End	95.2999	1.8926
Full activity control	Chemical*Portion	PCZ	Begin	109.26	2.0732
Full activity control	Chemical*Portion	PCZ	End	92.2798	1.8926
Full activity control	Chemical*Portion	NYP	Begin	103.63	1.8926
Full activity control	Chemical*Portion	NYP	End	96.3656	1.8926
Full activity control	Chemical*Portion	DBA	Begin	103.63	1.8926
Full activity control	Chemical*Portion	DBA	End	96.3656	1.8926
Full activity control	Chemical*Portion	FRM	Begin	109.26	2.0732
Full activity control	Chemical*Portion	FRM	End	92.2798	1.8926
Full activity control	Chemical*Portion	ECZ	Begin	110.51	1.8926
Full activity control	Chemical*Portion	ECZ	End	89.4899	1.8926
Full activity control	Chemical*Portion	CYN	Begin	110.51	1.8926
Full activity control	Chemical*Portion	CYN	End	89.4899	1.8926
Full activity control	Chemical*Portion	DCF	Begin	107.31	1.8926
Full activity control	Chemical*Portion	DCF	End	92.6919	1.8926
Full activity control	Chemical*Portion	ATZ	Begin	107.31	1.8926
Full activity control	Chemical*Portion	ATZ	End	92.6919	1.8926

Control	Effect	Chemical	Portion	Estimate	Standard Error
Negative Control	Chemical*Portion	AG	Begin	97.2068	3.6343
Negative Control	Chemical*Portion	AG	End	90.7479	3.6343
Negative Control	Chemical*Portion	KCZ	Begin	95.4767	3.6343
Negative Control	Chemical*Portion	KCZ	End	93.9611	3.6343
Negative Control	Chemical*Portion	PCZ	Begin	110.69	3.6343
Negative Control	Chemical*Portion	PCZ	End	93.3131	3.6343
Negative Control	Chemical*Portion	NYP	Begin	105.51	3.6343
Negative Control	Chemical*Portion	NYP	End	96.9927	3.6343
Negative Control	Chemical*Portion	DBA	Begin	105.51	3.6343
Negative Control	Chemical*Portion	DBA	End	96.9927	3.6343
Negative Control	Chemical*Portion	FRM	Begin	110.69	3.6343
Negative Control	Chemical*Portion	FRM	End	93.3131	3.6343
Negative Control	Chemical*Portion	ECZ	Begin	102.37	3.6343
Negative Control	Chemical*Portion	ECZ	End	96.8226	3.6343
Negative Control	Chemical*Portion	CYN	Begin	102.37	3.6343
Negative Control	Chemical*Portion	CYN	End	96.8226	3.6343
Negative Control	Chemical*Portion	DCF	Begin	101.80	3.6343
Negative Control	Chemical*Portion	DCF	End	96.9818	3.6343
Negative Control	Chemical*Portion	ATZ	Begin	101.80	3.6343
Negative Control	Chemical*Portion	ATZ	End	96.9818	3.6343
Positive control	Chemical*Portion	AG	Begin	58.5147	2.1271
Positive control	Chemical*Portion	AG	End	54.1086	2.1271
Positive control	Chemical*Portion	KCZ	Begin	59.5386	2.1271
Positive control	Chemical*Portion	KCZ	End	58.3089	2.1271
Positive control	Chemical*Portion	PCZ	Begin	64.8324	2.1271
Positive control	Chemical*Portion	PCZ	End	54.0075	2.1271
Positive control	Chemical*Portion	NYP	Begin	55.1533	2.1271
Positive control	Chemical*Portion	NYP	End	52.1118	2.1271
Positive control	Chemical*Portion	DBA	Begin	55.1533	2.1271
Positive control	Chemical*Portion	DBA	End	52.1118	2.1271
Positive control	Chemical*Portion	FRM	Begin	64.8324	2.1271
Positive control	Chemical*Portion	FRM	End	54.0075	2.1271
Positive control	Chemical*Portion	ECZ	Begin	60.1730	2.1271
Positive control	Chemical*Portion	ECZ	End	55.4083	2.1271

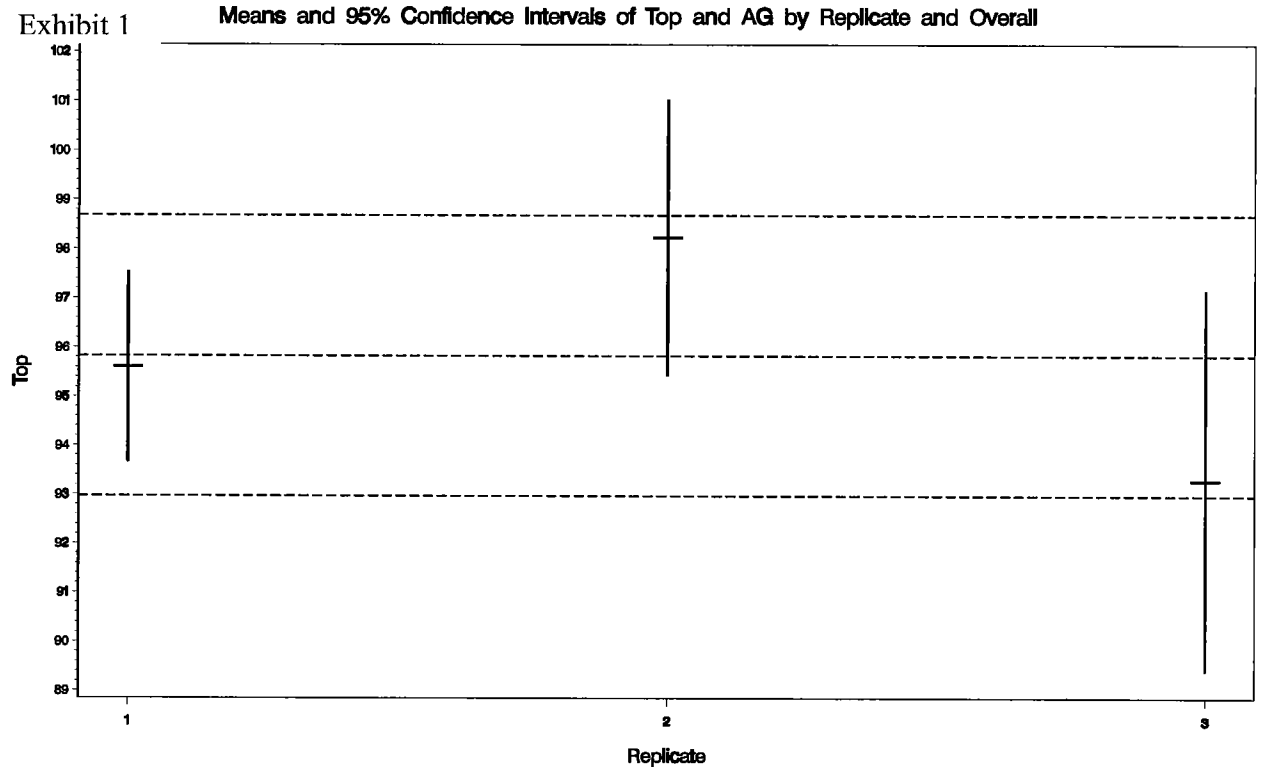
Control	Effect	Chemical	Portion	Estimate	Standard Error
Positive control	Chemical*Portion	CYN	Begin	60.1730	2.1271
Positive control	Chemical*Portion	CYN	End	55.4083	2.1271
Positive control	Chemical*Portion	DCF	Begin	57.3814	2.1271
Positive control	Chemical*Portion	DCF	End	56.7954	2.1271
Positive control	Chemical*Portion	ATZ	Begin	57.3814	2.1271
Positive control	Chemical*Portion	ATZ	End	56.7954	2.1271

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	2.22	0.1516
Background control	Chemical*Portion	KCZ	1	20	0.15	0.7017
Background control	Chemical*Portion	PCZ	1	20	0.16	0.6925
Background control	Chemical*Portion	NYP	1	20	7.54	0.0125
Background control	Chemical*Portion	DBA	1	20	7.54	0.0125
Background control	Chemical*Portion	FRM	1	20	0.16	0.6925
Background control	Chemical*Portion	ECZ	1	20	3.98	0.0598
Background control	Chemical*Portion	CYN	1	20	3.98	0.0598
Background control	Chemical*Portion	DCF	1	20	0.13	0.7223
Background control	Chemical*Portion	ATZ	1	20	0.13	0.7223
Full activity control	Chemical*Portion	AG	1	20	23.91	<.0001
Full activity control	Chemical*Portion	KCZ	1	20	12.33	0.0022
Full activity control	Chemical*Portion	PCZ	1	20	36.61	<.0001
Full activity control	Chemical*Portion	NYP	1	20	7.38	0.0133
Full activity control	Chemical*Portion	DBA	1	20	7.38	0.0133
Full activity control	Chemical*Portion	FRM	1	20	36.61	<.0001
Full activity control	Chemical*Portion	ECZ	1	20	61.68	<.0001
Full activity control	Chemical*Portion	CYN	1	20	61.68	<.0001
Full activity control	Chemical*Portion	DCF	1	20	29.82	<.0001
Full activity control	Chemical*Portion	ATZ	1	20	29.82	<.0001
Negative Control	Chemical*Portion	AG	1	20	2.53	0.1276
Negative Control	Chemical*Portion	KCZ	1	20	0.14	0.7131
Negative Control	Chemical*Portion	PCZ	1	20	18.28	0.0004
Negative Control	Chemical*Portion	NYP	1	20	4.40	0.0489
Negative Control	Chemical*Portion	DBA	1	20	4.40	0.0489

Control	Effect	Chemical	Num DF	Den DF	F Value	Pr > F
Negative Control	Chemical*Portion	FRM	1	20	18.28	0.0004
Negative Control	Chemical*Portion	ECZ	1	20	1.87	0.1872
Negative Control	Chemical*Portion	CYN	1	20	1.87	0.1872
Negative Control	Chemical*Portion	DCF	1	20	1.41	0.2492
Negative Control	Chemical*Portion	ATZ	1	20	1.41	0.2492
Positive control	Chemical*Portion	AG	1	20	6.73	0.0174
Positive control	Chemical*Portion	KCZ	1	20	0.52	0.4776
Positive control	Chemical*Portion	PCZ	1	20	40.60	<.0001
Positive control	Chemical*Portion	NYP	1	20	3.21	0.0886
Positive control	Chemical*Portion	DBA	1	20	3.21	0.0886
Positive control	Chemical*Portion	FRM	1	20	40.60	<.0001
Positive control	Chemical*Portion	ECZ	1	20	7.87	0.0109
Positive control	Chemical*Portion	CYN	1	20	7.87	0.0109
Positive control	Chemical*Portion	DCF	1	20	0.12	0.7338
Positive control	Chemical*Portion	ATZ	1	20	0.12	0.7338

Exhibit 1



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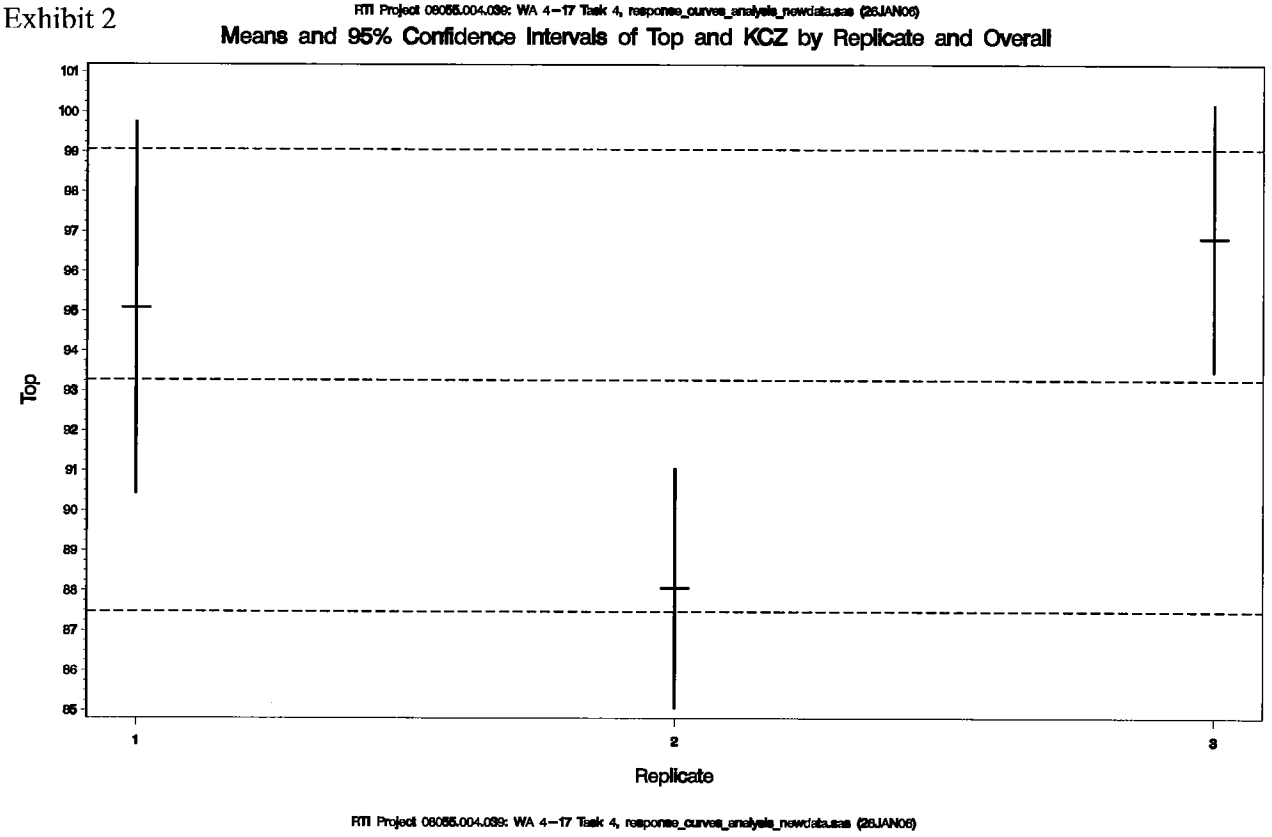

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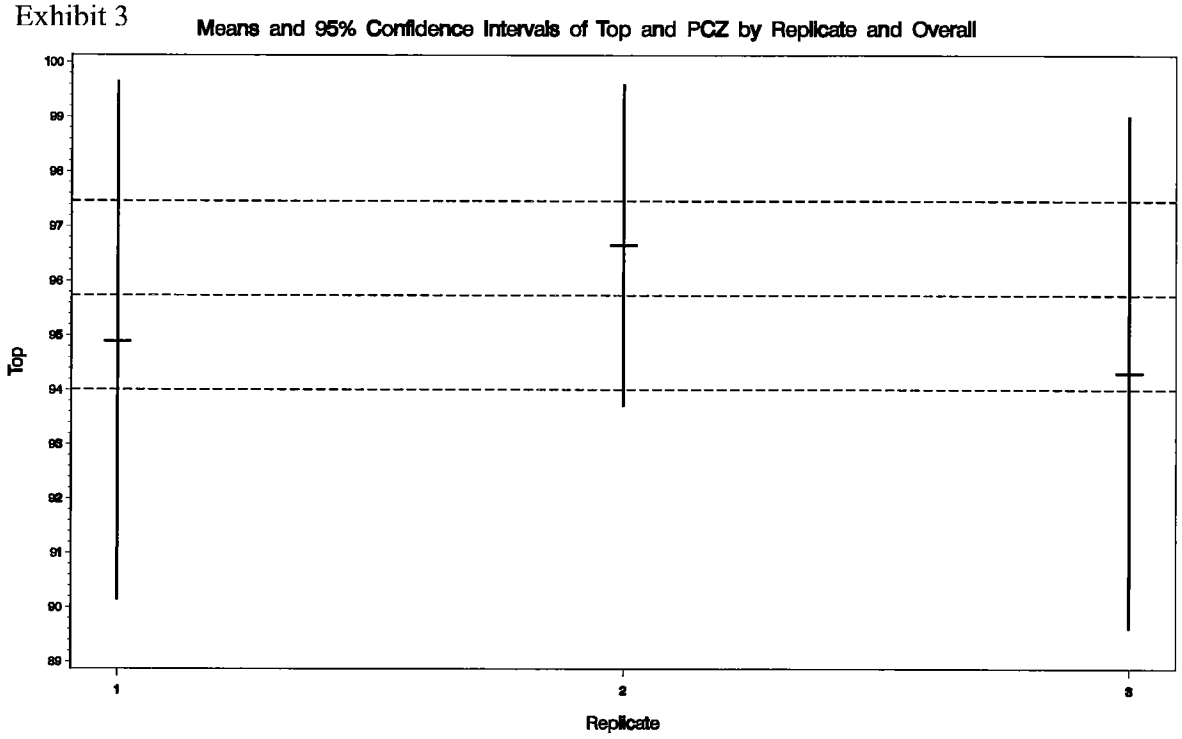

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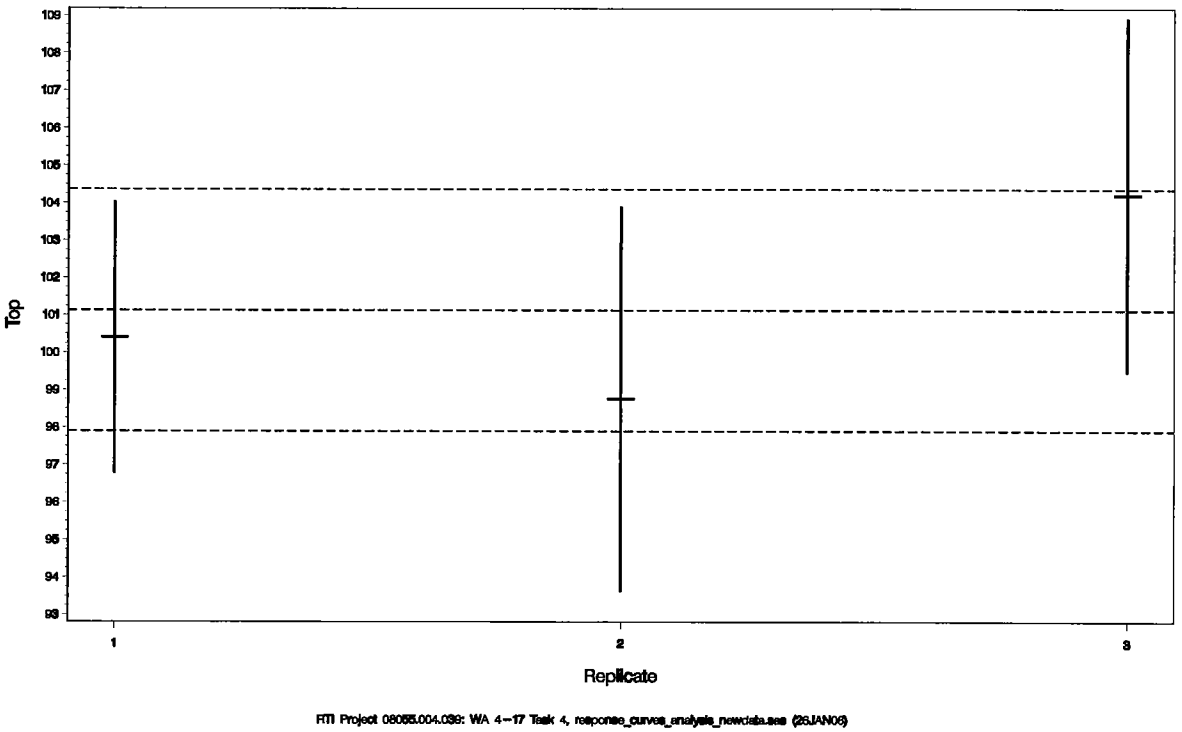

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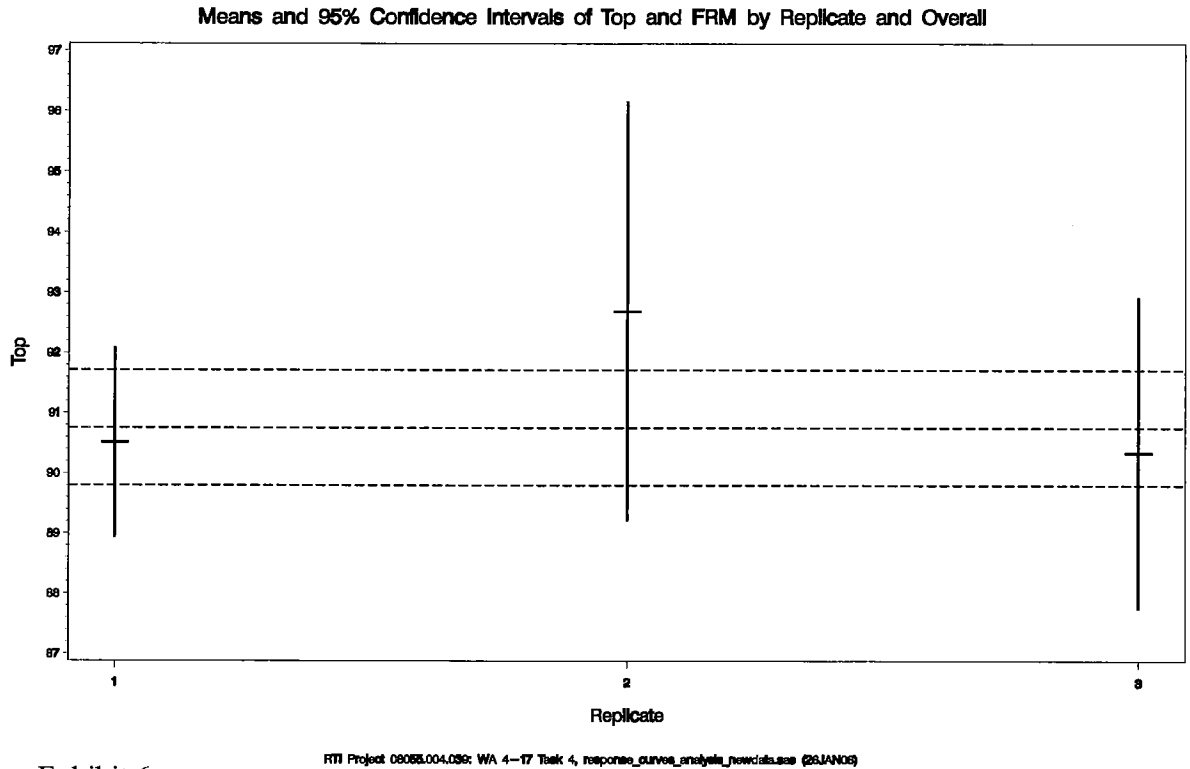

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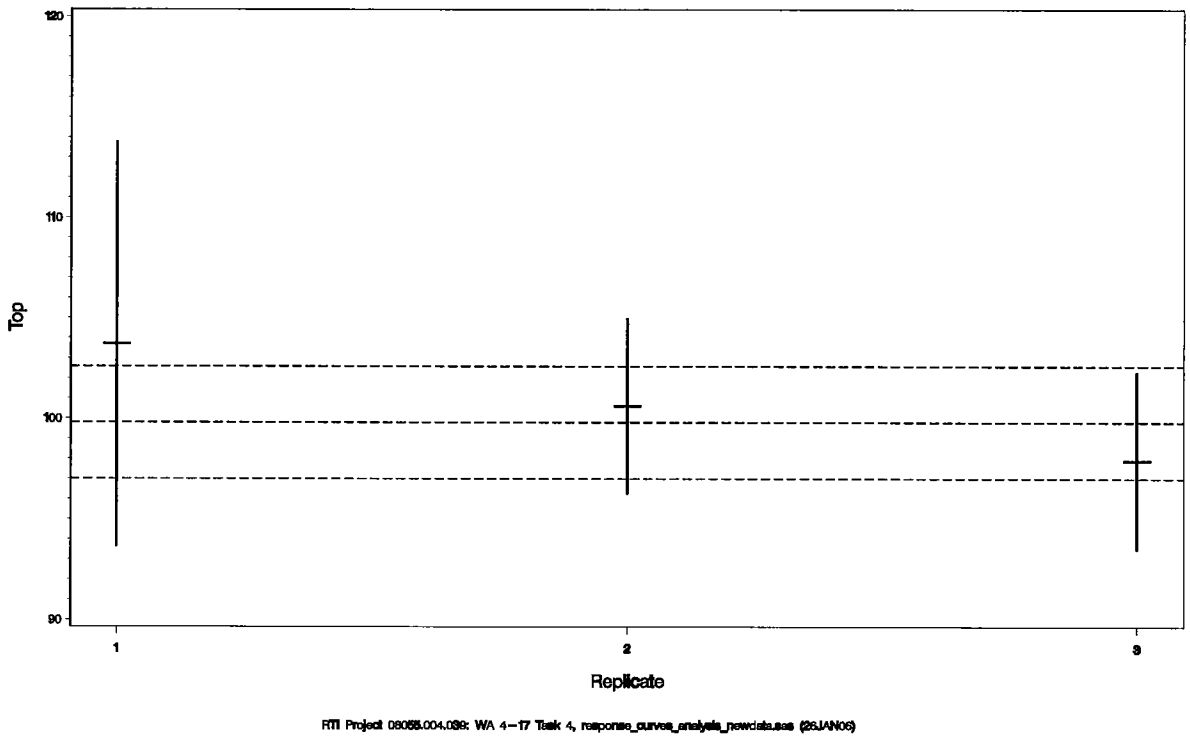

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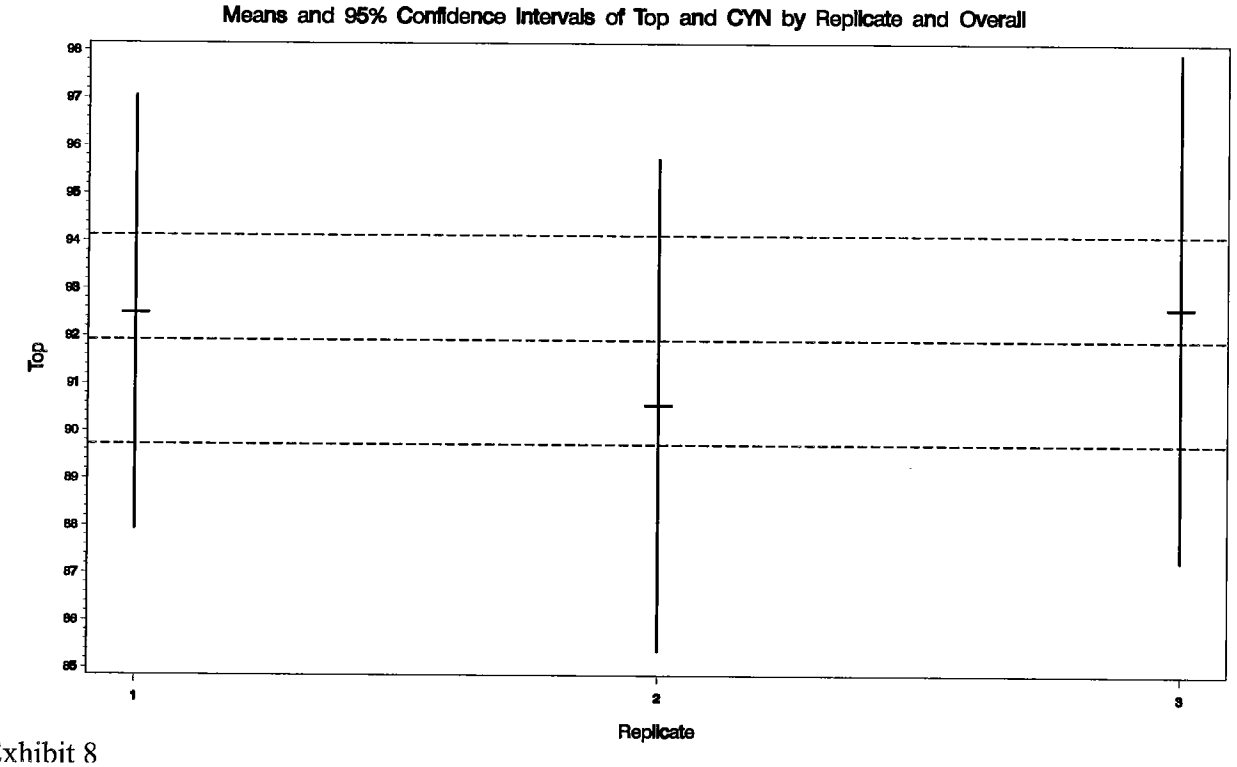

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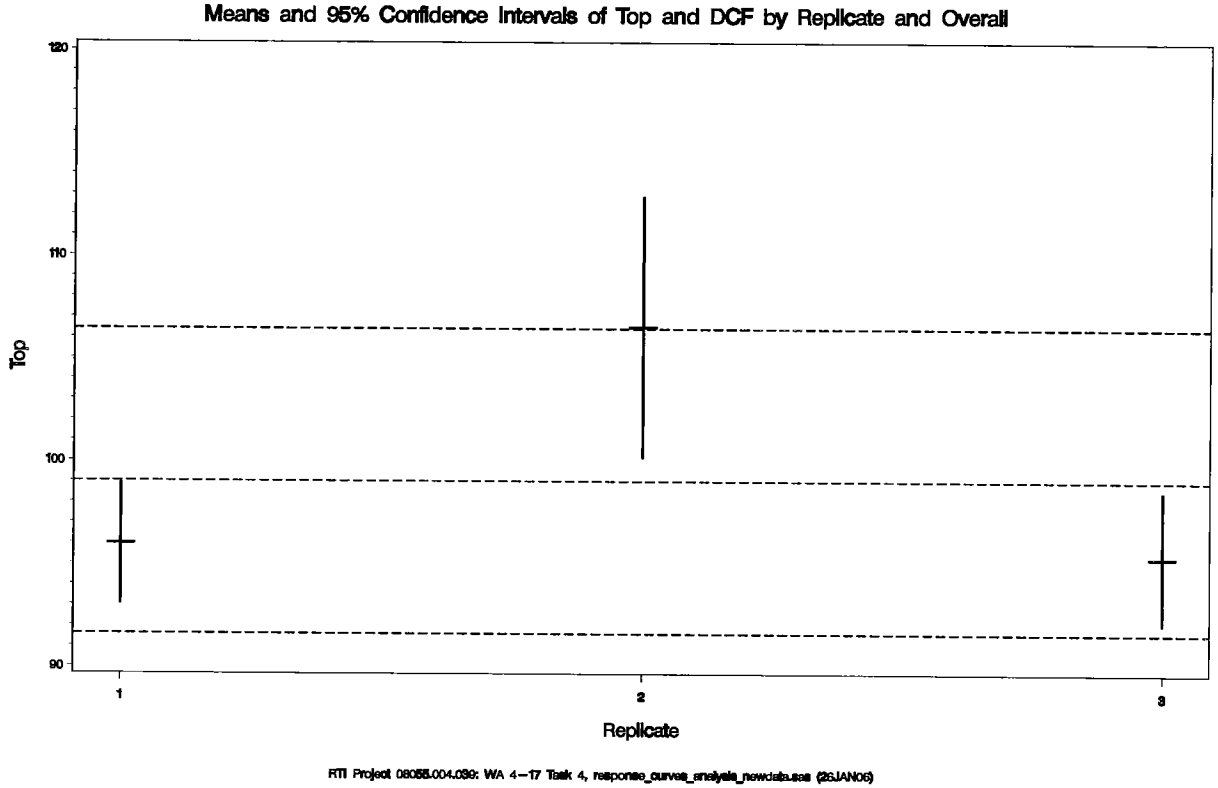
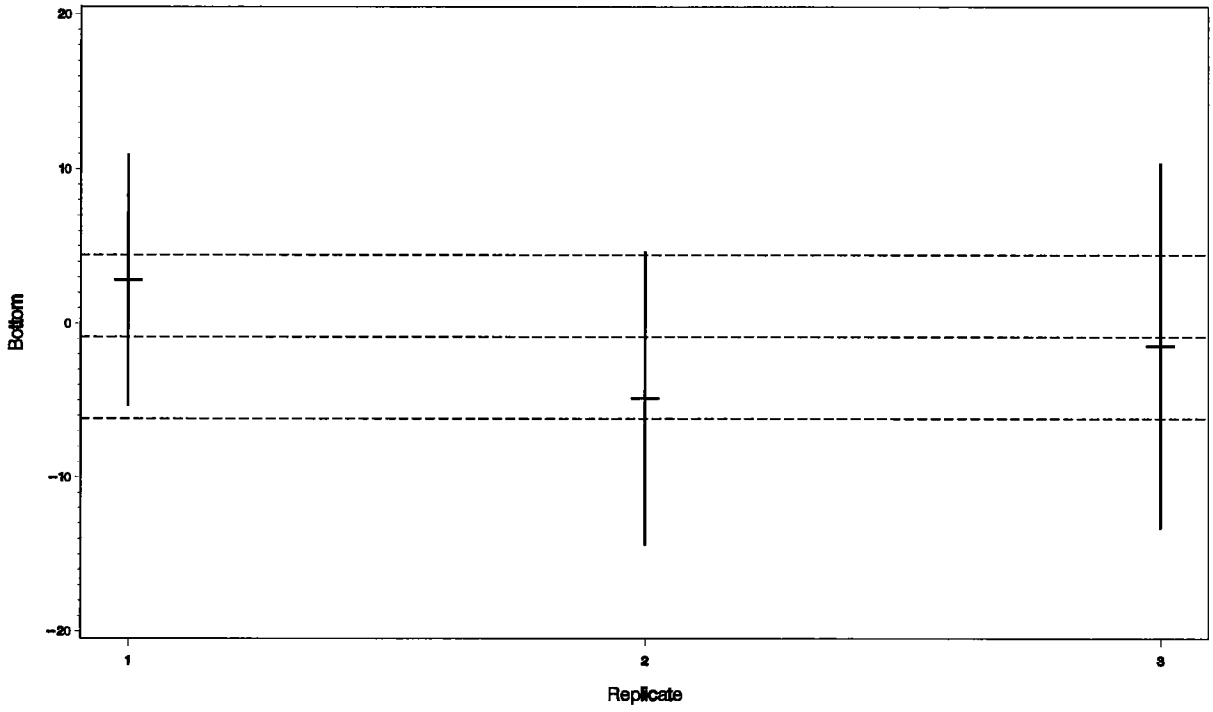
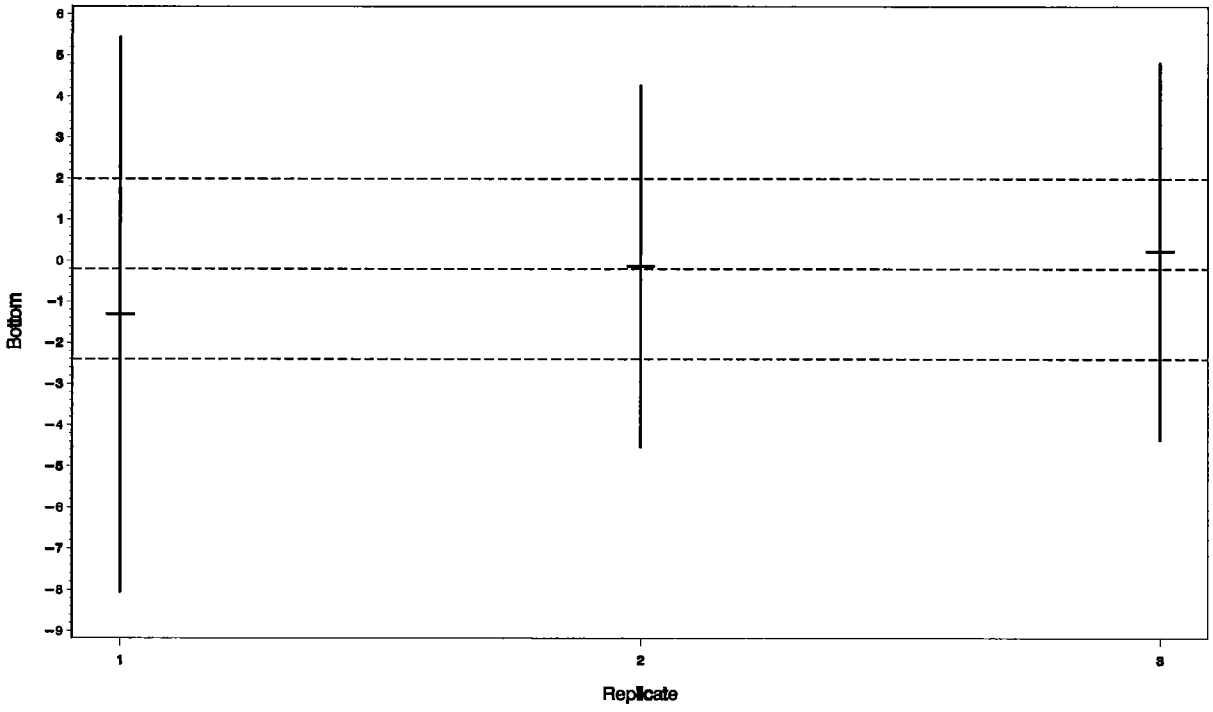


Exhibit 9 Means and 95% Confidence Intervals of Bottom and AG by Replicate and Overall



RTI Project 08055.004.036: WA 4-17 Task 4, response_curves_analysis_newdata.sas (26JAN06)

Exhibit 10 Means and 95% Confidence Intervals of Bottom and KCZ by Replicate and Overall



RTI Project 08055.004.036: WA 4-17 Task 4, response_curves_analysis_newdata.sas (26JAN06)

Exhibit 11 **Means and 95% Confidence Intervals of Bottom and PCZ by Replicate and Overall**

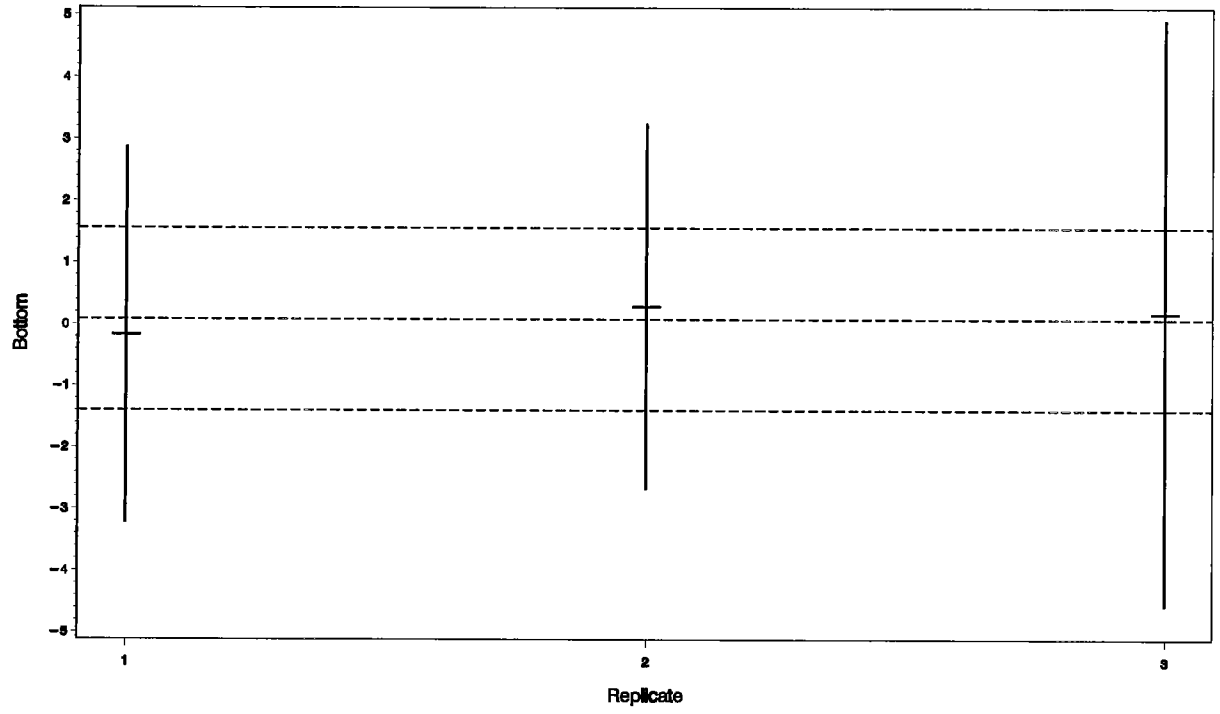
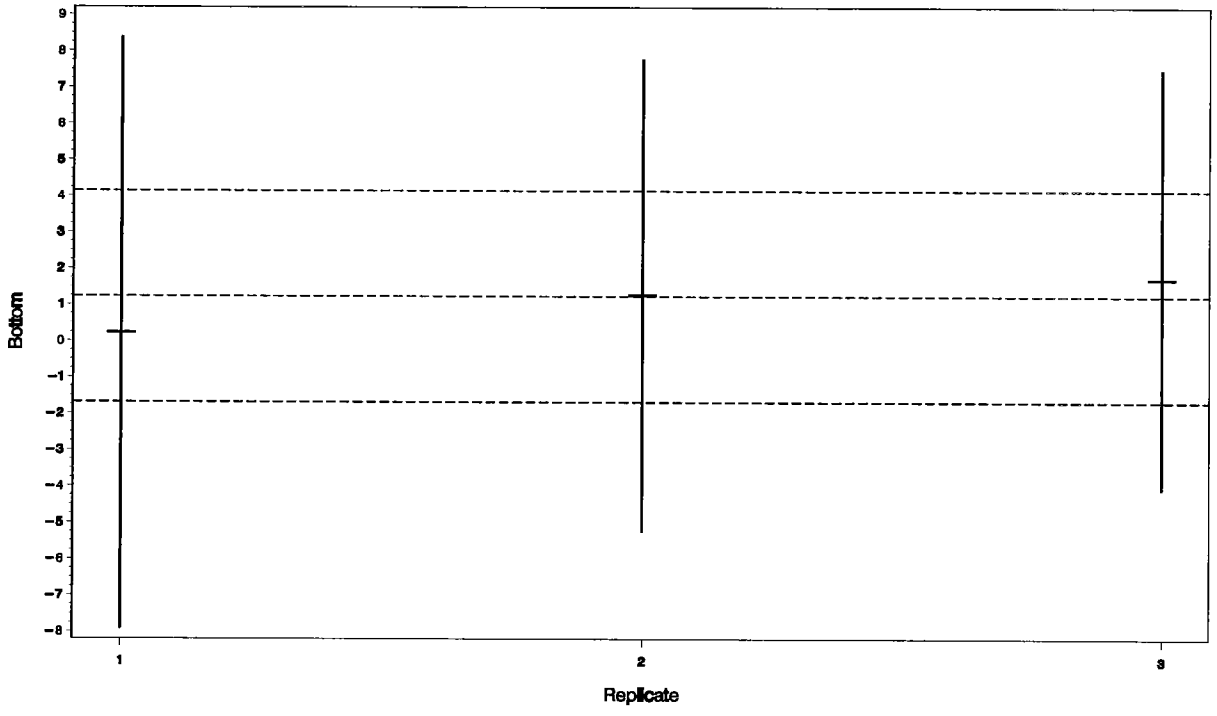


Exhibit 12

RTI Project 08058.004.039: WA 4-17 Task 4, response_curves_analysis_newdata.sas (26JAN06)

Means and 95% Confidence Intervals of Bottom and NYP by Replicate and Overall



RTI Project 08058.004.039: WA 4-17 Task 4, response_curves_analysis_newdata.sas (26JAN06)

Exhibit 13 ...eans and 95% Confidence Intervals of Bottom and FRM by Replicate and Overall

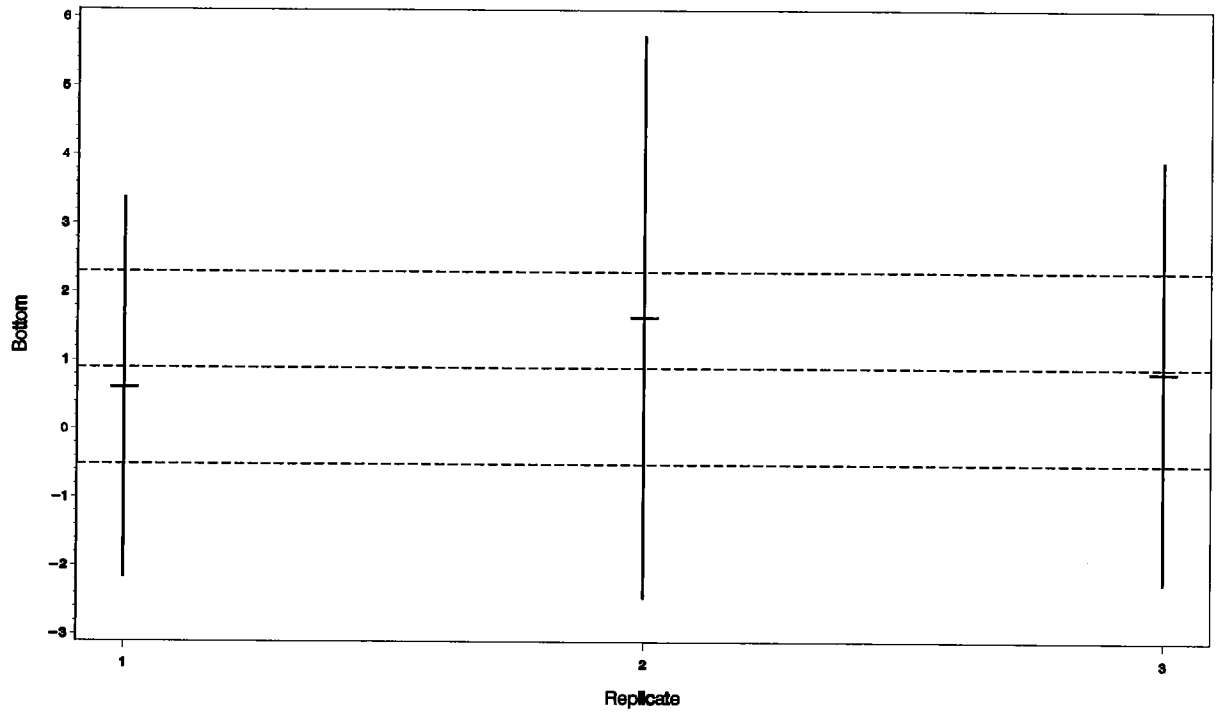


Exhibit 14

Means and 95% Confidence Intervals of Bottom and ECZ by Replicate and Overall

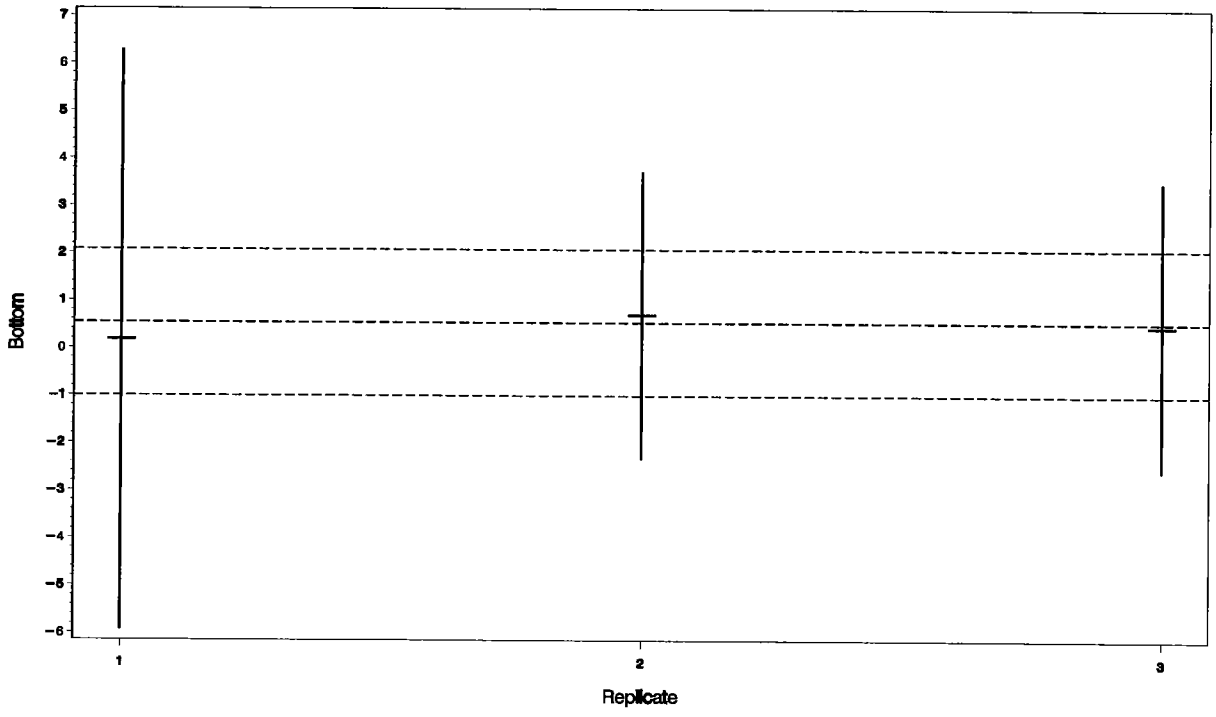


Exhibit 15

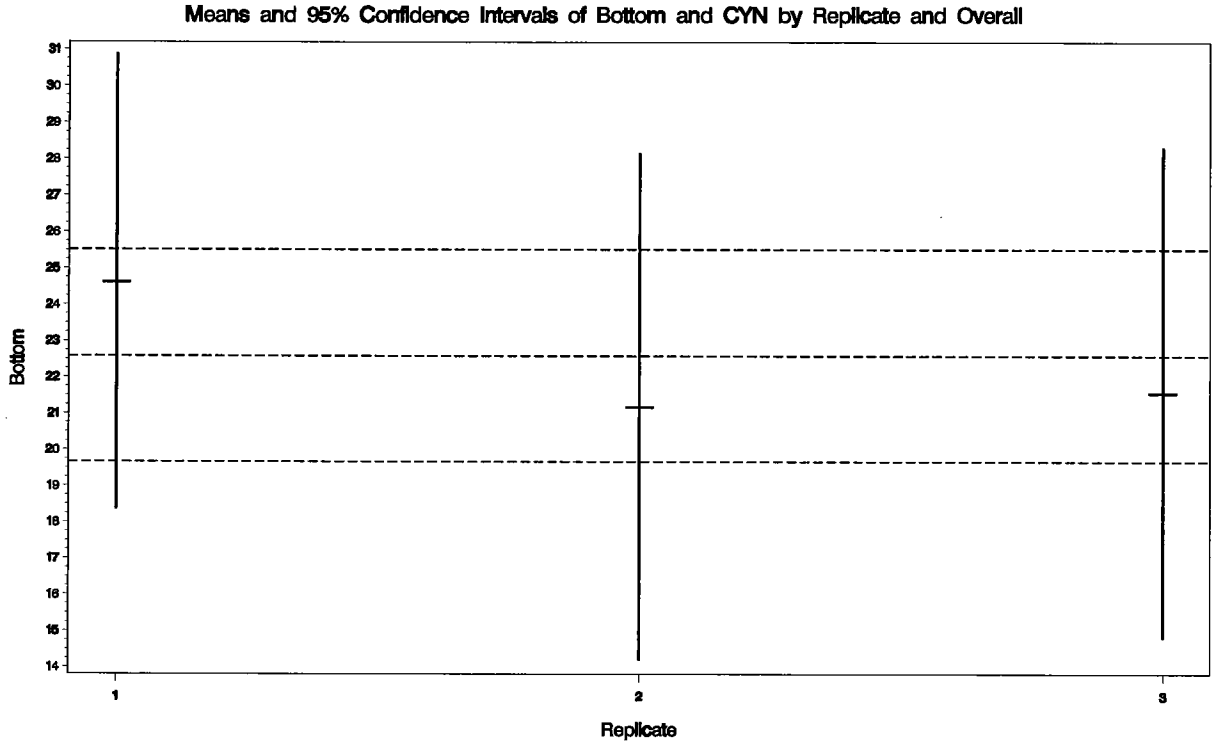


Exhibit 16

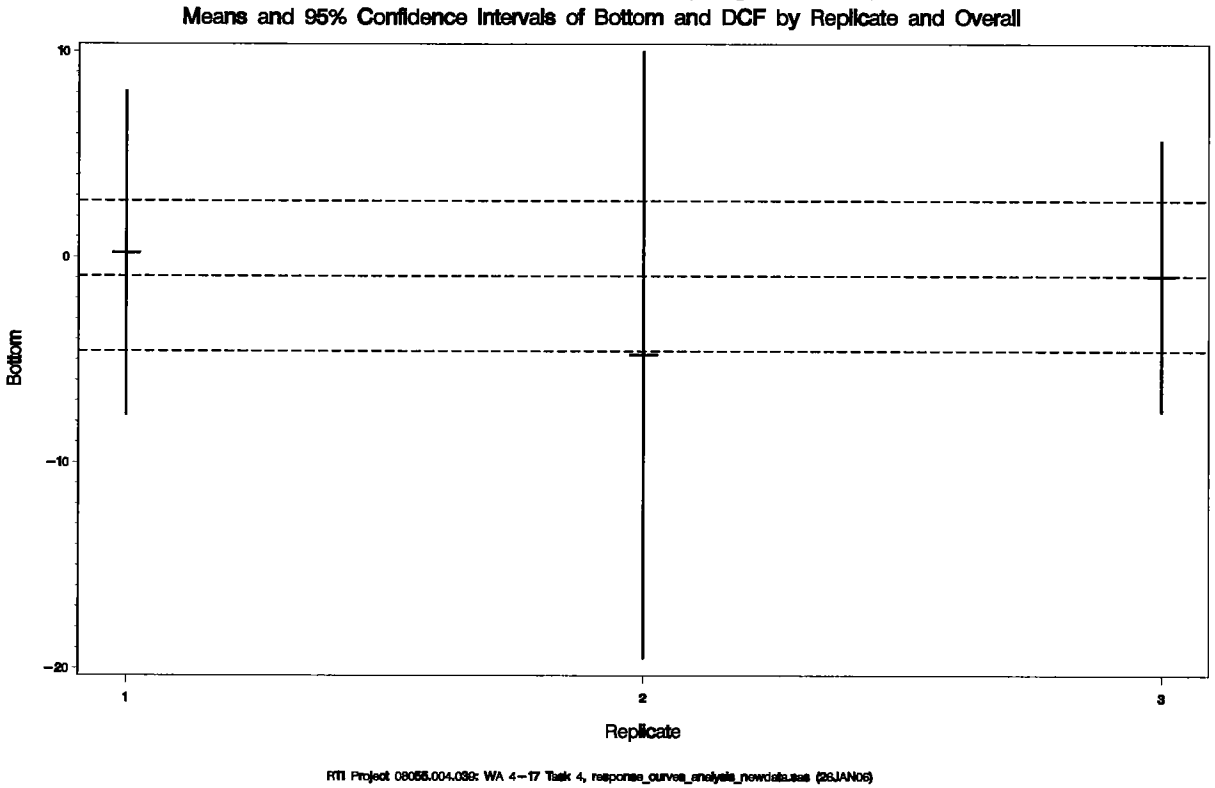


Exhibit 17

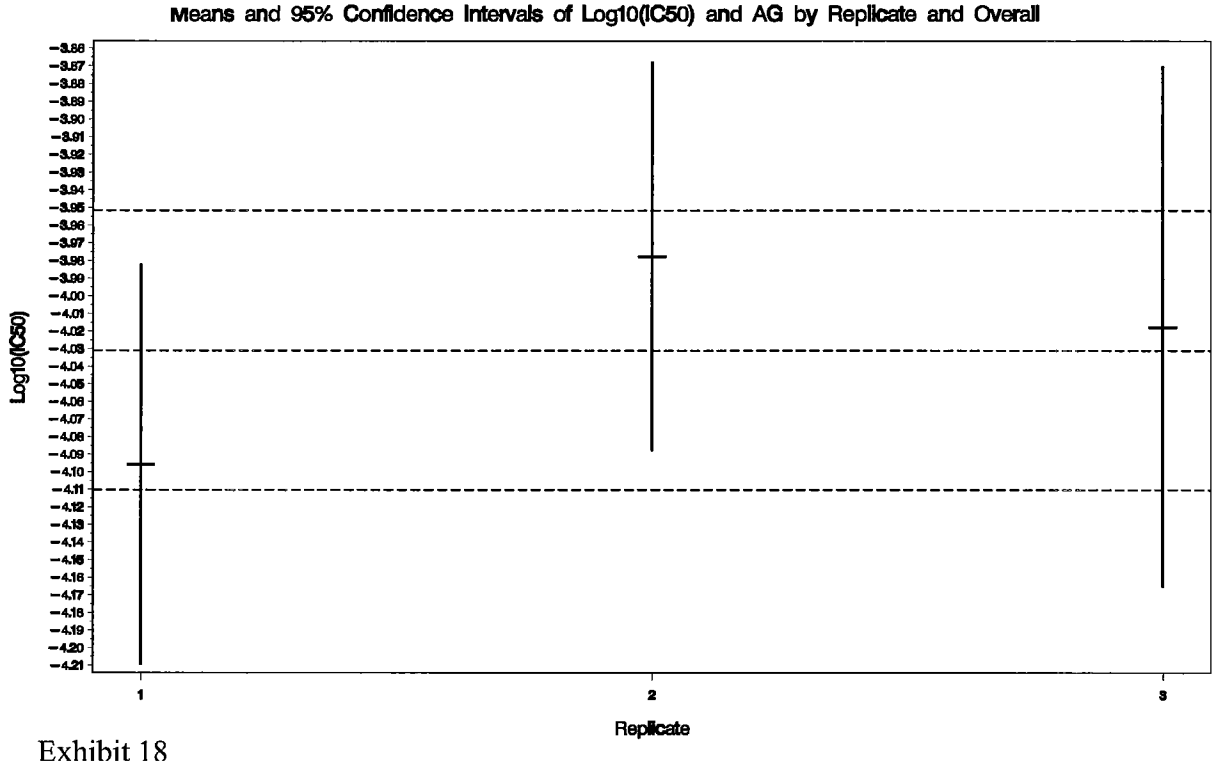


Exhibit 18

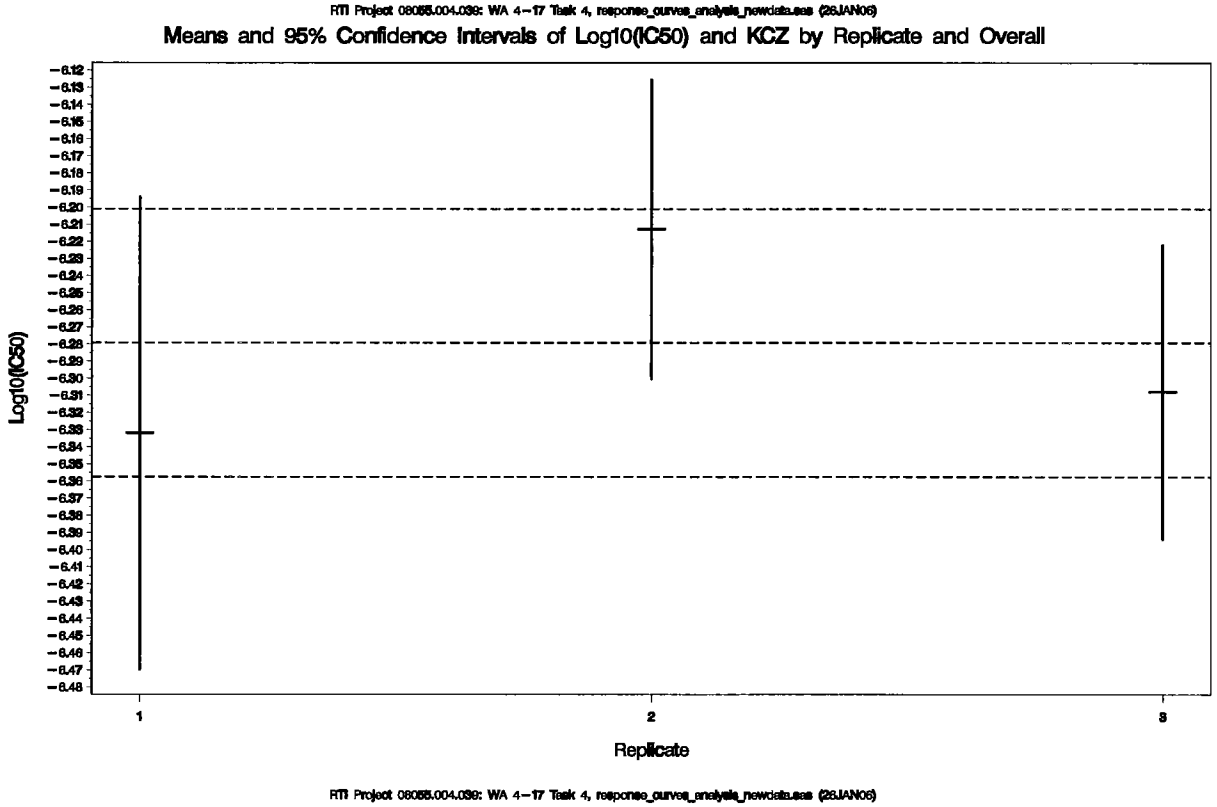


Exhibit 19

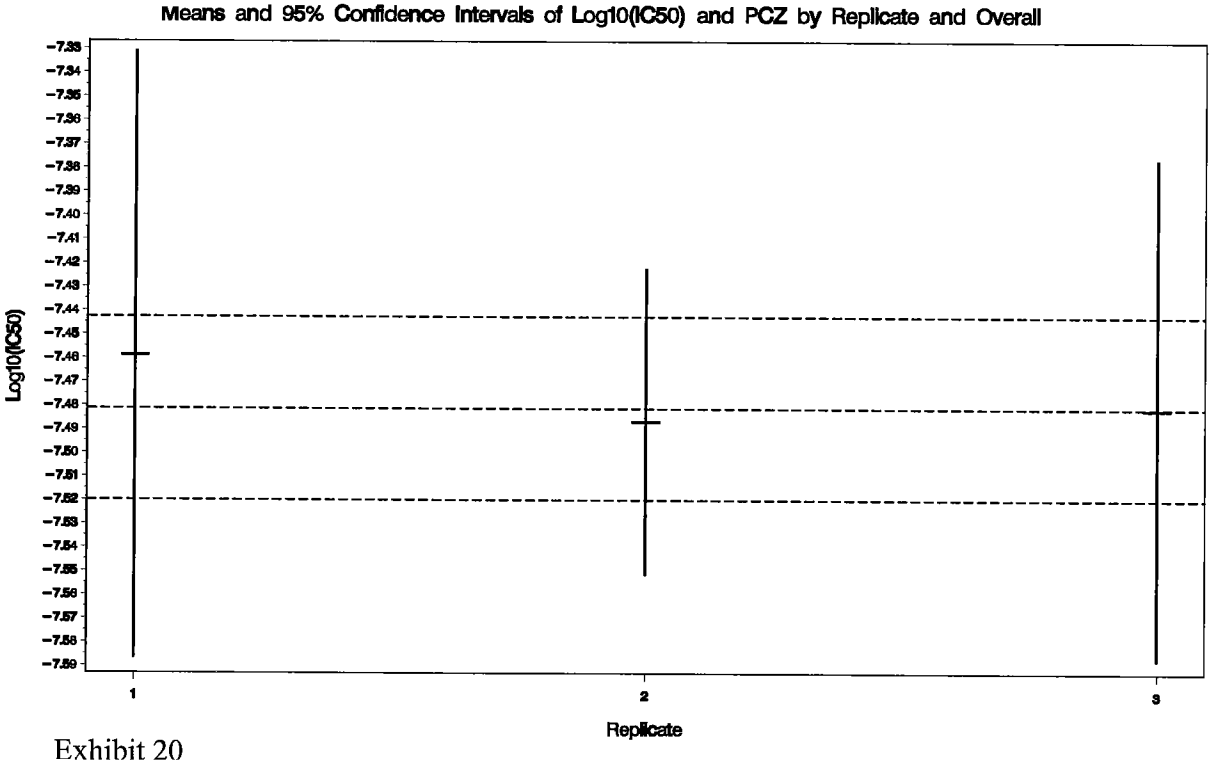


Exhibit 20

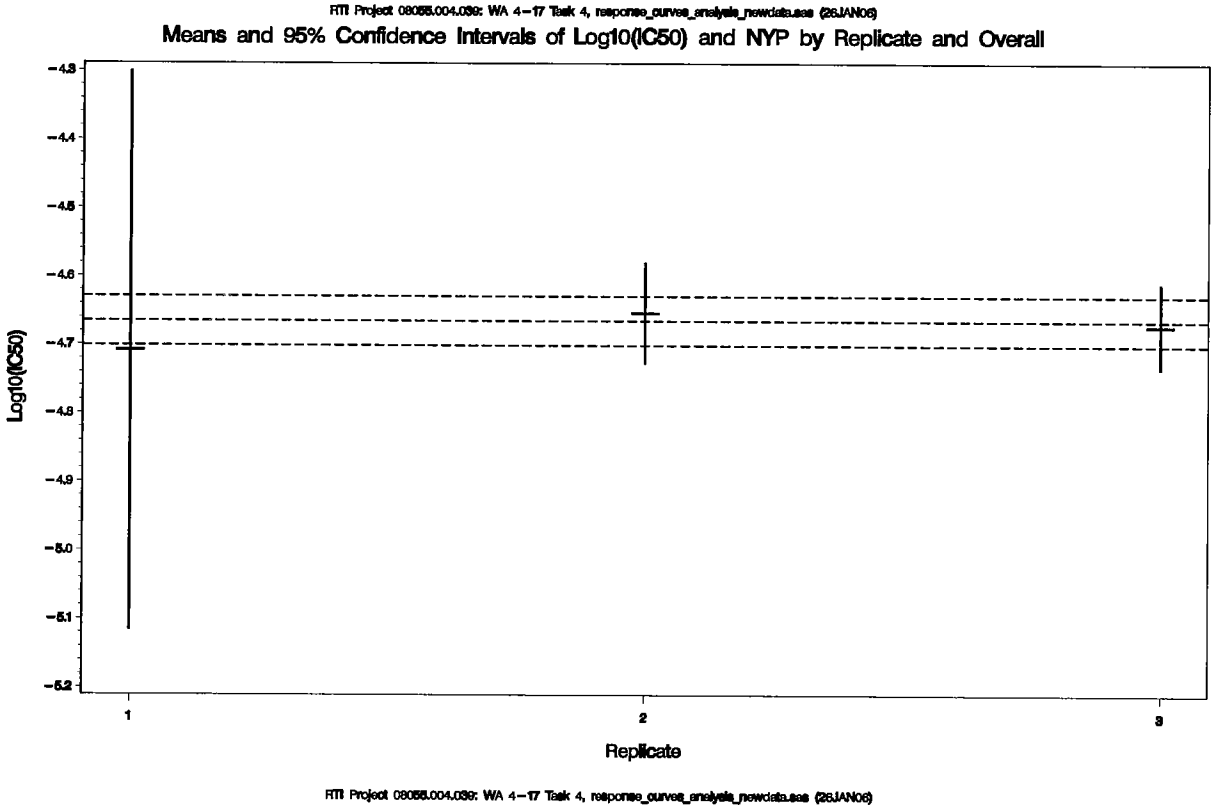


Exhibit 21

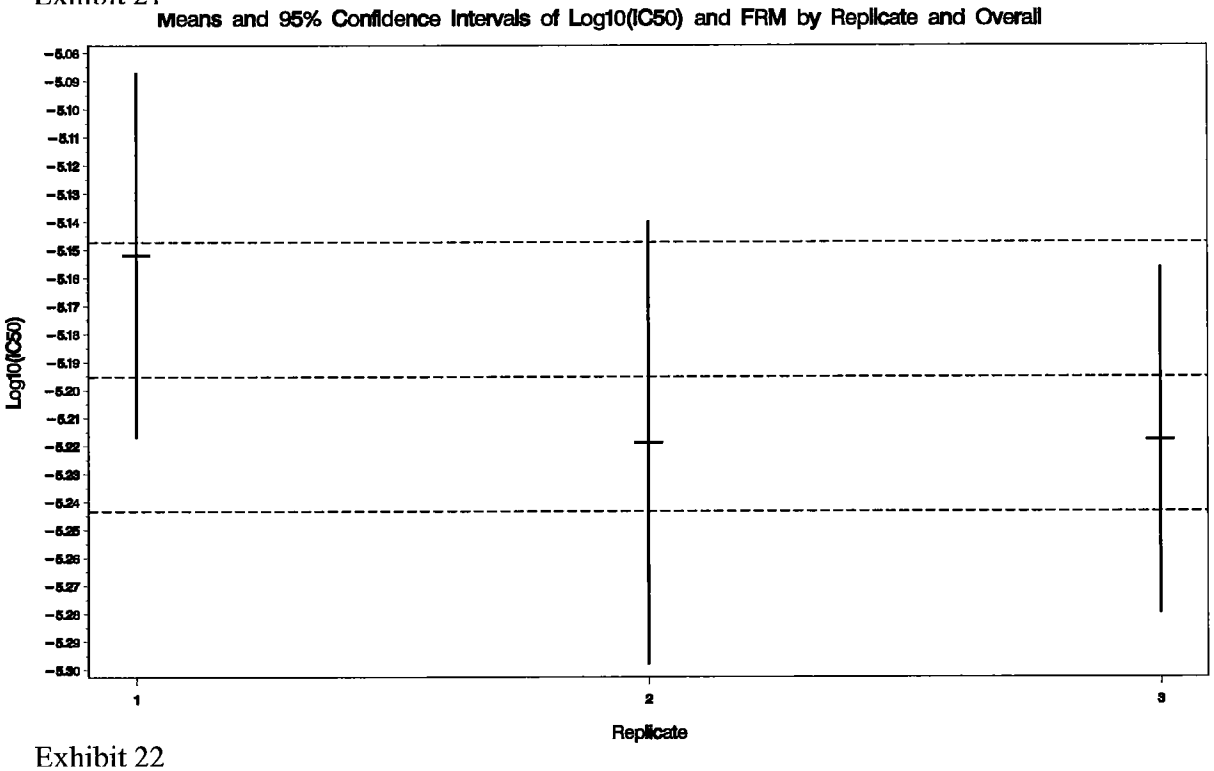


Exhibit 22

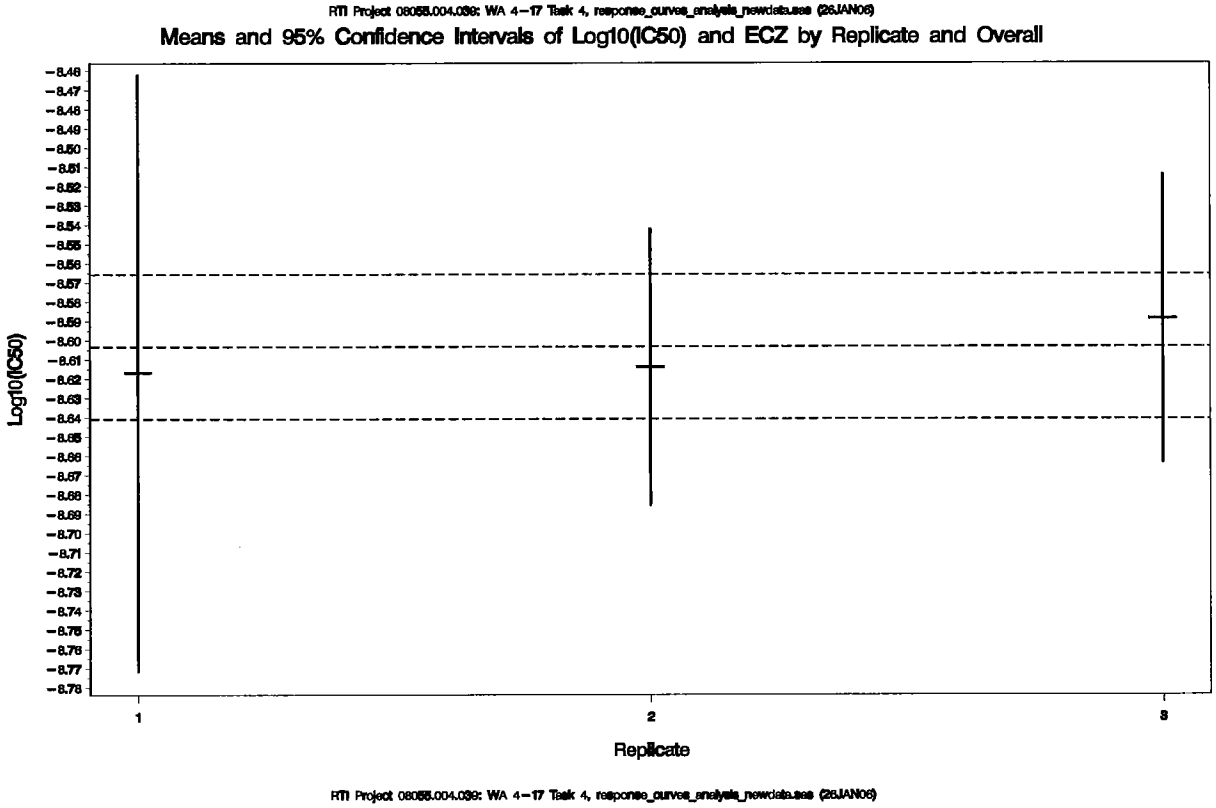


Exhibit 23

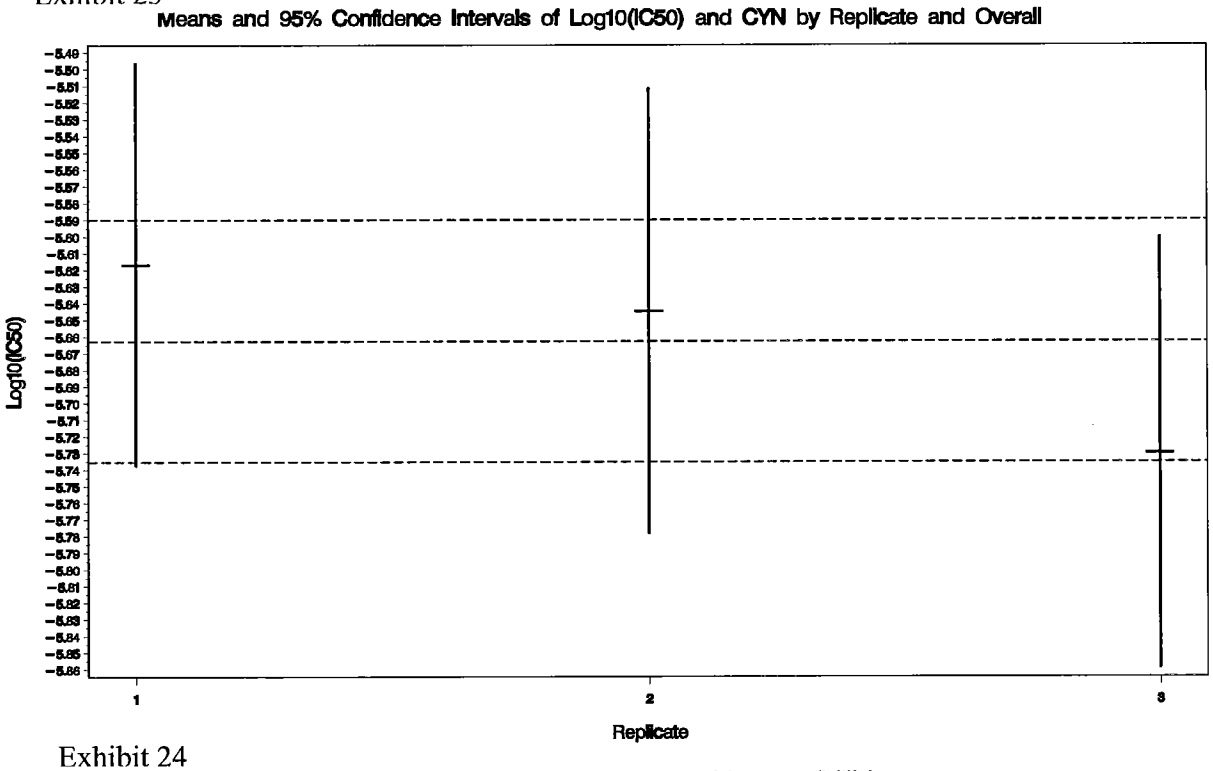


Exhibit 24

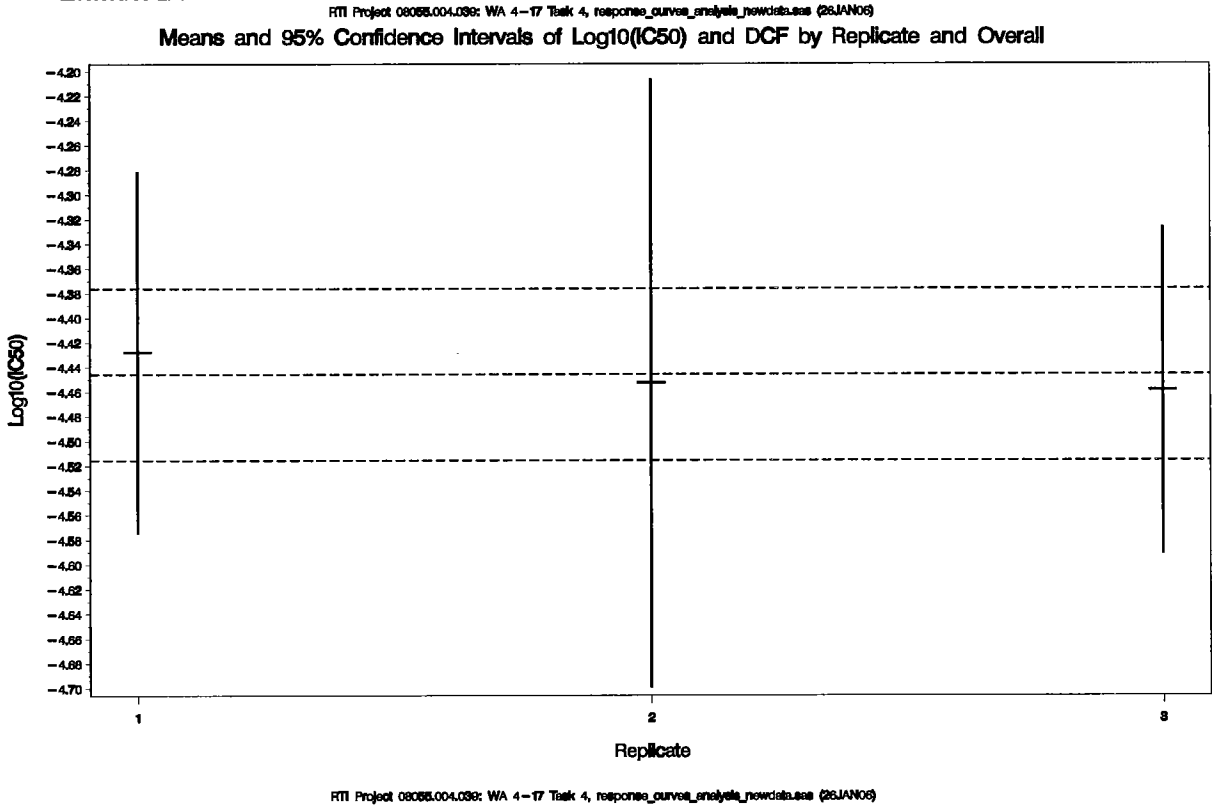


Exhibit 25

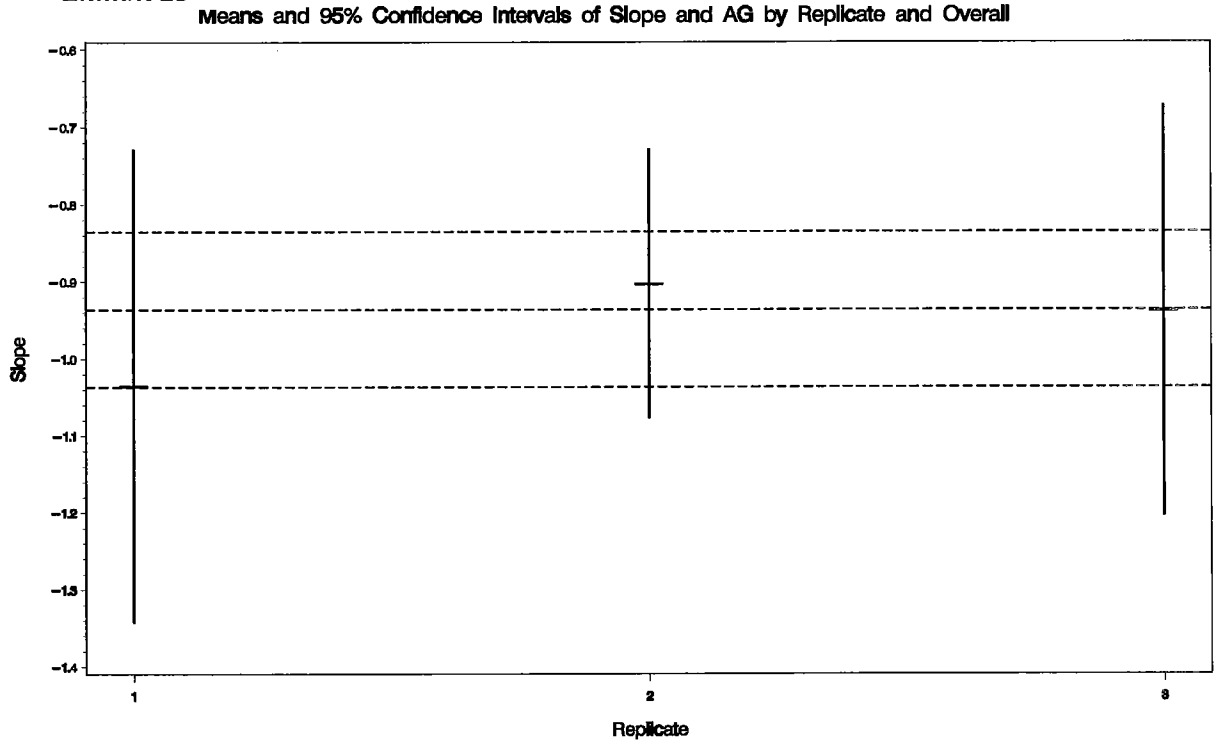


Exhibit 26

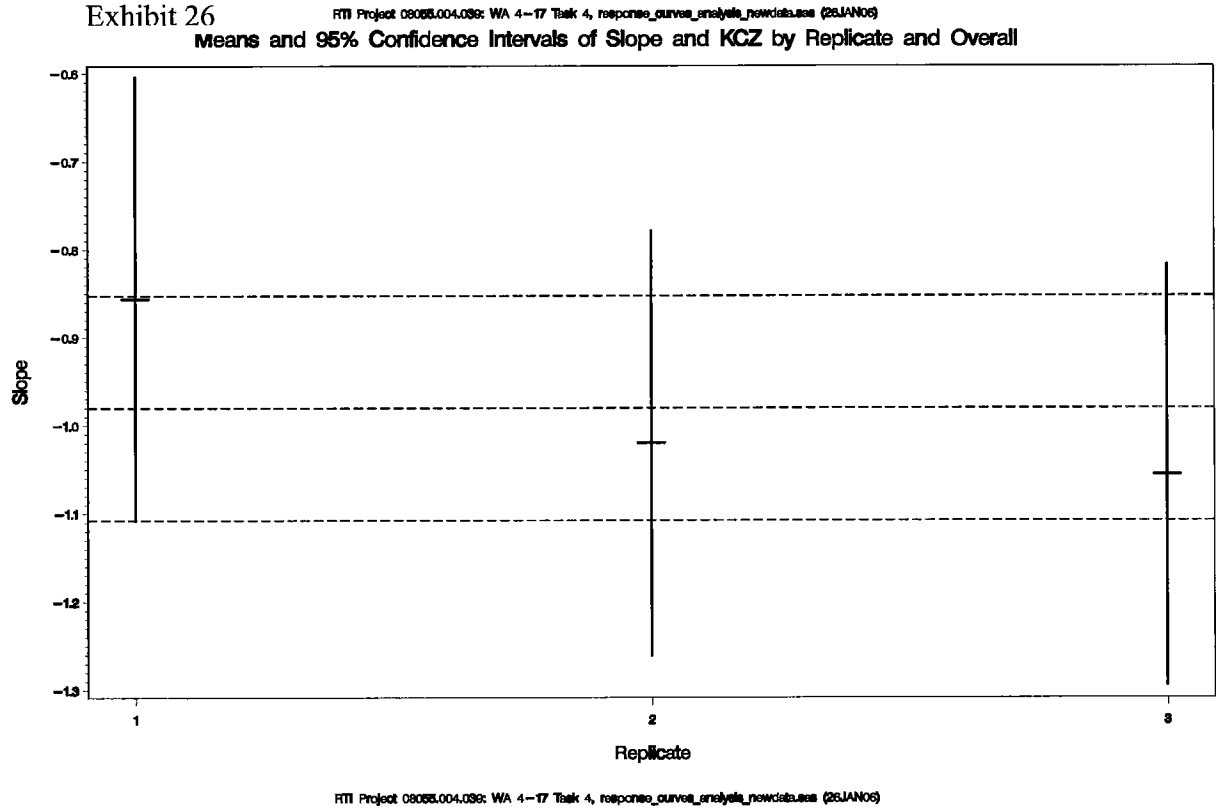


Exhibit 27

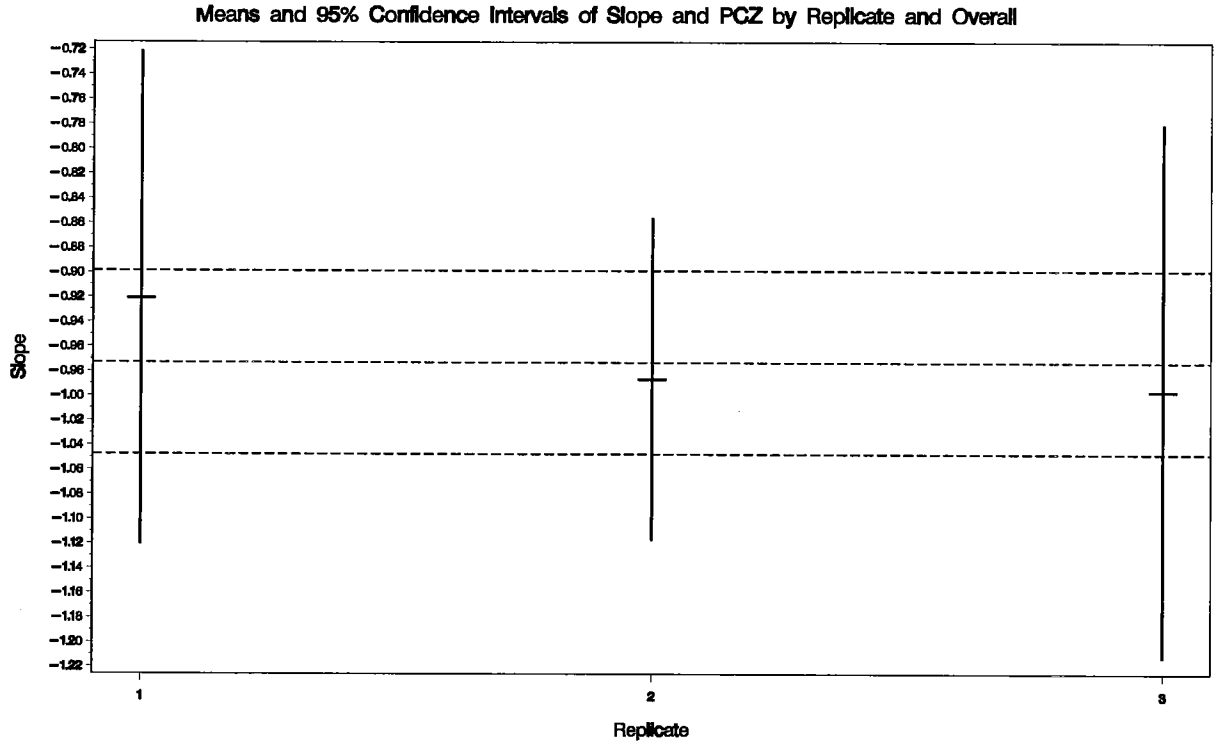


Exhibit 28

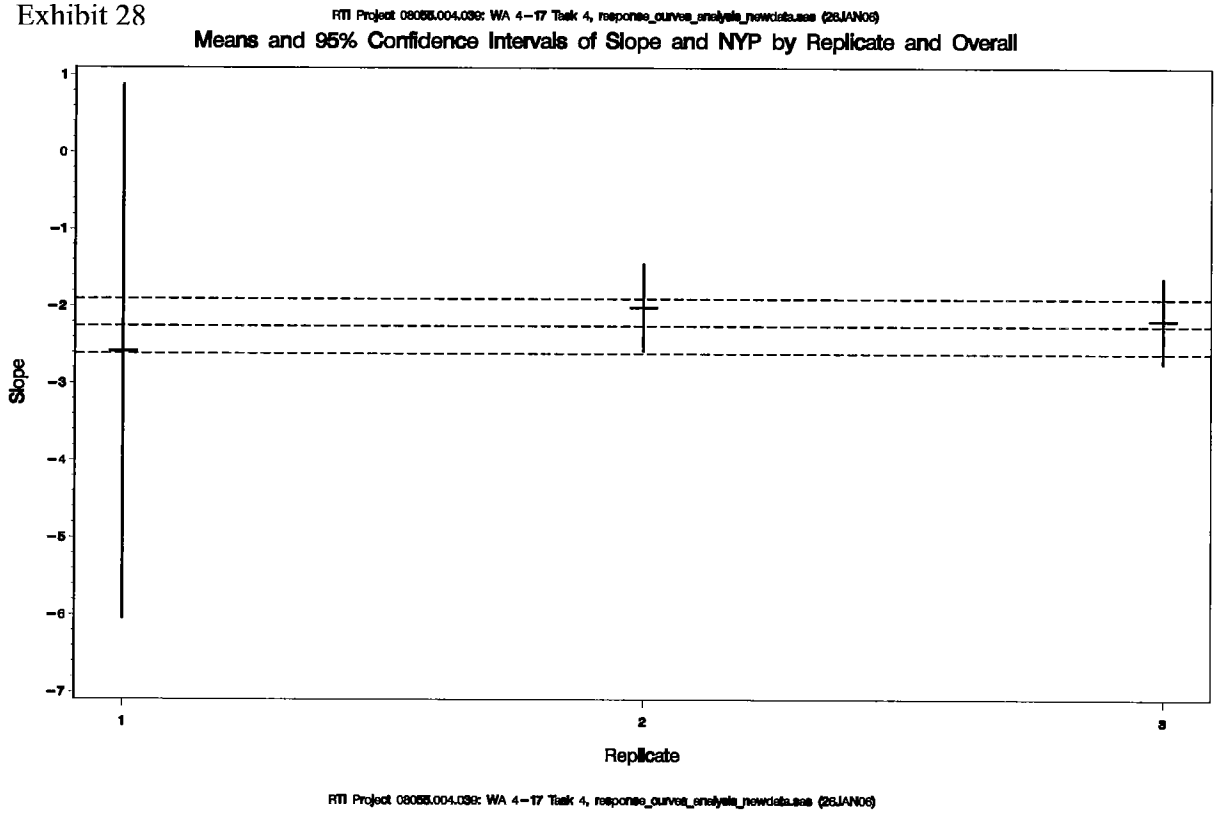
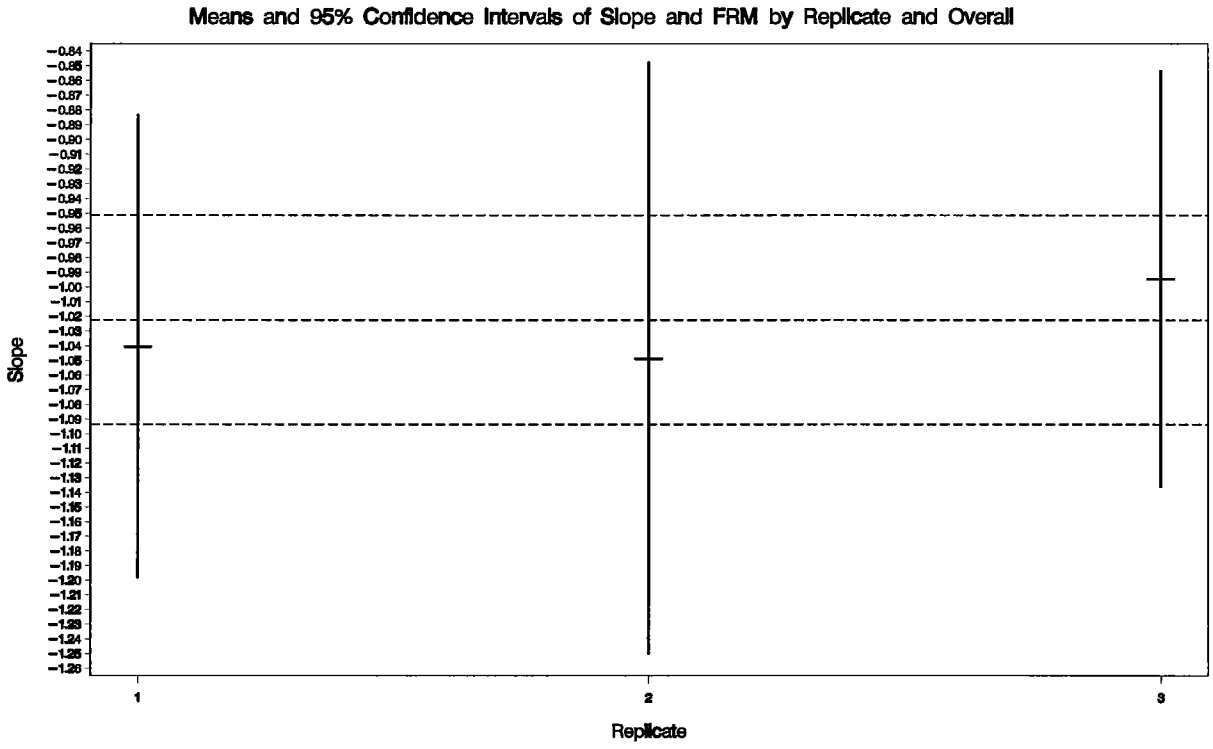
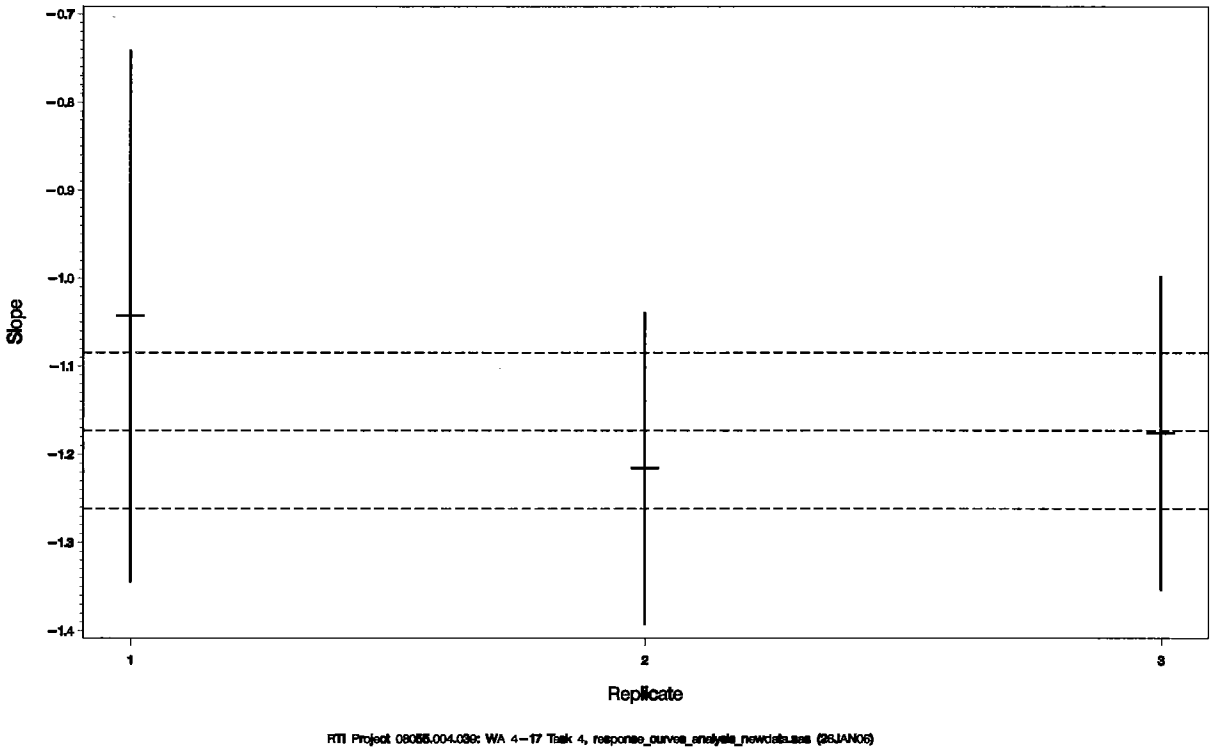


Exhibit 29



RTI Project 08055.004.030: WA 4-17 Task 4, response_curves_analysis_newdata.sas (26JAN06)

Exhibit 30



RTI Project 08055.004.030: WA 4-17 Task 4, response_curves_analysis_newdata.sas (26JAN06)

Exhibit 31

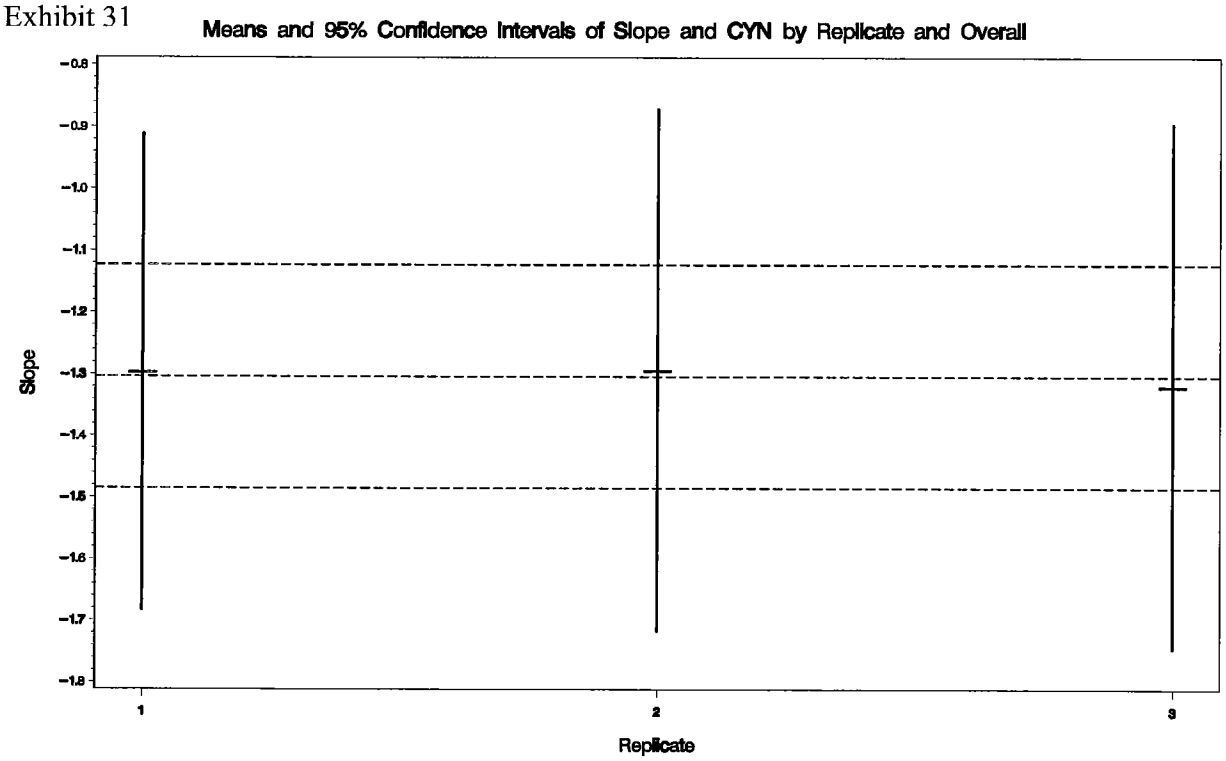


Exhibit 32

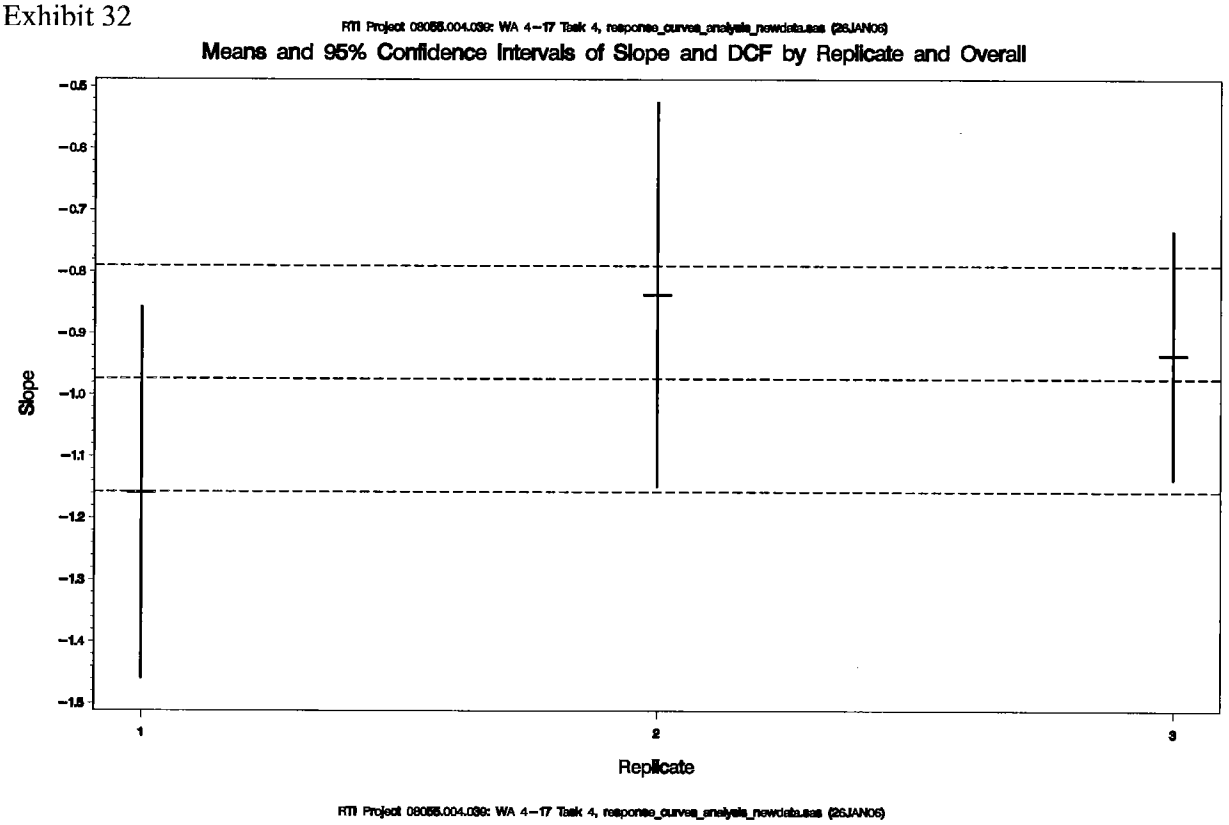
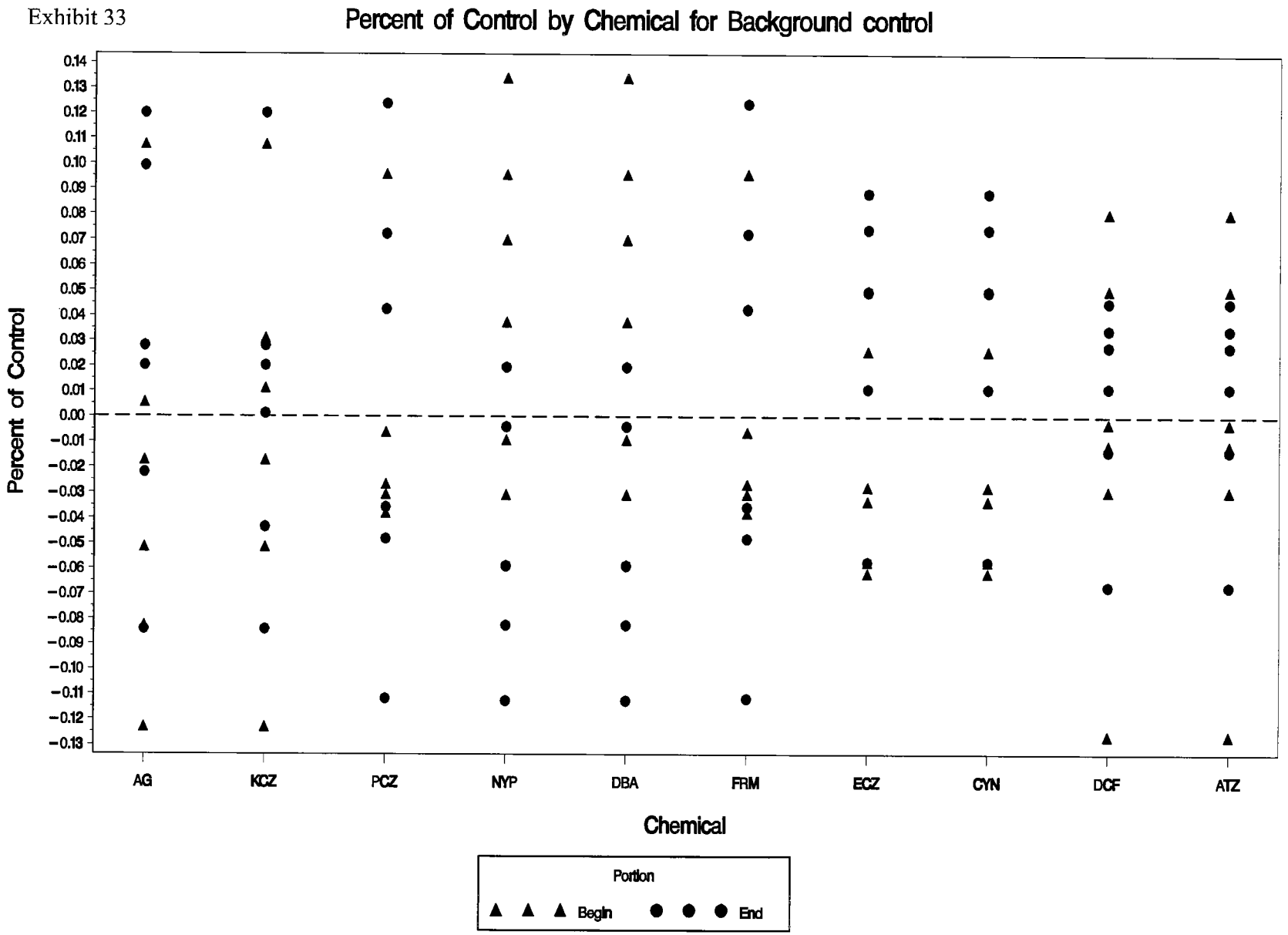


Exhibit 33



RTI Project 08055.004.039: WA 4−17 Task 4, Eor[illegible]5_analysis.sas (09JAN06)

Exhibit 34

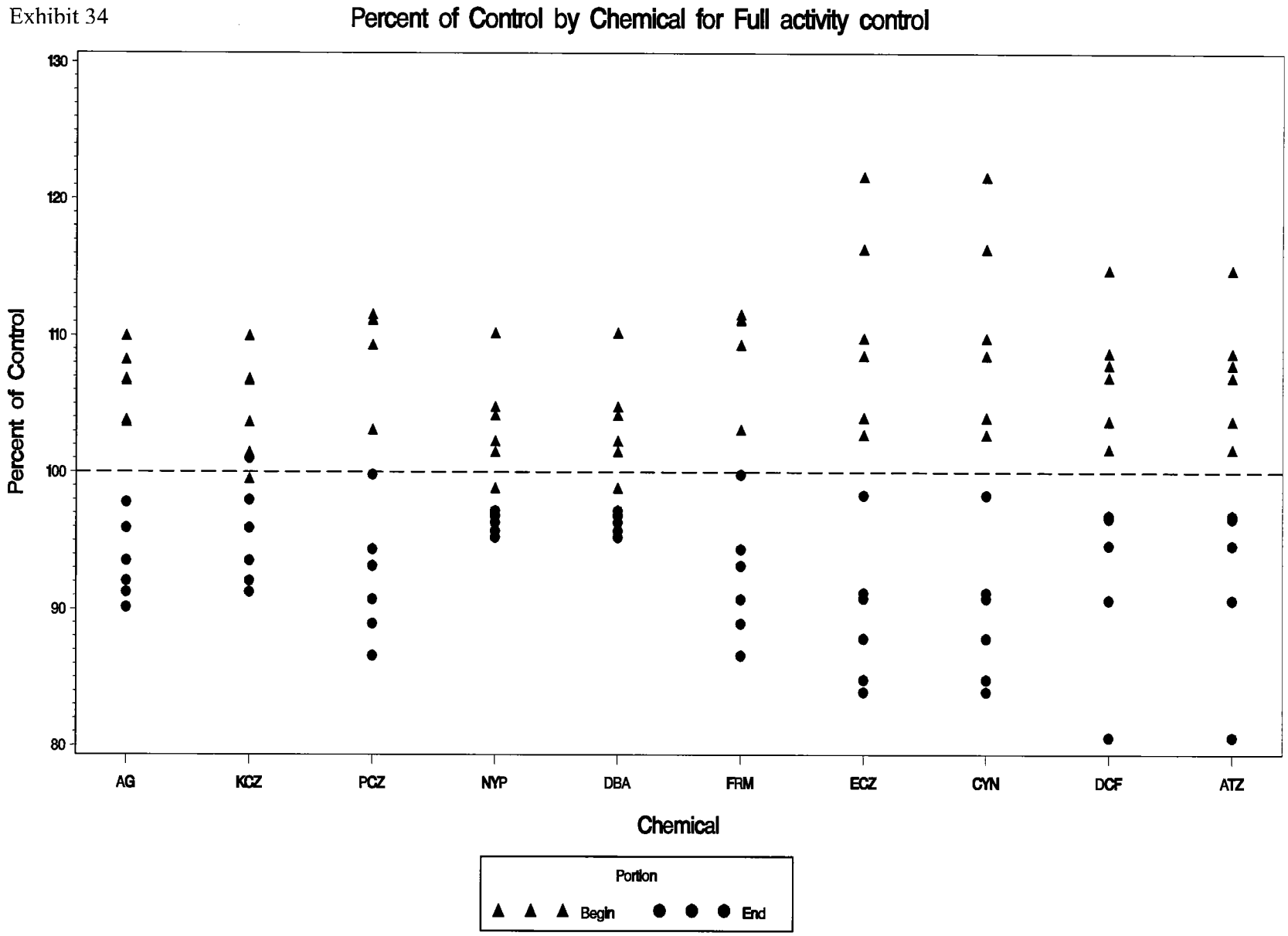
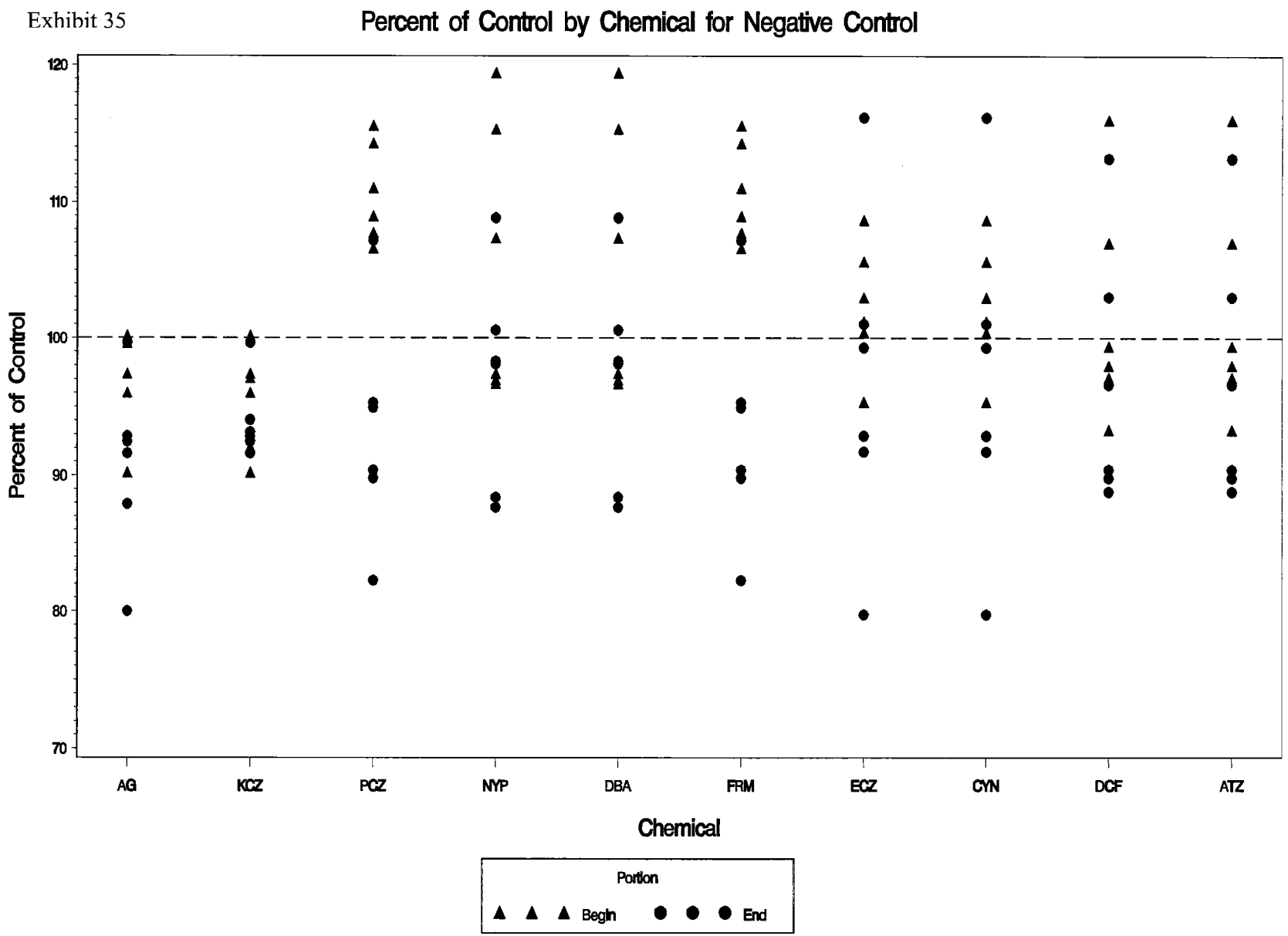

Percent of Control by Chemical for Full activity control
Percent of Control
130
120
110
100
90
80
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
Portion
Begin
End

Exhibit 35



RTI Project 08055.004.039: WA 4-17 Task 4, Exh35_analysis.sas (09JAN06)

Exhibit 36

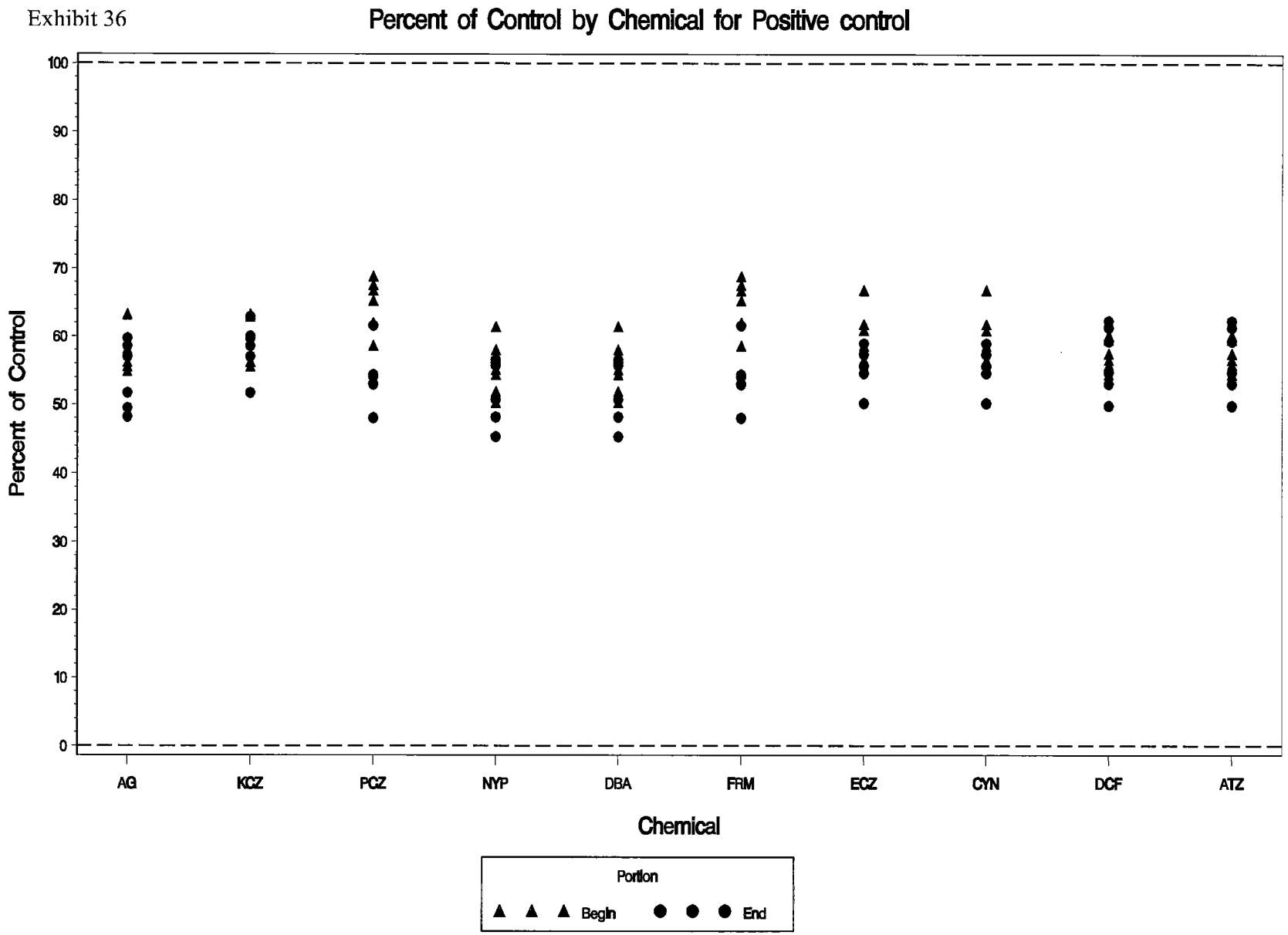
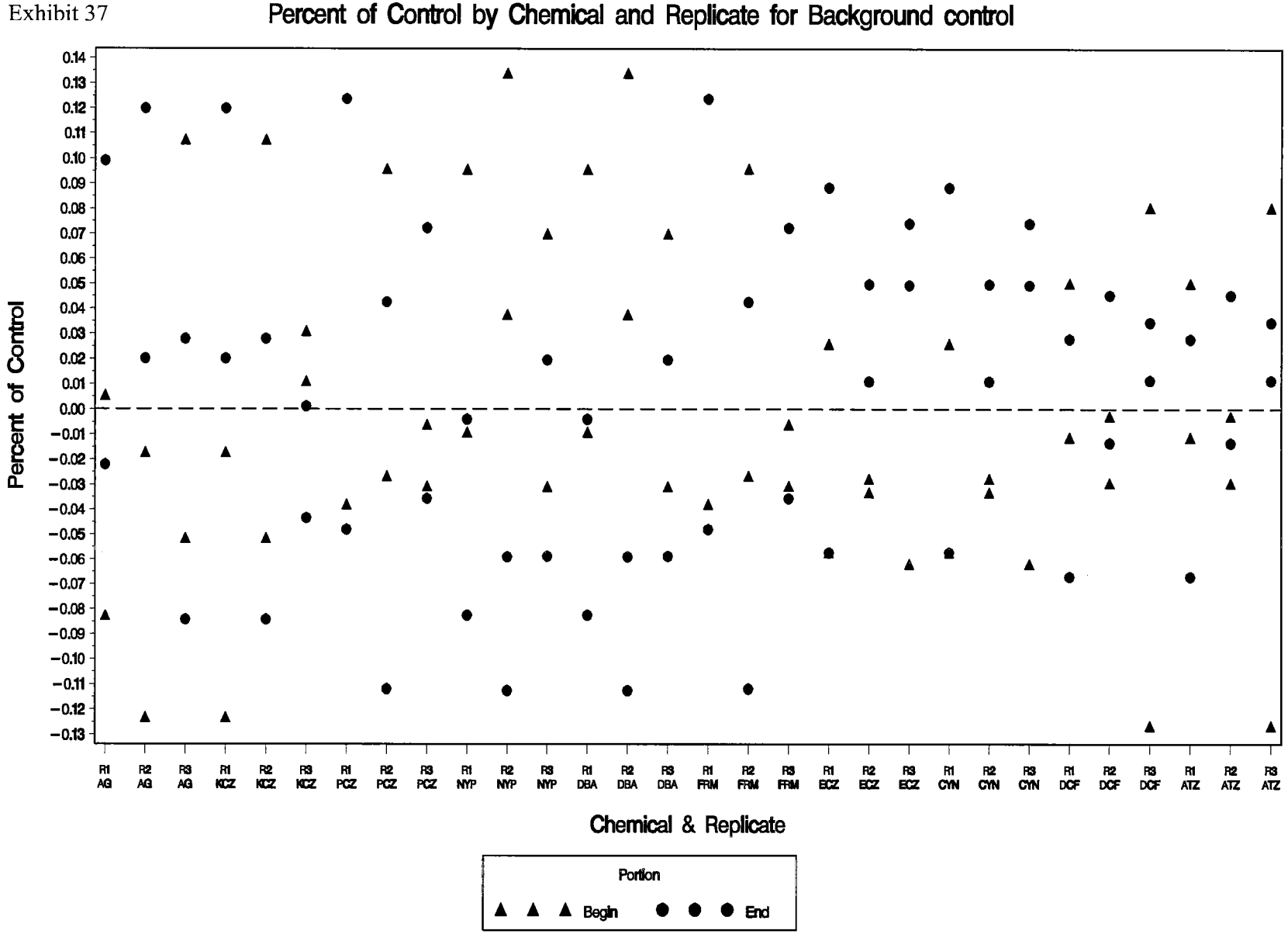
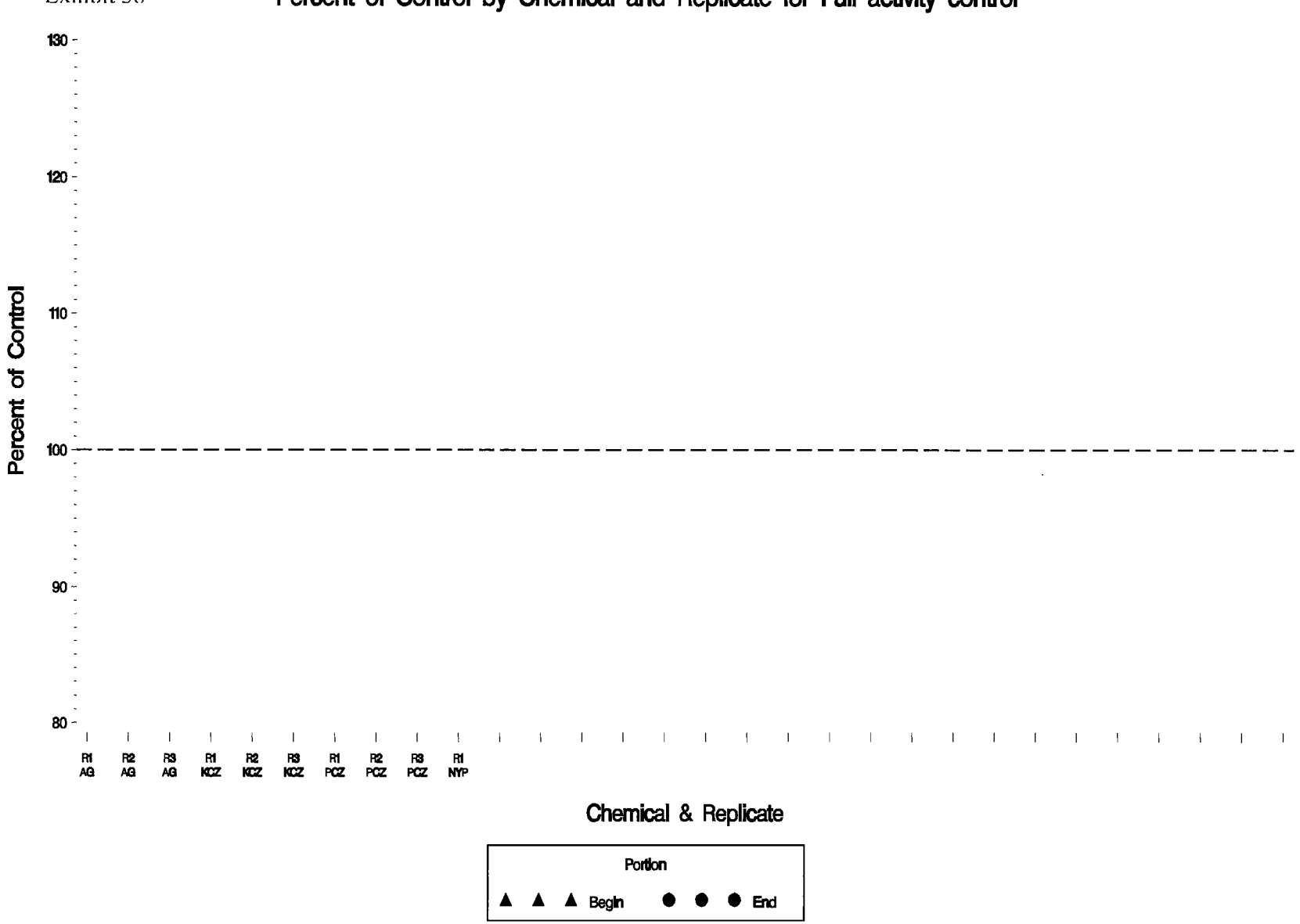

Percent of Control by Chemical for Positive control
Percent of Control
100
90
80
70
60
50
40
30
20
10
0
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
Portion
Begin
End

Exhibit 37



RTI Project 08055.004.039: WA 4−17 Task 4, Exh37_analysis.sas (09JAN06)

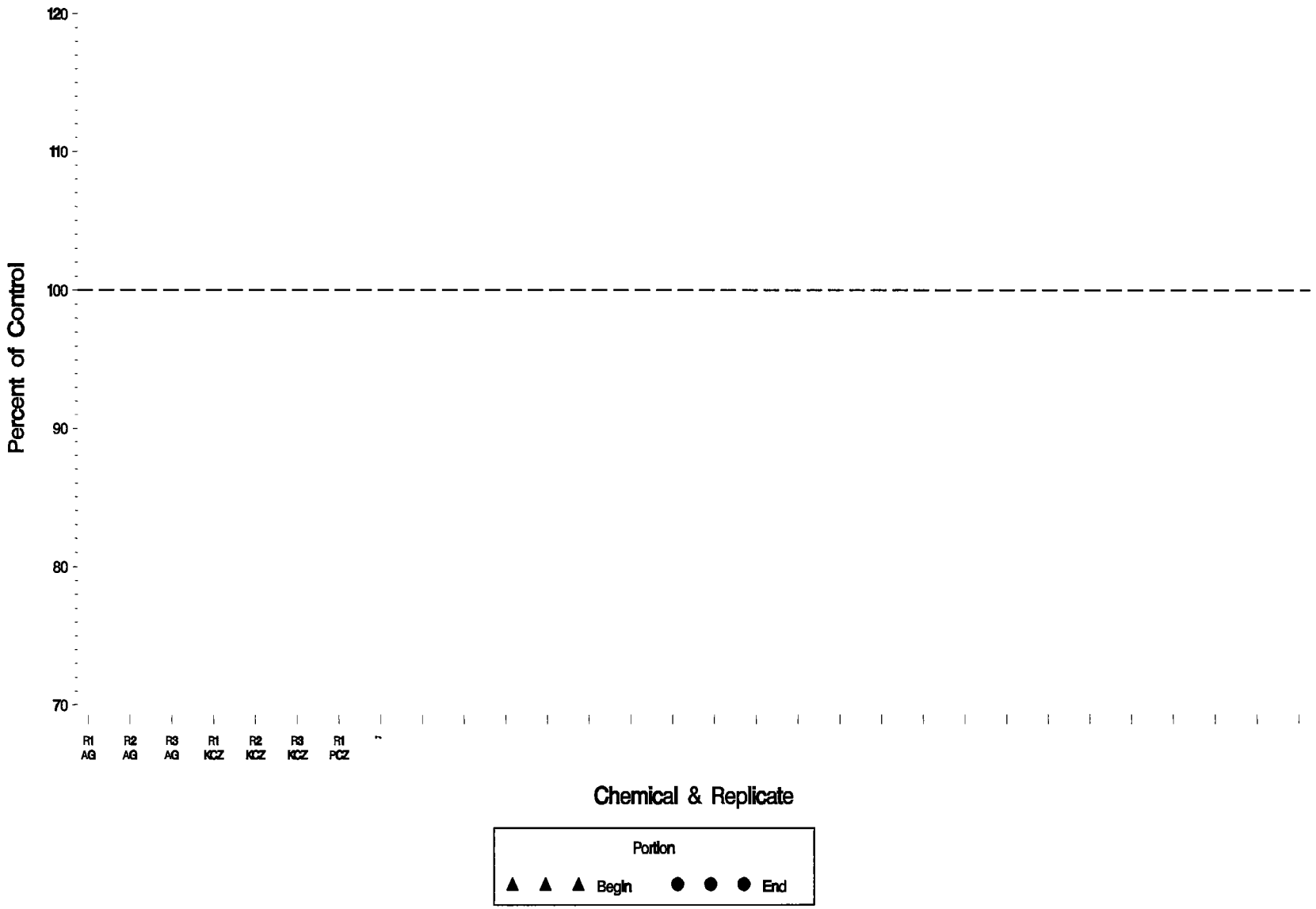
Exhibit 38



RTI Project 08055.004.039: WA 4-17 Task 4, Epi@G_analysis.sas (09JAN06)

Exhibit 39

Percent of Control by Chemical and Replicate for Negative Control



RTI Project 08055.004.039: WA 4-17 Task 4, Eor@rdl_analysis.sas (09JAN06)

Exhibit 40

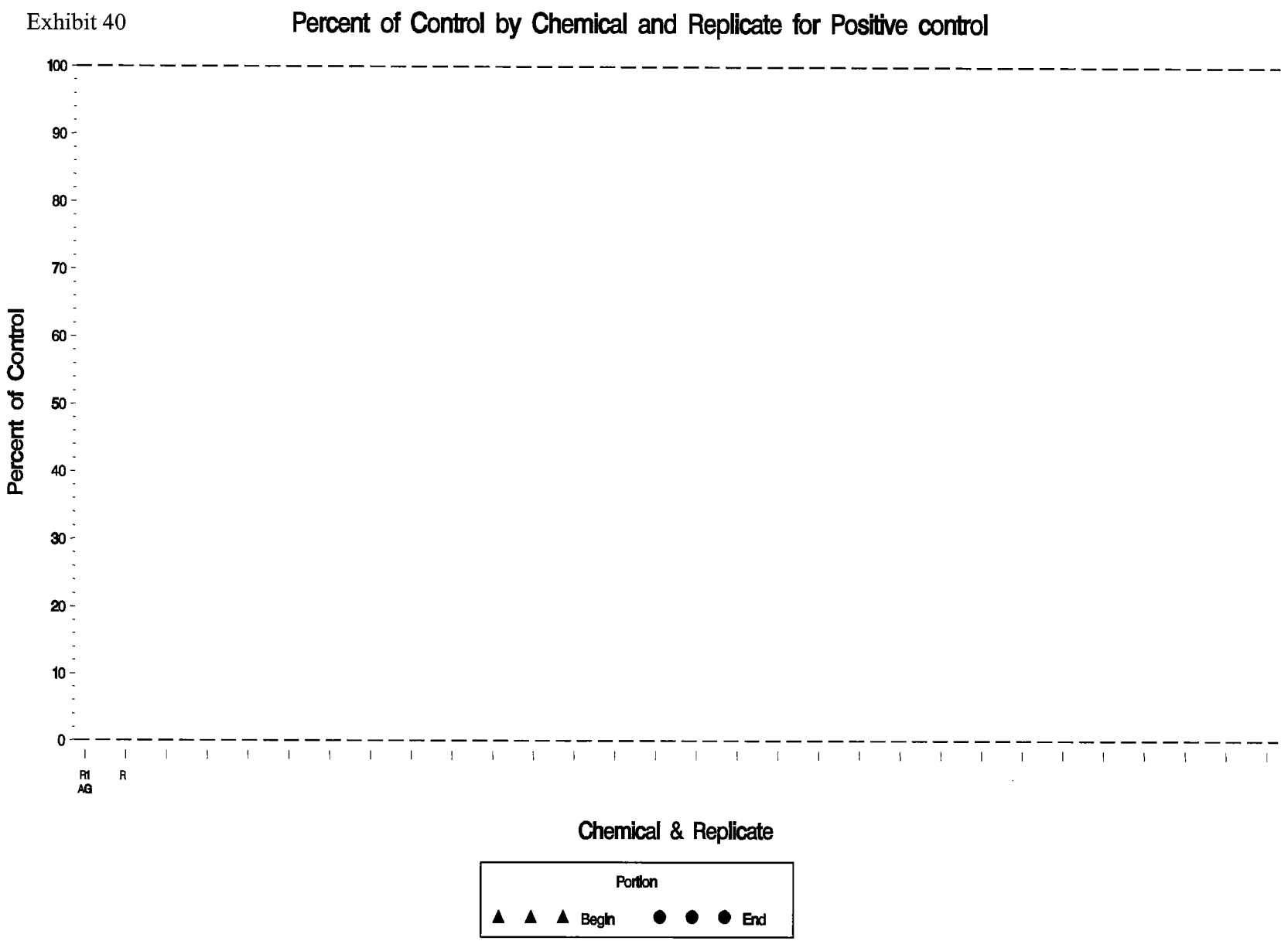

Percent of Control by Chemical and Replicate for Positive control
Percent of Control
100
90
80
70
60
50
40
30
20
10
0
R1
AG
R
Chemical & Replicate
Portion
Begin
End

Exhibit 41

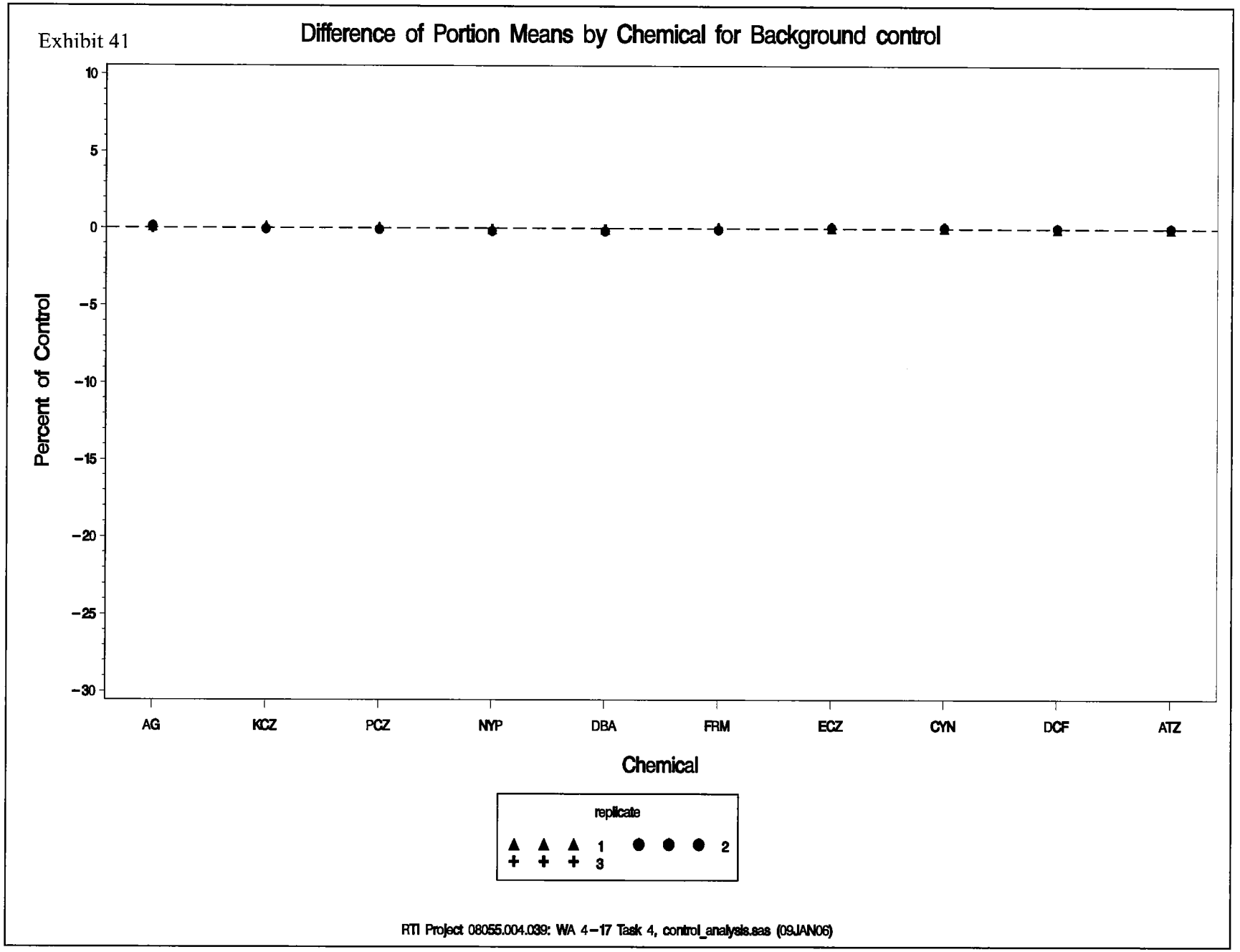

Difference of Portion Means by Chemical for Background control
Percent of Control
10
5
0
-5
-10
-15
-20
-25
-30
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
replicate
1
2
3
RTI Project 08055.004.039: WA 4-17 Task 4, control_analysis.sas (09JAN06)

Exhibit 42

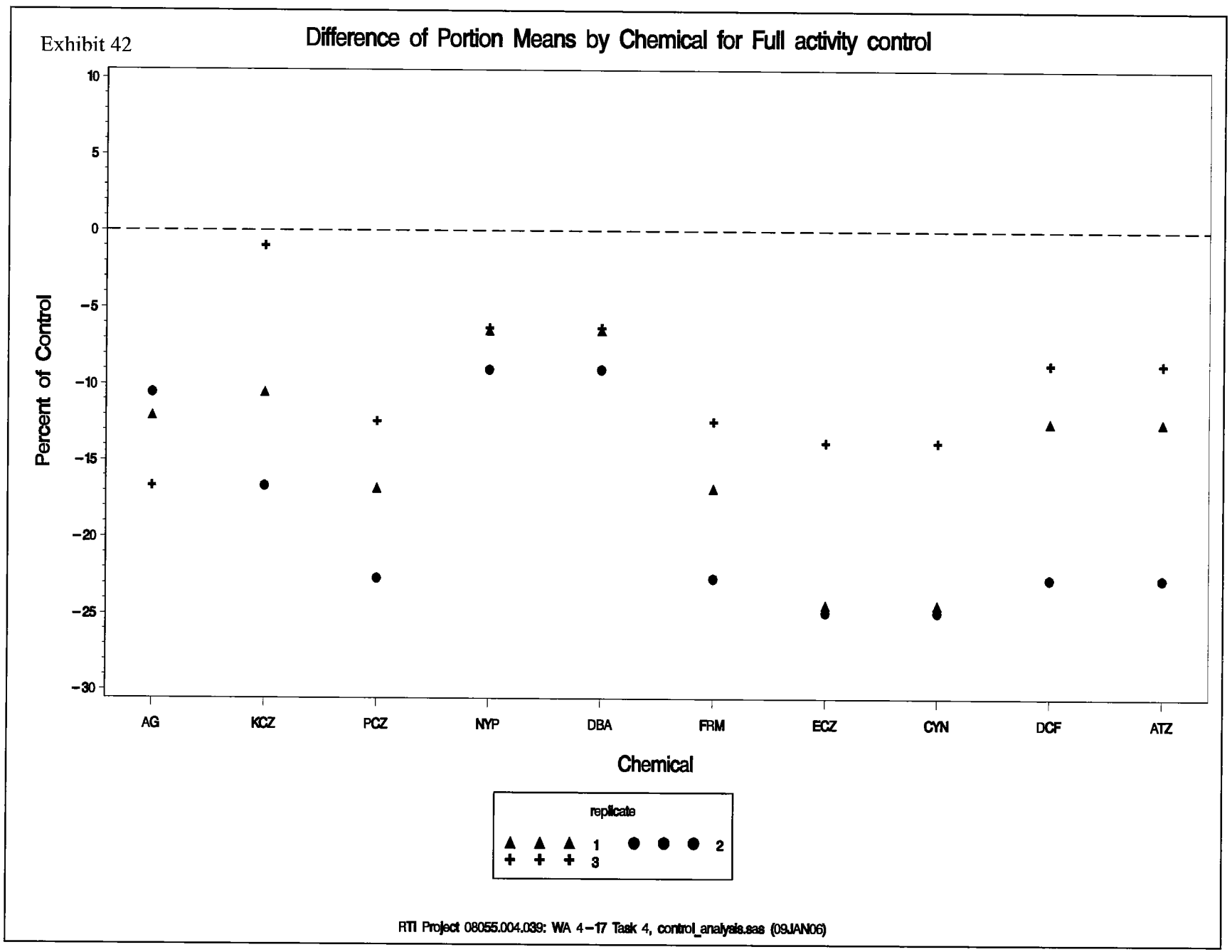

Difference of Portion Means by Chemical for Full activity control
Percent of Control
10
5
0
-5
-10
-15
-20
-25
-30
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
replicate
1
2
3
RTI Project 08055.004.039: WA 4-17 Task 4, control_analysis.sas (09JAN06)

Exhibit 43

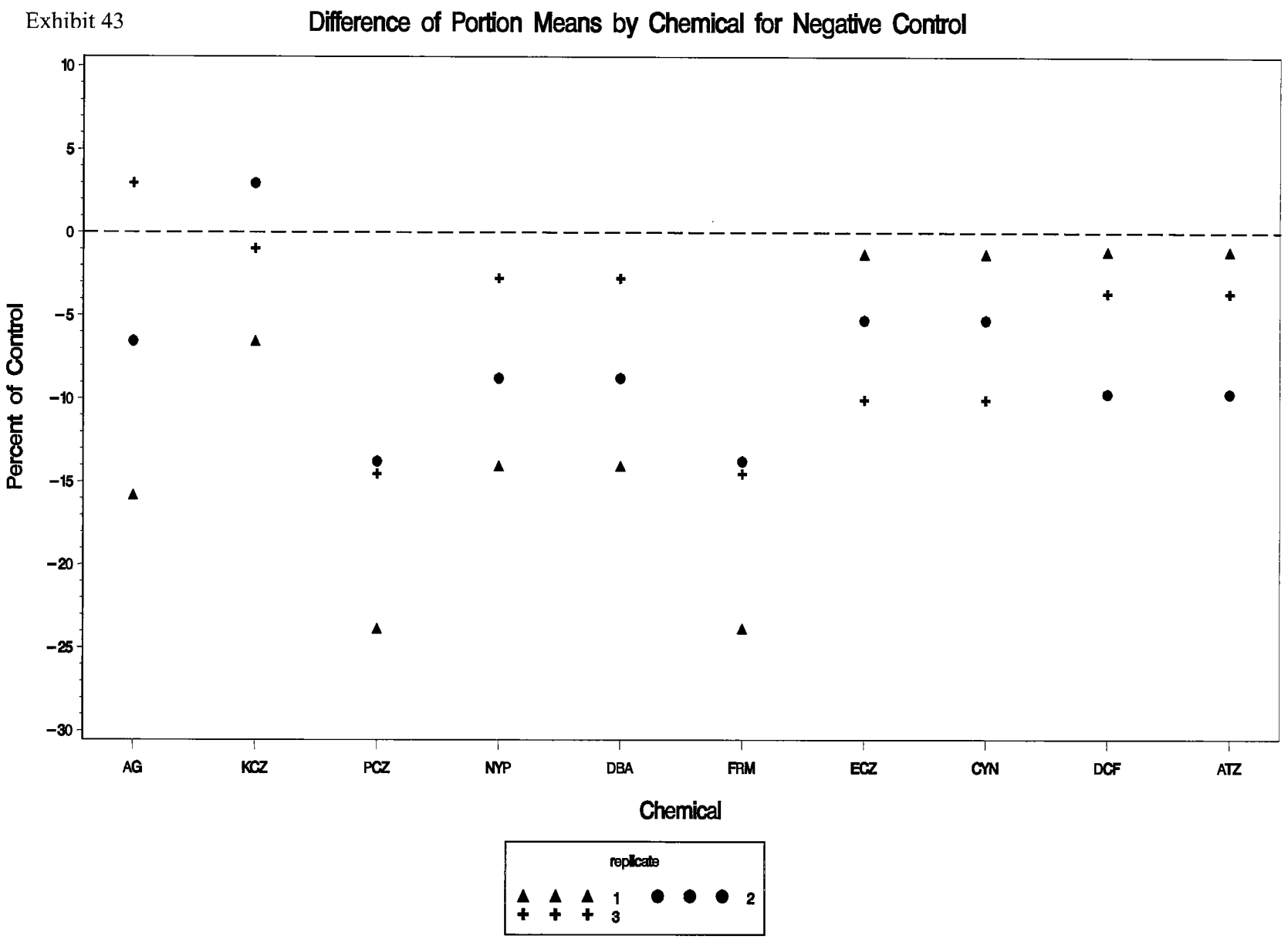
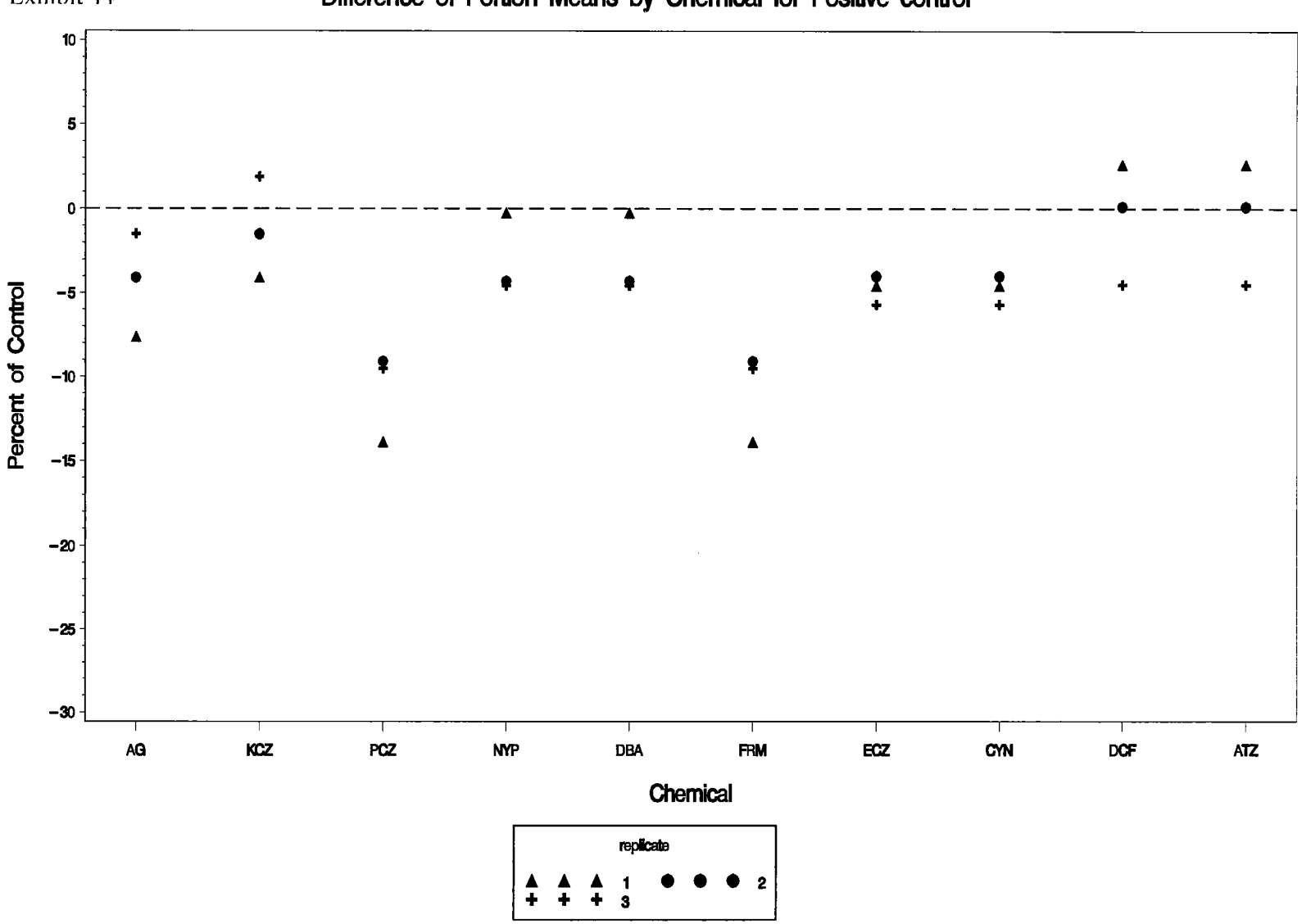

Difference of Portion Means by Chemical for Negative Control
Percent of Control
10
5
0
-5
-10
-15
-20
-25
-30
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
replicate
1
2
3
RTI Project 08055.004.039: WA 4-17 Task 4, Epr@GI_analysis.sas (09JAN06)

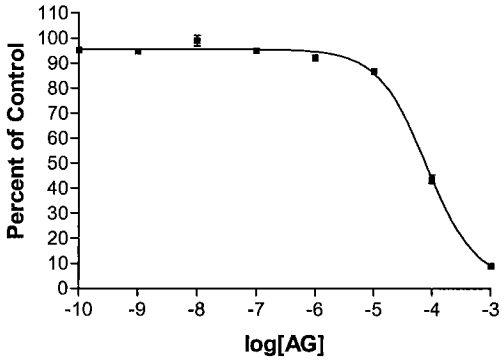
Exhibit 44


Difference of Portion Means by Chemical for Positive control
Percent of Control
10
5
0
-5
-10
-15
-20
-25
-30
AG
KCZ
PCZ
NYP
DBA
FRM
ECZ
CYN
DCF
ATZ
Chemical
replicate
1
2
3
RTI Project 08055.004.039: WA 4-17 Task 4, control_analysis.sas (09JAN06)

Appendix F

Concentration Response Curves

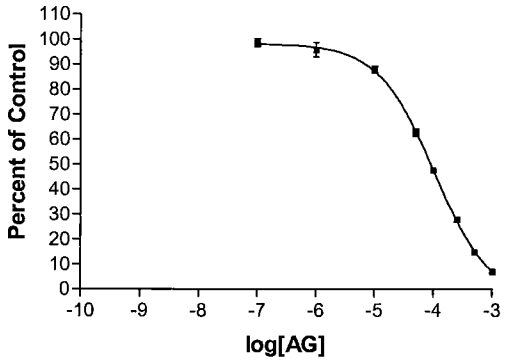
Aminoglutethimide, Replicate 1



log[AG]	Replicate 1		
	Y1	Y2	Y3
-3.0	9.7	9.3	8.5
-3.3			
-3.6			
-4.0	40.5	46.2	44.7
-4.3			
-5.0	86.9	~~56.6~~	86.8
-6.0	93.5	89.9	92.7
-7.0	94.0	94.4	97.1
-8.0	97.6	96.2	103.3
-9.0	94.4	93.6	96.6
-10.0	95.2	94.5	96.3

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	2.781
TOP	95.60
LOGEC50	-4.096
HILLSLOPE	-1.036
Std. Error	
BOTTOM	2.934
TOP	0.6988
LOGEC50	0.04096
HILLSLOPE	0.1105
95% Confidence Intervals	
BOTTOM	-3.361 to 8.923
TOP	94.14 to 97.06
LOGEC50	-4.181 to -4.010
HILLSLOPE	-1.268 to -0.8049
Goodness of Fit	
Degrees of Freedom	19
R^2	0.9944
Absolute Sum of Squares	125.5
Sy.x	2.570
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	23
Number of missing values	10

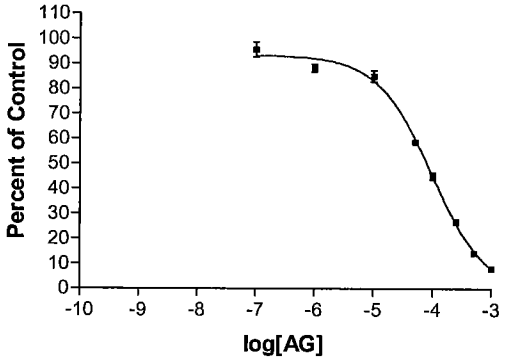
Aminoglutethimide, Replicate 2



log[AG]	Replicate 2		
	Y1	Y2	Y3
-3.0	6.8	7.0	7.5
-3.3	14.8	15.0	14.9
-3.6	28.0	28.2	27.7
-4.0	48.1	46.4	48.7
-4.3	62.6	60.5	65.5
-5.0	87.0	90.4	86.4
-6.0	95.7	90.8	100.8
-7.0	95.6	100.8	99.4
-8.0			
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	-4.887
TOP	98.23
LOGEC50	-3.978
HILLSLOPE	-0.9035
Std. Error	
BOTTOM	3.433
TOP	1.009
LOGEC50	0.03964
HILLSLOPE	0.06290
95% Confidence Intervals	
BOTTOM	-12.05 to 2.274
TOP	96.13 to 100.3
LOGEC50	-4.061 to -3.896
HILLSLOPE	-1.035 to -0.7723
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9966
Absolute Sum of Squares	96.87
Sy.x	2.201
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

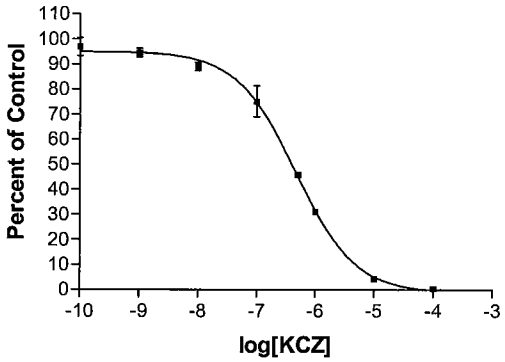
Aminoglutethimide, Replicate 3



log[AG]	Replicate 3		
	Y1	Y2	Y3
-3.0	8.4	8.1	7.9
-3.3	13.4	15.4	14.2
-3.6	27.7	26.6	26.0
-4.0	45.5	47.2	42.5
-4.3	58.8	58.8	58.8
-5.0	89.4	83.3	82.3
-6.0	89.0	85.2	90.7
-7.0	92.7	92.6	101.6
-8.0			
-9.0			
-10.0			

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	-1.499
TOP	93.28
LOGEC50	-4.018
HILLSLOPE	-0.9384
Std. Error	
BOTTOM	4.268
TOP	1.397
LOGEC50	0.05313
HILLSLOPE	0.09583
95% Confidence Intervals	
BOTTOM	-10.40 to 7.404
TOP	90.36 to 96.19
LOGEC50	-4.129 to -3.907
HILLSLOPE	-1.138 to -0.7385
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9924
Absolute Sum of Squares	191.0
Sy.x	3.090
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

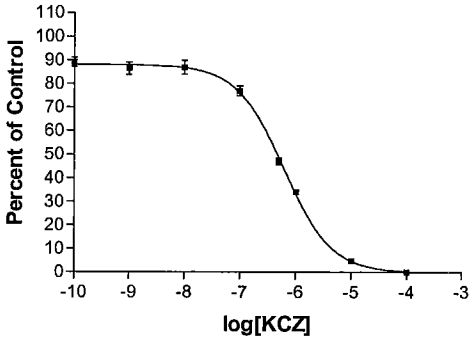
Ketoconazole, Replicate 1



log[KCZ]	Replicate 1		
	Y1	Y2	Y3
-4.0	0.5	0.3	0.4
-5.0	4.4	4.2	4.4
-6.0	30.1	32.3	31.2
-6.3	45.3	46.7	45.8
-7.0	82.6	80.1	63.0
-8.0	88.6	92.1	86.7
-9.0	98.0	92.8	92.8
-10.0	90.5	97.4	102.9

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	-1.319
TOP	95.08
LOGEC50	-6.332
HILLSLOPE	-0.8562
Std. Error	
BOTTOM	2.433
TOP	1.680
LOGEC50	0.04977
HILLSLOPE	0.09097
95% Confidence Intervals	
BOTTOM	-6.394 to 3.757
TOP	91.57 to 98.58
LOGEC50	-6.436 to -6.228
HILLSLOPE	-1.046 to -0.6665
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9888
Absolute Sum of Squares	377.6
Sy.x	4.345
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

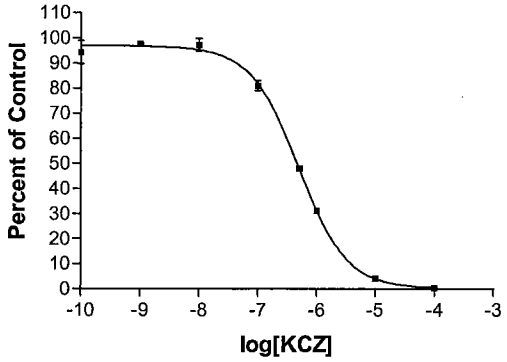
Ketoconazole, Replicate 2



log[KCZ]	Replicate 2		
	Y1	Y2	Y3
-4.0	0.1	0.1	-1.0e-002
-5.0	5.2	4.7	4.7
-6.0	35.4	34.9	32.2
-6.3	44.2	48.2	48.6
-7.0	81.1	75.5	74.5
-8.0	89.4	80.8	90.4
-9.0	84.0	83.5	91.5
-10.0	89.8	85.5	91.9

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	-0.1461
TOP	88.06
LOGEC50	-6.213
HILLSLOPE	-1.020
Std. Error	
BOTTOM	1.587
TOP	1.080
LOGEC50	0.03158
HILLSLOPE	0.08696
95% Confidence Intervals	
BOTTOM	-3.457 to 3.164
TOP	85.81 to 90.31
LOGEC50	-6.279 to -6.147
HILLSLOPE	-1.201 to -0.8382
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9937
Absolute Sum of Squares	183.9
Sy.x	3.032
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

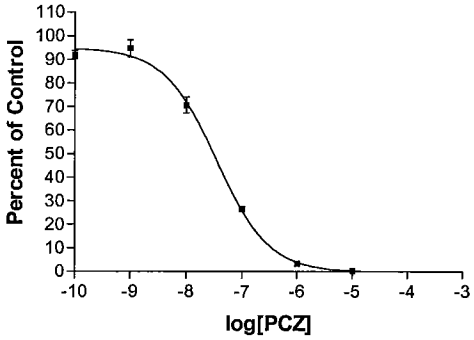
Ketoconazole, Replicate 3



log[KCZ]	Replicate 3		
	Y1	Y2	Y3
-4.0	0.4	0.4	0.5
-5.0	4.1	4.0	4.5
-6.0	31.3	29.9	32.5
-6.3	48.7	48.5	47.5
-7.0	77.2	84.0	82.2
-8.0	99.1	92.4	100.3
-9.0	95.8	99.0	98.1
-10.0	90.4	89.1	103.3

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	0.2179
TOP	96.83
LOGEC50	-6.308
HILLSLOPE	-1.056
Std. Error	
BOTTOM	1.656
TOP	1.211
LOGEC50	0.03105
HILLSLOPE	0.08612
95% Confidence Intervals	
BOTTOM	-3.237 to 3.673
TOP	94.31 to 99.36
LOGEC50	-6.373 to -6.243
HILLSLOPE	-1.235 to -0.8761
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9937
Absolute Sum of Squares	229.1
Sy.x	3.385
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

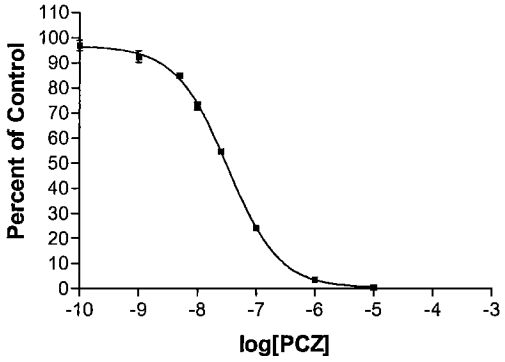
Prochloraz, Replicate 1



log[PCZ]	Replicate 1		
	Y1	Y2	Y3
-3.0	-0.1	0.0	-0.1
-4.0	1.0e-002	-0.1	-1.0e-002
-5.0	0.3	0.2	0.3
-6.0	3.2	3.2	3.8
-7.0	28.6	25.0	26.0
-7.6			
-8.0	64.9	70.5	76.9
-8.3			
-9.0	101.8	90.4	92.1
-10.0	91.6	89.2	95.2

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	-0.1794
TOP	94.89
LOGEC50	-7.459
HILLSLOPE	-0.9214
Std. Error	
BOTTOM	1.098
TOP	1.711
LOGEC50	0.04605
HILLSLOPE	0.07189
95% Confidence Intervals	
BOTTOM	-2.469 to 2.110
TOP	91.32 to 98.46
LOGEC50	-7.555 to -7.363
HILLSLOPE	-1.071 to -0.7715
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9940
Absolute Sum of Squares	232.3
Sy.x	3.408
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

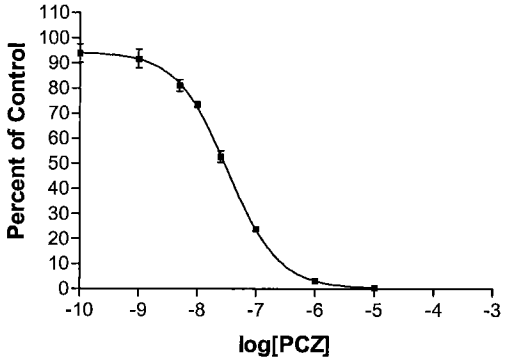
Prochloraz, Replicate 2



log[PCZ]	Replicate 2		
	Y1	Y2	Y3
-3.0			
-4.0			
-5.0	0.5	0.5	0.6
-6.0	3.6	3.5	3.5
-7.0	24.7	23.1	25.0
-7.6	55.4	53.1	55.8
-8.0	75.7	70.5	72.3
-8.3	83.3	84.7	87.0
-9.0	88.4	92.7	96.4
-10.0	92.9	97.9	99.7

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	0.2822
TOP	96.65
LOGEC50	-7.487
HILLSLOPE	-0.9868
Std. Error	
BOTTOM	1.067
TOP	1.060
LOGEC50	0.02328
HILLSLOPE	0.04696
95% Confidence Intervals	
BOTTOM	-1.944 to 2.508
TOP	94.43 to 98.86
LOGEC50	-7.535 to -7.438
HILLSLOPE	-1.085 to -0.8889
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9970
Absolute Sum of Squares	97.76
Sy.x	2.211
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

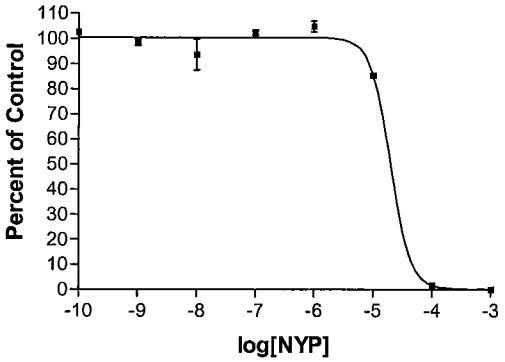
Prochloraz, Replicate 3



log[PCZ]	Replicate 3		
	Y1	Y2	Y3
-3.0			
-4.0			
-5.0	0.6	0.4	0.4
-6.0	2.5	3.3	3.5
-7.0	24.8	21.9	25.0
-7.6	57.3	49.5	51.7
-8.0	74.2	71.3	74.9
-8.3	78.0	79.7	85.8
-9.0	93.5	97.2	84.6
-10.0	94.4	100.1	87.3

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	0.1699
TOP	94.31
LOGEC50	-7.482
HILLSLOPE	-0.9968
Std. Error	
BOTTOM	1.709
TOP	1.692
LOGEC50	0.03800
HILLSLOPE	0.07788
95% Confidence Intervals	
BOTTOM	-3.395 to 3.735
TOP	90.79 to 97.84
LOGEC50	-7.561 to -7.403
HILLSLOPE	-1.159 to -0.8344
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9920
Absolute Sum of Squares	252.8
Sy.x	3.555
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

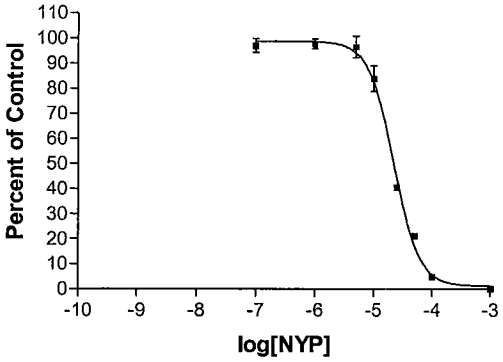
4-Nonylphenol, Replicate 1



log[NYP]	Replicate 1		
	Y1	Y2	Y3
-3.0	0.1	0.1	0.1
-4.0	1.6	1.9	1.9
-4.3			
-4.6			
-5.0	84.1	87.3	84.7
-5.3			
-6.0	104.4	108.8	101.3
-7.0	104.5	101.8	100.7
-8.0	97.3	81.7	102.0
-9.0	99.6	100.2	95.9
-10.0	102.6	102.9	102.4

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	0.2218
TOP	100.4
LOGEC50	-4.709
HILLSLOPE	-2.588
Std. Error	
BOTTOM	2.936
TOP	1.308
LOGEC50	0.1469
HILLSLOPE	1.248
95% Confidence Intervals	
BOTTOM	-5.902 to 6.345
TOP	97.69 to 103.1
LOGEC50	-5.015 to -4.402
HILLSLOPE	-5.192 to 0.01606
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9882
Absolute Sum of Squares	512.0
Sy.x	5.060
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

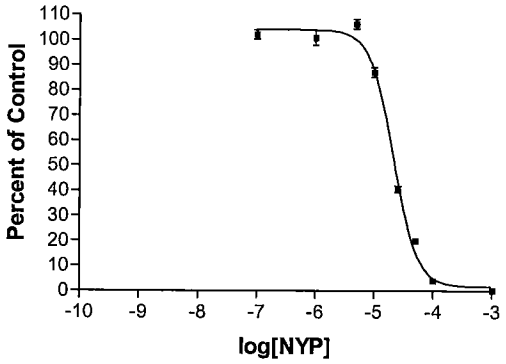
4-Nonylphenol, Replicate 2



log[NYP]	Replicate 2		
	Y1	Y2	Y3
-3.0	0.1	0.2	0.1
-4.0	4.1	4.6	5.9
-4.3	21.1	21.4	21.5
-4.6	39.8	40.0	41.9
-5.0	92.7	74.9	84.0
-5.3	101.5	88.1	100.0
-6.0	94.2	100.8	98.1
-7.0	91.8	101.3	98.0
-8.0			
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	1.255
TOP	98.75
LOGEC50	-4.654
HILLSLOPE	-2.018
Std. Error	
BOTTOM	2.345
TOP	1.850
LOGEC50	0.02659
HILLSLOPE	0.2053
95% Confidence Intervals	
BOTTOM	-3.637 to 6.147
TOP	94.90 to 102.6
LOGEC50	-4.709 to -4.598
HILLSLOPE	-2.446 to -1.590
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9881
Absolute Sum of Squares	469.1
Sy.x	4.843
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

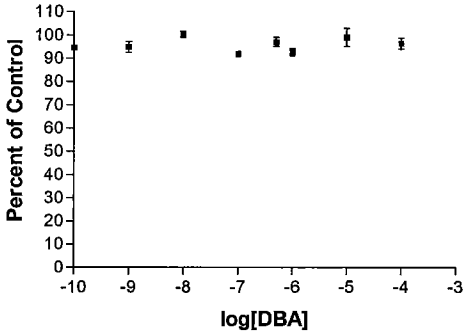
4-Nonylphenol, Replicate 3



log[NYP]	Replicate 3		
	Y1	Y2	Y3
-3.0	0.1	0.1	0.1
-4.0	5.5	2.8	3.8
-4.3	20.1	21.8	18.2
-4.6	39.4	43.1	39.5
-5.0	90.9	85.0	85.4
-5.3	107.5	102.6	108.9
-6.0	97.1	98.7	107.0
-7.0	102.2	98.8	105.5
-8.0			
-9.0			
-10.0			

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	1.698
TOP	104.2
LOGEC50	-4.673
HILLSLOPE	-2.186
Std. Error	
BOTTOM	2.084
TOP	1.705
LOGEC50	0.02229
HILLSLOPE	0.2014
95% Confidence Intervals	
BOTTOM	-2.650 to 6.046
TOP	100.6 to 107.7
LOGEC50	-4.720 to -4.627
HILLSLOPE	-2.606 to -1.766
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9909
Absolute Sum of Squares	408.2
Sy.x	4.518
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

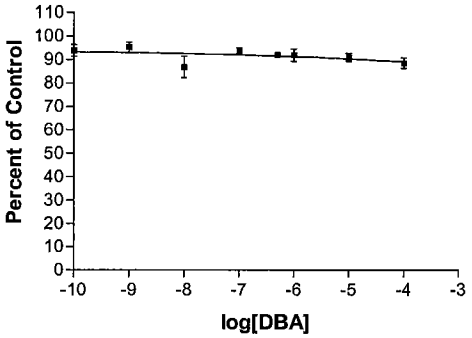
Dibenz[a,h]anthracene, Replicate 1



log[DBA]	Replicate 1		
	Y1	Y2	Y3
-4.0	92.1	96.4	100.3
-5.0	106.6	96.6	93.7
-6.0	91.8	90.6	95.9
-6.3	93.1	99.3	98.5
-7.0	91.8	92.9	91.0
-8.0	98.7	103.0	99.2
-9.0	99.4	92.0	93.1
-10.0	95.8	93.6	94.3

	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	

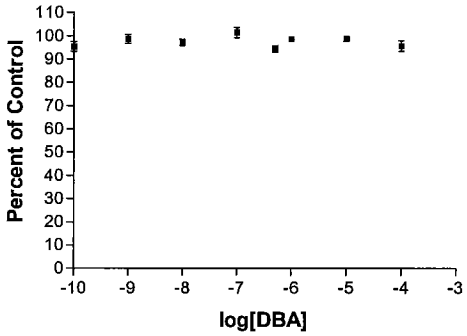
Dibenz[a,h]anthracene, Replicate 2



log[DBA]	Replicate 2		
	Y1	Y2	Y3
-4.0	83.9	90.6	90.9
-5.0	87.6	91.5	93.7
-6.0	97.1	89.8	88.6
-6.3	91.6	92.4	92.3
-7.0	94.9	91.3	95.1
-8.0	92.2	90.8	77.6
-9.0	93.6	99.5	92.8
-10.0	97.3	95.4	89.1

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	-34180
TOP	94.10
LOGEC50	25.19
HILLSLOPE	-0.1312
Std. Error	
BOTTOM	4.9980e+009
TOP	23.61
LOGEC50	483297
HILLSLOPE	2.869
95% Confidence Intervals	
BOTTOM	-10430000000 to 1.0430e+010
TOP	44.85 to 143.3
LOGEC50	-1008000 to 1.0080e+006
HILLSLOPE	-6.117 to 5.854
Goodness of Fit	
Degrees of Freedom	20
R^2	0.09070
Absolute Sum of Squares	421.7
Sy.x	4.592
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

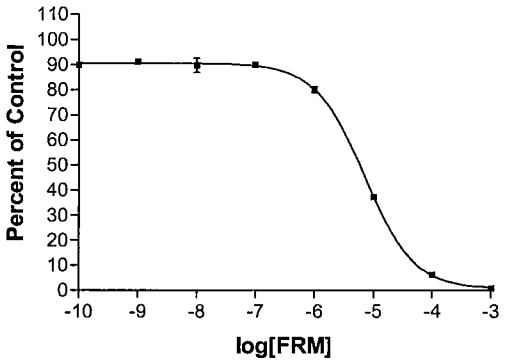
Dibenz[a,h]anthracene, Replicate 3



log[DBA]	Replicate 3		
	Y1	Y2	Y3
-4.0	93.3	93.6	100.2
-5.0	100.3	98.4	97.8
-6.0	97.5	98.4	100.5
-6.3	96.6	94.7	92.0
-7.0	100.1	105.8	98.7
-8.0	99.6	97.0	94.8
-9.0	97.1	102.7	96.4
-10.0	91.5	96.6	98.2

	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	

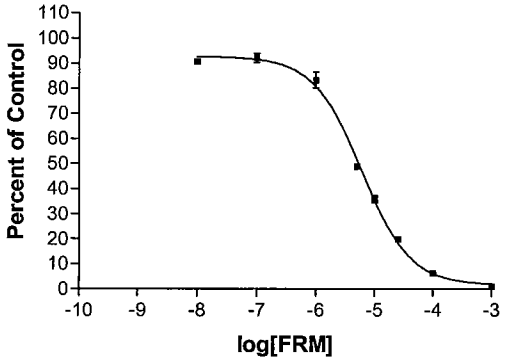
Fenarimol, Replicate 1



log[FRM]	Replicate 1		
	Y1	Y2	Y3
-3.0	0.9	0.9	0.8
-4.0	6.5	6.2	6.1
-4.6			
-5.0	36.8	37.2	38.0
-5.3			
-6.0	77.8	81.0	81.6
-7.0	89.2	89.9	90.9
-8.0	92.9	92.1	84.2
-9.0	90.0	91.0	92.7
-10.0	88.8	89.2	91.4

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	0.6013
TOP	90.51
LOGEC50	-5.152
HILLSLOPE	-1.041
Std. Error	
BOTTOM	0.9991
TOP	0.5684
LOGEC50	0.02339
HILLSLOPE	0.05676
95% Confidence Intervals	
BOTTOM	-1.483 to 2.685
TOP	89.32 to 91.69
LOGEC50	-5.201 to -5.103
HILLSLOPE	-1.159 to -0.9222
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9979
Absolute Sum of Squares	70.28
Sy.x	1.875
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

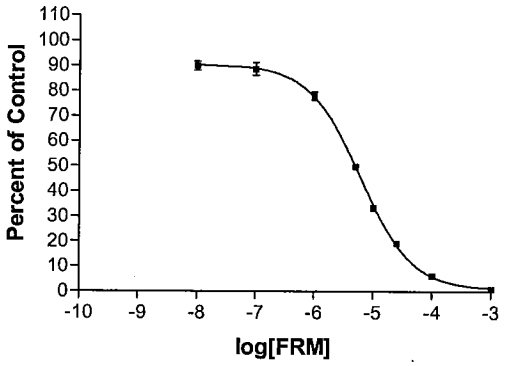
Fenarimol, Replicate 2



log[FRM]	Replicate 2		
	Y1	Y2	Y3
-3.0	1.0	1.0	1.0
-4.0	6.4	6.4	6.4
-4.6	19.6	21.0	19.4
-5.0	37.5	37.1	33.1
-5.3	50.2	47.7	48.9
-6.0	77.0	86.7	86.0
-7.0	93.3	94.4	88.7
-8.0	90.1	92.6	89.3
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	1.631
TOP	92.68
LOGEC50	-5.219
HILLSLOPE	-1.049
Std. Error	
BOTTOM	1.477
TOP	1.252
LOGEC50	0.02851
HILLSLOPE	0.07255
95% Confidence Intervals	
BOTTOM	-1.450 to 4.711
TOP	90.06 to 95.29
LOGEC50	-5.278 to -5.159
HILLSLOPE	-1.201 to -0.8979
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9945
Absolute Sum of Squares	164.6
Sy.x	2.869
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

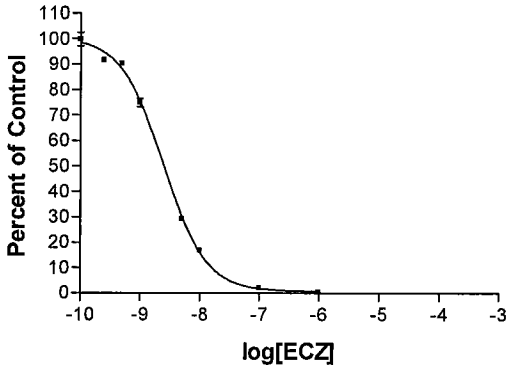
Fenarimol, Replicate 3



log[FRM]	Replicate 3		
	Y1	Y2	Y3
-3.0	0.9	1.0	1.0
-4.0	6.1	6.1	6.3
-4.6	19.5	18.9	19.5
-5.0	35.0	33.4	32.1
-5.3	49.7	50.3	49.7
-6.0	76.6	76.3	81.2
-7.0	93.4	84.7	87.9
-8.0	87.9	88.5	93.6
-9.0			
-10.0			

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	0.8316
TOP	90.33
LOGEC50	-5.218
HILLSLOPE	-0.9949
Std. Error	
BOTTOM	1.111
TOP	0.9310
LOGEC50	0.02225
HILLSLOPE	0.05099
95% Confidence Intervals	
BOTTOM	-1.485 to 3.148
TOP	88.39 to 92.27
LOGEC50	-5.265 to -5.172
HILLSLOPE	-1.101 to -0.8886
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9969
Absolute Sum of Squares	86.88
Sy.x	2.084
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

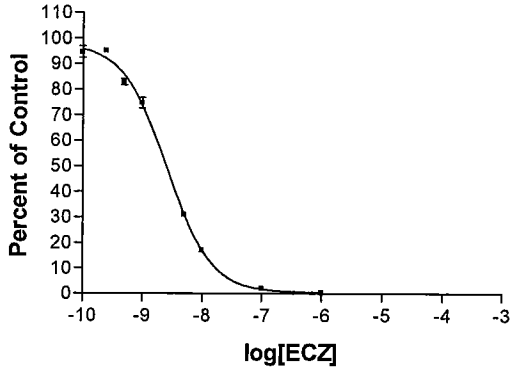
Econazole, Replicate 2



log[ECZ]	Replicate 2		
	Y1	Y2	Y3
-6.0	0.3	0.2	0.3
-7.0	2.3	1.9	2.0
-8.0	15.7	16.6	17.8
-8.3	30.1	30.3	27.4
-9.0	77.2	75.9	71.7
-9.3	90.5	88.9	91.2
-9.6	91.9	91.3	91.2
-10.0	94.8	104.0	100.6

	Replicate 2
Top down to Bottom, variable slope	
Best-fit values	
BOTTOM	0.6946
TOP	100.6
LOGEC50	-8.614
HILLSLOPE	-1.216
Std. Error	
BOTTOM	1.089
TOP	1.571
LOGEC50	0.02592
HILLSLOPE	0.06375
95% Confidence Intervals	
BOTTOM	-1.576 to 2.966
TOP	97.29 to 103.8
LOGEC50	-8.668 to -8.560
HILLSLOPE	-1.349 to -1.083
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9967
Absolute Sum of Squares	127.6
Sy.x	2.526
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

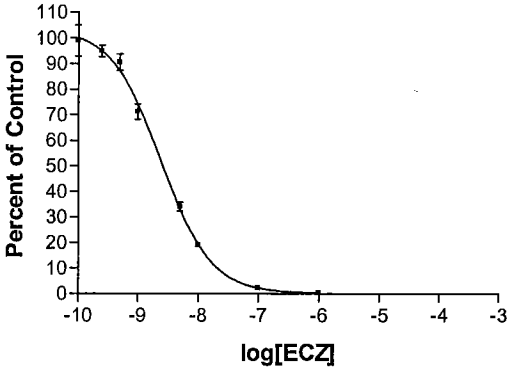
Econazole, Replicate 3



log[ECZ]	Replicate 3		
	Y1	Y2	Y3
-6.0	0.3	0.3	0.3
-7.0	1.9	2.1	2.0
-8.0	15.3	18.0	17.8
-8.3	30.2	31.3	31.1
-9.0	77.0	70.7	76.4
-9.3	82.2	81.3	85.2
-9.6	95.6	95.1	94.2
-10.0	94.9	90.5	98.3

	Replicate 3
Top down to Bottom, variable slope	
Best-fit values	
BOTTOM	0.4549
TOP	97.88
LOGEC50	-8.589
HILLSLOPE	-1.176
Std. Error	
BOTTOM	1.094
TOP	1.591
LOGEC50	0.02698
HILLSLOPE	0.06406
95% Confidence Intervals	
BOTTOM	-1.826 to 2.736
TOP	94.57 to 101.2
LOGEC50	-8.645 to -8.532
HILLSLOPE	-1.309 to -1.042
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9965
Absolute Sum of Squares	125.0
Sy.x	2.500
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

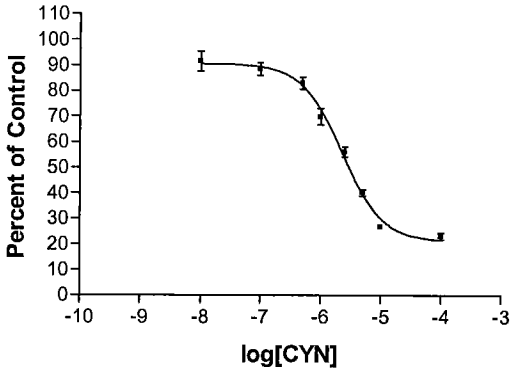
Econazole, Replicate 4



log[ECZ]	Replicate 4		
	Y1	Y2	Y3
-6.0	0.2	0.3	0.2
-7.0	2.1	2.2	2.5
-8.0	17.7	19.9	19.6
-8.3	32.0	37.5	32.8
-9.0	71.0	76.4	66.0
-9.3	89.7	85.4	96.3
-9.6	99.2	94.0	91.4
-10.0	98.4	88.7	109.8

	Replicate 4
Top down to Bottom, variable slope	
Best-fit values	
BOTTOM	0.1701
TOP	103.7
LOGEC50	-8.617
HILLSLOPE	-1.043
Std. Error	
BOTTOM	2.199
TOP	3.627
LOGEC50	0.05578
HILLSLOPE	0.1087
95% Confidence Intervals	
BOTTOM	-4.418 to 4.758
TOP	96.16 to 111.3
LOGEC50	-8.733 to -8.501
HILLSLOPE	-1.270 to -0.8160
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9877
Absolute Sum of Squares	463.2
Sy.x	4.813
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

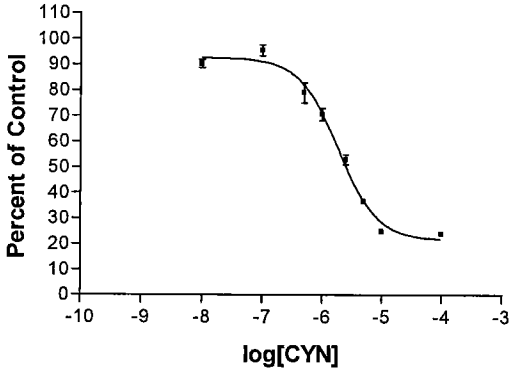
Chrysin, Replicate 2



log[CYN]	Replicate 2		
	Y1	Y2	Y3
-4.0	25.3	21.0	23.3
-5.0	28.5	26.7	25.2
-5.3	41.2	37.8	41.6
-5.6	52.3	57.2	58.8
-6.0	63.5	72.7	73.4
-6.3	84.3	86.3	78.5
-7.0	92.5	89.1	84.1
-8.0	89.1	99.2	86.0

	Replicate 2
Top down to Bottom, variable slope	
Best-fit values	
BOTTOM	21.16
TOP	90.54
LOGEC50	-5.645
HILLSLOPE	-1.295
Std. Error	
BOTTOM	2.513
TOP	1.870
LOGEC50	0.04807
HILLSLOPE	0.1526
95% Confidence Intervals	
BOTTOM	15.92 to 26.40
TOP	86.64 to 94.44
LOGEC50	-5.745 to -5.544
HILLSLOPE	-1.614 to -0.9768
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9776
Absolute Sum of Squares	361.2
Sy.x	4.250
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

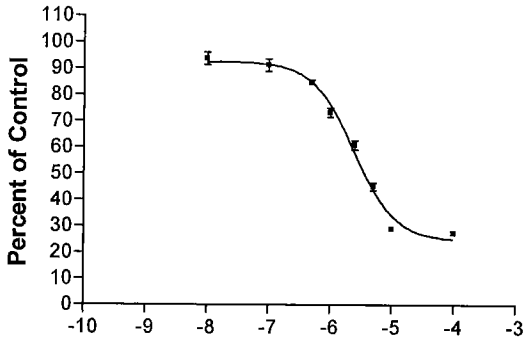
Chrysin, Replicate 3



log[CYN]	Replicate 3		
	Y1	Y2	Y3
-4.0	25.0	24.3	22.6
-5.0	23.7	26.0	25.0
-5.3	35.7	37.8	36.5
-5.6	49.7	56.3	52.7
-6.0	70.6	74.7	66.3
-6.3	86.6	73.9	76.6
-7.0	99.8	92.6	94.2
-8.0	90.8	87.3	92.6

	Replicate 3
Top down to Bottom, variable slope	
Best-fit values	
BOTTOM	21.55
TOP	92.60
LOGEC50	-5.730
HILLSLOPE	-1.322
Std. Error	
BOTTOM	2.430
TOP	1.927
LOGEC50	0.04673
HILLSLOPE	0.1532
95% Confidence Intervals	
BOTTOM	16.48 to 26.62
TOP	88.58 to 96.62
LOGEC50	-5.828 to -5.633
HILLSLOPE	-1.641 to -1.002
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9788
Absolute Sum of Squares	370.5
Sy.x	4.304
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

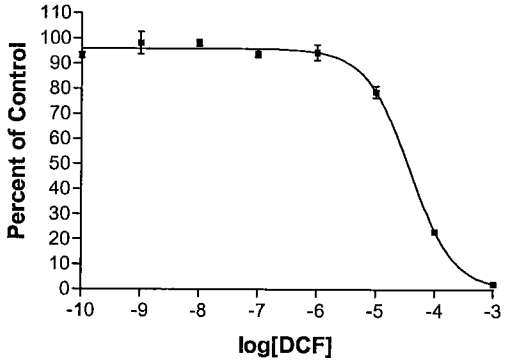
Chrysin, Replicate 4



log[CYN]	Replicate 4		
	Y1	Y2	Y3
-4.0	27.7	27.7	26.8
-5.0	29.1	27.2	30.5
-5.3	42.7	45.0	47.8
-5.6	64.2	59.5	58.7
-6.0	73.1	70.3	76.3
-6.3	82.4	85.2	85.9
-7.0	93.5	86.4	93.7
-8.0	97.5	94.1	89.3

	Replicate 4
Top down to Bottom, variable slope	
Best-fit values	
BOTTOM	24.62
TOP	92.48
LOGEC50	-5.617
HILLSLOPE	-1.298
Std. Error	
BOTTOM	2.255
TOP	1.646
LOGEC50	0.04351
HILLSLOPE	0.1392
95% Confidence Intervals	
BOTTOM	19.91 to 29.32
TOP	89.05 to 95.91
LOGEC50	-5.708 to -5.527
HILLSLOPE	-1.589 to -1.008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9814
Absolute Sum of Squares	284.8
Sy.x	3.773
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

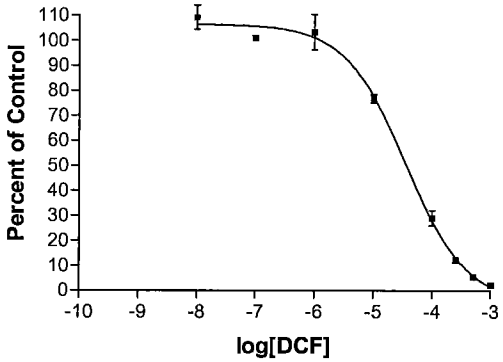
Dicofol, Replicate 1



log[DCF]	Replicate 1		
	Y1	Y2	Y3
-3.0	2.5	2.1	2.2
-3.3			
-3.6			
-4.0	24.6	22.2	23.2
-5.0	82.6	79.7	74.3
-6.0	90.5	92.4	100.7
-7.0	92.1	97.3	92.9
-8.0	100.7	95.9	97.8
-9.0	106.9	92.2	95.5
-10.0	91.2	93.6	94.9

	Replicate 1
Equation 1	
Best-fit values	
BOTTOM	0.1887
TOP	95.95
LOGEC50	-4.428
HILLSLOPE	-1.159
Std. Error	
BOTTOM	2.846
TOP	1.061
LOGEC50	0.05282
HILLSLOPE	0.1085
95% Confidence Intervals	
BOTTOM	-5.748 to 6.126
TOP	93.73 to 98.16
LOGEC50	-4.539 to -4.318
HILLSLOPE	-1.386 to -0.9331
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9898
Absolute Sum of Squares	312.0
Sy.x	3.950
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

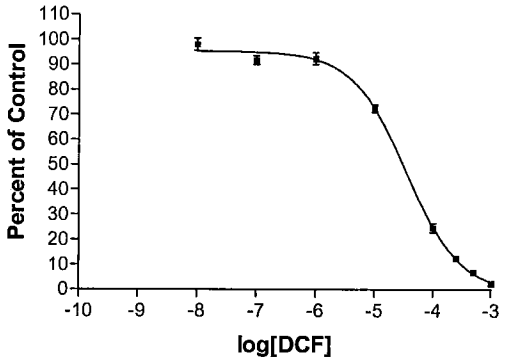
Dicofol, Replicate 2



log[DCF]	Replicate 2		
	Y1	Y2	Y3
-3.0	2.8	1.8	2.1
-3.3	4.8	6.7	5.3
-3.6	11.1	11.5	14.4
-4.0	23.8	29.3	34.2
-5.0	74.1	77.0	79.6
-6.0	113.8	106.7	89.7
-7.0	100.7	102.2	100.5
-8.0	115.1	99.7	113.2
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	-4.776
TOP	106.5
LOGEC50	-4.453
HILLSLOPE	-0.8375
Std. Error	
BOTTOM	5.315
TOP	2.287
LOGEC50	0.08875
HILLSLOPE	0.1125
95% Confidence Intervals	
BOTTOM	-15.86 to 6.311
TOP	101.8 to 111.3
LOGEC50	-4.638 to -4.268
HILLSLOPE	-1.072 to -0.6028
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9865
Absolute Sum of Squares	640.8
Sy.x	5.661
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

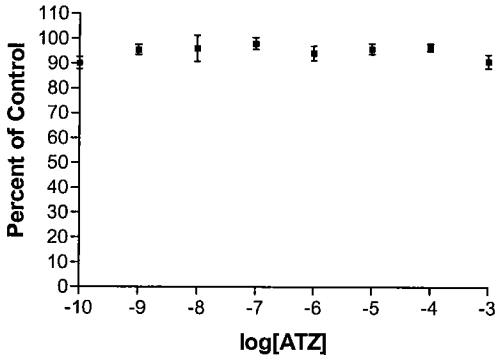
Dicofol, Replicate 3



log[DCF]	Replicate 3		
	Y1	Y2	Y3
-3.0	2.6	2.7	2.1
-3.3	7.1	7.4	6.3
-3.6	11.8	11.7	14.2
-4.0	23.0	22.8	28.2
-5.0	75.7	70.8	71.2
-6.0	92.3	96.6	88.3
-7.0	94.2	92.7	88.3
-8.0	96.3	94.8	102.7
-9.0			
-10.0			

	Replicate 3
Equation 1	
Best-fit values	
BOTTOM	-0.9486
TOP	95.29
LOGEC50	-4.459
HILLSLOPE	-0.9354
Std. Error	
BOTTOM	2.385
TOP	1.157
LOGEC50	0.04765
HILLSLOPE	0.07271
95% Confidence Intervals	
BOTTOM	-5.924 to 4.026
TOP	92.88 to 97.71
LOGEC50	-4.559 to -4.360
HILLSLOPE	-1.087 to -0.7837
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9951
Absolute Sum of Squares	182.7
Sy.x	3.023
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

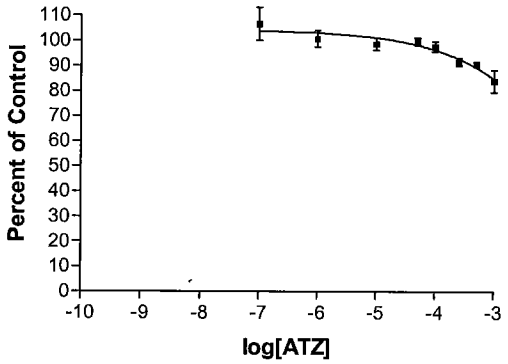
Atrazine, Replicate 1



log[ATZ]	Replicate 1		
	Y1	Y2	Y3
-3.0	93.2	94.0	85.3
-3.3			
-3.6			
-4.0	97.4	93.6	98.7
-4.3			
-5.0	99.9	95.1	92.7
-6.0	97.9	88.4	95.9
-7.0	99.2	101.2	93.6
-8.0	92.2	106.4	89.5
-9.0	91.6	96.7	98.2
-10.0	94.1	90.6	85.7

	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	

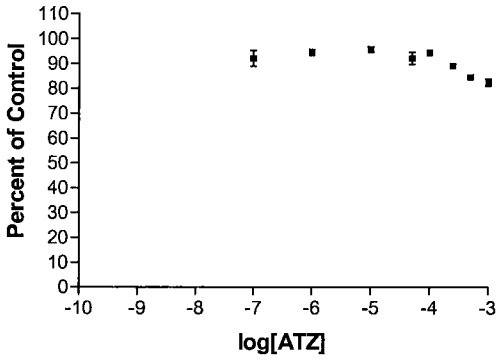
Atrazine, Replicate 2



log[ATZ]	Replicate 2		
	Y1	Y2	Y3
-3.0	92.8	78.7	80.5
-3.3	91.2	92.0	88.4
-3.6	88.8	94.5	91.8
-4.0	95.3	101.6	96.0
-4.3	102.6	99.2	97.5
-5.0	100.4	101.9	93.8
-6.0	100.7	94.9	106.6
-7.0	93.6	112.0	114.4
-8.0			
-9.0			
-10.0			

	Replicate 2
Equation 1	
Best-fit values	
BOTTOM	-111.7
TOP	104.3
LOGEC50	-0.5980
HILLSLOPE	-0.4175
Std. Error	
BOTTOM	5617
TOP	4.787
LOGEC50	34.71
HILLSLOPE	0.8322
95% Confidence Intervals	
BOTTOM	-11830 to 11605
TOP	94.30 to 114.3
LOGEC50	-73.00 to 71.80
HILLSLOPE	-2.153 to 1.318
Goodness of Fit	
Degrees of Freedom	20
R^2	0.6101
Absolute Sum of Squares	623.4
Sy.x	5.583
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

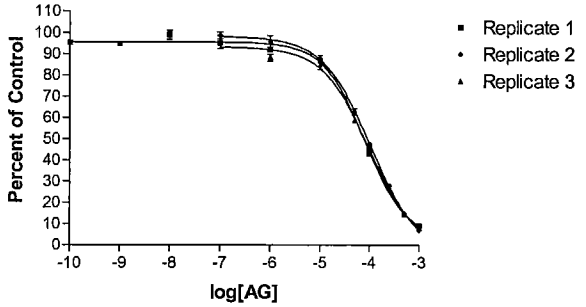
Atrazine, Replicate 3



log[ATZ]	Replicate 3		
	Y1	Y2	Y3
-3.0	79.8	83.0	84.4
-3.3	85.2	85.5	83.2
-3.6	90.6	87.4	89.5
-4.0	95.9	93.3	94.2
-4.3	87.6	95.3	93.6
-5.0	95.0	97.7	94.0
-6.0	96.9	93.1	93.5
-7.0	98.1	88.6	89.5
-8.0			
-9.0			
-10.0			

	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	

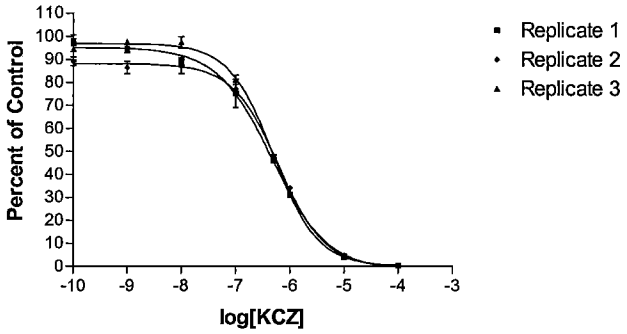
Aminoglutethimide



log[AG]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	9.7	9.3	8.5	6.8	7.0	7.5	8.4	8.1	7.9
-3.3				14.8	15.0	14.9	13.4	15.4	14.2
-3.6				28.0	28.2	27.7	27.7	26.6	26.0
-4.0	40.5	46.2	44.7	48.1	46.4	48.7	45.5	47.2	42.5
-4.3				62.6	60.5	65.5	58.8	58.8	58.8
-5.0	86.9	~~56.6~~	86.8	87.0	90.4	86.4	89.4	83.3	82.3
-6.0	93.5	89.9	92.7	95.7	90.8	100.8	89.0	85.2	90.7
-7.0	94.0	94.4	97.1	95.6	100.8	99.4	92.7	92.6	101.6
-8.0	97.6	96.2	103.3						
-9.0	94.4	93.6	96.6						
-10.0	95.2	94.5	96.3						

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	2.781	-4.887	-1.499
TOP	95.60	98.23	93.28
LOGEC50	-4.096	-3.978	-4.018
HILLSLOPE	-1.036	-0.9035	-0.9384
Std. Error			
BOTTOM	2.934	3.433	4.268
TOP	0.6988	1.009	1.397
LOGEC50	0.04096	0.03964	0.05313
HILLSLOPE	0.1105	0.06290	0.09583
95% Confidence Intervals			
BOTTOM	-3.361 to 8.923	-12.05 to 2.274	-10.40 to 7.404
TOP	94.14 to 97.06	96.13 to 100.3	90.36 to 96.19
LOGEC50	-4.181 to -4.010	-4.061 to -3.896	-4.129 to -3.907
HILLSLOPE	-1.268 to -0.8049	-1.035 to -0.7723	-1.138 to -0.7385
Goodness of Fit			
Degrees of Freedom	19	20	20
R^2	0.9944	0.9966	0.9924
Absolute Sum of Squares	125.5	96.87	191.0
Sy.x	2.570	2.201	3.090
Data			
Number of X values	11	8	8
Number of Y replicates	3	3	3
Total number of values	23	24	24
Number of missing values	10	0	0

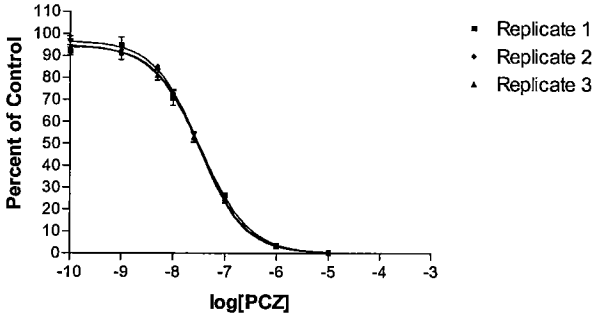
Ketoconazole



log[KCZ]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.0	0.5	0.3	0.4	0.1	0.1	-1.0e-002	0.4	0.4	0.5
-5.0	4.4	4.2	4.4	5.2	4.7	4.7	4.1	4.0	4.5
-6.0	30.1	32.3	31.2	35.4	34.9	32.2	31.3	29.9	32.5
-6.3	45.3	46.7	45.8	44.2	48.2	48.6	48.7	48.5	47.5
-7.0	82.6	80.1	63.0	81.1	75.5	74.5	77.2	84.0	82.2
-8.0	88.6	92.1	86.7	89.4	80.8	90.4	99.1	92.4	100.3
-9.0	98.0	92.8	92.8	84.0	83.5	91.5	95.8	99.0	98.1
-10.0	90.5	97.4	102.9	89.8	85.5	91.9	90.4	89.1	103.3

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	-1.319	-0.1461	0.2179
TOP	95.08	88.06	96.83
LOGEC50	-6.332	-6.213	-6.308
HILLSLOPE	-0.8562	-1.020	-1.056
Std. Error			
BOTTOM	2.433	1.587	1.656
TOP	1.680	1.080	1.211
LOGEC50	0.04977	0.03158	0.03105
HILLSLOPE	0.09097	0.08696	0.08612
95% Confidence Intervals			
BOTTOM	-6.394 to 3.757	-3.457 to 3.164	-3.237 to 3.673
TOP	91.57 to 98.58	85.81 to 90.31	94.31 to 99.36
LOGEC50	-6.436 to -6.228	-6.279 to -6.147	-6.373 to -6.243
HILLSLOPE	-1.046 to -0.6665	-1.201 to -0.8382	-1.235 to -0.8761
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9888	0.9937	0.9937
Absolute Sum of Squares	377.6	183.9	229.1
Sy.x	4.345	3.032	3.385
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

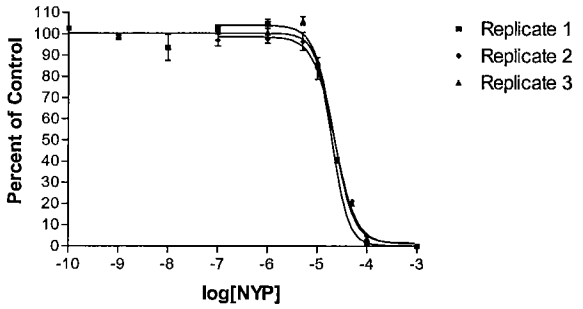
Prochloraz



log[PCZ]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	-0.1	0.0	-0.1						
-4.0	1.0e-002	-0.1	-1.0e-002						
-5.0	0.3	0.2	0.3	0.5	0.5	0.6	0.6	0.4	0.4
-6.0	3.2	3.2	3.8	3.6	3.5	3.5	2.5	3.3	3.5
-7.0	28.6	25.0	26.0	24.7	23.1	25.0	24.8	21.9	25.0
-7.6				55.4	53.1	55.8	57.3	49.5	51.7
-8.0	64.9	70.5	76.9	75.7	70.5	72.3	74.2	71.3	74.9
-8.3				83.3	84.7	87.0	78.0	79.7	85.8
-9.0	101.8	90.4	92.1	88.4	92.7	96.4	93.5	97.2	84.6
-10.0	91.6	89.2	95.2	92.9	97.9	99.7	94.4	100.1	87.3

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	-0.1794	0.2822	0.1699
TOP	94.89	96.65	94.31
LOGEC50	-7.459	-7.487	-7.482
HILLSLOPE	-0.9214	-0.9868	-0.9968
Std. Error			
BOTTOM	1.098	1.067	1.709
TOP	1.711	1.060	1.692
LOGEC50	0.04605	0.02328	0.03800
HILLSLOPE	0.07189	0.04696	0.07788
95% Confidence Intervals			
BOTTOM	-2.469 to 2.110	-1.944 to 2.508	-3.395 to 3.735
TOP	91.32 to 98.46	94.43 to 98.86	90.79 to 97.84
LOGEC50	-7.555 to -7.363	-7.535 to -7.438	-7.561 to -7.403
HILLSLOPE	-1.071 to -0.7715	-1.085 to -0.8889	-1.159 to -0.8344
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9940	0.9970	0.9920
Absolute Sum of Squares	232.3	97.76	252.8
Sy.x	3.408	2.211	3.555
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

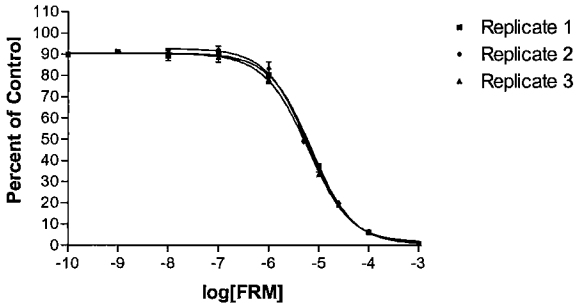
4-Nonylphenol



log[NYP]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
-4.0	1.6	1.9	1.9	4.1	4.6	5.9	5.5	2.8	3.8
-4.3				21.1	21.4	21.5	20.1	21.8	18.2
-4.6				39.8	40.0	41.9	39.4	43.1	39.5
-5.0	84.1	87.3	84.7	92.7	74.9	84.0	90.9	85.0	85.4
-5.3				101.5	88.1	100.0	107.5	102.6	108.9
-6.0	104.4	108.8	101.3	94.2	100.8	98.1	97.1	98.7	107.0
-7.0	104.5	101.8	100.7	91.8	101.3	98.0	102.2	98.8	105.5
-8.0	97.3	81.7	102.0						
-9.0	99.6	100.2	95.9						
-10.0	102.6	102.9	102.4						

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	0.2218	1.255	1.698
TOP	100.4	98.75	104.2
LOGEC50	-4.709	-4.654	-4.673
HILLSLOPE	-2.588	-2.018	-2.186
Std. Error			
BOTTOM	2.936	2.345	2.084
TOP	1.308	1.850	1.705
LOGEC50	0.1469	0.02659	0.02229
HILLSLOPE	1.248	0.2053	0.2014
95% Confidence Intervals			
BOTTOM	-5.902 to 6.345	-3.637 to 6.147	-2.650 to 6.046
TOP	97.69 to 103.1	94.90 to 102.6	100.6 to 107.7
LOGEC50	-5.015 to -4.402	-4.709 to -4.598	-4.720 to -4.627
HILLSLOPE	-5.192 to 0.01606	-2.446 to -1.590	-2.606 to -1.766
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9882	0.9881	0.9909
Absolute Sum of Squares	512.0	469.1	408.2
Sy.x	5.060	4.843	4.518
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

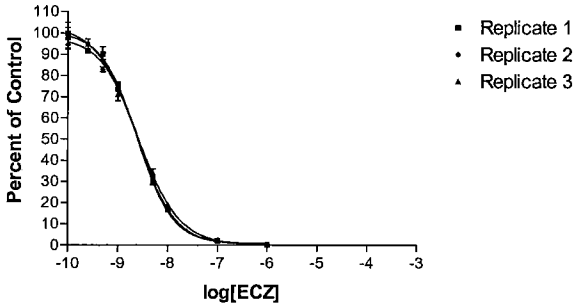
Fenarimol



log[FRM]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	0.9	0.9	0.8	1.0	1.0	1.0	0.9	1.0	1.0
-4.0	6.5	6.2	6.1	6.4	6.4	6.4	6.1	6.1	6.3
-4.6				19.6	21.0	19.4	19.5	18.9	19.5
-5.0	36.8	37.2	38.0	37.5	37.1	33.1	35.0	33.4	32.1
-5.3				50.2	47.7	48.9	49.7	50.3	49.7
-6.0	77.8	81.0	81.6	77.0	86.7	86.0	76.6	76.3	81.2
-7.0	89.2	89.9	90.9	93.3	94.4	88.7	93.4	84.7	87.9
-8.0	92.9	92.1	84.2	90.1	92.6	89.3	87.9	88.5	93.6
-9.0	90.0	91.0	92.7						
-10.0	88.8	89.2	91.4						

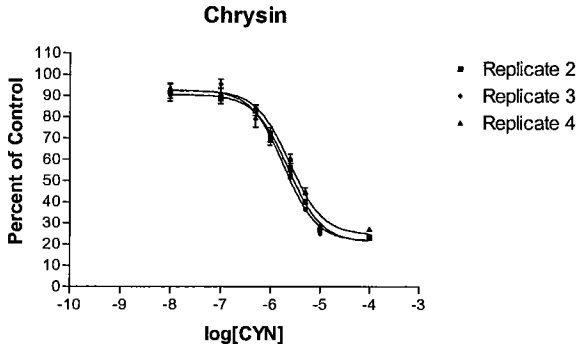
	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	0.6013	1.631	0.8316
TOP	90.51	92.68	90.33
LOGEC50	-5.152	-5.219	-5.218
HILLSLOPE	-1.041	-1.049	-0.9949
Std. Error			
BOTTOM	0.9991	1.477	1.111
TOP	0.5684	1.252	0.9310
LOGEC50	0.02339	0.02851	0.02225
HILLSLOPE	0.05676	0.07255	0.05099
95% Confidence Intervals			
BOTTOM	-1.483 to 2.685	-1.450 to 4.711	-1.485 to 3.148
TOP	89.32 to 91.69	90.06 to 95.29	88.39 to 92.27
LOGEC50	-5.201 to -5.103	-5.278 to -5.159	-5.265 to -5.172
HILLSLOPE	-1.159 to -0.9222	-1.201 to -0.8979	-1.101 to -0.8886
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9979	0.9945	0.9969
Absolute Sum of Squares	70.28	164.6	86.88
Sy.x	1.875	2.869	2.084
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



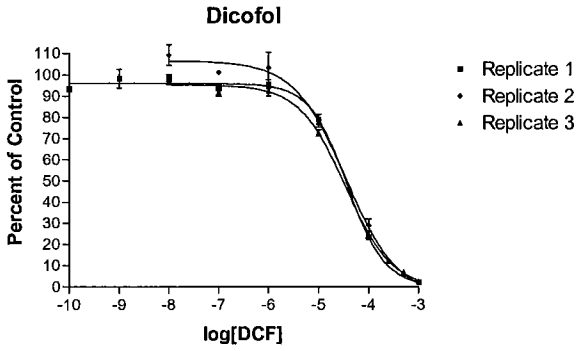
log[ECZ]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-6.0	0.3	0.2	0.3	0.3	0.3	0.3	0.2	0.3	0.2
-7.0	2.3	1.9	2.0	1.9	2.1	2.0	2.1	2.2	2.5
-8.0	15.7	16.6	17.8	15.3	18.0	17.8	17.7	19.9	19.6
-8.3	30.1	30.3	27.4	30.2	31.3	31.1	32.0	37.5	32.8
-9.0	77.2	75.9	71.7	77.0	70.7	76.4	71.0	76.4	66.0
-9.3	90.5	88.9	91.2	82.2	81.3	85.2	89.7	85.4	96.3
-9.6	91.9	91.3	91.2	95.6	95.1	94.2	99.2	94.0	91.4
-10.0	94.8	104.0	100.6	94.9	90.5	98.3	98.4	88.7	109.8

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	0.6946	0.4549	0.1701
TOP	100.6	97.88	103.7
LOGEC50	-8.614	-8.589	-8.617
HILLSLOPE	-1.216	-1.176	-1.043
Std. Error			
BOTTOM	1.089	1.094	2.199
TOP	1.571	1.591	3.627
LOGEC50	0.02592	0.02698	0.05578
HILLSLOPE	0.06375	0.06406	0.1087
95% Confidence Intervals			
BOTTOM	-1.576 to 2.966	-1.826 to 2.736	-4.418 to 4.758
TOP	97.29 to 103.8	94.57 to 101.2	96.16 to 111.3
LOGEC50	-8.668 to -8.560	-8.645 to -8.532	-8.733 to -8.501
HILLSLOPE	-1.349 to -1.083	-1.309 to -1.042	-1.270 to -0.8160
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9967	0.9965	0.9877
Absolute Sum of Squares	127.6	125.0	463.2
Sy.x	2.526	2.500	4.813
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



log[CYN]	Replicate 2			Replicate 3			Replicate 4		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.0	25.3	21.0	23.3	25.0	24.3	22.6	27.7	27.7	26.8
-5.0	28.5	26.7	25.2	23.7	26.0	25.0	29.1	27.2	30.5
-5.3	41.2	37.8	41.6	35.7	37.8	36.5	42.7	45.0	47.8
-5.6	52.3	57.2	58.8	49.7	56.3	52.7	64.2	59.5	58.7
-6.0	63.5	72.7	73.4	70.6	74.7	66.3	73.1	70.3	76.3
-6.3	84.3	86.3	78.5	86.6	73.9	76.6	82.4	85.2	85.9
-7.0	92.5	89.1	84.1	99.8	92.6	94.2	93.5	86.4	93.7
-8.0	89.1	99.2	86.0	90.8	87.3	92.6	97.5	94.1	89.3

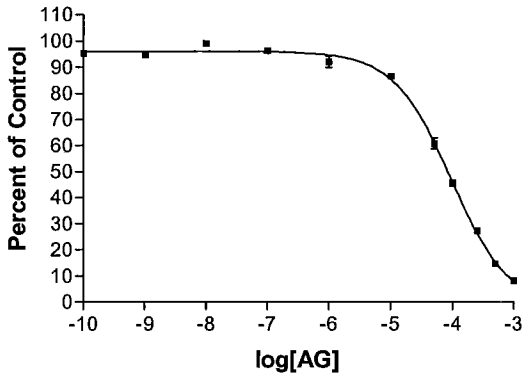
	Replicate 2	Replicate 3	Replicate 4
Equation 1			
Best-fit values			
BOTTOM	21.16	21.55	24.61
TOP	90.54	92.60	92.48
LOGEC50	-5.645	-5.730	-5.617
HILLSLOPE	-1.295	-1.321	-1.298
Std. Error			
BOTTOM	2.513	2.429	2.254
TOP	1.870	1.927	1.645
LOGEC50	0.04807	0.04671	0.04349
HILLSLOPE	0.1526	0.1533	0.1393
95% Confidence Intervals			
BOTTOM	15.92 to 26.40	16.48 to 26.62	19.91 to 29.31
TOP	86.64 to 94.44	88.58 to 96.62	89.05 to 95.91
LOGEC50	-5.745 to -5.544	-5.828 to -5.633	-5.708 to -5.527
HILLSLOPE	-1.614 to -0.9768	-1.641 to -1.002	-1.589 to -1.007
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9776	0.9788	0.9814
Absolute Sum of Squares	361.2	370.5	284.8
Sy.x	4.250	4.304	3.773
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



log[DCF]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.0	2.5	2.1	2.2	2.8	1.8	2.1	2.6	2.7	2.1
-3.3				4.8	6.7	5.3	7.1	7.4	6.3
-3.6				11.1	11.5	14.4	11.8	11.7	14.2
-4.0	24.6	22.2	23.2	23.8	29.3	34.2	23.0	22.8	28.2
-5.0	82.6	79.7	74.3	74.1	77.0	79.6	75.7	70.8	71.2
-6.0	90.5	92.4	100.7	113.8	106.7	89.7	92.3	96.6	88.3
-7.0	92.1	97.3	92.9	100.7	102.2	100.5	94.2	92.7	88.3
-8.0	100.7	95.9	97.8	115.1	99.7	113.2	96.3	94.8	102.7
-9.0	106.9	92.2	95.5						
-10.0	91.2	93.6	94.9						

	Replicate 1	Replicate 2	Replicate 3
Equation 1			
Best-fit values			
BOTTOM	0.1887	-4.776	-0.9486
TOP	95.95	106.5	95.29
LOGEC50	-4.428	-4.453	-4.459
HILLSLOPE	-1.159	-0.8375	-0.9354
Std. Error			
BOTTOM	2.846	5.315	2.385
TOP	1.061	2.287	1.157
LOGEC50	0.05282	0.08875	0.04765
HILLSLOPE	0.1085	0.1125	0.07271
95% Confidence Intervals			
BOTTOM	-5.748 to 6.126	-15.86 to 6.311	-5.924 to 4.026
TOP	93.73 to 98.16	101.8 to 111.3	92.88 to 97.71
LOGEC50	-4.539 to -4.318	-4.638 to -4.268	-4.559 to -4.360
HILLSLOPE	-1.386 to -0.9331	-1.072 to -0.6028	-1.087 to -0.7837
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9898	0.9865	0.9951
Absolute Sum of Squares	312.0	640.8	182.7
Sy.x	3.950	5.661	3.023
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

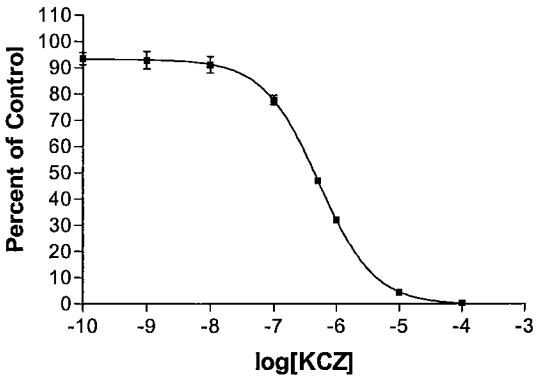
Aminoglutethimide



log[AG]	Data Set-A		
	Y	SEM	N
-3.0	8.1	0.6	3
-3.3	14.6	0.3	2
-3.6	27.4	0.6	2
-4.0	45.5	1.2	3
-4.3	60.8	2.0	2
-5.0	86.6	0.9	3
-6.0	92.0	2.2	3
-7.0	96.5	1.1	3
-8.0	99.0	0.0	1
-9.0	94.8	0.0	1
-10.0	95.3	0.0	1

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	-1.858
TOP	96.02
LOGEC50	-4.021
HILLSLOPE	-0.9315
Std. Error	
BOTTOM	4.027
TOP	0.8202
LOGEC50	0.05118
HILLSLOPE	0.08181
95% Confidence Intervals	
BOTTOM	-11.38 to 7.666
TOP	94.08 to 97.96
LOGEC50	-4.142 to -3.900
HILLSLOPE	-1.125 to -0.7380
Goodness of Fit	
Degrees of Freedom	7
R^2	0.9983
Absolute Sum of Squares	21.65
Sy.x	1.759
Data	
Number of X values	11
# of Y replicates (mean analyzed)	1
Total number of values	11
Number of missing values	0

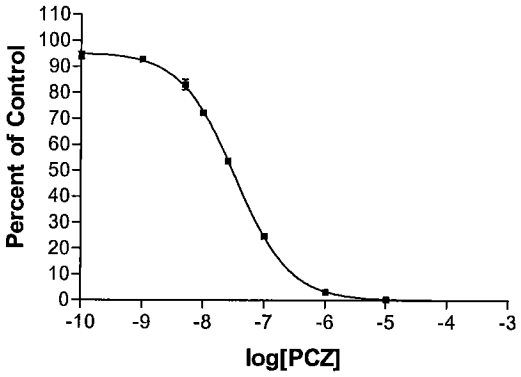
Ketoconazole



log[KCZ]	Data Set-A		
	Y	SEM	N
-4.0	0.3	0.1	3
-5.0	4.5	0.2	3
-6.0	32.2	1.0	3
-6.3	47.0	0.7	3
-7.0	77.8	1.7	3
-8.0	91.1	3.2	3
-9.0	92.8	3.4	3
-10.0	93.4	2.3	3

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	-0.3191
TOP	93.20
LOGEC50	-6.284
HILLSLOPE	-0.9778
Std. Error	
BOTTOM	0.2353
TOP	0.1654
LOGEC50	0.004634
HILLSLOPE	0.01124
95% Confidence Intervals	
BOTTOM	-0.9723 to 0.3340
TOP	92.74 to 93.66
LOGEC50	-6.297 to -6.271
HILLSLOPE	-1.009 to -0.9466
Goodness of Fit	
Degrees of Freedom	4
R^2	1.000
Absolute Sum of Squares	0.2742
Sy.x	0.2618
Data	
Number of X values	8
# of Y replicates (mean analyzed)	1
Total number of values	8
Number of missing values	0

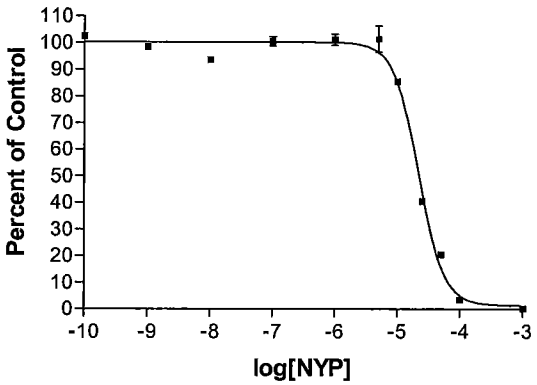
Prochloraz



log[PCZ]	Data Set-A		
	Y	SEM	N
-3.0	-0.1	0.0	1
-4.0	-1.7e-002	0.0	1
-5.0	0.4	0.1	3
-6.0	3.4	0.1	3
-7.0	24.9	0.8	3
-7.6	53.8	0.9	2
-8.0	72.3	0.8	3
-8.3	83.1	1.9	2
-9.0	93.0	0.9	3
-10.0	94.2	1.4	3

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	0.02071
TOP	95.38
LOGEC50	-7.477
HILLSLOPE	-0.9710
Std. Error	
BOTTOM	0.3366
TOP	0.5120
LOGEC50	0.01070
HILLSLOPE	0.02101
95% Confidence Intervals	
BOTTOM	-0.8030 to 0.8445
TOP	94.13 to 96.64
LOGEC50	-7.503 to -7.451
HILLSLOPE	-1.022 to -0.9196
Goodness of Fit	
Degrees of Freedom	6
R^2	0.9998
Absolute Sum of Squares	2.296
Sy.x	0.6186
Data	
Number of X values	10
# of Y replicates (mean analyzed)	1
Total number of values	10
Number of missing values	0

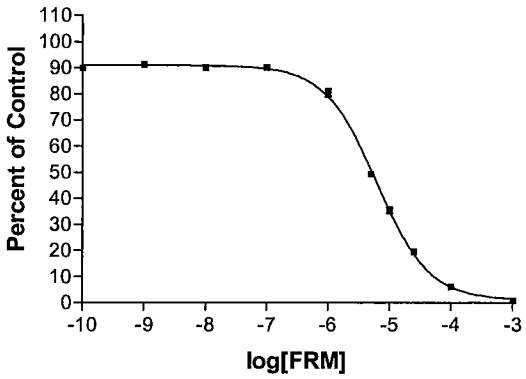
4-Nonylphenol



log[NYP]	Data Set-A		
	Y	SEM	N
-3.0	0.1	8.7e-003	3
-4.0	3.6	0.9	3
-4.3	20.7	0.6	2
-4.6	40.6	0.1	2
-5.0	85.5	0.9	3
-5.3	101.4	4.9	2
-6.0	101.1	2.1	3
-7.0	100.5	1.7	3
-8.0	93.7	0.0	1
-9.0	98.6	0.0	1
-10.0	102.6	0.0	1

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	1.346
TOP	100.2
LOGEC50	-4.656
HILLSLOPE	-2.161
Std. Error	
BOTTOM	3.317
TOP	1.771
LOGEC50	0.03474
HILLSLOPE	0.3064
95% Confidence Intervals	
BOTTOM	-6.498 to 9.191
TOP	96.03 to 104.4
LOGEC50	-4.738 to -4.574
HILLSLOPE	-2.886 to -1.437
Goodness of Fit	
Degrees of Freedom	7
R^2	0.9935
Absolute Sum of Squares	117.7
Sy.x	4.101
Data	
Number of X values	11
# of Y replicates (mean analyzed)	1
Total number of values	11
Number of missing values	0

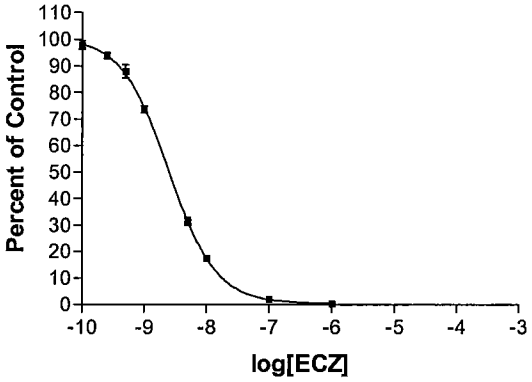
Fenarimol



log[FRM]	Data Set-A		
	Y	SEM	N
-3.0	1.0	4.5e-002	3
-4.0	6.3	0.1	3
-4.6	19.6	0.3	2
-5.0	35.6	1.1	3
-5.3	49.4	0.5	2
-6.0	80.5	1.5	3
-7.0	90.3	1.0	3
-8.0	90.1	0.3	3
-9.0	91.3	0.0	1
-10.0	89.8	0.0	1

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	1.118
TOP	90.91
LOGEC50	-5.205
HILLSLOPE	-1.022
Std. Error	
BOTTOM	1.090
TOP	0.6260
LOGEC50	0.02042
HILLSLOPE	0.04890
95% Confidence Intervals	
BOTTOM	-1.549 to 3.786
TOP	89.38 to 92.44
LOGEC50	-5.255 to -5.155
HILLSLOPE	-1.142 to -0.9028
Goodness of Fit	
Degrees of Freedom	6
R^2	0.9993
Absolute Sum of Squares	8.744
Sy.x	1.207
Data	
Number of X values	10
# of Y replicates (mean analyzed)	1
Total number of values	10
Number of missing values	0

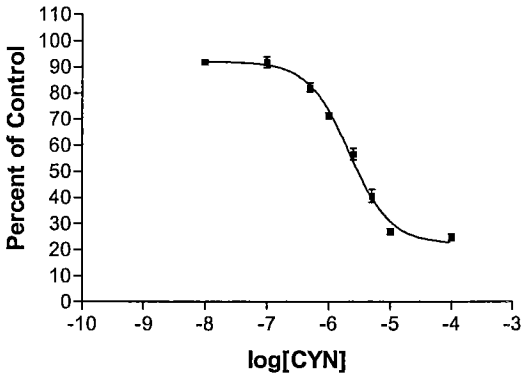
Econazole



log[ECZ]	Data Set-A		
	Y	SEM	N
-6.0	0.3	1.9e-002	3
-7.0	2.1	0.1	3
-8.0	17.6	0.7	3
-8.3	31.4	1.4	3
-9.0	73.6	1.2	3
-9.3	87.8	2.5	3
-9.6	93.8	1.1	3
-10.0	97.8	1.6	3

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	0.4486
TOP	100.7
LOGEC50	-8.607
HILLSLOPE	-1.139
Std. Error	
BOTTOM	0.5694
TOP	0.8624
LOGEC50	0.01401
HILLSLOPE	0.03138
95% Confidence Intervals	
BOTTOM	-1.132 to 2.029
TOP	98.32 to 103.1
LOGEC50	-8.646 to -8.568
HILLSLOPE	-1.226 to -1.052
Goodness of Fit	
Degrees of Freedom	4
R^2	0.9998
Absolute Sum of Squares	2.221
Sy.x	0.7451
Data	
Number of X values	8
# of Y replicates (mean analyzed)	1
Total number of values	8
Number of missing values	0

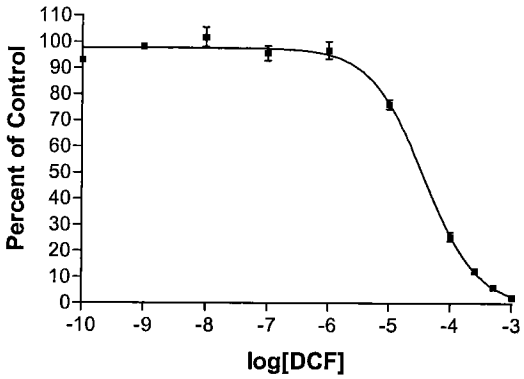
Chrysin



log[CYN]	Data Set-A		
	Y	SEM	N
-4.0	24.8	1.3	3
-5.0	26.9	1.2	3
-5.3	40.7	2.5	3
-5.6	56.6	2.3	3
-6.0	71.2	1.0	3
-6.3	82.2	1.6	3
-7.0	91.7	2.0	3
-8.0	91.8	1.0	3

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	22.45
TOP	91.90
LOGEC50	-5.666
HILLSLOPE	-1.298
Std. Error	
BOTTOM	2.922
TOP	2.208
LOGEC50	0.05631
HILLSLOPE	0.1791
95% Confidence Intervals	
BOTTOM	14.34 to 30.56
TOP	85.77 to 98.03
LOGEC50	-5.822 to -5.509
HILLSLOPE	-1.796 to -0.8011
Goodness of Fit	
Degrees of Freedom	4
R^2	0.9938
Absolute Sum of Squares	33.21
Sy.x	2.882
Data	
Number of X values	8
# of Y replicates (mean analyzed)	1
Total number of values	8
Number of missing values	0

Dicofol



log[DCF]	Data Set-A		
	Y	SEM	N
-3.0	2.3	0.1	3
-3.3	6.3	0.7	2
-3.6	12.4	0.1	2
-4.0	25.7	1.7	3
-5.0	76.1	1.9	3
-6.0	96.8	3.4	3
-7.0	95.6	2.8	3
-8.0	101.8	3.8	3
-9.0	98.2	0.0	1
-10.0	93.2	0.0	1

	Data Set-A
Equation 1	
Best-fit values	
BOTTOM	-0.7286
TOP	97.64
LOGEC50	-4.434
HILLSLOPE	-0.9959
Std. Error	
BOTTOM	3.422
TOP	1.295
LOGEC50	0.06696
HILLSLOPE	0.1097
95% Confidence Intervals	
BOTTOM	-9.103 to 7.645
TOP	94.48 to 100.8
LOGEC50	-4.598 to -4.270
HILLSLOPE	-1.264 to -0.7274
Goodness of Fit	
Degrees of Freedom	6
R^2	0.9974
Absolute Sum of Squares	44.34
Sy.x	2.718
Data	
Number of X values	10
# of Y replicates (mean analyzed)	1
Total number of values	10
Number of missing values	0

APPENDIX A

BATTELLE REPORT

DRAFT REPORT

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES

Prepared By:
Battelle Memorial Institute
505 King Avenue
Columbus, Ohio 43201-2693

Prepared For:
EPA Contract: 68-W-01-023
Work Assignment: WA 4-17, Task 4

Battelle Study No. G608317

DRAFT REPORT

Title:	AROMATSE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES
Author:	Bozena D. Lusiak, Ph.D.
Performing Laboratory:	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693
Sponsor:	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693
Sponsor's Representatives:	David P. Houchens, Ph.D. EDSP Program Manager Battelle Jerry D. Johnson, Ph.D. Diplomate, A.B.T. Work Assignment Leader Battelle
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	7/5/2005	7/5/2005
Sample preparation	8/8/2005	8/9/2005
Dilution	8/8/2005	8/9/2005
Dispensing	8/8/2005	8/9/2005
Centrifugation	8/8/2005	8/9/2005
Test system preparation	8/8/2005	8/9/2005
Test substance preparation	8/8/2005	8/9/2005
Protein analysis	8/8/2005	8/9/2005
Extraction	8/8/2005	8/9/2005
Solution preparation	8/9/2005	8/9/2005
Protocol amendment review	11/22/2005	11/22/2005
Protocol amendment review	12/16/2005	12/16/2005
Audit study file	3/15/2006	3/15/2006
Audit draft report	3/15/2006	3/15/2006
Audit draft report	6/14/2006	6/14/2006

This report provides an accurate record of the results obtained

Quality Assurance Unit Date

COMPLIANCE STATEMENT

This study, Battelle Study No. G608317, 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 human recombinant microsomes.

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 recombinants microsomes using the classic ^3H_2O method. Briefly, each inhibitor was incubated with recombinant microsomes in the presence of ^{3}H-androstenedione (substrate for aromatase) at pH 7.4 and 37 ± 1°C, for 15 minutes. Eight concentrations of each inhibitor 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 110.7 µM, for ketoconazole 571.2 nM, for prochloraz 32.53 nM, for 4-nonylphenol 20.21 µM, for fenarimol 5.415 µM, for econazole 2.392 nM, for chrysin 1.477 µM, and for dicofol 17.54 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 β-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. IC_{50} for each replicate were calculated using Prism (Version 4.0) software as specified in the protocol.

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 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	>99.5	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 was 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 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	Final Concentration in Assay
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)

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

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 solution 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 Name	Mfr. Purity	CAS No.	Molecular Formula	Molecular Weight (g/mol)	Stock Solution ID	Vehicle	Storage Conditions (°C)
4- hydroxyandrostenedione	99%	566-48-3	$C_{19}H_{26}O_3$	302.4	4-ASDN-1	95% ethanol	2-8
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 (0.01M)	0.0001	NA
2	1900	100	Diln.1	0.000005	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)	0.001	NA
2	900	100	Diln.1	0.0001	1.00E-06

3.4 Microsomes

Human recombinant microsomes were obtained through RTI International (Research Triangle, NC), Lot No.5, from BD GENTEST (Woburn, MA) and were stored at approximately -70°C until the time of 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 45 to 50 minutes.

The protein concentration in stock microsomes was approximately 4.8 mg/mL. Microsomes were diluted in assay buffer in two serial dilutions. For the replicate 1 and 2 of aminoglutethimide (RC# 1) the first dilution was achieved by gently mixing 0.1 mL of microsomes stock solution with 4.9 mL of buffer (total volume 5.0 mL, 1:50 dilution). Next 4.0 mL of the first dilution was gently mixed with 46.0 mL of buffer (total volume 50.0 mL, 1:12.5 dilution). The first dilution for the all other reference chemicals was achieved by gently mixing 0.08 mL of microsomes stock solutions with 4.72 mL of buffer (total volume 4.8 mL, 1:60 dilution). The second dilution (1:10.5) was obtained by gently mixing 4.0 mL of first microsomes dilution with 38.0 mL of buffer. The first dilution was kept on ice until the protein concentration was measured. In the second dilution, the target protein concentration was ca. 0.008 mg/mL to achieve a final protein concentration in the incubation mixture ca. 0.004 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.

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/ Battelle CR	SW0045
Vehicle (DMSO) for Reference Chemicals 1-7, 9, 10, and Lindane	Mallinckrodt	2969A46428
Vehicle (DMSO) for Reference 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 β-NADPH in 4 mL of assay buffer and was 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 concentration 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 and samples were gently mixed. The samples were incubated in room temperature for at least 15 minutes. Each sample (standard and unknown) was transferred to disposable polystyrene cuvettes and the absorbance at 750 nm was measured using a spectrophotometer. The protein concentration of each microsomal sample 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 (or more) 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 mL, and the tubes were incubated for 15 minutes. The incubations were stopped by 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 Recombinant Microsomes

Assay Component	Component Volume Added to the Assay	Final Concentration in the Assay
Microsomal protein	1.0 mL	0.004 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 as:

- 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 Statistical Analysis System (SAS), 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.

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 WA4-17, Task 4
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	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 (for replicate 1 and 2 for aminoglutethimide (RC#1)) and 1:60 (for the all other reference chemicals) microsomes dilution in assay buffer was processed. The results for measuring protein concentration in recombinant microsomes for each day of use are presented in Table 22. The average measured protein content of the recombinant microsomes stock solution was 5.17 mg/mL (4.16% CV), with the range of 4.59 to 5.61 mg/mL.

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; nominal value 0.012 mg/mL) and overall mean value for the high QC standard was determined as 0.107 ± 0.003 mg/mL (2.4% CV; nominal value 0.110 mg/mL).

Table 22 - Protein Concentration Data

Run by Reference Chemical	Protein Concentration (mg/mL)			Protein Concentration (mg/mL)			
	Rep. 1	Rep. 2	Rep. 3	Mean	SD	SEM	%CV
Aminoglutethimide (RC #1)	5.12	5.26	5.53	5.17	0.21	0.04	4.16
Ketoconazole (RC #2)	5.00	5.16	5.28				
Prochloraz (RC #3)	5.04	4.99	4.97				
4-Nonylphenol (RC #4)	5.27	5.20	5.35				
Dibenz[a,h]anthracene (RC #5)	5.61	5.44	5.46				
Fenarimol (RC #6)	5.13	5.13	4.77				
Econazole (RC #7)	4.98	4.92	5.08				
Chrysin (RC #8)	5.23	5.18	5.18				
Dicofol (RC #9)	4.59	5.25	5.29				
Atrazine (RC #10)	5.30	5.08	5.31				

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	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	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					
	8/4/2005	0.104					
	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 of 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	5.12	1.122	1.00E-04 to 1.00E-11
RC# 1	Aminoglutethimide	2	7/21/2005	TD	5.26	1.146	1.00E-03 to 1.00E-06
RC# 1	Aminoglutethimide	3	7/22/2005	TD	5.53	1.234	1.00E-03 to 1.00E-06
RC# 2	Ketoconazole	1	8/11/2005	TD	5.00	1.212	1.00E-04 to 1.00E-10
RC# 2	Ketoconazole	2	8/12/2005	TD	5.16	1.203	1.00E-04 to 1.00E-09
RC# 2	Ketoconazole	3	8/15/2005	TD	5.28	1.176	1.00E-04 to 1.00E-09
RC# 3	Prochloraz	1	8/2/2005	TD	5.04	1.182	1.00E-04 to 1.00E-11
RC# 3	Prochloraz	2	8/3/2005	TD	4.99	1.129	1.00E-05 to 1.00E-10
RC# 3	Prochloraz	3	8/4/2005	TD	4.97	1.147	1.00E-05 to 1.00E-10
RC# 4	4-Nonylphenol	1	9/27/2005	TD	5.27	0.927	1.00E-03 to 1.00E-10
RC# 4	4-Nonylphenol	2	9/28/2005	TD	5.20	0.895	1.00E-03 to 1.00E-08
RC# 4	4-Nonylphenol	3	9/29/2005	TD	5.35	0.948	1.00E-03 to 1.00E-08
RC# 5	Dibenz[a,h]anthracene	1	9/30/2005	TD	5.61	0.954	1.00E-04 to 1.00E-10
RC# 5	Dibenz[a,h]anthracene	2	10/3/2005	TD	5.44	0.869	1.00E-04 to 1.00E-10
RC# 5	Dibenz[a,h]anthracene	3	10/4/2005	TD	5.46	0.864	1.00E-04 to 1.00E-10
RC# 6	Fenarimol	1	8/5/2005	TD	5.13	1.157	1.00E-03 to 1.00E-10
RC# 6	Fenarimol	2	8/8/2005	TD	5.13	1.094	1.00E-03 to 1.00E-09
RC# 6	Fenarimol	3	8/9/2005	TD	4.77	1.177	1.00E-03 to 1.00E-09
RC# 7	Econazole	1	8/17/2005	TD	4.98	1.221	1.00E-03 to 1.00E-10
RC# 7	Econazole	2	8/18/2005	TD	4.92	1.039	1.00E-04 to 1.00E-10
RC# 7	Econazole	3	8/19/2005	TD	5.08	1.151	1.00E-04 to 1.00E-10
RC# 8	Chrysin	1	8/23/2005	TD	5.23	1.196	1.00E-04 to 1.00E-10
RC# 8	Chrysin	2	8/24/2005	TD	5.18	1.151	1.00E-04 to 1.00E-09
RC# 8	Chrysin	3	8/25/2005	TD	5.18	1.140	1.00E-04 to 1.00E-09
RC# 9	Dicofol	1	8/29/2005	TD	4.59	1.085	1.00E-03 to 1.00E-10
RC# 9	Dicofol	2	8/30/2005	TD	5.25	1.092	1.00E-03 to 1.00E-09
RC# 9	Dicofol	3	8/31/2005	TD	5.29	1.222	1.00E-03 to 1.00E-09
RC# 10	Atrazine	1	9/6/2005	TD	5.30	1.151	1.00E-03 to 1.00E-10
RC# 10	Atrazine	2	9/7/2005	TD	5.08	0.919	1.00E-03 to 1.00E-06
RC# 10	Atrazine	3	9/8/2005	TD	5.31	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 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 of control values for background activity were consistent (0.0000) across the replicates and chemicals. The overall control values for full enzyme activity control and negative control were very close, 0.3123 ± 0.0502 nmol/mg/min (16.1% CV) and 0.3051 ± 0.0512 nmol/mg/min (16.8% CV), respectively. The overall negative control activity value was approximately 98% of the full activity value. The overall positive control activity value 0.1688 ± 0.0251 nmol/mg/min (14.9% CV) was approximately 54% of the full enzyme activity control.

Table 25 - Aromatase Activity in Control

Reference Chemical	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	1	0.5066	0.4987	0.5027	0.0163	0.0082	3.24				
	2	0.2825	0.3534	0.3180	0.0726	0.0363	22.83				
	3	0.3593	0.3517	0.3555	0.0060	0.0030	1.69	0.3921	0.0976	0.0564	24.90
Ketoconazole	1	0.3761	0.3922	0.3841	0.0159	0.0080	4.14				
	2	0.3779	0.3450	0.3615	0.0217	0.0109	6.00				
	3	0.2754	0.2409	0.2581	0.0209	0.0105	8.10	0.3346	0.0672	0.0388	20.08
Prochloraz	1	0.4086	0.3822	0.3954	0.0153	0.0077	3.87				
	2	0.3372	0.3302	0.3337	0.0094	0.0047	2.82				
	3	0.2733	0.2623	0.2678	0.0082	0.0041	3.06	0.3323	0.0638	0.0368	19.20
4-Nonylphenol	1	0.2462	0.2462	0.2462	0.0111	0.0056	4.51				
	2	0.3500	0.3224	0.3362	0.0215	0.0108	6.40				
	3	0.3249	0.2729	0.2989	0.0307	0.0154	10.30	0.2938	0.0452	0.0261	15.39
Dibenz[a,h]anthracene	1	0.3005	0.3057	0.3031	0.0047	0.0024	1.55				
	2	0.2811	0.2688	0.2749	0.0132	0.0066	4.80				
	3	0.3095	0.2801	0.2948	0.0210	0.0105	7.12	0.2909	0.0145	0.0084	4.98
Fenarimol	1	0.3711	0.3320	0.3516	0.0256	0.0128	7.28				
	2	0.3672	0.3482	0.3577	0.0145	0.0073	4.05				
	3	0.3773	0.3704	0.3738	0.0041	0.0021	1.10	0.3610	0.0115	0.0066	3.18
Econazole	1	0.3654	0.2689	0.3171	0.0561	0.0281	17.7				
	2	0.3434	0.3231	0.3333	0.0217	0.0109	6.51				
	3	0.3393	0.3102	0.3248	0.0183	0.0092	5.63	0.3251	0.0081	0.0047	2.49
Chrysin	1	0.3225	0.3044	0.3135	0.0150	0.0075	4.78				
	2	0.3000	0.3017	0.3008	0.0096	0.0048	3.19				
	3	0.3338	0.3465	0.3401	0.0112	0.0056	3.29	0.3181	0.0201	0.0116	6.30
Dicofol	1	0.2502	0.2402	0.2452	0.0090	0.0045	3.67				
	2	0.2817	0.2564	0.2691	0.0159	0.0080	5.91				
	3	0.2764	0.2418	0.2591	0.0203	0.0102	7.83	0.2578	0.0120	0.0069	4.66
Atrazine	1	0.2515	0.2237	0.2376	0.0161	0.0081	6.78				
	2	0.2140	0.1753	0.1946	0.0325	0.0163	16.70				
	3	0.2210	0.2169	0.2190	0.0039	0.0020	1.78	0.2171	0.0216	0.0125	9.93
Overall								0.3123	0.0502	0.0159	16.07

[a] Units are nmol/mg protein/min.

Table 25 – Aromatase Activity in Control (Continued)

Reference Chemical	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											
Aminoglutethimide	1	0.2653	0.2519	0.2586	0.0088	0.0044	3.40				
	2	0.1964	0.1955	0.1960	0.0023	0.0012	1.17				
	3	0.1834	0.1810	0.1822	0.0058	0.0029	3.18	0.2123	0.0407	0.0235	19.18
Ketoconazole	1	0.1948	0.1981	0.1964	0.0035	0.0018	1.78				
	2	0.1907	0.1741	0.1824	0.0117	0.0059	6.41				
	3	0.1517	0.1432	0.1475	0.0053	0.0027	3.59	0.1754	0.0252	0.0145	14.35
Prochloraz	1	0.2117	0.2083	0.2100	0.0027	0.0014	1.29				
	2	0.1986	0.1866	0.1926	0.0078	0.0039	4.05				
	3	0.1733	0.1579	0.1656	0.0090	0.0045	5.43	0.1894	0.0224	0.0129	11.81
4-Nonylphenol	1	0.1532	0.1480	0.1506	0.0064	0.0032	4.25				
	2	0.1794	0.1641	0.1718	0.0130	0.0065	7.57				
	3	0.1731	0.1529	0.1630	0.0123	0.0062	7.55	0.1618	0.0107	0.0061	6.58
Dibenz[a,h]anthracene	1	0.1677	0.1579	0.1628	0.0070	0.0035	4.30				
	2	0.1625	0.1553	0.1589	0.0068	0.0034	4.28				
	3	0.1796	0.1630	0.1713	0.0112	0.0056	6.54	0.1643	0.0063	0.0037	3.86
Fenarimol	1	0.1641	0.1852	0.1747	0.0130	0.0065	7.44				
	2	0.1756	0.1879	0.1818	0.0084	0.0042	4.62				
	3	0.1944	0.1856	0.1900	0.0085	0.0043	4.47	0.1822	0.0077	0.0044	4.20
Econazole	1	0.2023	0.1421	0.1722	0.0351	0.0176	20.38				
	2	0.1842	0.1841	0.1842	0.0040	0.0020	2.17				
	3	0.1736	0.1637	0.1687	0.0062	0.0031	3.68	0.1750	0.0081	0.0047	4.64
Chrysin	1	0.1684	0.1642	0.1663	0.0038	0.0019	2.29				
	2	0.1612	0.1520	0.1566	0.0072	0.0036	4.60				
	3	0.1863	0.1730	0.1797	0.0106	0.0053	5.90	0.1675	0.0116	0.0067	6.92
Dicofol	1	0.1236	0.1411	0.1324	0.0107	0.0054	8.08				
	2	0.1431	0.1392	0.1411	0.0032	0.0016	2.27				
	3	0.1298	0.1211	0.1254	0.0069	0.0035	5.50	0.1330	0.0079	0.0045	5.92
Atrazine	1	0.1329	0.1210	0.1269	0.0071	0.0036	5.59				
	2	0.1250	0.1179	0.1214	0.0059	0.0030	4.86				
	3	0.1419	0.1234	0.1327	0.0108	0.0054	8.14	0.1270	0.0057	0.0033	4.45
Overall								0.1688	0.0251	0.0079	14.88

[a] Units are nmol/mg protein/min.

Table 25 – Aromatase Activity in Control (Continued)

Reference Chemical	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	1	0.5298	0.4939	0.5118	0.0256	0.0128	5.00				
	2	0.3376	0.3271	0.3323	0.0063	0.0032	1.90				
	3	0.3319	0.3296	0.3308	0.0056	0.0028	1.69	0.3916	0.1041	0.0601	26.57
Ketoconazole	1	0.3966	0.3770	0.3868	0.0162	0.0081	4.19				
	2	0.3678	0.3466	0.3572	0.0126	0.0063	3.53				
	3	0.2710	0.2454	0.2582	0.0162	0.0081	6.27	0.3341	0.0673	0.0389	20.16
Prochloraz	1	0.3797	0.3593	0.3695	0.0135	0.0068	3.65				
	2	0.2990	0.3116	0.3053	0.0209	0.0105	6.85				
	3	0.2535	0.2413	0.2474	0.0097	0.0049	3.35	0.3074	0.0611	0.0353	19.87
4-Nonylphenol	1	0.2425	0.2474	0.2449	0.0039	0.0020	1.59				
	2	0.2936	0.3006	0.2971	0.0052	0.0026	1.75				
	3	0.3058	0.2737	0.2897	0.0194	0.0097	6.70	0.2772	0.0282	0.0163	10.19
Dibenz[a,h]anthracene	1	0.2992	0.2908	0.2950	0.0060	0.0030	2.03				
	2	0.2667	0.2688	0.2692	0.0174	0.0087	6.46				
	3	0.3202	0.3033	0.3118	0.0113	0.0058	3.62	0.2920	0.0215	0.0124	7.35
Fenarimol	1	0.3623	0.3429	0.3526	0.0117	0.0083	3.32				
	2	0.3690	0.3607	0.3649	0.0048	0.0024	1.32				
	3	0.3706	0.3537	0.3622	0.0165	0.0083	4.56	0.3599	0.0065	0.0037	1.80
Econazole	1	0.3459	0.2849	0.3154	0.0382	0.0191	12.11				
	2	0.3188	0.3199	0.3194	0.0087	0.0044	2.72				
	3	0.3217	0.3057	0.3137	0.0120	0.0060	3.83	0.3162	0.0029	0.0017	0.93
Chrysin	1	0.3307	0.3092	0.3200	0.0127	0.0064	3.97				
	2	0.2764	0.2967	0.2865	0.0219	0.0110	7.64				
	3	0.3347	0.3157	0.3252	0.0137	0.0069	4.21	0.3106	0.0210	0.0121	6.76
Dicofol	1	0.2661	0.2443	0.2552	0.0165	0.0083	6.47				
	2	0.2298	0.2432	0.2365	0.0294	0.0147	12.43				
	3	0.2337	0.2224	0.2280	0.0128	0.0064	5.61	0.2399	0.0139	0.0080	5.80
Atrazine	1	0.2637	0.2402	0.2520	0.0145	0.0073	5.75				
	2	0.2050	0.1930	0.1990	0.0118	0.0059	5.93				
	3	0.2197	0.2116	0.2156	0.0131	0.0066	6.08	0.2222	0.0271	0.0157	12.20
Overall								0.3051	0.0512	0.0162	16.77

[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

		Percent of Control Values							
Reference Chemical	Replicate	Log [RC]	Replicate Tube 1	Replicate Tube 2	Replicate Tube 3	Mean	± SD	SEM	% CV
Aminoglutethimide	1	-4.00	41.23	39.23	39.76	40.07	1.04	0.60	2.59
		-5.00	85.93	81.34	76.21	81.16	4.86	2.81	5.99
		-6.00	92.61	93.18	90.53	92.11	1.40	0.81	1.52
		-7.00	93.24	87.68	92.85	91.26	3.10	1.79	3.40
		-8.00	96.15	95.72	95.05	95.64	0.55	0.32	0.58
		-9.00	96.97	94.43	92.76	94.72	2.12	1.22	2.24
		-10.00	95.91	95.47	92.10	94.49	2.09	1.20	2.21
		-11.00	84.98	96.22	92.97	91.39	5.79	3.34	6.33
	2	-3.00	8.49	8.75	8.29	8.51	0.23	0.13	2.72
		-3.60	27.78	27.82	27.30	27.63	0.29	0.17	1.05
		-4.00	50.69	47.45	49.86	49.33	1.68	0.97	3.41
		-4.30	68.56	69.01	67.34	68.30	0.86	0.50	1.26
		-4.60	86.73	84.05	84.00	84.93	1.56	0.90	1.84
		-5.00	94.45	97.92	94.61	95.66	1.96	1.13	2.05
		-5.60	109.43	102.17	106.75	106.11	3.67	2.12	3.46
		-6.00	106.30	107.05	106.86	106.74	0.39	0.23	0.37
	3	-3.00	5.71	5.81	5.54	5.69	0.14	0.08	2.41
		-3.60	23.60	22.97	23.16	23.24	0.33	0.19	1.40
		-4.00	42.65	42.61	43.00	42.76	0.21	0.12	0.50
		-4.30	58.23	58.81	58.69	58.58	0.30	0.17	0.52
		-4.60	73.64	75.12	73.78	74.18	0.81	0.47	1.10
		-5.00	84.36	88.62	88.16	87.05	2.34	1.35	2.69
		-5.60	98.83	98.51	94.83	97.39	2.23	1.28	2.28
		-6.00	99.96	99.14	102.47	100.52	1.73	1.00	1.73
Ketoconazole	1	-4.00	0.48	0.37	0.45	0.43	0.06	0.03	12.86
		-5.00	5.21	4.66	4.75	4.87	0.30	0.17	6.08
		-6.00	32.69	33.59	33.03	33.10	0.45	0.26	1.37
		-6.60	67.57	65.72	73.08	68.79	3.83	2.21	5.57
		-7.00	83.61	83.55	84.54	83.90	0.56	0.32	0.66
		-8.00	96.91	99.31	99.82	98.68	1.55	0.89	1.57
		-9.00	102.17	99.47	102.20	101.28	1.57	0.90	1.55
		-10.00	101.73	104.21	104.06	103.34	1.39	0.80	1.35

Table 26 – Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [RC]	Percent of Control Values Replicate Tube 1	Replicate Tube 2	Replicate Tube 3	Mean	± SD	SEM	% CV
Ketoconazole (Continued)	2	-4.00	0.46	0.49	0.37	0.44	0.06	0.03	13.36
		-5.00	5.12	5.09	4.89	5.04	0.12	0.07	2.44
		-6.00	32.55	32.61	32.56	32.57	0.03	0.02	0.10
		-6.30	49.39	48.93	48.70	49.01	0.35	0.20	0.71
		-6.60	62.72	65.23	65.23	64.39	1.45	0.84	2.25
		-7.00	77.17	77.49	83.32	79.33	3.46	2.00	4.37
		-8.00	88.26	98.13	95.28	93.89	5.08	2.93	5.41
		-9.00	94.80	93.14	98.55	95.49	2.77	1.60	2.90
	3	-4.00	0.63	0.80	0.52	0.65	0.14	0.08	21.66
		-5.00	6.31	6.35	6.27	6.31	0.04	0.02	0.58
		-6.00	39.33	39.40	37.57	38.77	1.04	0.60	2.67
		-6.30	54.31	54.60	58.04	55.65	2.07	1.20	3.72
		-6.60	68.43	69.41	71.21	69.69	1.41	0.81	2.02
		-7.00	82.22	80.59	86.39	83.07	2.99	1.73	3.60
		-8.00	90.00	91.05	94.22	91.76	2.20	1.27	2.39
		-9.00	99.74	92.95	97.93	96.87	3.52	2.03	3.63
Prochloraz	1	-4.00	0.17	0.07	0.13	0.12	0.05	0.03	40.18
		-5.00	0.33	0.30	0.28	0.30	0.03	0.01	8.48
		-6.00	2.66	2.84	2.92	2.81	0.13	0.08	4.72
		-7.00	21.45	20.98	21.43	21.29	0.27	0.15	1.26
		-8.00	67.23	68.48	68.55	68.09	0.75	0.43	1.09
		-9.00	97.35	99.28	96.68	97.77	1.35	0.78	1.38
		-10.00	99.30	98.28	98.79	98.79	0.51	0.29	0.52
		-11.00	101.69	100.59	98.94	100.41	1.39	0.80	1.38
	2	-5.00	0.32	0.40	0.32	0.35	0.05	0.03	13.43
		-6.00	3.38	3.42	3.35	3.38	0.04	0.02	1.04
		-7.00	25.06	23.06	24.31	24.14	1.02	0.59	4.21
		-7.60	55.99	54.78	57.25	56.00	1.23	0.71	2.20
		-8.00	75.21	75.96	78.09	76.42	1.49	0.86	1.96
		-8.60	95.38	89.08	92.67	92.37	3.16	1.83	3.42
		-9.00	94.50	95.41	66.39	85.43	16.50	9.53	19.31
		-10.00	96.90	93.90	98.84	96.54	2.49	1.44	2.58
	3	-5.00	0.56	0.30	0.32	0.39	0.14	0.08	36.59
		-6.00	3.87	3.85	3.57	3.76	0.17	0.10	4.41
		-7.00	27.04	26.77	25.72	26.51	0.69	0.40	2.62
		-7.60	58.88	60.85	59.06	59.59	1.09	0.63	1.83
		-8.00	76.15	76.84	79.26	77.42	1.64	0.94	2.11
		-8.60	95.22	92.63	92.36	93.40	1.58	0.91	1.69
		-9.00	93.67	97.70	97.19	96.19	2.20	1.27	2.29
		-10.00	96.06	97.70	99.20	97.65	1.57	0.91	1.61

Table 26 – Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [RC]	Percent of Control Values Replicate Tube 1	Replicate Tube 2	Replicate Tube 3	Mean	± SD	SEM	% CV
4-Nonylphenol	1	-3.00	1.18	1.11	0.54	0.95	0.35	0.20	37.07
		-4.00	7.23	4.27	4.48	5.33	1.65	0.95	31.04
		-5.00	87.15	87.55	86.23	86.98	0.68	0.39	0.78
		-6.00	105.83	100.03	112.84	106.23	6.41	3.70	6.03
		-7.00	106.53	107.89	96.49	103.64	6.23	3.59	6.01
		-8.00	107.48	106.24	110.45	108.06	2.16	1.25	2.00
		-9.00	105.67	107.07	107.71	106.82	1.04	0.60	0.98
		-10.00	93.82	111.52	111.25	105.53	10.14	5.85	9.61
	2	-3.00	0.59	1.17	0.98	0.91	0.29	0.17	32.27
		-4.00	4.96	3.20	3.39	3.85	0.96	0.56	25.03
		-4.30	10.70	9.97	9.68	10.12	0.53	0.30	5.20
		-4.60	34.45	32.88	32.15	33.16	1.17	0.68	3.54
		-5.00	74.57	71.91	71.69	72.73	1.60	0.93	2.21
		-6.00	96.79	88.73	94.58	93.37	4.16	2.40	4.46
		-7.00	87.51	99.95	88.67	92.04	6.88	3.97	7.47
		-8.00	95.74	87.79	95.91	93.15	4.64	2.68	4.98
	3	-3.00	0.76	0.32	0.40	0.49	0.24	0.14	47.63
		-4.00	2.89	2.73	3.25	2.96	0.27	0.15	9.07
		-4.30	8.77	12.98	12.91	11.55	2.41	1.39	20.86
		-4.60	41.67	36.40	36.93	38.33	2.91	1.68	7.58
		-5.00	76.46	77.68	75.07	76.40	1.30	0.75	1.71
		-6.00	97.41	91.83	57.36	82.20	21.69	12.52	26.39
		-7.00	91.65	90.77	94.47	92.29	1.93	1.12	2.09
		-8.00	102.30	103.21	105.29	103.60	1.54	0.89	1.48
Dibenz[a,h]anthracene	1	-4.00	102.00	101.10	102.45	101.85	0.69	0.40	0.68
		-5.00	94.39	95.05	96.09	95.18	0.86	0.50	0.90
		-6.00	97.79	96.15	96.05	96.66	0.97	0.56	1.01
		-6.60	94.55	97.05	101.18	97.59	3.35	1.93	3.43
		-7.00	91.69	92.71	96.66	93.69	2.62	1.51	2.80
		-8.00	99.44	95.77	96.46	97.22	1.95	1.12	2.00
		-9.00	90.71	98.46	98.58	95.92	4.51	2.61	4.70
		-10.00	103.63	96.09	98.01	99.24	3.92	2.26	3.95
	2	-4.00	106.49	108.54	104.76	106.60	1.89	1.09	1.77
		-5.00	107.33	108.89	109.41	108.55	1.08	0.62	1.00
		-6.00	112.20	102.86	110.82	108.63	5.05	2.91	4.64
		-6.60	111.10	96.04	102.50	103.21	7.56	4.36	7.32
		-7.00	105.57	106.72	108.45	106.91	1.45	0.84	1.36
		-8.00	101.41	101.55	103.84	102.27	1.36	0.79	1.33
		-9.00	99.74	112.68	97.42	103.28	8.22	4.75	7.96
		-10.00	105.88	113.05	99.11	106.01	6.97	4.03	6.58

Table 26 – Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [RC]	Percent of Control Values Replicate Tube 1	Replicate Tube 2	Replicate Tube 3	Mean	± SD	SEM	% CV
Dibenz[a,h]anthracene (Continued)	3	-4.00	108.97	112.83	110.79	110.86	1.93	1.12	1.74
		-5.00	107.07	109.34	103.88	106.77	2.74	1.58	2.57
		-6.00	115.30	110.14	112.92	112.79	2.58	1.49	2.29
		-6.60	113.84	99.12	110.35	107.77	7.69	4.44	7.14
		-7.00	98.92	105.02	108.41	104.12	4.81	2.78	4.62
		-8.00	109.50	103.98	104.98	106.15	2.94	1.70	2.77
		-9.00	102.26	110.09	109.02	107.12	4.25	2.45	3.96
		-10.00	104.55	100.68	102.18	102.47	1.95	1.13	1.91
Fenarimol	1	-3.00	1.44	1.64	1.47	1.52	0.10	0.06	6.85
		-4.00	5.54	5.59	5.55	5.56	0.03	0.02	0.48
		-5.00	35.18	35.55	33.23	34.65	1.25	0.72	3.59
		-6.00	81.48	82.52	81.94	81.98	0.52	0.30	0.63
		-7.00	95.57	97.22	98.00	96.93	1.24	0.72	1.28
		-8.00	101.59	101.55	97.50	100.22	2.35	1.36	2.35
		-9.00	98.83	93.30	97.52	96.55	2.89	1.67	2.99
		-10.00	98.84	98.26	99.92	99.01	0.84	0.49	0.85
	2	-3.00	1.65	1.46	1.43	1.51	0.12	0.07	7.99
		-4.00	5.89	5.71	5.71	5.77	0.10	0.06	1.75
		-5.00	37.22	37.92	37.11	37.42	0.44	0.25	1.18
		-5.60	69.23	69.01	68.79	69.01	0.22	0.13	0.32
		-6.00	81.88	81.35	84.46	82.56	1.67	0.96	2.02
		-7.00	101.14	103.86	97.10	100.70	3.40	1.96	3.38
		-8.00	102.81	98.90	100.61	100.77	1.96	1.13	1.94
		-9.00	97.15	99.22	97.91	98.09	1.04	0.60	1.06
	3	-3.00	1.29	1.28	1.36	1.31	0.04	0.03	3.41
		-4.00	5.68	5.71	5.55	5.65	0.08	0.05	1.50
		-5.00	36.13	35.02	36.91	36.02	0.95	0.55	2.64
		-5.60	66.94	67.55	66.46	66.98	0.55	0.32	0.82
		-6.00	81.76	81.07	80.49	81.11	0.63	0.37	0.78
		-7.00	99.93	94.84	92.13	95.63	3.96	2.29	4.14
		-8.00	98.07	104.13	99.88	100.70	3.11	1.79	3.09
		-9.00	105.67	88.41	99.37	97.82	8.73	5.04	8.93
Econazole	1	-3.00	0.27	0.01	0.13	0.14	0.13	0.08	96.17
		-4.00	0.14	0.12	0.03	0.10	0.06	0.04	65.23
		-5.00	-0.12	-0.10	-0.09	-0.10	0.02	0.01	-17.71
		-6.00	0.06	0.22	0.55	0.28	0.25	0.14	90.29
		-7.00	1.66	1.41	1.38	1.48	0.16	0.09	10.60
		-8.00	12.33	12.77	13.20	12.76	0.43	0.25	3.40
		-9.00	52.80	58.50	57.49	56.26	3.04	1.76	5.40
		-10.00	78.65	81.56	83.42	81.21	2.40	1.39	2.96

Table 26 – Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [RC]	Percent of Control Values Replicate Tube 1	Replicate Tube 2	Replicate Tube 3	Mean	± SD	SEM	% CV
Econazole (Continued)	2	-4.00	0.22	-0.07	-0.05	0.03	0.16	0.09	465.76
		-5.00	0.18	0.01	-0.05	0.05	0.11	0.07	246.59
		-6.00	0.15	0.21	0.06	0.14	0.08	0.04	54.70
		-7.00	1.96	1.92	1.90	1.93	0.03	0.02	1.55
		-8.00	17.55	17.60	17.31	17.49	0.16	0.09	0.90
		-8.60	51.86	50.96	51.88	51.57	0.53	0.30	1.02
		-9.00	78.31	75.66	77.15	77.04	1.33	0.77	1.72
		-10.00	90.61	93.48	91.82	91.97	1.44	0.83	1.56
	3	-4.00	-0.01	0.25	0.02	0.09	0.14	0.08	163.35
		-5.00	0.16	-0.07	0.01	0.03	0.12	0.07	374.53
		-6.00	0.36	0.10	0.10	0.19	0.15	0.09	80.99
		-7.00	2.06	1.81	2.00	1.96	0.13	0.08	6.68
		-8.00	16.12	15.78	16.25	16.05	0.24	0.14	1.50
		-8.60	48.12	46.88	45.22	46.74	1.46	0.84	3.11
		-9.00	74.73	68.15	70.96	71.28	3.30	1.91	4.63
		-10.00	96.87	97.74	96.90	97.17	0.50	0.29	0.51
Chrysin	1	-4.00	27.69	25.82	27.85	27.12	1.13	0.65	4.15
		-5.00	25.08	25.72	26.20	25.67	0.56	0.32	2.19
		-6.00	40.14	49.62	53.76	47.84	6.98	4.03	14.59
		-6.60	53.90	68.94	71.96	64.93	9.67	5.58	14.90
		-7.00	74.73	75.85	76.40	75.66	0.85	0.49	1.12
		-8.00	75.11	69.43	75.92	73.49	3.54	2.04	4.81
		-9.00	71.68	74.54	76.19	74.14	2.28	1.32	3.08
		-10.00	73.85	74.82	76.39	75.02	1.28	0.74	1.70
	2	-4.00	22.41	22.07	25.37	23.28	1.82	1.05	7.80
		-5.00	26.08	28.20	26.76	27.01	1.08	0.63	4.01
		-5.30	39.45	34.62	40.19	38.09	3.03	1.75	7.95
		-5.60	55.09	58.13	31.77	48.33	14.42	8.33	29.84
		-6.00	55.84	64.43	69.39	63.22	6.85	3.96	10.84
		-7.00	72.43	77.45	81.19	77.02	4.39	2.54	5.70
		-8.00	91.67	91.75	94.34	92.59	1.52	0.88	1.64
		-9.00	85.88	92.28	88.20	88.79	3.24	1.87	3.65
	3	-4.00	22.44	22.94	19.10	21.49	2.09	1.21	9.71
		-5.00	25.37	23.73	25.45	24.85	0.97	0.56	3.91
		-5.30	33.52	36.14	41.25	36.97	3.93	2.27	10.64
		-5.60	58.45	60.79	54.12	57.79	3.39	1.96	5.86
		-6.00	67.08	67.76	68.06	67.63	0.50	0.29	0.74
		-7.00	86.33	92.82	91.57	90.24	3.44	1.99	3.82
		-8.00	80.52	91.74	97.25	89.84	8.53	4.92	9.49
		-9.00	98.66	98.62	100.25	99.18	0.93	0.54	0.94

Table 26 – Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [RC]	Percent of Control Values Replicate Tube 1	Replicate Tube 2	Replicate Tube 3	Mean	± SD	SEM	% CV
Dicofol	1	-3.00	2.01	2.27	1.80	2.03	0.24	0.14	11.61
		-4.00	15.10	16.33	15.86	15.77	0.62	0.36	3.94
		-5.00	59.22	61.59	61.10	60.64	1.26	0.72	2.07
		-6.00	103.16	104.65	100.14	102.65	2.30	1.33	2.24
		-7.00	96.20	91.16	99.83	95.73	4.36	2.51	4.55
		-8.00	78.10	98.21	101.84	92.72	12.79	7.38	13.79
		-9.00	102.94	107.43	93.69	101.35	7.01	4.04	6.91
		-10.00	96.18	94.95	89.52	93.55	3.54	2.05	3.79
	2	-3.00	1.99	2.10	2.42	2.17	0.22	0.13	10.26
		-4.00	16.74	16.96	16.24	16.65	0.37	0.21	2.20
		-4.30	23.16	24.40	25.13	24.23	1.00	0.58	4.13
		-5.00	55.31	56.15	57.27	56.24	0.99	0.57	1.76
		-6.00	88.97	90.94	91.51	90.48	1.33	0.77	1.47
		-7.00	92.31	86.30	93.30	90.64	3.79	2.19	4.18
		-8.00	93.60	100.11	100.45	98.05	3.86	2.23	3.94
		-9.00	92.40	94.05	97.64	94.70	2.68	1.55	2.83
	3	-3.00	2.99	3.20	2.37	2.85	0.43	0.25	15.02
		-4.00	16.48	17.48	17.65	17.20	0.63	0.36	3.66
		-4.30	20.01	22.05	22.96	21.68	1.51	0.87	6.97
		-5.00	67.48	63.36	73.98	68.27	5.35	3.09	7.84
		-6.00	74.58	85.40	93.86	84.61	9.67	5.58	11.42
		-7.00	86.07	83.47	90.54	86.69	3.58	2.06	4.13
		-8.00	91.84	93.82	91.90	92.52	1.13	0.65	1.22
		-9.00	94.03	87.98	92.41	91.47	3.13	1.81	3.42
Atrazine	1	-3.00	90.89	94.05	95.37	93.44	2.30	1.33	2.46
		-4.00	100.83	110.86	102.64	104.78	5.35	3.09	5.10
		-5.00	109.31	106.15	104.74	106.73	2.34	1.35	2.20
		-6.00	102.45	109.54	111.11	107.70	4.61	2.66	4.28
		-7.00	111.87	106.29	112.12	110.10	3.30	1.90	2.99
		-8.00	110.71	104.59	106.15	107.15	3.18	1.84	2.97
		-9.00	104.57	106.68	106.29	105.84	1.12	0.65	1.06
		-10.00	106.22	102.11	106.98	105.10	2.62	1.51	2.49
	2	-3.00	99.96	99.58	91.77	97.10	4.63	2.67	4.76
		-3.05	97.95	93.96	96.61	96.17	2.03	1.17	2.11
		-3.10	92.58	98.55	92.79	94.64	3.39	1.96	3.58
		-3.12	95.16	97.12	95.55	95.94	1.04	0.60	1.08
		-3.30	96.20	100.18	100.02	98.80	2.26	1.30	2.28
		-4.00	107.11	108.38	106.85	107.45	0.82	0.48	0.77
		-5.00	114.70	106.74	107.51	109.65	4.39	2.54	4.00
		-6.00	108.15	108.45	112.38	109.66	2.36	1.36	2.15

Table 26 – Percent of Control Aromatase Activity Detected in Assays with Reference Chemicals (Continued)

Reference Chemical	Replicate	Log [RC]	Percent of Control Values Replicate Tube 1	Replicate Tube 2	Replicate Tube 3	Mean	± SD	SEM	% CV
Atrazine (Continued)	3	-3.00	82.56	87.74	86.88	85.73	2.78	1.60	3.24
		-3.05	85.82	84.74	84.86	85.14	0.59	0.34	0.70
		-3.10	78.65	84.45	82.70	81.93	2.98	1.72	3.63
		-3.12	83.74	80.34	84.57	82.88	2.24	1.29	2.71
		-3.30	81.41	85.11	77.95	81.49	3.58	2.07	4.40
		-4.00	92.82	93.11	86.87	90.93	3.52	2.03	3.87
		-5.00	105.42	99.38	94.34	99.71	5.55	3.20	5.56
		-6.00	102.92	94.43	94.58	97.31	4.86	2.81	4.99

The % CV at all tested concentrations for aminoglutethimide, dibenz[a,h]anthracene, fenarimol and atrazine were below 10%, for dicofol below 15.02%. The high % CV values were observed at the highest concentration levels (first or second dilution) for ketoconazole, prochloraz, 4-nonylphenol, and chrysin. For all three replicates of econazole, the lowest three concentrations were observed to have very high % CV. Those high % CV values are the results 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 [RC]	Mean Percent of Control Values Replicate 1	Replicate 2	Replicate 3	Mean	± SD	SEM	% CV
Aminoglutethimide	-3.00	NA	8.51	5.69	7.10	NC	NC	NC
	-3.60	NA	27.63	23.24	25.44	NC	NC	NC
	-4.00	40.07	49.33	42.76	44.05	4.77	2.75	10.82
	-4.30	NA	68.30	58.58	63.44	NC	NC	NC
	-4.60	NA	84.93	74.18	79.55	NC	NC	NC
	-5.00	81.16	95.66	87.05	87.96	7.29	4.21	8.29
	-5.60	NA	106.11	97.39	101.75	NC	NC	NC
	-6.00	92.11	106.74	100.52	99.79	7.34	4.24	7.36
	-7.00	91.26	NA	NA	NC	NC	NC	NC
	-8.00	95.64	NA	NA	NC	NC	NC	NC
	-9.00	94.72	NA	NA	NC	NC	NC	NC
	-10.00	94.49	NA	NA	NC	NC	NC	NC
	-11.00	91.39	NA	NA	NC	NC	NC	NC
Ketoconazole	-4.00	0.43	0.44	0.65	0.51	0.12	0.07	23.94
	-5.00	4.87	5.04	6.31	5.41	0.79	0.45	14.57
	-6.00	33.10	32.57	38.77	34.82	3.44	1.98	9.87
	-6.30	NA	49.01	55.65	52.33	NC	NC	NC
	-6.60	68.79	64.39	69.69	67.62	2.83	1.64	4.19
	-7.00	83.90	79.33	83.07	82.10	2.44	1.41	2.97
	-8.00	98.68	93.89	91.76	94.77	3.55	2.05	3.74
	-9.00	101.28	95.49	96.87	97.88	3.02	1.75	3.09
	-10.00	103.34	NA	NA	NC	NC	NC	NC

Table 27 - Mean Percent of Control per Replicate (Continued)

Mean Percent of Control Values								
Reference Chemical	Log [RC]	Replicate 1	Replicate 2	Replicate 3	Mean	± SD	SEM	% CV
Prochloraz	-4.00	0.12	NA	NA	NC	NC	NC	NC
	-5.00	0.30	0.35	0.39	0.35	0.04	0.03	12.83
	-6.00	2.81	3.38	3.76	3.32	0.48	0.28	14.54
	-7.00	21.29	24.14	26.51	23.98	2.62	1.51	10.91
	-7.60	NA	56.00	59.59	57.80	NC	NC	NC
	-8.00	68.09	76.42	77.42	73.97	5.12	2.96	6.92
	-8.60	NA	92.37	93.40	92.89	NC	NC	NC
	-9.00	97.77	85.43	96.19	93.13	6.71	3.88	7.21
	-10.00	98.79	96.54	97.65	97.66	1.12	0.65	1.15
	-11.00	100.41	NA	NA	NC	NC	NC	NC
4-Nonylphenol	-3.00	0.95	0.91	0.49	0.78	0.25	0.15	32.10
	-4.00	5.33	3.85	2.96	4.04	1.20	0.69	29.58
	-4.30	NA	10.12	11.55	10.84	NC	NC	NC
	-4.60	NA	33.16	38.33	35.75	NC	NC	NC
	-5.00	86.98	72.73	76.40	78.70	7.40	4.27	9.40
	-6.00	106.23	93.37	82.20	93.93	12.03	6.94	12.80
	-7.00	103.64	92.04	92.29	95.99	6.62	3.82	6.90
	-8.00	108.06	93.15	103.60	101.60	7.65	4.42	7.53
	-9.00	106.82	NA	NA	NC	NC	NC	NC
	-10.00	105.53	NA	NA	NC	NC	NC	NC
Dibenz[a,h]anthracene	-4.00	101.85	106.60	110.86	106.44	4.51	2.60	4.24
	-5.00	95.18	108.55	106.77	103.50	7.26	4.19	7.01
	-6.00	96.66	108.63	112.79	106.03	8.37	4.83	7.89
	-6.60	97.59	103.21	107.77	102.86	5.10	2.94	4.95
	-7.00	93.69	106.91	104.12	101.57	6.97	4.02	6.86
	-8.00	97.22	102.27	106.15	101.88	4.48	2.59	4.40
	-9.00	95.92	103.28	107.12	102.11	5.70	3.29	5.58
	-10.00	99.24	106.01	102.47	102.58	3.39	1.95	3.30
Fenarimol	-3.00	1.52	1.51	1.31	1.45	0.12	0.07	8.17
	-4.00	5.56	5.77	5.65	5.66	0.10	0.06	1.84
	-5.00	34.65	37.42	36.02	36.03	1.38	0.80	3.84
	-5.60	NA	69.01	66.98	68.00	NC	NC	NC
	-6.00	81.98	82.56	81.11	81.88	0.73	0.42	0.89
	-7.00	96.93	100.70	95.63	97.75	2.63	1.52	2.69
	-8.00	100.22	100.77	100.70	100.56	0.30	0.17	0.30
	-9.00	96.55	98.09	97.82	97.49	0.82	0.48	0.84
	-10.00	99.01	NA	NA	NC	NC	NC	NC
Econazole	-3.00	0.14	NA	NA	NC	NC	NC	NC
	-4.00	0.10	0.03	0.09	0.07	0.03	0.02	45.33
	-5.00	-0.10	0.05	0.03	-0.01	0.08	0.05	-998.02
	-6.00	0.28	0.14	0.19	0.20	0.07	0.04	34.69
	-7.00	1.48	1.93	1.96	1.79	0.26	0.15	14.79
	-8.00	12.76	17.49	16.05	15.43	2.42	1.40	15.69
	-8.60	NA	51.57	46.74	49.15	NC	NC	NC
	-9.00	56.26	77.04	71.28	68.20	10.73	6.19	15.73
	-10.00	81.21	91.97	97.17	90.12	8.14	4.70	9.03

Table 27 - Mean Percent of Control per Replicate (Continued)

Reference Chemical	Log [RC]	Mean Percent of Control Values						
		Replicate 1	Replicate 2	Replicate 3	Mean	± SD	SEM	% CV
Chrysin	-4.00	27.12	23.28	21.49	23.97	2.88	1.66	12.00
	-5.00	25.67	27.01	24.85	25.84	1.09	0.63	4.23
	-5.30	NA	38.09	36.97	37.53	NC	NC	NC
	-5.60	NA	48.33	57.79	53.06	NC	NC	NC
	-6.00	47.84	63.22	67.63	59.56	10.39	6.00	17.45
	-6.60	64.93	NA	NA	NC	NC	NC	NC
	-7.00	75.66	77.02	90.24	80.97	8.05	4.65	9.94
	-8.00	73.49	92.59	89.84	85.30	10.33	5.96	12.11
	-9.00	74.14	88.79	99.18	87.37	12.58	7.26	14.40
	-10.00	75.02	NA	NA	NC	NC	NC	NC
Dicofol	-3.00	2.03	2.17	2.85	2.35	0.44	0.25	18.76
	-4.00	15.77	16.65	17.20	16.54	0.72	0.42	4.37
	-4.30	NA	24.23	21.68	22.95	NC	NC	NC
	-5.00	60.64	56.24	68.27	61.72	6.09	3.51	9.86
	-6.00	102.65	90.48	84.61	92.58	9.20	5.31	9.94
	-7.00	95.73	90.64	86.69	91.02	4.53	2.62	4.98
	-8.00	92.72	98.05	92.52	94.43	3.14	1.81	3.32
	-9.00	101.35	94.70	91.47	95.84	5.04	2.91	5.26
	-10.00	93.55	NA	NA	NC	NC	NC	NC
Atrazine	-3.00	93.44	97.10	85.73	92.09	5.81	3.35	6.31
	-3.05	NA	96.17	85.14	90.66	NC	NC	NC
	-3.10	NA	94.64	81.93	88.29	NC	NC	NC
	-3.12	NA	95.94	82.88	89.41	NC	NC	NC
	-3.30	NA	98.80	81.49	90.15	NC	NC	NC
	-4.00	104.78	107.45	90.93	101.05	8.86	5.12	8.77
	-5.00	106.73	109.65	99.71	105.36	5.11	2.95	4.85
	-6.00	107.70	109.66	97.31	104.89	6.64	3.83	6.33
	-7.00	110.10	NA	NA	NC	NC	NC	NC
	-8.00	107.15	NA	NA	NC	NC	NC	NC
	-9.00	105.84	NA	NA	NC	NC	NC	NC
	-10.00	105.10	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 or % CV 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 the IC_{50} and slope were calculated. The concentration response curves for all ten reference chemicals are presented in Figures 1 to 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 are from approximately 20 to 90%, 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 very low. In case of 4-nonylphenol, ketoconazole and fenarimol, the concentration curve fits for second and third replicate were almost identical. The first replicate for those reference chemicals was a little different because they were run in different concentrations range 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 and chrysin, most of it is associated with the first replicate.

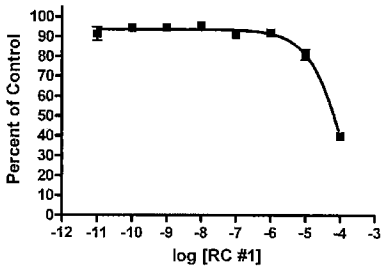
Figure 12 presents the results of curve fitting across the replicates for each reference chemical. The mean percent of control data for the repetitions within a replicate (as presented in Table 27) were fit to the model.

The IC_{50} and slope data are summarized in Table 28 by reference chemical and replicate. The status of each response 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.

Recombinant Microsomes Assay 7-20-05

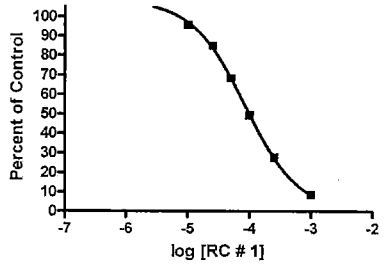
Replicate 1 (Aminoglutethimide)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-42.74
TOP	93.67
LOGEC50	-3.766
HILLSLOPE	-0.8087
EC50	0.0001712
Std. Error	
BOTTOM	295.2
TOP	0.9012
LOGEC50	2.057
HILLSLOPE	0.5081
95% Confidence Intervals	
BOTTOM	-658.5 to 573.0
TOP	91.79 to 95.55
LOGEC50	-8.057 to 0.5244
HILLSLOPE	-1.869 to 0.2512
EC50	8.766e-009 to 3.345
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9728
Absolute Sum of Squares	204.9
Sy.x	3.201
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant Microsomes Assay 7-21-05

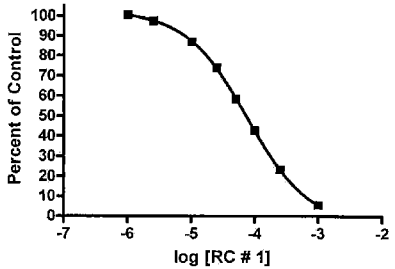
Replicate 2 (Aminoglutethimide)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.2759
TOP	108.2
LOGEC50	-4.070
HILLSLOPE	-1.009
EC50	8.503e-005
Std. Error	
BOTTOM	2.013
TOP	0.9190
LOGEC50	0.02117
HILLSLOPE	0.04640
95% Confidence Intervals	
BOTTOM	-3.923 to 4.475
TOP	106.3 to 110.1
LOGEC50	-4.115 to -4.026
HILLSLOPE	-1.106 to -0.9126
EC50	7.681e-005 to 9.413e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9979
Absolute Sum of Squares	60.46
Sy.x	1.739
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant Microsomes Assay 7-22-05

Replicate 3 (Aminoglutethimide)

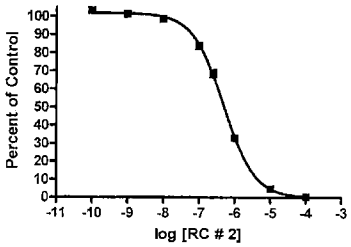


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-4.090
TOP	102.5
LOGEC50	-4.119
HILLSLOPE	-0.8902
EC50	7.599e-005
Std. Error	
BOTTOM	1.671
TOP	0.8010
LOGEC50	0.01835
HILLSLOPE	0.03348
95% Confidence Intervals	
BOTTOM	-7.577 to -0.6042
TOP	100.8 to 104.2
LOGEC50	-4.158 to -4.081
HILLSLOPE	-0.9600 to -0.8203
EC50	6.958e-005 to 8.299e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9988
Absolute Sum of Squares	31.23
Sy.x	1.250
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

Recombinant microsomes 08-11-05

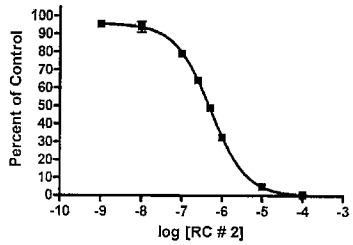
Replicate 1 (Ketoconazole)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.1264
TOP	101.8
LOGEC50	-6.304
HILLSLOPE	-0.9977
EC50	4.969e-007
Std. Error	
BOTTOM	0.9069
TOP	0.6383
LOGEC50	0.01778
HILLSLOPE	0.03745
95% Confidence Intervals	
BOTTOM	-2.018 to 1.765
TOP	100.5 to 103.2
LOGEC50	-6.341 to -6.267
HILLSLOPE	-1.076 to -0.9196
EC50	4.563e-007 to 5.412e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9984
Absolute Sum of Squares	63.01
Sy.x	1.775
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-12-05

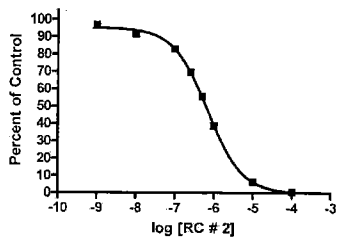
Replicate 2 (Ketoconazole)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.09930
TOP	95.71
LOGEC50	-6.284
HILLSLOPE	-0.9779
EC50	5.196e-007
Std. Error	
BOTTOM	1.146
TOP	1.014
LOGEC50	0.02116
HILLSLOPE	0.05108
95% Confidence Intervals	
BOTTOM	-2.490 to 2.291
TOP	93.59 to 97.82
LOGEC50	-6.328 to -6.240
HILLSLOPE	-1.084 to -0.8714
EC50	4.694e-007 to 5.752e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9967
Absolute Sum of Squares	97.15
Sy.x	2.204
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-15-05

Replicate 3 (Ketoconazole)

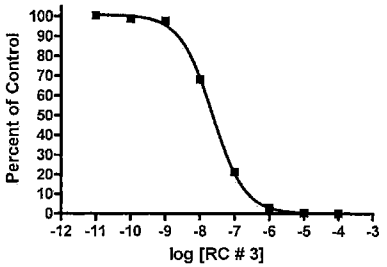


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.1560
TOP	95.25
LOGEC50	-6.157
HILLSLOPE	-0.9826
EC50	6.972e-007
Std. Error	
BOTTOM	1.172
TOP	0.9456
LOGEC50	0.02177
HILLSLOPE	0.05159
95% Confidence Intervals	
BOTTOM	-2.601 to 2.289
TOP	93.27 to 97.22
LOGEC50	-6.202 to -6.111
HILLSLOPE	-1.090 to -0.8750
EC50	6.280e-007 to 7.741e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9969
Absolute Sum of Squares	90.21
Sy.x	2.124
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

Recombinant microsomes 08-02-05

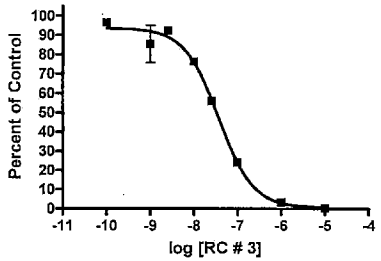
Replicate 1 (Prochloraz)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.06698
TOP	100.8
LOGEC50	-7.637
HILLSLOPE	-0.9271
EC50	2.307e-008
Std. Error	
BOTTOM	0.4862
TOP	0.5067
LOGEC50	0.01572
HILLSLOPE	0.02568
95% Confidence Intervals	
BOTTOM	-0.9473 to 1.081
TOP	99.70 to 101.8
LOGEC50	-7.670 to -7.604
HILLSLOPE	-0.9807 to -0.8735
EC50	2.139e-008 to 2.488e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9993
Absolute Sum of Squares	33.46
Sy.x	1.293
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-03-05

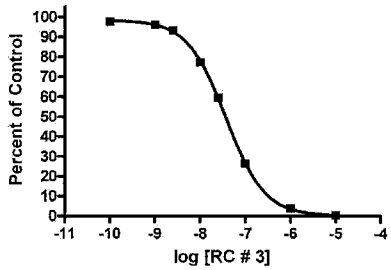
Replicate 2 (Prochloraz)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.5819
TOP	93.60
LOGEC50	-7.432
HILLSLOPE	-1.101
EC50	3.697e-008
Std. Error	
BOTTOM	2.908
TOP	2.614
LOGEC50	0.06147
HILLSLOPE	0.1579
95% Confidence Intervals	
BOTTOM	-5.484 to 6.648
TOP	88.15 to 99.06
LOGEC50	-7.560 to -7.304
HILLSLOPE	-1.431 to -0.7720
EC50	2.752e-008 to 4.967e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9771
Absolute Sum of Squares	778.1
Sy.x	6.237
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-04-05

Replicate 3 (Prochloraz)

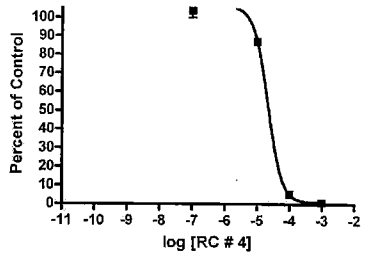


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.2406
TOP	98.41
LOGEC50	-7.426
HILLSLOPE	-1.021
EC50	3.749e-008
Std. Error	
BOTTOM	0.6303
TOP	0.5727
LOGEC50	0.01301
HILLSLOPE	0.02955
95% Confidence Intervals	
BOTTOM	-1.074 to 1.555
TOP	97.21 to 99.60
LOGEC50	-7.453 to -7.399
HILLSLOPE	-1.083 to -0.9596
EC50	3.522e-008 to 3.991e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9991
Absolute Sum of Squares	33.78
Sy.x	1.300
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

Recombinant microsomes 09-27-2005

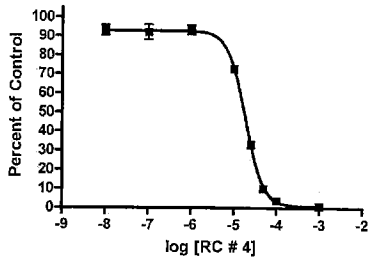
Replicate 1 (4-Nonylphenol)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.9143
TOP	106.1
LOGEC50	-4.675
HILLSLOPE	-2.014
EC50	2.111e-005
Std. Error	
BOTTOM	2.686
TOP	1.184
LOGEC50	0.06991
HILLSLOPE	0.3821
95% Confidence Intervals	
BOTTOM	-4.689 to 6.517
TOP	103.6 to 108.6
LOGEC50	-4.821 to -4.530
HILLSLOPE	-2.811 to -1.217
EC50	1.509e-005 to 2.954e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9910
Absolute Sum of Squares	416.3
Sy.x	4.562
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (09-28-2005)

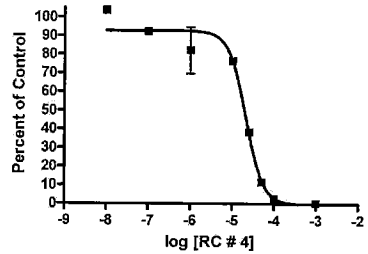
Replicate 2 (4-Nonylphenol)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.8241
TOP	92.88
LOGEC50	-4.732
HILLSLOPE	-2.096
EC50	1.852e-005
Std. Error	
BOTTOM	1.403
TOP	1.035
LOGEC50	0.01731
HILLSLOPE	0.1438
95% Confidence Intervals	
BOTTOM	-2.103 to 3.751
TOP	90.72 to 95.03
LOGEC50	-4.769 to -4.696
HILLSLOPE	-2.396 to -1.796
EC50	1.704e-005 to 2.012e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9949
Absolute Sum of Squares	190.4
Sy.x	3.085
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (09-29-2005)

Replicate 3 (4-Nonylphenol)

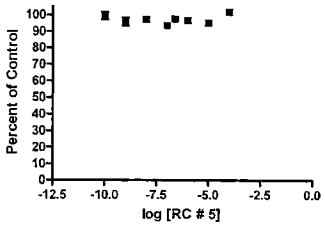


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.1897
TOP	92.73
LOGEC50	-4.678
HILLSLOPE	-2.112
EC50	2.099e-005
Std. Error	
BOTTOM	4.287
TOP	3.048
LOGEC50	0.04987
HILLSLOPE	0.4305
95% Confidence Intervals	
BOTTOM	-9.132 to 8.752
TOP	86.37 to 99.09
LOGEC50	-4.782 to -4.574
HILLSLOPE	-3.010 to -1.214
EC50	1.652e-005 to 2.668e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9572
Absolute Sum of Squares	1670
Sy.x	9.138
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

Recombinant microsomes (09-30-2005)

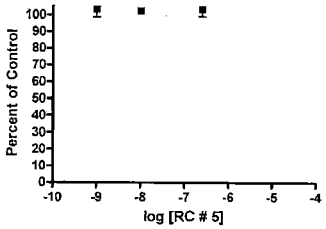
Replicate 1 (Dibenz[a,h]anthracene)



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	

Recombinant microsomes (10-03-2005)

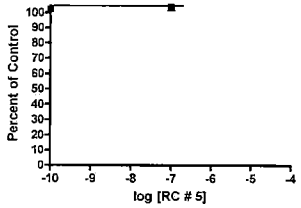
Replicate 2 (Dibenz[a,h]anthracene)



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	

Recombinant microsomes (10-04-2005)

Replicate 3 (Dibenz[a,h]anthracene)

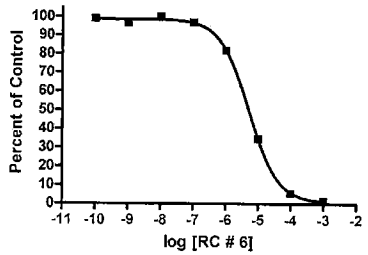


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	105.0
TOP	110.1
LOGEC50	-6.604
HILLSLOPE	20.96
EC50	2.492e-007
Std. Error	
BOTTOM	1.409
TOP	1.409
LOGEC50	18631
HILLSLOPE	1.104e+008
95% Confidence Intervals	
BOTTOM	102.0 to 107.9
TOP	107.2 to 113.1
LOGEC50	-38870 to 38857
HILLSLOPE	-230200000 to 2.302e+008
EC50	
Goodness of Fit	
Degrees of Freedom	20
R^2	0.2794
Absolute Sum of Squares	357.2
Sy.x	4.226
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Figure 5 - Fitted Concentration Response for Dibenz[a,h]anthracene

Recombinant microsomes 08-05-05

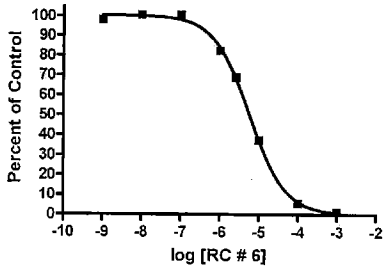
Replicate 1 (Fenarimol)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.7102
TOP	98.71
LOGEC50	-5.287
HILLSLOPE	-0.9738
EC50	5.165e-006
Std. Error	
BOTTOM	0.9017
TOP	0.5373
LOGEC50	0.02144
HILLSLOPE	0.03955
95% Confidence Intervals	
BOTTOM	-1.171 to 2.591
TOP	97.59 to 99.83
LOGEC50	-5.332 to -5.242
HILLSLOPE	-1.056 to -0.8913
EC50	4.660e-006 to 5.725e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9985
Absolute Sum of Squares	59.83
Sy.x	1.730
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-08-05

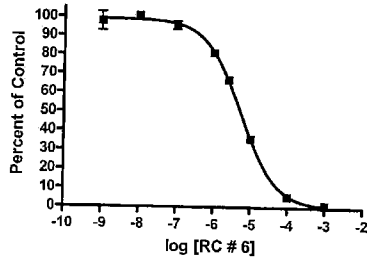
Replicate 2 (Fenarimol)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.3027
TOP	100.4
LOGEC50	-5.250
HILLSLOPE	-0.9460
EC50	5.626e-006
Std. Error	
BOTTOM	1.110
TOP	0.7436
LOGEC50	0.02250
HILLSLOPE	0.04279
95% Confidence Intervals	
BOTTOM	-2.013 to 2.618
TOP	98.83 to 101.9
LOGEC50	-5.297 to -5.203
HILLSLOPE	-1.035 to -0.8567
EC50	5.049e-006 to 6.268e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9977
Absolute Sum of Squares	83.85
Sy.x	2.048
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-09-05

Replicate 3 (Fenarimol)

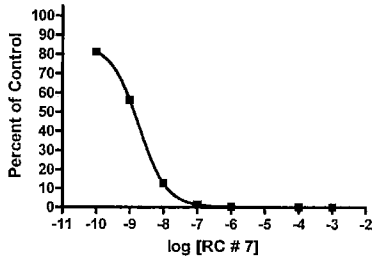


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.05869
TOP	98.94
LOGEC50	-5.263
HILLSLOPE	-0.9191
EC50	5.454e-006
Std. Error	
BOTTOM	1.868
TOP	1.245
LOGEC50	0.03860
HILLSLOPE	0.07001
95% Confidence Intervals	
BOTTOM	-3.955 to 3.837
TOP	96.34 to 101.5
LOGEC50	-5.344 to -5.183
HILLSLOPE	-1.065 to -0.7730
EC50	4.531e-006 to 6.566e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9936
Absolute Sum of Squares	229.6
Sy.x	3.388
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

Recombinant microsomes (08-17-2005)

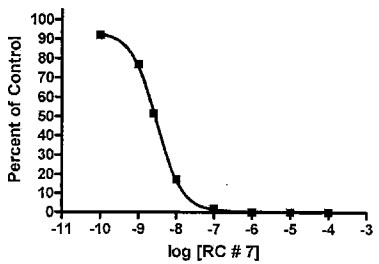
Replicate 1 (Econazole)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.08361
TOP	84.94
LOGEC50	-8.721
HILLSLOPE	-1.046
EC50	1.901e-009
Std. Error	
BOTTOM	0.3373
TOP	1.060
LOGEC50	0.01999
HILLSLOPE	0.03953
95% Confidence Intervals	
BOTTOM	-0.6201 to 0.7873
TOP	82.73 to 87.15
LOGEC50	-8.763 to -8.679
HILLSLOPE	-1.129 to -0.9638
EC50	1.727e-009 to 2.093e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9985
Absolute Sum of Squares	30.82
Sy.x	1.241
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-18-2005)

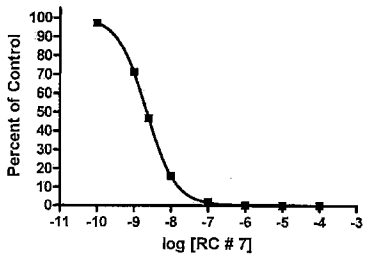
Replicate 2 (Econazole)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.3425
TOP	93.54
LOGEC50	-8.516
HILLSLOPE	-1.297
EC50	3.049e-009
Std. Error	
BOTTOM	0.3082
TOP	0.6593
LOGEC50	0.009402
HILLSLOPE	0.03138
95% Confidence Intervals	
BOTTOM	-0.3004 to 0.9855
TOP	92.17 to 94.92
LOGEC50	-8.535 to -8.496
HILLSLOPE	-1.362 to -1.231
EC50	2.915e-009 to 3.190e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9993
Absolute Sum of Squares	21.43
Sy.x	1.035
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-19-2005)

Replicate 3 (Econazole)

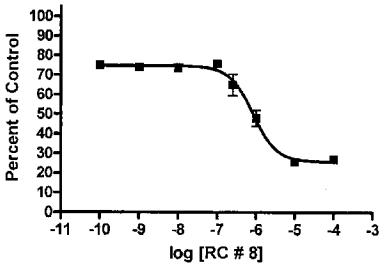


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.1713
TOP	100.3
LOGEC50	-8.652
HILLSLOPE	-1.112
EC50	2.226e-009
Std. Error	
BOTTOM	0.3541
TOP	0.8884
LOGEC50	0.01166
HILLSLOPE	0.03149
95% Confidence Intervals	
BOTTOM	-0.5673 to 0.9099
TOP	98.44 to 102.1
LOGEC50	-8.677 to -8.628
HILLSLOPE	-1.178 to -1.046
EC50	2.105e-009 to 2.355e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9991
Absolute Sum of Squares	27.39
Sy.x	1.170
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

Recombinant microsomes (08-23-2005)

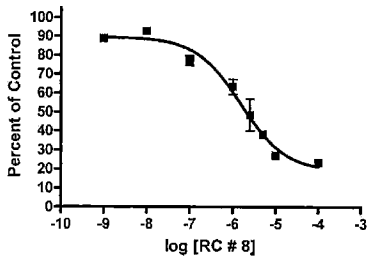
Replicate 1 (Chrysin)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	25.83
TOP	74.73
LOGEC50	-6.081
HILLSLOPE	-1.426
EC50	8.299e-007
Std. Error	
BOTTOM	2.033
TOP	1.443
LOGEC50	0.06977
HILLSLOPE	0.3208
95% Confidence Intervals	
BOTTOM	21.59 to 30.07
TOP	71.72 to 77.74
LOGEC50	-6.227 to -5.935
HILLSLOPE	-2.095 to -0.7572
EC50	5.935e-007 to 1.160e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9610
Absolute Sum of Squares	393.2
Sy.x	4.434
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-24-2005)

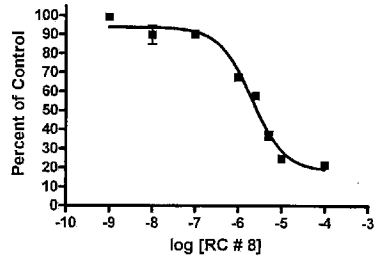
Replicate 2 (Chrysin)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	19.03
TOP	89.36
LOGEC50	-5.814
HILLSLOPE	-0.8334
EC50	1.536e-006
Std. Error	
BOTTOM	4.980
TOP	2.890
LOGEC50	0.1152
HILLSLOPE	0.1911
95% Confidence Intervals	
BOTTOM	8.638 to 29.42
TOP	83.34 to 95.39
LOGEC50	-6.054 to -5.573
HILLSLOPE	-1.232 to -0.4347
EC50	8.829e-007 to 2.670e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9472
Absolute Sum of Squares	852.7
Sy.x	6.530
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-25-2005)

Replicate 3 (Chrysin)

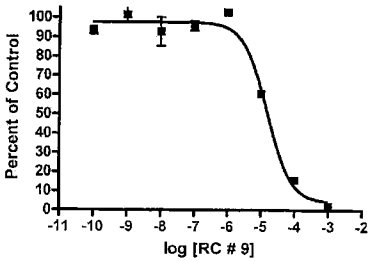


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	18.31
TOP	93.75
LOGEC50	-5.685
HILLSLOPE	-1.145
EC50	2.066e-006
Std. Error	
BOTTOM	3.429
TOP	1.975
LOGEC50	0.06139
HILLSLOPE	0.1804
95% Confidence Intervals	
BOTTOM	11.16 to 25.46
TOP	89.63 to 97.87
LOGEC50	-5.813 to -5.557
HILLSLOPE	-1.521 to -0.7685
EC50	1.538e-006 to 2.774e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9712
Absolute Sum of Squares	581.4
Sy.x	5.392
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

Recombinant microsomes (08-29-2005)

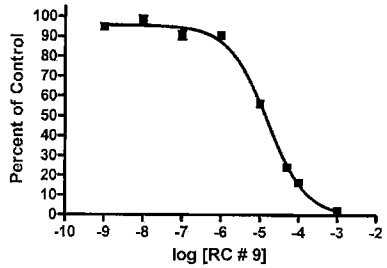
Replicate 1 (Dicofol)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	4.122
TOP	97.57
LOGEC50	-4.817
HILLSLOPE	-1.246
EC50	1.525e-005
Std. Error	
BOTTOM	4.327
TOP	1.887
LOGEC50	0.08487
HILLSLOPE	0.3011
95% Confidence Intervals	
BOTTOM	-4.904 to 13.15
TOP	93.64 to 101.5
LOGEC50	-4.994 to -4.640
HILLSLOPE	-1.874 to -0.6178
EC50	1.014e-005 to 2.292e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9729
Absolute Sum of Squares	941.3
Sy.x	6.860
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-30-2005)

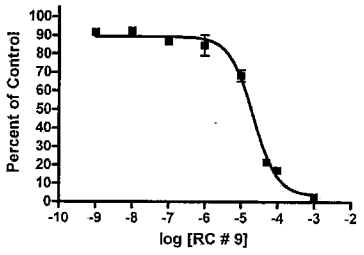
Replicate 2 (Dicofol)



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.3419
TOP	95.54
LOGEC50	-4.808
HILLSLOPE	-0.8659
EC50	1.557e-005
Std. Error	
BOTTOM	0.5575
TOP	1.115
LOGEC50	0.02623
HILLSLOPE	0.04279
95% Confidence Intervals	
BOTTOM	-1.505 to 0.8210
TOP	93.22 to 97.87
LOGEC50	-4.862 to -4.753
HILLSLOPE	-0.9552 to -0.7766
EC50	1.373e-005 to 1.766e-005
Goodness of Fit	
Degrees of Freedom	20
R^2 (unweighted)	0.9946
Weighted Sum of Squares (1/Y)	2.349
Absolute Sum of Squares	180.4
Sy.x	3.004
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24

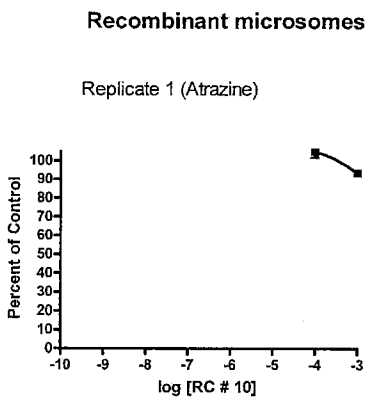
Recombinant microsomes (08-31-2005)

Replicate 3 (Dicofol)

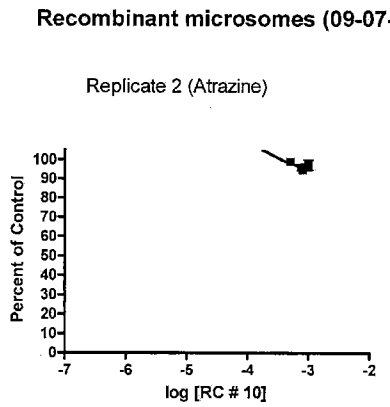


Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	3.439
TOP	89.40
LOGEC50	-4.662
HILLSLOPE	-1.321
EC50	2.179e-005
Std. Error	
BOTTOM	2.945
TOP	1.467
LOGEC50	0.05258
HILLSLOPE	0.1554
95% Confidence Intervals	
BOTTOM	-2.705 to 9.582
TOP	86.34 to 92.46
LOGEC50	-4.771 to -4.552
HILLSLOPE	-1.645 to -0.9972
EC50	1.693e-005 to 2.806e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9843
Absolute Sum of Squares	475.7
Sy.x	4.877
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

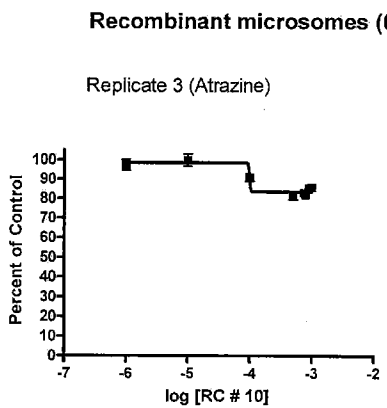
Figure 9 - Fitted Concentration Response for Dicofol



Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	81.45
TOP	107.1
LOGEC50	-3.056
HILLSLOPE	-1.048
EC50	0.0008797
Std. Error	
BOTTOM	240.7
TOP	0.9018
LOGEC50	8.262
HILLSLOPE	4.487
95% Confidence Intervals	
BOTTOM	-420.7 to 583.6
TOP	105.3 to 109.0
LOGEC50	-20.29 to 14.18
HILLSLOPE	-10.41 to 8.312
EC50	5.134e-021 to 1.507e+014
Goodness of Fit	
Degrees of Freedom	20
R^2	0.6820
Absolute Sum of Squares	224.2
Sy.x	3.348
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0



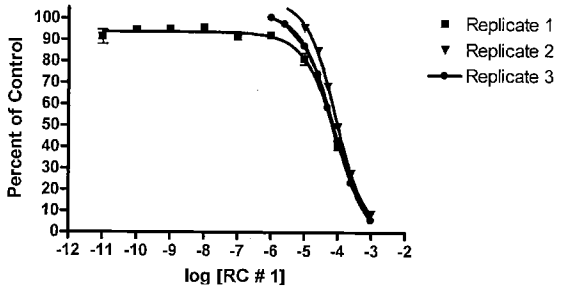
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	94.66
TOP	109.6
LOGEC50	-3.578
HILLSLOPE	-1.899
EC50	0.0002642
Std. Error	
BOTTOM	3.057
TOP	1.152
LOGEC50	0.2127
HILLSLOPE	1.024
95% Confidence Intervals	
BOTTOM	88.28 to 101.0
TOP	107.2 to 112.0
LOGEC50	-4.022 to -3.134
HILLSLOPE	-4.036 to 0.2379
EC50	9.509e-005 to 0.0007340
Goodness of Fit	
Degrees of Freedom	20
R^2	0.8523
Absolute Sum of Squares	153.4
Sy.x	2.770
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0



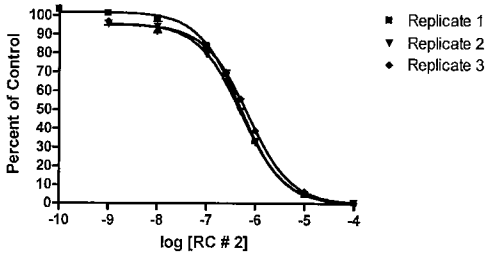
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	83.44
TOP	98.51
LOGEC50	-4.000
HILLSLOPE	-57.36
EC50	9.998e-005
Std. Error	
BOTTOM	0.9224
TOP	1.458
LOGEC50	351046
HILLSLOPE	2.455e+011
95% Confidence Intervals	
BOTTOM	81.51 to 85.36
TOP	95.47 to 101.6
LOGEC50	-732300 to 732277
HILLSLOPE	-512100000000 to 5.121e+011
EC50	
Goodness of Fit	
Degrees of Freedom	20
R^2	0.7968
Absolute Sum of Squares	255.2
Sy.x	3.572
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Figure 10 - Fitted Concentration Response for Atrazine

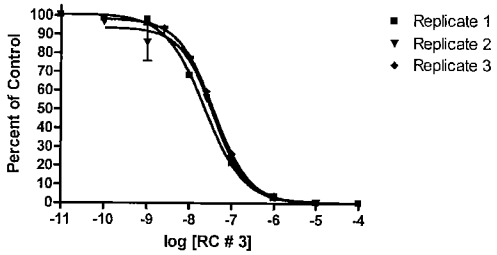
Replicates 1, 2, and 3 (Aminoglutethimide)



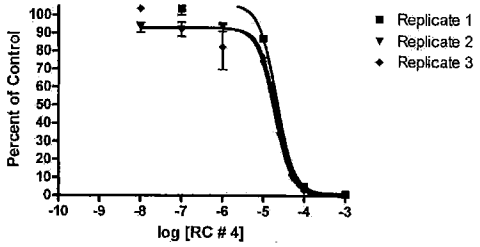
Ketoconazole Replicates 1, 2, and 3



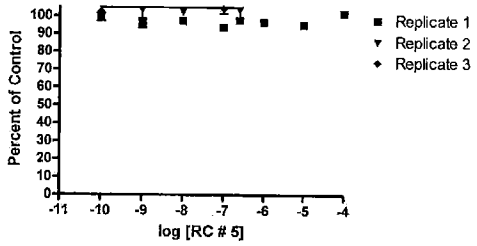
Prochloraz Replicates 1, 2, and 3



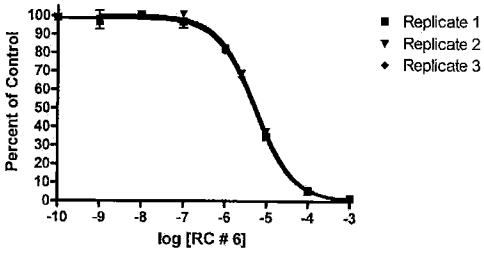
4-Nonylphenol Replicates 1, 2, and 3



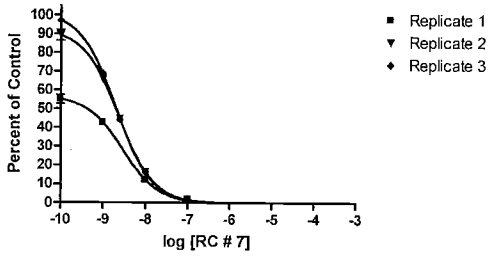
Dibenz[a,h]anthracene Replicates 1, 2, and 3



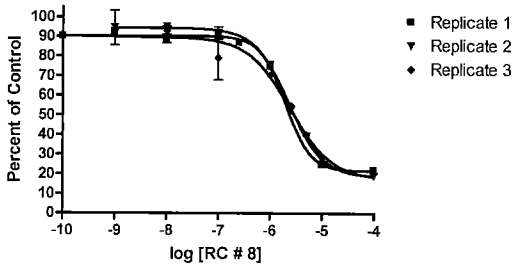
Fenarimol Replicates 1, 2, and 3



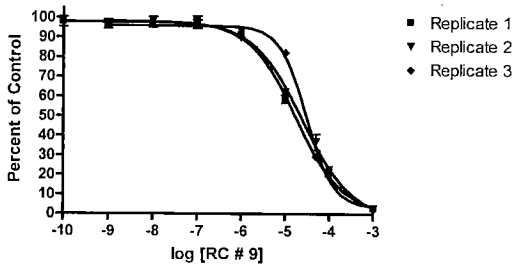
Econazole Replicates 1, 2, and 3



Chrysin Replicates 1, 2, and 3



Dicofol Replicates 1, 2, and 3



Atrazine Replicates 1, 2, and 3

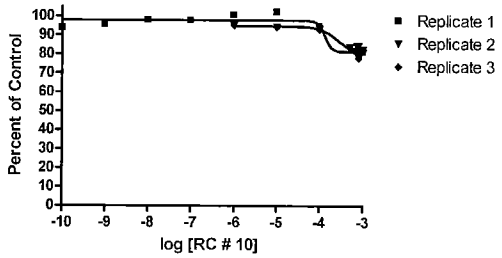
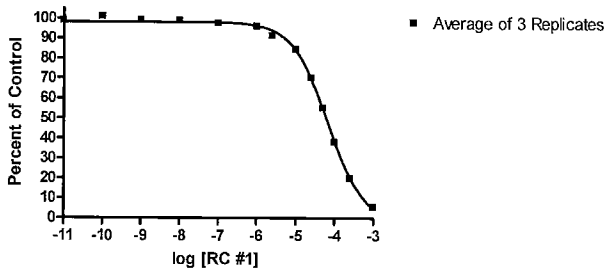
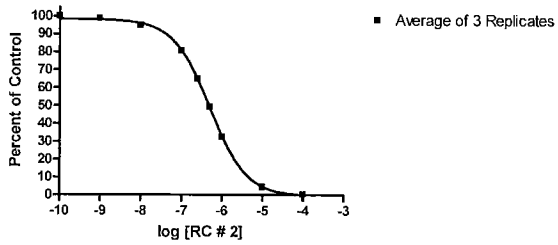


Figure 11 - Concentration Response Curve Fits – Overlay of Individual Replicate Results

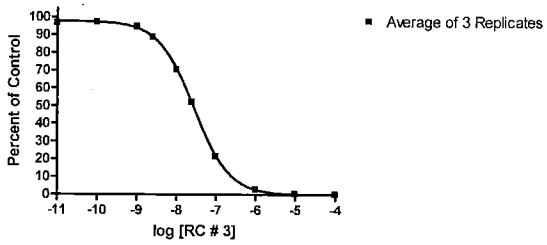
Average of Replicates 1, 2, 3 (Aminoglutethimide)



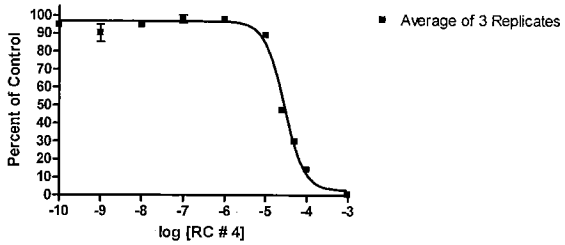
Average of Ketoconazole Replicates 1, 2, and 3



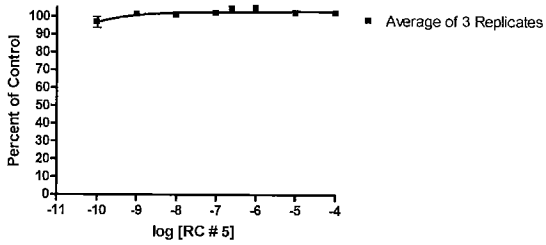
Average of Prochloraz Replicates 1, 2, and 3



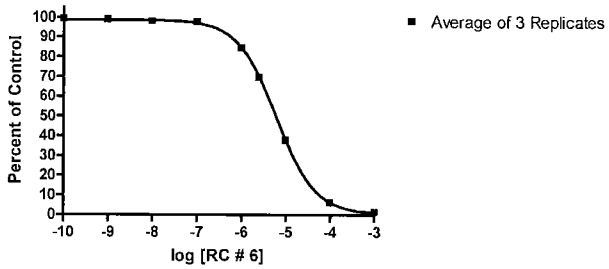
Average of 4-Nonylphenol Replicates 1, 2, and 3



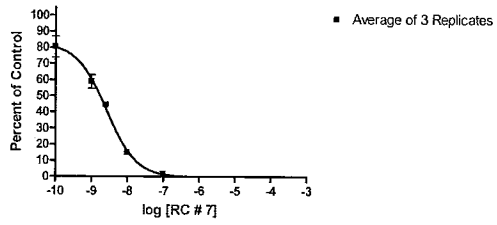
Average of Dibenz[a,h]anthracene Replicates 1, 2, and 3



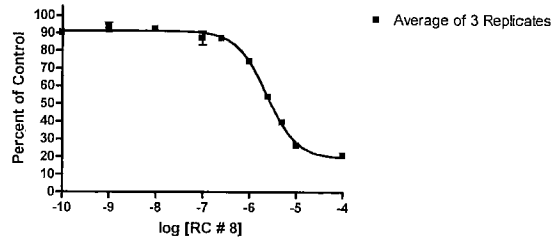
Average of Fenarimol Replicates 1, 2, and 3



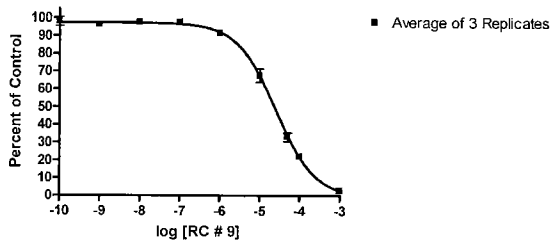
Average of Econazole Replicates 1, 2, and 3



Average of Chrysin Replicates 1, 2, and 3



Average of Dicofol Replicates 1, 2, and 3



Average of Atrazine Replicates 1, 2, and 3

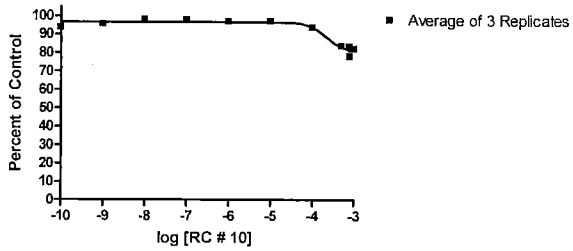


Figure 12 - Average Concentration Response Curve Fits

Table 28 - Summary of LogIC_{50} and Slope and their Associated Standard Errors (SE)

Reference Chemical	Chemical Code	Replicate	Log IC_{50}	SE Log IC_{50}	Slope	SE Slope	Status	IC_{50} (µM)
Aminoglutethimide	1	1	-3.766	2.057	-0.8087	0.5081	Inhibitor	171.2
		2	-4.070	0.02117	-1.009	0.04640	Inhibitor	85.03
		3	-4.119	0.01835	-0.8902	0.03348	Inhibitor	75.99
Ketoconazole	2	1	-6.304	0.01778	-0.9977	0.03745	Inhibitor	0.4969
		2	-6.284	0.02116	-0.9779	0.05108	Inhibitor	0.5196
		3	-6.157	0.02177	-0.9826	0.05159	Inhibitor	0.6972
Prochloraz	3	1	-7.637	0.01572	-0.9271	0.02568	Inhibitor	0.0231
		2	-7.432	0.06147	-1.101	0.1579	Inhibitor	0.0370
		3	-7.426	0.01301	-1.021	0.02955	Inhibitor	0.0375
4-Nonylphenol	4	1	-4.675	0.06991	-2.014	0.3821	Inhibitor	21.11
		2	-4.732	0.01731	-2.096	0.1438	Inhibitor	18.52
		3	-4.678	0.04987	-2.112	0.4305	Inhibitor	20.99
Dibenz[a,h]anthracene*	5						No inhibition	
Fenarimol	6	1	-5.287	0.02144	-0.9738	0.03955	Inhibitor	5.165
		2	-5.250	0.02250	-0.9460	0.04279	Inhibitor	5.626
		3	-5.263	0.03860	-0.9191	0.07001	Inhibitor	5.454
Econazole	7	1	-8.721	0.01999	-1.046	0.03953	Inhibitor	0.001901
		2	-8.516	0.009402	-1.297	0.03138	Inhibitor	0.003049
		3	-8.652	0.01166	-1.112	0.03149	Inhibitor	0.002226
Chrysin	8	1	-6.081	0.06977	-1.426	0.3208	Inhibitor	0.8299
		2	-5.814	0.1152	-0.8334	0.1911	Inhibitor	1.536
		3	-5.685	0.06139	-1.145	0.1804	Inhibitor	2.066
Dicofol	9	1	-4.817	0.08487	-1.246	0.3011	Inhibitor	15.25
		2	-4.808	0.02623	-0.8659	0.04279	Inhibitor	15.57
		3	-4.662	0.05258	-1.321	0.1554	Inhibitor	21.79
Atrazine*	10						No inhibition	

* No inhibition – no data available above 20% inhibition - IC_{50} was not calculated.

Table 29 - Summary Results

Reference Chemical	Replicate	IC_{50}	Mean* IC_{50}	SD	SEM	% CV
Aminoglutethimide	1	171.2 µM	110.7 µM	52.6	30.3	47.5
	2	85.03 µM				
	3	75.99 µM				
Ketoconazole	1	496.9 nM	571.2 nM	109.7	63.3	19.20
	2	519.6 nM				
	3	697.2 nM				
Prochloraz	1	23.10 nM	32.53 nM	8.17	4.7	25.12
	2	37.00 nM				
	3	37.50 nM				
4-Nonylphenol	1	21.11µM	20.21 µM	1.46	0.8	7.23
	2	18.52 µM				
	3	20.99 µM				
Fenarimol	1	5.165 µM	5.415 µM	0.233	0.1	4.30
	2	5.626 µM				
	3	5.454 µM				
Econazole	1	1.901 nM	2.392 nM	0.592	0.3	24.7
	2	3.049 nM				
	3	2.226 nM				
Chrysin	1	0.8299 µM	1.477 µM	0.620	0.4	42.0
	2	1.536 µM				
	3	2.066 µM				
Dicofol	1	15.25 µM	17.54 µM	3.69	2.1	21.0
	2	15.57 µM				
	3	21.79 µM				

* Calculated (arithmetic) average value from three measurements.

5.0 DISCUSSION

The presented study (Task 4) involved testing the response of recombinant microsomes to the presence of ten reference chemicals. Eight different concentrations of each reference chemical generally ranging from 1×10^{-10} to 1×10^{-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 all eight reference chemicals are presented in Table 29.

The microsomes used in presented study were obtained from BD GENTEST via RTI. The protein content of the recombinant microsomes was measured each time the aromatase assay was run (total 30 runs). The average determined protein concentration was 5.17 ± 0.21 mg/mL (4.16% CV). In order to better characterize the protein assay, QC samples were included in all runs (total 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 necessarily was zero (0). The average full aromatase activity across all 30 runs of the aromatase assay was 0.3123 ± 0.0502 nmol/mg/min (16.1% CV). The positive control activity across 30 runs averaged 0.1688 ± 0.0251 nmol/mg/min (14.9% CV), approximately 54% of full aromatase activity and the negative control activity averaged 0.3051 ± 0.0512 (16.8% 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 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 0% to almost 100% for aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, fenarimol, econazole and dicofol. For chrysin, the percent of control values ranged from about 20 to about 90%. 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.

Concentrations response curve 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. Generally, the obtained curves fit the data well ($r^2 > 0.99$).

Replicate-to-replicate variation in IC_{50} was low (4.30 %CV and 7.23 %CV, respectively) for fenarimol and 4-nonylphenol. For the rest of reference chemicals replicate-to-replicate variation was much higher, from 19.20 %CV for ketoconazole up to 47.46 %CV for aminoglutethimide and 41.98 %CV for chrysin; for the remaining three inhibitors % CV was 25.16, 24.74 and 21.02. 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 range between first and remaining two replicates. The IC_{50} values

measured for 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 appears to be good inhibitors of the aromatase activity while two chemicals, dibenz[a,h]anthracene and atrazine, had no inhibitors 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

- The responsiveness (in a concentration dependent manner) of the human recombinant microsome aromatase assay to aminglutethimide, ketoconazole, prochloraz, 4-nonylphenol, fenarimol, econazole, chrysin, econazole, and dicofol were confirmed.

- It was determined that econazole was the most potent aromatase activity inhibitor (IC_{50} = 2.4 nM) from the group of eight studied reference chemicals.

- Dibenz[a,h]anthracene and atrazine did not inhibit the aromatase activity in the studied range of concentration.

APPENDIX A

Protocol (with Amendments and Deviations) A-1

BATTELLE STUDY PROTOCOL

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES

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 4-17, Task 4

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 RECOMBINANT MICROSOMES

APPROVED, BATTELLE:

Bozena D. Lusiak, Ph.D.
Study Director, Battelle Memorial Institute — Date: 07-11-05

Steven W. Graves
CTC Manager, Battelle Memorial Institute — Date: 7/11/05

REVIEWED BY, BATTELLE:

Hillary A.G. Flory, B.S.
Quality Assurance Auditor, Battelle Memorial Institute — Date: 7-11-05

APPROVED, SPONSOR:

Jerry D. Johnson, Ph.D., D.A.B.T.
Work Assignment Leader
Endocrine Disruptor Screening Program
Battelle Memorial Institute — Date: 7-11-05

REVIEVED BY, SPONSOR:

Terri L. Pollock, B.A.
EDSP Quality Assurance Manager
Battelle Memorial Institute — Date: 7-11-05

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AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES

1.0 OBJECTIVES

The objectives of this task are to test 10 reference chemicals using recombinant microsomes.

1.1 Justification for Test System

The test system for this study is recombinant microsomes. This test system was selected because it provides a commercially available source of the aromatase enzyme and since the assay is being evaluated for its potential to serve as a screening assay, the use of recombinant human enzyme 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 recombinant microsomal preparation 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 4, 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.

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 Microsomes

Recombinant microsomes will be supplied to each laboratory by RTI. 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 recombinant 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 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.

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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 0.008 mg/mL. The addition of 1 mL of that microsomes dilution will result in a final approximate protein concentration of 0.004 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 laboratory and distributed to each participating laboratory. Each of these QC standards will be run in duplicate with each run of the protein assay.

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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)	Recombinant
Microsomal Protein (mg/mL)[a]	0.004
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

Ten reference chemicals will be tested. 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. Each reference chemical will be tested at eight concentrations and there will be three (triplicate) repetitions for each concentration of a given replicate. 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. Multiple reference chemicals may be conducted by a single technician in a given day. 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. A single replicate study of a given reference chemical is described in Table 4.

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.

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- The lowest concentration to be tested is 10^{-10} M.

Table 4. 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 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.

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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: **G608317**

STUDY TITLE: **Aromatase Assay Validation: Conduct Multiple Chemical Studies with Recombinant Microsomes (WA 4-17, Task 4)**

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 12, 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 11 and page 12, 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 21, 2005

APPROVED BY:

Bozena D. Lusiak, Study Director — 11-28-05 Date

Sponsor Representative — 11-28-05 Date

PROTOCOL AMENDMENT 2

STUDY NUMBER: **G608317**

STUDY TITLE: **Aromatase Assay Validation: Conduct Multiple Chemical Studies with Recombinant Microsomes (WA 4-17, Task 4)**

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
Bozena D. Lusiak, Study Director

12-16-05
Date

Jerry D. Josh
Sponsor Representative

12-16-05
Date

DEVIATION REPORT

G608317

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES (TASK 4)

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: Bryan D. Livinski Date: 02-02-06

Original: Study File
Copies: H. Flory

DEVIATION REPORT

G608317

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES (TASK 4)

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: Brynn D. Linnel Date: 07-07-06

Original: Study File
Copies : H. Flory

DEVIATION REPORT

G608317

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES (TASK 4)

Type of Deviation:	Protocol
Date of Deviation:	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 two assays, the microsomes thaw and use time was not recorded.
Impact of Deviation on the Study:	None.
Corrective Action:	None

Approved By: Bryna D Juniak Date: 03-23-06

Original: Study File
Copies : H. Flory

PROTOCOL AMENDMENT 3

STUDY NUMBER: **G608317**

STUDY TITLE: **Aromatase Assay Validation: Conduct Multiple Chemical Studies with Recombinant Microsomes (WA 4-17, Task 4)**

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 27, 2006

APPROVED BY:

Bozena D. Lusiak

Bozena D. Lusiak, Study Director

03-27-06
Date

Sponsor Representative

3-27-06
Date

DEVIATION REPORT

G608317

AROMATASE ASSAY VALIDATION: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES (TASK 4)

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: Bryan D. Hurst Date: 05-11-06

Original: Study File
Copies : H. Flory

Appendix B

Spreadsheets - Aromatase Activity Calculation Page of the Spreadsheet for Each Chemical/Replicate. B-1

Assay Date	7/20/2005	Test Chemical ID	1	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	recombinar	Microsome ID	lot # 5

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	recombinant	Microsome ID	lot # 5			
									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	0.138	0.069	0.028	0.015	0.000				
Samples:	QC 100	QC 10	Microsomes								
	0.263	0.037	0.254								
	0.262	0.038	0.254								
			0.246								

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	
M, LE 05-22-06 BSC													
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	0.254	0.254	0.023	200	100	5000	0.006	0.006	5.667	
Microsomes	0.254	0.254	0.023	200	100	5000	0.006			
Microsomes	0.246	0.246	0.022	200	100	5000	0.006			

Assay Date	7/20/2005	Test Chemical ID	1	# Concentrations tested	8						
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	lot # 5				

								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	0.138	0.069	0.028	0.015	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.263	0.037	0.254
	0.262	0.038	0.254
			0.246

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	0.513	0.513	0.0426	m, b	0.084	-0.001
125	100	200	0.00013	200	0.0250	0.301	0.301	0.0246	se_m, se_b	0.003	0.000
50	200	1000	0.00005	200	0.0100	0.137	0.137	0.0108	r^2, se_y	0.997	0.001
25	100	1000	0.00003	200	0.0050	0.071	0.071	0.0053	F, df	1089	3
10	40	1000	0.00001	200	0.0020	0.029	0.029	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.016	0.016	0.0006			
				Blank	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 100.	037	0.037	0.002	200	1	1	0.000	0.000	0.012
QC 100.	038	0.038	0.002	200	1	1	0.000		
QC 10									
Microsomes	254	0.254	0.021	200	100	5000	0.005	0.005	5.116
Microsomes	0.254	0.254	0.021	200	100	5000	0.005		
Microsomes	0.246	0.246	0.020	200	100	5000	0.005		

Assay Date	7/20/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	recombinai	Microsome ID lot #	5	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	4	mL microsome Dilution A used
	50	mL total volume
	12.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	625	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.115792
Protein Concentration (dilution added to assay, mg/mL):	0.008185

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	recombinant	Microsome ID	lot # 5	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	11411	22821.4	22941.7	45883.4	0.1	147548	31.10	45740	0.0641	1	0.004	15	0.5223
			0.5	2	11531	23062											
	2	2	0.5	1	11041	22081.2	21561.9	43123.8	0.1	147548	29.23	42981	0.0603	1	0.004	15	0.4908
			0.5	2	10521	21042.6											
	3	2	0.5	1	11225	22450	22388.4	44776.8	0.1	147548	30.35	44634	0.0626	1	0.004	15	0.5097
			0.5	2	11163	22326.8											
	4	2	0.5	1	10921	21842.2	21428.8	42857.6	0.1	147548	29.05	42714	0.0599	1	0.004	15	0.4878
			0.5	2	10508	21015.4											
Background control	1	2	0.5	1	35	69.06	71.03	142.06	0.1	147548	0.10	-1	0.0000	1	0.004	15	0.0000
			0.5	2	37	73											
	2	2	0.5	1	32	64.06	65.6	131.2	0.1	147548	0.09	-12	0.0000	1	0.004	15	-0.0001
			0.5	2	34	67.14											
	3	2	0.5	1	41	82.28	76.37	152.74	0.1	147548	0.10	10	0.0000	1	0.004	15	0.0001
			0.5	2	35	70.46											
	4	2	0.5	1	33	65.36	73.32	146.64	0.1	147548	0.10	3	0.0000	1	0.004	15	0.0000
			0.5	2	41	81.28											
Positive control	1	2	0.5	1	5903	11805.8	11698.8	23397.6	0.1	147548	15.86	23254	0.0326	1	0.004	15	0.2656
			0.5	2	5796	11591.8											
	2	2	0.5	1	5885	11770.2	11674.4	23348.8	0.1	147548	15.82	23206	0.0325	1	0.004	15	0.2650
			0.5	2	5789	11578.6											
	3	2	0.5	1	5662	11324	11325.7	22651.4	0.1	147548	15.35	22508	0.0316	1	0.004	15	0.2570
			0.5	2	5664	11327.4											
	4	2	0.5	1	5462	10924.4	10877.1	21754.2	0.1	147548	14.74	21611	0.0303	1	0.004	15	0.2468
			0.5	2	5415	10829.8											
Negative Control	1	2	0.5	1	11668	23336	23165.2	46330.4	0.1	147548	31.40	46187	0.0648	1	0.004	15	0.5274
			0.5	2	11497	22994.4											
	2	2	0.5	1	11812	23624.6	23372.2	46744.4	0.1	147548	31.68	46601	0.0653	1	0.004	15	0.5322
			0.5	2	11560	23119.8											
	3	2	0.5	1	10634	21268	20897.8	41795.6	0.1	147548	28.33	41652	0.0584	1	0.004	15	0.4755
			0.5	2	10264	20527.6											
	4	2	0.5	1	11289	22577.6	22493.3	44986.6	0.1	147548	30.49	44843	0.0629	1	0.004	15	0.5121
			0.5	2	11205	22409											
1	1-1	2	0.5	1	4499	8998	9145.8	18291.6	0.1	147548	12.40	18148	0.0254	1	0.004	15	0.2072
			0.5	2	4647	9293.6											
	1-2	2	0.5	1	4406	8811.6	8704.7	17409.4	0.1	147548	11.80	17266	0.0242	1	0.004	15	0.1972
			0.5	2	4299	8597.8											
	1-3	2	0.5	1	4458	8915.4	8821.7	17643.4	0.1	147548	11.96	17500	0.0245	1	0.004	15	0.1998
			0.5	2	4364	8728											
	2-1	2	0.5	1	9522	19043.2	18982.8	37965.6	0.1	147548	25.73	37822	0.0530	1	0.004	15	0.4319
			0.5	2	9461	18922.4											
	2-2	2	0.5	1	8947	17893.4	17974	35948	0.1	147548	24.36	35805	0.0502	1	0.004	15	0.4089
			0.5	2	9027	18054.6											
	2-3	2	0.5	1	8995	17989.4	16844	33688	0.1	147548	22.83	33545	0.0470	1	0.004	15	0.3831
			0.5	2	7849	15698.6											
	3-1	2	0.5	1	10372	20743.4	20454.4	40908.8	0.1	147548	27.73	40766	0.0572	1	0.004	15	0.4655
			0.5	2	10083	20165.4											
	3-2	2	0.5	1	10194	20387	20580.3	41160.6	0.1	147548	27.90	41017	0.0575	1	0.004	15	0.4684
			0.5	2	10387	20773.6											
	3-3	2	0.5	1	9910	19820.8	19996.2	39992.4	0.1	147548	27.10	39849	0.0559	1	0.004	15	0.4551
			0.5	2	10086	20171.6											
	4-1	2	0.5	1	10312	20623.6	20593.5	41187	0.1	147548	27.91	41044	0.0575	1	0.004	15	0.4687
			0.5	2	10282	20563.4											
	4-2	2	0.5	1	9612	19223.2	19369	38738	0.1	147548	26.25	38595	0.0541	1	0.004	15	0.4407
			0.5	2	9757	19514.8											
	4-3	2	0.5	1	10287	20574.6	20506.1	41012.2	0.1	147548	27.80	40869	0.0573	1	0.004	15	0.4667
			0.5	2	10219	20437.6											
	5-1	2	0.5	1	10505	21210.4	21233.3	42466.6	0.1	147548	28.78	42323	0.0593	1	0.004	15	0.4833
			0.5	2	10628	21256.2											
	5-2	2	0.5	1	10552	21103.6	21138.5	42277	0.1	147548	28.65	42134	0.0591	1	0.004	15	0.4811
			0.5	2	10587	21173.4											
	5-3	2	0.5	1	10568	21135.6	20991	41982	0.1	147548	28.45	41839	0.0587	1	0.004	15	0.4778
			0.5	2	10423	20846.4											
	6-1	2	0.5	1	10834	21668	21412.3	42824.6	0.1	147548	29.02	42681	0.0598	1	0.004	15	0.4874
			0.5	2	10578	21156.6											
	6-2	2	0.5	1	10468	20936.4	20853.7	41707.4	0.1	147548	28.27	41564	0.0583	1	0.004	15	0.4746
			0.5	2	10386	20771											
	6-3	2	0.5	1	10172	20343	20486.8	40973.6	0.1	147548	27.77	40830	0.0572	1	0.004	15	0.4663
			0.5	2	10315	20630.6											
	7-1	2	0.5	1	10817	21634.8	21180.3	42360.6	0.1	147548	28.71	42217	0.0592	1	0.004	15	0.4821
			0.5	2	10363	20725.8											
	7-2	2	0.5	1	10495	20990.6	21083	42166	0.1	147548	28.58	42023	0.0589	1	0.004	15	0.4799
			0.5	2	10588	21175.4											
	7-3	2	0.5	1	10049	20097.4	20341.3	40682.6	0.1	147548	27.57	40539	0.0568	1	0.004	15	0.4629
			0.5	2	10293	20585.2											
	8-1	2	0.5	1	10148	20295.2	18773.8	37547.6	0.1	147548	25.45	37404	0.0524	1	0.004	15	0.4271
			0.5	2	8626	17252.4											
	8-2	2	0.5	1	10712	21423	21249.1	42498.2	0.1	147548	28.80	42355	0.0594	1	0.004	15	0.4837
			0.5	2	10538	21075.2											
	8-3	2	0.5	1	10400	20799	20532.2	41064.4	0.1	147548	27.83	40921	0.0574	1	0.004	15	0.4673
			0.5	2	10133	20265.4											

Assay Date	7/20/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	recombinant	Microsome ID lot #	5	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.5066	0.0223
Full activity	End	0.4987	0.0155
Full activity	Overall	0.5027	0.0163
Background	Beginning	-0.0001	8.76918E-05
Background	End	0.0001	4.9256E-05
Background	Overall	0.0000	0.000103856
Positive	Beginning	0.2653	0.0004
Positive	End	0.2519	0.0072
Positive	Overall	0.2586	0.0088
Negative	Beginning	0.5298	0.0033
Negative	End	0.4939	0.0258
Negative	Overall	0.5118	0.0256

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
1	1	1	1.00E-04	-4.00	0.2072
1	1	2	1.00E-04	-4.00	0.1972
1	1	3	1.00E-04	-4.00	0.1998
1	2	1	1.00E-05	-5.00	0.4319
1	2	2	1.00E-05	-5.00	0.4089
1	2	3	1.00E-05	-5.00	0.3831
1	3	1	1.00E-06	-6.00	0.4655
1	3	2	1.00E-06	-6.00	0.4684
1	3	3	1.00E-06	-6.00	0.4551
1	4	1	1.00E-07	-7.00	0.4687
1	4	2	1.00E-07	-7.00	0.4407
1	4	3	1.00E-07	-7.00	0.4667
1	5	1	1.00E-08	-8.00	0.4833
1	5	2	1.00E-08	-8.00	0.4811
1	5	3	1.00E-08	-8.00	0.4778
1	6	1	1.00E-09	-9.00	0.4874
1	6	2	1.00E-09	-9.00	0.4746
1	6	3	1.00E-09	-9.00	0.4663
1	7	1	1.00E-10	-10.00	0.4821
1	7	2	1.00E-10	-10.00	0.4799
1	7	3	1.00E-10	-10.00	0.4629
1	8	1	1.00E-11	-11.00	0.4271
1	8	2	1.00E-11	-11.00	0.4837
1	8	3	1.00E-11	-11.00	0.4673

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	41.23	39.23	39.76
2	-5.00	85.93	81.34	76.21
3	-6.00	92.61	93.18	90.53
4	-7.00	93.24	87.68	92.85
5	-8.00	96.15	95.72	95.05
6	-9.00	96.97	94.43	92.76
7	-10.00	95.91	95.47	92.10
8	-11.00	84.98	96.22	92.97

Assay Date	7/21/2005	Test Chemical ID	1	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5

								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	0.206
	0.261	0.028	0.209
			0.207

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	
In LE 03-22-06 BJC														
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	0.206	0.206	0.019	200	80	4800	0.006	0.006	5.738
Microsomes	0.209	0.209	0.019	200	80	4800	0.006		
Microsomes	0.207	0.207	0.019	200	80	4800	0.006		

Assay Date	7/21/2005	Test Chemical ID	1	# Concentrations tested	8						
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	Lot # 5				

								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	0.206
	0.261	0.028	0.209
			0.207

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	0.515	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 100.	031	0.031	0.003	200	1	1	0.000	0.000	0.014
QC 100.	028	0.028	0.003	200	1	1	0.000		
QC 10									
Microsomes	206	0.206	0.017	200	80	4800	0.005	0.005	5.260
Microsomes	0.209	0.209	0.018	200	80	4800	0.005		
Microsomes	0.207	0.207	0.018	200	80	4800	0.005		

Assay Date	7/21/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	recombinai	Microsome ID Lot # 5	Technician ID	TD	Replicate # 2

Microsome Dilution Details

Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.259778
Protein Concentration (dilution added to assay, mg/mL):	0.008349

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	recombinant Microsome ID	Lot # 5	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	8177	16354.8	16298.3	32596.6	0.1	149301	21.83	32418	0.0445	1	0.004	15	0.3553
			0.5	2	8121	16241.8											
	2	2	0.5	1	4850	9699	9657.3	19314.6	0.1	149301	12.94	19136	0.0263	1	0.004	15	0.2097
			0.5	2	4808	9615.6											
	3	2	0.5	1	7917	15833.6	15786.3	31572.6	0.1	149301	21.15	31394	0.0431	1	0.004	15	0.3441
			0.5	2	7870	15739											
	4	2	0.5	1	8220	16440.2	16641.3	33282.6	0.1	149301	22.29	33104	0.0454	1	0.004	15	0.3628
			0.5	2	8421	16842.4											
Background control	1	2	0.5	1	50	100.14	93.86	187.72	0.1	149301	0.13	10	0.0000	1	0.004	15	0.0001
			0.5	2	44	87.58											
	2	2	0.5	1	44	88.18	87.89	175.78	0.1	149301	0.12	-2	0.0000	1	0.004	15	0.0000
			0.5	2	44	87.6											
	3	2	0.5	1	37	74.9	88.13	176.26	0.1	149301	0.12	-2	0.0000	1	0.004	15	0.0000
			0.5	2	51	101.36											
	4	2	0.5	1	48	96.59	86.37	172.74	0.1	149301	0.12	-5	0.0000	1	0.004	15	-0.0001
			0.5	2	38	76.16											
Positive control	1	2	0.5	1	4648	9295.6	9166.8	18333.6	0.1	149301	12.28	18155	0.0249	1	0.004	15	0.1990
			0.5	2	4519	9038											
	2	2	0.5	1	4425	8849.4	8932.6	17865.2	0.1	149301	11.97	17687	0.0243	1	0.004	15	0.1938
			0.5	2	4508	9015.8											
	3	2	0.5	1	4469	8937.8	8966.3	17932.6	0.1	149301	12.01	17754	0.0244	1	0.004	15	0.1946
			0.5	2	4497	8994.8											
	4	2	0.5	1	4553	9106.8	9050.4	18100.8	0.1	149301	12.12	17923	0.0246	1	0.004	15	0.1964
			0.5	2	4497	8994											
Negative Control	1	2	0.5	1	7651	15301.8	15396.9	30793.8	0.1	149301	20.63	30616	0.0420	1	0.004	15	0.3355
			0.5	2	7746	15492											
	2	2	0.5	1	7858	15715.6	15584	31168	0.1	149301	20.88	30990	0.0425	1	0.004	15	0.3396
			0.5	2	7726	15452.4											
	3	2	0.5	1	7589	15177.8	15044.5	30089	0.1	149301	20.15	29911	0.0411	1	0.004	15	0.3278
			0.5	2	7456	14911.2											
	4	2	0.5	1	7461	14922.8	14976.2	29952.4	0.1	149301	20.06	29774	0.0409	1	0.004	15	0.3263
			0.5	2	7515	15029.6											
1	1-1	2	0.5	1	653	1306.76	1321.36	2642.72	0.1	149301	1.77	2465	0.0034	1	0.004	15	0.0270
			0.5	2	668	1335.96											
	1-2	2	0.5	1	685	1369.62	1358.77	2717.54	0.1	149301	1.82	2539	0.0035	1	0.004	15	0.0278
			0.5	2	674	1347.72											
	1-3	2	0.5	1	642	1283.82	1291.65	2583.3	0.1	149301	1.73	2405	0.0033	1	0.004	15	0.0264
			0.5	2	650	1299.48											
	2-1	2	0.5	1	2070	4139.8	4118.9	8237.8	0.1	149301	5.52	8060	0.0111	1	0.004	15	0.0883
			0.5	2	2049	4098											
	2-2	2	0.5	1	2076	4151	4124.8	8249.6	0.1	149301	5.53	8071	0.0111	1	0.004	15	0.0885
			0.5	2	2049	4098.6											
	2-3	2	0.5	1	2018	4035.8	4048.8	8097.6	0.1	149301	5.42	7919	0.0109	1	0.004	15	0.0868
			0.5	2	2031	4061.8											
	3-1	2	0.5	1	3692	7383.8	7442.7	14885.4	0.1	149301	9.97	14707	0.0202	1	0.004	15	0.1612
			0.5	2	3751	7501.6											
	3-2	2	0.5	1	3471	6942	6972.6	13945.2	0.1	149301	9.34	13767	0.0189	1	0.004	15	0.1509
			0.5	2	3502	7003.2											
	3-3	2	0.5	1	3682	7363.6	7322.2	14644.4	0.1	149301	9.81	14466	0.0199	1	0.004	15	0.1585
			0.5	2	3640	7280.8											
	4-1	2	0.5	1	4942	9884.4	10034.7	20069.4	0.1	149301	13.44	19891	0.0273	1	0.004	15	0.2180
			0.5	2	5093	10185											
	4-2	2	0.5	1	5108	10215.8	10100	20200	0.1	149301	13.53	20022	0.0275	1	0.004	15	0.2194
			0.5	2	4992	9984.2											
	4-3	2	0.5	1	4890	9779.2	9858.2	19716.4	0.1	149301	13.21	19538	0.0268	1	0.004	15	0.2141
			0.5	2	4969	9937.2											
	5-1	2	0.5	1	6225	12449.8	12671.3	25342.6	0.1	149301	16.97	25164	0.0345	1	0.004	15	0.2758
			0.5	2	6446	12892.8											
	5-2	2	0.5	1	6227	12454.6	12281.6	24563.2	0.1	149301	16.45	24385	0.0335	1	0.004	15	0.2673
			0.5	2	6054	12108.6											
	5-3	2	0.5	1	6113	12225.4	12275.4	24550.8	0.1	149301	16.44	24373	0.0335	1	0.004	15	0.2671
			0.5	2	6163	12325.4											
	6-1	2	0.5	1	6884	13767.2	13790.5	27581	0.1	149301	18.47	27403	0.0376	1	0.004	15	0.3003
			0.5	2	6907	13813.8											
	6-2	2	0.5	1	7188	14375.8	14294.2	28588.4	0.1	149301	19.15	28410	0.0390	1	0.004	15	0.3114
			0.5	2	7106	14212.6											
	6-3	2	0.5	1	6853	13706.6	13814.5	27629	0.1	149301	18.51	27451	0.0377	1	0.004	15	0.3009
			0.5	2	6961	13922.4											
	7-1	2	0.5	1	7980	15959.6	15963.4	31926.8	0.1	149301	21.38	31749	0.0436	1	0.004	15	0.3480
			0.5	2	7984	15967.2											
	7-2	2	0.5	1	7443	14886.6	14909.9	29819.8	0.1	149301	19.97	29642	0.0407	1	0.004	15	0.3249
			0.5	2	7467	14933.2											
	7-3	2	0.5	1	7704	15408.4	15574.6	31149.2	0.1	149301	20.86	30971	0.0425	1	0.004	15	0.3394
			0.5	2	7870	15740.8											
	8-1	2	0.5	1	7782	15563.2	15509	31018	0.1	149301	20.78	30840	0.0423	1	0.004	15	0.3380
			0.5	2	7727	15454.8											
	8-2	2	0.5	1	7890	15779.4	15618.5	31237	0.1	149301	20.92	31059	0.0426	1	0.004	15	0.3404
			0.5	2	7729	15457.6											
	8-3	2	0.5	1	7851	15702.4	15591.1	31182.2	0.1	149301	20.89	31004	0.0426	1	0.004	15	0.3396
			0.5	2	7740	15479.8											

Assay Date	7/21/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot #	5	Technician ID TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.2825	0.1029
Full activity	End	0.3534	0.0133
Full activity	Overall	0.3180	0.0726
Background	Beginning	0.0000	9.25309E-05
Background	End	0.0000	2.72788E-05
Background	Overall	0.0000	7.21563E-05
Positive	Beginning	0.1964	0.0036
Positive	End	0.1955	0.0013
Positive	Overall	0.1960	0.0023
Negative	Beginning	0.3376	0.0029
Negative	End	0.3271	0.0011
Negative	Overall	0.3323	0.0063

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
1	1	1	1.00E-03	-3.00	0.0270
1	1	2	1.00E-03	-3.00	0.0278
1	1	3	1.00E-03	-3.00	0.0264
1	2	1	2.50E-04	-3.60	0.0883
1	2	2	2.50E-04	-3.60	0.0885
1	2	3	2.50E-04	-3.60	0.0868
1	3	1	1.00E-04	-4.00	0.1612
1	3	2	1.00E-04	-4.00	0.1509
1	3	3	1.00E-04	-4.00	0.1585
1	4	1	5.00E-05	-4.30	0.2180
1	4	2	5.00E-05	-4.30	0.2194
1	4	3	5.00E-05	-4.30	0.2141
1	5	1	2.50E-05	-4.60	0.2758
1	5	2	2.50E-05	-4.60	0.2673
1	5	3	2.50E-05	-4.60	0.2671
1	6	1	1.00E-05	-5.00	0.3003
1	6	2	1.00E-05	-5.00	0.3114
1	6	3	1.00E-05	-5.00	0.3009
1	7	1	2.50E-06	-5.60	0.3480
1	7	2	2.50E-06	-5.60	0.3249
1	7	3	2.50E-06	-5.60	0.3394
1	8	1	1.00E-06	-6.00	0.3380
1	8	2	1.00E-06	-6.00	0.3404
1	8	3	1.00E-06	-6.00	0.3398

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	8.49	8.75	8.29
2	-3.60	27.78	27.82	27.30
3	-4.00	50.69	47.45	49.86
4	-4.30	68.56	69.01	67.34
5	-4.60	86.73	84.05	84.00
6	-5.00	94.45	97.92	94.61
7	-5.60	109.43	102.17	106.75
8	-6.00	106.30	107.05	106.86

Assay Date	7/22/2005	Test Chemical ID	1	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5			

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.504	0.300	0.133	0.076	0.035	0.020	0.000	25	100	11343-77
	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	0.232
	0.280	0.042	0.232
			0.220

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	
in LE, 3-22-06 RRC														
250	200	200	0.00025		200		0.0500		0.508	0.508	0.0482	m, b	0.100	-0.002
125	100	200	0.00013		200		0.0250		0.300	0.300	0.0276	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200		0.0100		0.136	0.136	0.0112	r^2, se_y	0.992	0.002
25	100	1000	0.00003		200		0.0050		0.077	0.077	0.0053	F, df	520	4
10	40	1000	0.00001		200		0.0020		0.035	0.035	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.020	0.020	-0.0004			

Regression results are calculated using the function LINEST

Blank	0.000	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	0.232	0.232	0.021	200	80	4800	0.006	0.006	6.099
Microsomes	0.232	0.232	0.021	200	80	4800	0.006		
Microsomes	0.220	0.220	0.020	200	80	4800	0.006		

Assay Date	7/22/2005	Test Chemical ID	1	# Concentrations tested	8						
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	Lot # 5				

								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	0.232
	0.280	0.042	0.232
			0.220

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	
µ 3-22-06 KBL LE											
250	200	200	0.00025	200	0.0500	0.508	0.508	0.0426	m, b	0.086	-0.001
125	100	200	0.00013	200	0.0250	0.300	0.300	0.0247	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.136	0.136	0.0105	r^2, se_y	0.998	0.001
25	100	1000	0.00003	200	0.0050	0.077	0.077	0.0055	F, df	1377	3
10	40	1000	0.00001	200	0.0020	0.035	0.035	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
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

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 100.	041	0.041	0.002	200	1	1	0.000	0.000	0.012
QC 100.	042	0.042	0.002	200	1	1	0.000		
QC 10									
Microsomes	232	0.232	0.019	200	80	4800	0.006	0.006	5.534
Microsomes	0.232	0.232	0.019	200	80	4800	0.006		
Microsomes	0.220	0.220	0.018	200	80	4800	0.005		

Assay Date	7/22/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	recombinai Microsome ID	Lot # 5	Technician ID	TD	Replicate # 3

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.533851
Protein Concentration (dilution added to assay, mg/mL):	0.008784

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	recombinant	Microsome ID	Lot # 5	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	9323	18645.6	18601.9	37203.8	0.1	156329	23.80	36920	0.0471	1	0.004	15	0.3572
			0.5	2	9279	18558.2											
	2	2	0.5	1	9480	18960	18820.1	37640.2	0.1	156329	24.08	37356	0.0476	1	0.004	15	0.3614
			0.5	2	9340	18680.2											
	3	2	0.5	1	9005	18010.8	18084.7	36169.4	0.1	156329	23.14	35885	0.0457	1	0.004	15	0.3472
			0.5	2	9079	18158.6											
	4	2	0.5	1	9189	18377.4	18554.8	37109.6	0.1	156329	23.74	36825	0.0469	1	0.004	15	0.3563
			0.5	2	9366	18732.2											
Background control	1	2	0.5	1	81	161.26	161.95	323.9	0.1	156329	0.21	40	0.0001	1	0.004	15	0.0004
			0.5	2	81	162.64											
	2	2	0.5	1	77	153.08	146.87	293.74	0.1	156329	0.19	9	0.0000	1	0.004	15	0.0001
			0.5	2	70	140.66											
	3	2	0.5	1	66	132	129.42	258.84	0.1	156329	0.17	-25	0.0000	1	0.004	15	-0.0002
			0.5	2	63	125.84											
	4	2	0.5	1	71	141.62	130.33	260.66	0.1	156329	0.17	-24	0.0000	1	0.004	15	-0.0002
			0.5	2	60	119.04											
Positive control	1	2	0.5	1	4690	9380.8	9408.3	18816.6	0.1	156329	12.04	18532	0.0236	1	0.004	15	0.1793
			0.5	2	4718	9435.8											
	2	2	0.5	1	4901	9802.6	9836.6	19673.2	0.1	156329	12.58	19389	0.0247	1	0.004	15	0.1876
			0.5	2	4935	9870.6											
	3	2	0.5	1	4889	9777	9783.4	19566.8	0.1	156329	12.52	19283	0.0246	1	0.004	15	0.1865
			0.5	2	4895	9789.8											
	4	2	0.5	1	4663	9325	9207.4	18414.8	0.1	156329	11.78	18131	0.0231	1	0.004	15	0.1754
			0.5	2	4545	9089.8											
Negative Control	1	2	0.5	1	8599	17198.6	17347.4	34694.8	0.1	156329	22.19	34411	0.0439	1	0.004	15	0.3329
			0.5	2	8748	17496.2											
	2	2	0.5	1	8558	17116	17248.6	34497.2	0.1	156329	22.07	34213	0.0436	1	0.004	15	0.3310
			0.5	2	8691	17381.2											
	3	2	0.5	1	8690	17379.2	17516.1	35032.2	0.1	156329	22.41	34748	0.0443	1	0.004	15	0.3362
			0.5	2	8827	17653											
	4	2	0.5	1	8493	16985.6	16836.9	33673.8	0.1	156329	21.54	33390	0.0426	1	0.004	15	0.3230
			0.5	2	8344	16688.2											
1	1-1	2	0.5	1	586	1171.12	1192.11	2384.22	0.1	156329	1.53	2100	0.0027	1	0.004	15	0.0203
			0.5	2	607	1213.1											
	1-2	2	0.5	1	611	1222.16	1209.35	2418.7	0.1	156329	1.55	2134	0.0027	1	0.004	15	0.0206
			0.5	2	598	1196.54											
	1-3	2	0.5	1	582	1164.46	1159.72	2319.44	0.1	156329	1.48	2035	0.0026	1	0.004	15	0.0197
			0.5	2	577	1154.98											
	2-1	2	0.5	1	2254	4507.2	4479.1	8958.2	0.1	156329	5.73	8674	0.0111	1	0.004	15	0.0839
			0.5	2	2226	4451											
	2-2	2	0.5	1	2194	4387.6	4362.4	8724.8	0.1	156329	5.58	8441	0.0108	1	0.004	15	0.0817
			0.5	2	2169	4337.2											
	2-3	2	0.5	1	2186	4372	4397	8794	0.1	156329	5.63	8510	0.0108	1	0.004	15	0.0823
			0.5	2	2211	4422											
	3-1	2	0.5	1	4020	8040.6	7978.9	15957.8	0.1	156329	10.21	15674	0.0200	1	0.004	15	0.1516
			0.5	2	3959	7917.2											
	3-2	2	0.5	1	3997	7993.6	7971.6	15943.2	0.1	156329	10.20	15659	0.0200	1	0.004	15	0.1515
			0.5	2	3975	7949.6											
	3-3	2	0.5	1	4017	8034.6	8043.2	16086.4	0.1	156329	10.29	15802	0.0201	1	0.004	15	0.1529
			0.5	2	4026	8051.8											
	4-1	2	0.5	1	5373	10745.6	10841.4	21682.8	0.1	156329	13.87	21399	0.0273	1	0.004	15	0.2070
			0.5	2	5469	10937.2											
	4-2	2	0.5	1	5510	11020.6	10946.8	21893.6	0.1	156329	14.00	21609	0.0275	1	0.004	15	0.2091
			0.5	2	5437	10873											
	4-3	2	0.5	1	5475	10950	10925.1	21850.2	0.1	156329	13.98	21566	0.0275	1	0.004	15	0.2086
			0.5	2	5450	10900.2											
	5-1	2	0.5	1	6768	13536	13672.7	27345.4	0.1	156329	17.49	27061	0.0345	1	0.004	15	0.2618
			0.5	2	6905	13809.4											
	5-2	2	0.5	1	7031	14062.8	13943.2	27886.4	0.1	156329	17.84	27602	0.0352	1	0.004	15	0.2670
			0.5	2	6912	13823.6											
	5-3	2	0.5	1	6841	13681.8	13697	27394	0.1	156329	17.52	27110	0.0346	1	0.004	15	0.2623
			0.5	2	6856	13712.2											
	6-1	2	0.5	1	7824	15648.4	15641.3	31282.6	0.1	156329	20.01	30998	0.0395	1	0.004	15	0.2999
			0.5	2	7817	15634.2											
	6-2	2	0.5	1	8247	16493.8	16424.3	32848.6	0.1	156329	21.01	32564	0.0415	1	0.004	15	0.3150
			0.5	2	8177	16354.8											
	6-3	2	0.5	1	8259	16517.6	16340.1	32680.2	0.1	156329	20.90	32396	0.0413	1	0.004	15	0.3134
			0.5	2	8081	16162.6											
	7-1	2	0.5	1	9118	18236.2	18300.5	36601	0.1	156329	23.41	36317	0.0463	1	0.004	15	0.3513
			0.5	2	9182	18364.8											
	7-2	2	0.5	1	9085	18170.2	18242.2	36484.4	0.1	156329	23.34	36200	0.0461	1	0.004	15	0.3502
			0.5	2	9157	18314.2											
	7-3	2	0.5	1	8768	17536	17565	35130	0.1	156329	22.47	34846	0.0444	1	0.004	15	0.3371
			0.5	2	8797	17594											
	8-1	2	0.5	1	9340	18679.2	18508.9	37017.8	0.1	156329	23.68	36734	0.0468	1	0.004	15	0.3554
			0.5	2	9169	18338.6											
	8-2	2	0.5	1	9114	18228.4	18356.6	36713.2	0.1	156329	23.46	36429	0.0464	1	0.004	15	0.3524
			0.5	2	9242	18484.8											
	8-3	2	0.5	1	9421	18841.2	18968.6	37937.2	0.1	156329	24.27	37653	0.0480	1	0.004	15	0.3643
			0.5	2	9548	19096											

Assay Date	7/22/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot #	5	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3593	0.0030
Full activity	End	0.3517	0.0064
Full activity	Overall	0.3555	0.0060
Background	Beginning	0.0002	0.000206313
Background	End	-0.0002	1.24499E-05
Background	Overall	0.0000	0.000298924
Positive	Beginning	0.1834	0.0059
Positive	End	0.1810	0.0079
Positive	Overall	0.1822	0.0058
Negative	Beginning	0.3319	0.0014
Negative	End	0.3296	0.0093
Negative	Overall	0.3308	0.0056

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
1	1	1	1.00E-03	-3.00	0.0203
1	1	2	1.00E-03	-3.00	0.0206
1	1	3	1.00E-03	-3.00	0.0197
1	2	1	2.50E-04	-3.60	0.0839
1	2	2	2.50E-04	-3.60	0.0817
1	2	3	2.50E-04	-3.60	0.0823
1	3	1	1.00E-04	-4.00	0.1516
1	3	2	1.00E-04	-4.00	0.1515
1	3	3	1.00E-04	-4.00	0.1529
1	4	1	5.00E-05	-4.30	0.2070
1	4	2	5.00E-05	-4.30	0.2091
1	4	3	5.00E-05	-4.30	0.2086
1	5	1	2.50E-05	-4.60	0.2618
1	5	2	2.50E-05	-4.60	0.2670
1	5	3	2.50E-05	-4.60	0.2623
1	6	1	1.00E-05	-5.00	0.2999
1	6	2	1.00E-05	-5.00	0.3150
1	6	3	1.00E-05	-5.00	0.3134
1	7	1	2.50E-06	-5.60	0.3513
1	7	2	2.50E-06	-5.60	0.3502
1	7	3	2.50E-06	-5.60	0.3371
1	8	1	1.00E-06	-6.00	0.3554
1	8	2	1.00E-06	-6.00	0.3524
1	8	3	1.00E-06	-6.00	0.3643

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	5.71	5.81	5.54
2	-3.60	23.60	22.97	23.16
3	-4.00	42.65	42.61	43.00
4	-4.30	58.23	58.81	58.69
5	-4.60	73.64	75.12	73.78
6	-5.00	84.36	88.62	88.16
7	-5.60	98.83	98.51	94.83
8	-6.00	99.96	99.14	102.47

Assay Date	8/11/2005	Test Chemical ID	2	# Concentrations tested	8	
Technician ID	TD	Replicate #	1	Microsome type	recombinar Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.506	0.297	0.136	0.062	0.024	0.012	0.000	25	100	11343-77
	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	0.200
	0.263	0.028	0.198
			0.206

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	
m LE-03-27-06 BSL														
250	200	200	0.00025		200		0.0500		0.503	0.503	0.0481	m, b	0.098	-0.001
125	100	200	0.00013		200		0.0250		0.296	0.296	0.0278	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200		0.0100		0.132	0.132	0.0116	r^2, se_y	0.991	0.002
25	100	1000	0.00003		200		0.0050		0.061	0.061	0.0047	F, df	443	4
10	40	1000	0.00001		200		0.0020		0.025	0.025	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.012	0.012	-0.0002			
				Blank	0.000		r^2=	0.991						
							m=	0.098						
							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	0.121
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	0.200	0.200	0.018	200	80	4800	0.005	0.006	5.522
Microsomes	0.198	0.198	0.018	200	80	4800	0.005		
Microsomes	0.206	0.206	0.019	200	80	4800	0.006		

Assay Date	8/11/2005	Test Chemical ID	2	# Concentrations tested	8				
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	Lot # 5		

								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	0.200
	0.263	0.028	0.198
			0.206

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
M LE, 3-22-06 BGL								
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

		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

Regression results are calculated using the function LINEST

Blank	0.000	r^2=	0.998
		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	0.257	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 100.	029	0.029	0.002	200	1	1	0.000	0.000	0.011
QC 100.	028	0.028	0.002	200	1	1	0.000		
QC 10									
Microsomes	200	0.200	0.017	200	80	4800	0.005	0.005	5.000
Microsomes	0.198	0.198	0.016	200	80	4800	0.005		
Microsomes	0.206	0.206	0.017	200	80	4800	0.005		

Assay Date	8/11/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	recombinai	Microsome ID	Lot # 5	Technician ID	TD	Replicate #	1

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.999886
Protein Concentration (dilution added to assay, mg/mL):	0.007936

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	recombinant	Microsome ID	Lot # 5	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	8930	17859.6	16058.5	36117	0.1	155567	23.22	35885	0.0466	1	0.004	15	0.3911
			0.5	2	9129	18257.4											
	2	2	0.5	1	8260	16520.4	16682.7	33365.4	0.1	155567	21.45	33133	0.0430	1	0.004	15	0.3611
			0.5	2	8423	16845											
	3	2	0.5	1	9254	18506.4	18345.4	36690.8	0.1	155567	23.59	36458	0.0473	1	0.004	15	0.3973
			0.5	2	9091	18182.4											
	4	2	0.5	1	8931	17862.8	17873.1	35746.2	0.1	155567	22.98	35514	0.0461	1	0.004	15	0.3870
			0.5	2	8942	17883.6											
Background control	1	2	0.5	1	56	111.36	113.95	227.9	0.1	155567	0.15	-5	0.0000	1	0.004	15	0.0000
			0.5	2	58	116.54											
	2	2	0.5	1	60	119.06	123.21	246.42	0.1	155567	0.16	14	0.0000	1	0.004	15	0.0002
			0.5	2	64	127.36											
	3	2	0.5	1	64	127.82	120.08	240.16	0.1	155567	0.15	8	0.0000	1	0.004	15	0.0001
			0.5	2	56	112.34											
	4	2	0.5	1	58	116.34	107.61	215.22	0.1	155567	0.14	-17	0.0000	1	0.004	15	-0.0002
			0.5	2	49	98.88											
Positive control	1	2	0.5	1	4436	8872.8	8892.4	17784.8	0.1	155567	11.43	17552	0.0228	1	0.004	15	0.1913
			0.5	2	4456	8912											
	2	2	0.5	1	4634	9267.4	9213	18426	0.1	155567	11.84	18194	0.0236	1	0.004	15	0.1983
			0.5	2	4579	9158.6											
	3	2	0.5	1	4541	9082.4	9159.9	18317.8	0.1	155567	11.77	18085	0.0235	1	0.004	15	0.1971
			0.5	2	4618	9235.4											
	4	2	0.5	1	4630	9260.8	9247.4	18494.8	0.1	155567	11.89	18262	0.0237	1	0.004	15	0.1990
			0.5	2	4617	9234											
Negative Control	1	2	0.5	1	9288	18571.6	18647.8	37295.6	0.1	155567	23.97	37063	0.0481	1	0.004	15	0.4039
			0.5	2	9362	18724											
	2	2	0.5	1	9068	18136.8	17973.6	35947.2	0.1	155567	23.11	35715	0.0463	1	0.004	15	0.3892
			0.5	2	8905	17810.4											
	3	2	0.5	1	8364	16728.6	16853.5	33707	0.1	155567	21.67	33475	0.0434	1	0.004	15	0.3648
			0.5	2	8489	16978.4											
	4	2	0.5	1	8979	17957.2	17971.5	35943	0.1	155567	23.10	35711	0.0463	1	0.004	15	0.3892
			0.5	2	8993	17985.8											
2	1-1	2	0.5	1	102	204.92	200.31	400.62	0.1	155567	0.26	168	0.0002	1	0.004	15	0.0018
			0.5	2	98	195.7											
	1-2	2	0.5	1	90	179.22	181.49	362.96	0.1	155567	0.23	131	0.0002	1	0.004	15	0.0014
			0.5	2	92	183.76											
	1-3	2	0.5	1	96	192.06	195.73	391.46	0.1	155567	0.25	159	0.0002	1	0.004	15	0.0017
			0.5	2	100	199.4											
	2-1	2	0.5	1	516	1032.82	1034.42	2068.84	0.1	155567	1.33	1836	0.0024	1	0.004	15	0.0200
			0.5	2	518	1036.02											
	2-2	2	0.5	1	468	935.34	937.26	1874.52	0.1	155567	1.20	1642	0.0021	1	0.004	15	0.0179
			0.5	2	470	939.18											
	2-3	2	0.5	1	479	958.42	952.83	1905.66	0.1	155567	1.22	1673	0.0022	1	0.004	15	0.0182
			0.5	2	474	947.24											
	3-1	2	0.5	1	2901	5802.8	5876.9	11753.8	0.1	155567	7.56	11521	0.0149	1	0.004	15	0.1256
			0.5	2	2976	5951											
	3-2	2	0.5	1	2990	5980.4	6035.6	12071.2	0.1	155567	7.76	11839	0.0154	1	0.004	15	0.1290
			0.5	2	3045	6090.8											
	3-3	2	0.5	1	2960	5919.4	5936.8	11873.6	0.1	155567	7.63	11641	0.0151	1	0.004	15	0.1269
			0.5	2	2977	5954.2											
	4-1	2	0.5	1	5969	11938.4	12024.1	24048.2	0.1	155567	15.46	23816	0.0309	1	0.004	15	0.2595
			0.5	2	6055	12109.8											
	4-2	2	0.5	1	5856	11711.6	11698.1	23396.2	0.1	155567	15.04	23184	0.0301	1	0.004	15	0.2524
			0.5	2	5842	11684.6											
	4-3	2	0.5	1	6480	12959	12996.4	25992.8	0.1	155567	16.71	25760	0.0334	1	0.004	15	0.2807
			0.5	2	6517	13033.8											
	5-1	2	0.5	1	7388	14776	14850.9	29701.8	0.1	155567	19.09	29469	0.0382	1	0.004	15	0.3212
			0.5	2	7463	14925.8											
	5-2	2	0.5	1	7354	14708.8	14840.4	29680.8	0.1	155567	19.08	29448	0.0382	1	0.004	15	0.3209
			0.5	2	7486	14972											
	5-3	2	0.5	1	7582	15163.4	15015.5	30031	0.1	155567	19.30	29799	0.0387	1	0.004	15	0.3248
			0.5	2	7434	14867.6											
	6-1	2	0.5	1	8647	17294.2	17196.2	34392.4	0.1	155567	22.11	34160	0.0443	1	0.004	15	0.3723
			0.5	2	8549	17098.2											
	6-2	2	0.5	1	8728	17455.8	17618.3	35236.6	0.1	155567	22.65	35004	0.0454	1	0.004	15	0.3815
			0.5	2	8890	17780.8											
	6-3	2	0.5	1	8838	17676.2	17707.6	35415.2	0.1	155567	22.77	35183	0.0456	1	0.004	15	0.3834
			0.5	2	8870	17739											
	7-1	2	0.5	1	9123	18245.8	18122.6	36245.2	0.1	155567	23.30	36013	0.0467	1	0.004	15	0.3925
			0.5	2	9000	17999.4											
	7-2	2	0.5	1	8820	17639.2	17646.6	35293.2	0.1	155567	22.69	35061	0.0455	1	0.004	15	0.3821
			0.5	2	8827	17654											
	7-3	2	0.5	1	9025	18050.8	18127.5	36255	0.1	155567	23.31	36023	0.0467	1	0.004	15	0.3926
			0.5	2	9102	18204.2											
	8-1	2	0.5	1	9052	18103.8	18044.9	36089.8	0.1	155567	23.20	35857	0.0465	1	0.004	15	0.3908
			0.5	2	8993	17986											
	8-2	2	0.5	1	9257	18514	18482.6	36965.2	0.1	155567	23.76	36733	0.0477	1	0.004	15	0.4003
			0.5	2	9226	18451.2											
	8-3	2	0.5	1	9270	18539.8	18456	36912	0.1	155567	23.73	36680	0.0476	1	0.004	15	0.3997
			0.5	2	9186	18372.2											

Assay Date	8/11/2005	Test Chemical ID	2	# Concentrations tested	Microsome 8 type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.3761	0.0212
Full activity	End	0.3922	0.0073
Full activity	Overall	0.3841	0.0159
Background	Beginning	0.0001	0.000142719
Background	End	-0.0001	0.000192192
Background	Overall	0.0000	0.000150508
Positive	Beginning	0.1948	0.0049
Positive	End	0.1981	0.0014
Positive	Overall	0.1964	0.0035
Negative	Beginning	0.3966	0.0104
Negative	End	0.3770	0.0172
Negative	Overall	0.3868	0.0162

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
2	1	1	1.00E-04	-4.00	0.0018
2	1	2	1.00E-04	-4.00	0.0014
2	1	3	1.00E-04	-4.00	0.0017
2	2	1	1.00E-05	-5.00	0.0200
2	2	2	1.00E-05	-5.00	0.0179
2	2	3	1.00E-05	-5.00	0.0182
2	3	1	1.00E-06	-6.00	0.1256
2	3	2	1.00E-06	-6.00	0.1290
2	3	3	1.00E-06	-6.00	0.1269
2	4	1	2.50E-07	-6.60	0.2595
2	4	2	2.50E-07	-6.60	0.2524
2	4	3	2.50E-07	-6.60	0.2807
2	5	1	1.00E-07	-7.00	0.3212
2	5	2	1.00E-07	-7.00	0.3209
2	5	3	1.00E-07	-7.00	0.3248
2	6	1	1.00E-08	-8.00	0.3723
2	6	2	1.00E-08	-8.00	0.3815
2	6	3	1.00E-08	-8.00	0.3834
2	7	1	1.00E-09	-9.00	0.3925
2	7	2	1.00E-09	-9.00	0.3821
2	7	3	1.00E-09	-9.00	0.3926
2	8	1	1.00E-10	-10.00	0.3908
2	8	2	1.00E-10	-10.00	0.4003
2	8	3	1.00E-10	-10.00	0.3997

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.48	0.37	0.45
2	-5.00	5.21	4.66	4.75
3	-6.00	32.69	33.59	33.03
4	-6.60	67.57	65.72	73.08
5	-7.00	83.61	83.55	84.54
6	-8.00	96.91	99.31	99.82
7	-9.00	102.17	99.47	102.20
8	-10.00	101.73	104.21	104.06

Assay Date	8/12/2005	Test Chemical ID	2	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinar	Microsome ID	Lot # 5

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

Assay Date	8/12/2005	Test Chemical ID		2	# Concentrations tested		8				
Technician ID	TD	Replicate #		2	Microsome type		recombinant	Microsome ID	Lot # 5		

								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	0.209
	0.261	0.032	0.209
			0.208

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL		mg Protein Used		mg Protein Measured		A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
M 03-22-06 BJR														
250	200	200	0.00025		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			
				Blank	0.000		r^2=	0.991						
							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	0.122
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	0.209	0.209	0.019	200	80	4800	0.006	0.006	5.713
Microsomes	0.209	0.209	0.019	200	80	4800	0.006		
Microsomes	0.208	0.208	0.019	200	80	4800	0.006		

Assay Date	8/12/2005	Test Chemical ID	2	# Concentrations tested	8						
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	Lot # 5				
								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	0.209								
	0.261	0.032	0.209								
			0.208								

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	
M; KE 3-27-06	µL										
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			
										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	0.264	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 100.	032	0.032	0.002	200	1	1	0.000	0.000	0.012
QC 100.	032	0.032	0.002	200	1	1	0.000		
QC 10									
Microsomes	209	0.209	0.017	200	80	4800	0.005	0.005	5.158
Microsomes	0.209	0.209	0.017	200	80	4800	0.005		
Microsomes	0.208	0.208	0.017	200	80	4800	0.005		

Assay Date	8/12/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type	recombinar	Microsome ID Lot # 5	Technician ID	TD	Replicate # 2

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.157533
Protein Concentration (dilution added to assay, mg/mL):	0.008187

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	8/12/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type	recombinant Microsome ID	Lot # 6	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	9104	18208.4	18132.7	36265.4	0.1	158734	22.85	36027	0.0471	1	0.004	15	0.3836
			0.5	2	9029	18057											
	2	2	0.5	1	8764	17528.2	17603.6	35207.2	0.1	158734	22.18	34969	0.0457	1	0.004	15	0.3723
			0.5	2	8840	17679											
	3	2	0.5	1	7938	15875	15777.2	31554.4	0.1	158734	19.86	31316	0.0409	1	0.004	15	0.3334
			0.5	2	7840	15679.4											
	4	2	0.5	1	8447	16893	16864.1	33728.2	0.1	158734	21.25	33490	0.0438	1	0.004	15	0.3566
			0.5	2	8418	16835.2											
Background control	1	2	0.5	1	60	120.1	119.24	238.48	0.1	158734	0.15	0	0.0000	1	0.004	15	0.0000
			0.5	2	59	118.38											
	2	2	0.5	1	55	110.78	110.78	221.56	0.1	158734	0.14	-17	0.0000	1	0.004	15	-0.0002
			0.5	2	55	110.78											
	3	2	0.5	1	67	133.88	121.2	242.4	0.1	158734	0.15	4	0.0000	1	0.004	15	0.0000
			0.5	2	54	108.52											
	4	2	0.5	1	67	134.86	125.23	250.46	0.1	158734	0.16	12	0.0000	1	0.004	15	0.0001
			0.5	2	58	115.6											
Positive control	1	2	0.5	1	4693	9386.8	9333.8	18667.6	0.1	158734	11.76	18429	0.0241	1	0.004	15	0.1962
			0.5	2	4640	9280.8											
	2	2	0.5	1	4391	8781.2	8816.7	17633.4	0.1	158734	11.11	17395	0.0227	1	0.004	15	0.1852
			0.5	2	4426	8852.2											
	3	2	0.5	1	3967	7934	8006	16012	0.1	158734	10.09	15774	0.0206	1	0.004	15	0.1679
			0.5	2	4039	8078											
	4	2	0.5	1	4298	8596.8	8586.2	17172.4	0.1	158734	10.82	16934	0.0221	1	0.004	15	0.1803
			0.5	2	4288	8575.6											
Negative Control	1	2	0.5	1	8863	17726.4	17548.5	35097	0.1	158734	22.11	34859	0.0456	1	0.004	15	0.3711
			0.5	2	8685	17370.6											
	2	2	0.5	1	8623	17245.4	17236.4	34472.8	0.1	158734	21.72	34235	0.0448	1	0.004	15	0.3645
			0.5	2	8614	17227.4											
	3	2	0.5	1	8349	16697	16463	32926	0.1	158734	20.74	32688	0.0427	1	0.004	15	0.3480
			0.5	2	8115	16229											
	4	2	0.5	1	8076	16152	16328.8	32657.6	0.1	158734	20.57	32419	0.0424	1	0.004	15	0.3452
			0.5	2	8253	16505.6											
2	1-1	2	0.5	1	103	205.78	197.25	394.5	0.1	158734	0.25	156	0.0002	1	0.004	15	0.0017
			0.5	2	94	188.72											
	1-2	2	0.5	1	106	212.3	201.84	403.68	0.1	158734	0.25	165	0.0002	1	0.004	15	0.0018
			0.5	2	96	191.38											
	1-3	2	0.5	1	93	186.86	182.7	365.4	0.1	158734	0.23	127	0.0002	1	0.004	15	0.0014
			0.5	2	89	178.54											
	2-1	2	0.5	1	505	1009.84	988.17	1976.34	0.1	158734	1.25	1738	0.0023	1	0.004	15	0.0185
			0.5	2	483	966.5											
	2-2	2	0.5	1	509	1017.04	983.56	1967.12	0.1	158734	1.24	1729	0.0023	1	0.004	15	0.0184
			0.5	2	475	950.08											
	2-3	2	0.5	1	468	936.64	949.97	1899.94	0.1	158734	1.20	1662	0.0022	1	0.004	15	0.0177
			0.5	2	482	963.3											
	3-1	2	0.5	1	2814	5628.6	5645.1	11290.2	0.1	158734	7.11	11052	0.0144	1	0.004	15	0.1177
			0.5	2	2831	5661.6											
	3-2	2	0.5	1	2796	5592.4	5655.3	11310.6	0.1	158734	7.13	11072	0.0145	1	0.004	15	0.1179
			0.5	2	2859	5718.2											
	3-3	2	0.5	1	2838	5675.4	5645.8	11291.6	0.1	158734	7.11	11053	0.0145	1	0.004	15	0.1177
			0.5	2	2808	5616.2											
	4-1	2	0.5	1	4205	8410.8	8502.7	17005.4	0.1	158734	10.71	16767	0.0219	1	0.004	15	0.1785
			0.5	2	4297	8594.6											
	4-2	2	0.5	1	4196	8392.4	8425.7	16851.4	0.1	158734	10.62	16613	0.0217	1	0.004	15	0.1769
			0.5	2	4230	8459											
	4-3	2	0.5	1	4186	8372.8	8386.5	16773	0.1	158734	10.57	16535	0.0216	1	0.004	15	0.1760
			0.5	2	4200	8400.2											
	5-1	2	0.5	1	5341	10681	10765.8	21531.6	0.1	158734	13.56	21293	0.0278	1	0.004	15	0.2267
			0.5	2	5425	10850.6											
	5-2	2	0.5	1	5619	11238	11192.9	22385.8	0.1	158734	14.10	22148	0.0290	1	0.004	15	0.2358
			0.5	2	5574	11147.8											
	5-3	2	0.5	1	5581	11161.2	11191.5	22383	0.1	158734	14.10	22145	0.0290	1	0.004	15	0.2358
			0.5	2	5611	11221.8											
	6-1	2	0.5	1	6579	13158.8	13218.6	26437.2	0.1	158734	16.66	26199	0.0343	1	0.004	15	0.2789
			0.5	2	6639	13278.4											
	6-2	2	0.5	1	6686	13371.4	13273.2	26546.4	0.1	158734	16.72	26308	0.0344	1	0.004	15	0.2801
			0.5	2	6588	13175											
	6-3	2	0.5	1	7140	14279.4	14262.9	28525.8	0.1	158734	17.97	28288	0.0370	1	0.004	15	0.3012
			0.5	2	7123	14246.4											
	7-1	2	0.5	1	7565	15130	15100.9	30201.8	0.1	158734	19.03	29964	0.0392	1	0.004	15	0.3190
			0.5	2	7536	15071.8											
	7-2	2	0.5	1	8420	16839.4	16777.1	33554.2	0.1	158734	21.14	33316	0.0436	1	0.004	15	0.3547
			0.5	2	8357	16714.8											
	7-3	2	0.5	1	8092	16184	16292.7	32585.4	0.1	158734	20.53	32347	0.0423	1	0.004	15	0.3444
			0.5	2	8201	16401.4											
	8-1	2	0.5	1	8103	16206.8	16211.8	32423.6	0.1	158734	20.43	32185	0.0421	1	0.004	15	0.3427
			0.5	2	8108	16216.8											
	8-2	2	0.5	1	7966	15931.6	15929.1	31858.2	0.1	158734	20.07	31620	0.0413	1	0.004	15	0.3367
			0.5	2	7963	15926.6											
	8-3	2	0.5	1	8347	16694.8	16847.6	33695.2	0.1	158734	21.23	33457	0.0437	1	0.004	15	0.3562
			0.5	2	8500	17000.4											

Assay Date	8/12/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3779	0.0080
Full activity	End	0.3450	0.0164
Full activity	Overall	0.3615	0.0217
Background	Beginning	-0.0001	0.000127384
Background	End	0.0001	6.06805E-05
Background	Overall	0.0000	0.00012966
Positive	Beginning	0.1907	0.0078
Positive	End	0.1741	0.0087
Positive	Overall	0.1824	0.0117
Negative	Beginning	0.3678	0.0047
Negative	End	0.3466	0.0020
Negative	Overall	0.3572	0.0126

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
2	1	1	1.00E-04	-4.00	0.0017
2	1	2	1.00E-04	-4.00	0.0018
2	1	3	1.00E-04	-4.00	0.0014
2	2	1	1.00E-05	-5.00	0.0185
2	2	2	1.00E-05	-5.00	0.0184
2	2	3	1.00E-05	-5.00	0.0177
2	3	1	1.00E-06	-6.00	0.1177
2	3	2	1.00E-06	-6.00	0.1179
2	3	3	1.00E-06	-6.00	0.1177
2	4	1	5.00E-07	-6.30	0.1785
2	4	2	5.00E-07	-6.30	0.1769
2	4	3	5.00E-07	-6.30	0.1760
2	5	1	2.50E-07	-6.60	0.2267
2	5	2	2.50E-07	-6.60	0.2358
2	5	3	2.50E-07	-6.60	0.2358
2	6	1	1.00E-07	-7.00	0.2789
2	6	2	1.00E-07	-7.00	0.2801
2	6	3	1.00E-07	-7.00	0.3012
2	7	1	1.00E-08	-8.00	0.3190
2	7	2	1.00E-08	-8.00	0.3547
2	7	3	1.00E-08	-8.00	0.3444
2	8	1	1.00E-09	-9.00	0.3427
2	8	2	1.00E-09	-9.00	0.3367
2	8	3	1.00E-09	-9.00	0.3562

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.46	0.49	0.37
2	-5.00	5.12	5.09	4.89
3	-6.00	32.55	32.61	32.56
4	-6.30	49.39	48.93	48.70
5	-6.60	62.72	65.23	65.23
6	-7.00	77.17	77.49	83.32
7	-8.00	88.26	98.13	95.28
8	-9.00	94.80	93.14	98.55

Assay Date	8/15/2005	Test Chemical ID	2	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.516	0.294	0.132	0.064	0.023	0.012	0.000	25	100	11343-77
	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	0.212
	0.260	0.028	0.216
			0.213

Standard concentration (~~mg~~/mL) EN,EC05-22-06 BJK	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	0.0483	m, b	0.097	-0.001
125	100	200	0.00013		200		0.0250		0.298	0.298	0.0274	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200		0.0100		0.134	0.134	0.0116	r^2, se_y	0.992	0.002
25	100	1000	0.00003		200		0.0050		0.063	0.063	0.0047	F, df	526	4
10	40	1000	0.00001		200		0.0020		0.024	0.024	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.013	0.013	-0.0001			
				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	0.264	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	0.212	0.212	0.019	200	80	4800	0.006	0.006	5.798
Microsomes	0.216	0.216	0.020	200	80	4800	0.006		
Microsomes	0.213	0.213	0.019	200	80	4800	0.006		

Assay Date	8/15/2005	Test Chemical ID	2	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	Lot # 5

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	250	125	50	25	10	5	0	25	100	11343-77
	0.516	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	0.212
	0.260	0.028	0.216
			0.213

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	
M, LE 3-22-06 KBC											
250	200	200	0.00025	200	0.0500	0.513	0.513	0.0427	m, b	0.084	0.000
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	0.0109	r^2, se_y	0.997	0.001
25	100	1000	0.00003	200	0.0050	0.063	0.063	0.0049	F, df	1010	3
10	40	1000	0.00001	200	0.0020	0.024	0.024	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.013	0.013	0.0008			

Regression results are calculated using the function LINEST

			r^2=	0.997
Blank	0.000		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	0.264	0.264	0.022	200	1	1	0.000	0.000	0.108
QC 100	0.260	0.260	0.021	200	1	1	0.000		
QC 100									
QC 100.	028	0.028	0.002	200	1	1	0.000	0.000	0.010
QC 100.	028	0.028	0.002	200	1	1	0.000		
QC 10									
Microsomes	212	0.212	0.017	200	80	4800	0.005	0.005	5.281
Microsomes	0.216	0.216	0.018	200	80	4800	0.005		
Microsomes	0.213	0.213	0.018	200	80	4800	0.005		

Assay Date	8/15/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type	recombinar	Microsome ID Lot # 5	Technician ID	TD	Replicate #	3

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.281081
Protein Concentration (dilution added to assay, mg/mL):	0.008383

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	6	Microsome type	recombinant	Microsome ID	Lot # 5	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	6590	13179.2	13168.5	26337	0.1	156933	16.78	26074	0.0349	1	0.004	15	0.2773
			0.5	2	6579	13157.8											
	2	2	0.5	1	6481	12961.8	12986.9	25973.8	0.1	156933	16.55	25710	0.0344	1	0.004	15	0.2735
			0.5	2	6506	13012											
	3	2	0.5	1	5603	11206	11110.4	22220.8	0.1	156933	14.16	21957	0.0294	1	0.004	15	0.2335
			0.5	2	5507	11014.8											
	4	2	0.5	1	5972	11944	11802.7	23605.4	0.1	156933	15.04	23342	0.0312	1	0.004	15	0.2483
			0.5	2	5831	11661.4											
Background control	1	2	0.5	1	55	110.66	115.38	230.76	0.1	156933	0.15	-33	0.0000	1	0.004	15	-0.0003
			0.5	2	60	120.1											
	2	2	0.5	1	66	131.34	146.27	292.54	0.1	156933	0.19	29	0.0000	1	0.004	15	0.0003
			0.5	2	81	161.2											
	3	2	0.5	1	67	134.32	124	248	0.1	156933	0.16	-15	0.0000	1	0.004	15	-0.0002
			0.5	2	57	113.68											
	4	2	0.5	1	66	131.34	141.06	282.12	0.1	156933	0.18	19	0.0000	1	0.004	15	0.0002
			0.5	2	75	150.78											
Positive control	1	2	0.5	1	3685	7370.8	7353.3	14706.6	0.1	156933	9.37	14443	0.0193	1	0.004	15	0.1536
			0.5	2	3668	7335.8											
	2	2	0.5	1	3596	7191.6	7175.2	14350.4	0.1	156933	9.14	14087	0.0188	1	0.004	15	0.1498
			0.5	2	3579	7158.8											
	3	2	0.5	1	3473	6946.4	6935.8	13871.6	0.1	156933	8.84	13608	0.0182	1	0.004	15	0.1447
			0.5	2	3463	6925.2											
	4	2	0.5	1	3397	5794.6	6793.3	13586.6	0.1	156933	8.66	13323	0.0178	1	0.004	15	0.1417
			0.5	2	3396	6792											
Negative Control	1	2	0.5	1	6512	13023.6	13101.2	26202.4	0.1	156933	16.70	25939	0.0347	1	0.004	15	0.2759
			0.5	2	6589	13178.8											
	2	2	0.5	1	6309	12617.2	12645.7	25291.4	0.1	156933	16.12	25028	0.0335	1	0.004	15	0.2662
			0.5	2	6337	12674.2											
	3	2	0.5	1	5676	11351.6	11371.3	22742.6	0.1	156933	14.49	22479	0.0301	1	0.004	15	0.2391
			0.5	2	5696	11391											
	4	2	0.5	1	6002	12004.4	11966.9	23933.8	0.1	156933	15.25	23670	0.0317	1	0.004	15	0.2518
			0.5	2	5965	11929.4											
2	1-1	2	0.5	1	102	204.74	207.53	415.06	0.1	156933	0.26	152	0.0002	1	0.004	15	0.0016
			0.5	2	105	210.32											
	1-2	2	0.5	1	114	227.66	228.36	456.72	0.1	156933	0.29	193	0.0003	1	0.004	15	0.0021
			0.5	2	115	229.04											
	1-3	2	0.5	1	94	188.12	194.67	389.34	0.1	156933	0.25	126	0.0002	1	0.004	15	0.0013
			0.5	2	101	201.22											
	2-1	2	0.5	1	444	888.3	897.81	1795.62	0.1	156933	1.14	1532	0.0020	1	0.004	15	0.0163
			0.5	2	454	907.32											
	2-2	2	0.5	1	452	903.06	901.69	1803.38	0.1	156933	1.15	1540	0.0021	1	0.004	15	0.0164
			0.5	2	450	900.32											
	2-3	2	0.5	1	450	899.46	892.83	1785.66	0.1	156933	1.14	1522	0.0020	1	0.004	15	0.0162
			0.5	2	443	886.2											
	3-1	2	0.5	1	2425	4850.4	4904.7	9809.4	0.1	156933	6.25	9546	0.0128	1	0.004	15	0.1015
			0.5	2	2480	4959											
	3-2	2	0.5	1	2449	4898.4	4913.6	9827.2	0.1	156933	6.26	9564	0.0128	1	0.004	15	0.1017
			0.5	2	2464	4928.8											
	3-3	2	0.5	1	2333	4666	4691.5	9383	0.1	156933	5.98	9120	0.0122	1	0.004	15	0.0970
			0.5	2	2359	4717											
	4-1	2	0.5	1	3360	6719.6	6722.5	13445	0.1	156933	8.57	13182	0.0176	1	0.004	15	0.1402
			0.5	2	3363	6725.4											
	4-2	2	0.5	1	3398	6796.8	6757.7	13515.4	0.1	156933	8.61	13252	0.0177	1	0.004	15	0.1409
			0.5	2	3359	6718.6											
	4-3	2	0.5	1	3568	7136.8	7174.5	14349	0.1	156933	9.14	14086	0.0188	1	0.004	15	0.1498
			0.5	2	3606	7212.2											
	5-1	2	0.5	1	4180	8360.2	8436.4	16872.8	0.1	156933	10.75	16609	0.0222	1	0.004	15	0.1767
			0.5	2	4256	8512.6											
	5-2	2	0.5	1	4226	8451.8	8555.1	17110.2	0.1	156933	10.90	16847	0.0225	1	0.004	15	0.1792
			0.5	2	4329	8658.4											
	5-3	2	0.5	1	4442	8883	8773.6	17547.2	0.1	156933	11.18	17284	0.0231	1	0.004	15	0.1838
			0.5	2	4332	8664.2											
	6-1	2	0.5	1	5052	10103	10109.5	20219	0.1	156933	12.88	19956	0.0267	1	0.004	15	0.2122
			0.5	2	5058	10116											
	6-2	2	0.5	1	4957	9914	9911.7	19823.4	0.1	156933	12.63	19560	0.0262	1	0.004	15	0.2080
			0.5	2	4955	9909.4											
	6-3	2	0.5	1	5289	10577.8	10615.6	21231.2	0.1	156933	13.53	20968	0.0280	1	0.004	15	0.2230
			0.5	2	5327	10653.4											
	7-1	2	0.5	1	5601	11201	11053.9	22107.8	0.1	156933	14.09	21844	0.0292	1	0.004	15	0.2323
			0.5	2	5453	10906.8											
	7-2	2	0.5	1	5637	11273.2	11180.4	22360.8	0.1	156933	14.25	22097	0.0296	1	0.004	15	0.2350
			0.5	2	5544	11087.6											
	7-3	2	0.5	1	5820	11640.4	11565.6	23131.2	0.1	156933	14.74	22868	0.0306	1	0.004	15	0.2432
			0.5	2	5745	11490.8											
	8-1	2	0.5	1	6125	12250.8	12235.2	24470.4	0.1	156933	15.59	24207	0.0324	1	0.004	15	0.2575
			0.5	2	6110	12219.6											
	8-2	2	0.5	1	5703	11405.8	11411.3	22822.6	0.1	156933	14.54	22559	0.0302	1	0.004	15	0.2399
			0.5	2	5708	11416.8											
	8-3	2	0.5	1	6035	12070	12015.8	24031.6	0.1	156933	15.31	23768	0.0318	1	0.004	15	0.2528
			0.5	2	5981	11961.6											

Assay Date	8/15/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.2754	0.0027
Full activity	End	0.2409	0.0104
Full activity	Overall	0.2581	0.0209
Background	Beginning	0.0000	0.000464634
Background	End	0.0000	0.000256609
Background	Overall	0.0000	0.000307164
Positive	Beginning	0.1517	0.0027
Positive	End	0.1432	0.0021
Positive	Overall	0.1475	0.0053
Negative	Beginning	0.2710	0.0069
Negative	End	0.2454	0.0090
Negative	Overall	0.2582	0.0162

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
2	1	1	1.00E-04	-4.00	0.0016
2	1	2	1.00E-04	-4.00	0.0021
2	1	3	1.00E-04	-4.00	0.0013
2	2	1	1.00E-05	-5.00	0.0163
2	2	2	1.00E-05	-5.00	0.0164
2	2	3	1.00E-05	-5.00	0.0162
2	3	1	1.00E-06	-6.00	0.1015
2	3	2	1.00E-06	-6.00	0.1017
2	3	3	1.00E-06	-6.00	0.0970
2	4	1	5.00E-07	-6.30	0.1402
2	4	2	5.00E-07	-6.30	0.1409
2	4	3	5.00E-07	-6.30	0.1498
2	5	1	2.50E-07	-6.60	0.1767
2	5	2	2.50E-07	-6.60	0.1792
2	5	3	2.50E-07	-6.60	0.1838
2	6	1	1.00E-07	-7.00	0.2122
2	6	2	1.00E-07	-7.00	0.2080
2	6	3	1.00E-07	-7.00	0.2230
2	7	1	1.00E-08	-8.00	0.2323
2	7	2	1.00E-08	-8.00	0.2350
2	7	3	1.00E-08	-8.00	0.2432
2	8	1	1.00E-09	-9.00	0.2575
2	8	2	1.00E-09	-9.00	0.2399
2	8	3	1.00E-09	-9.00	0.2528

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.63	0.80	0.52
2	-5.00	6.31	6.35	6.27
3	-6.00	39.33	39.40	37.57
4	-6.30	54.31	54.60	58.04
5	-6.60	68.43	69.41	71.21
6	-7.00	82.22	80.59	86.39
7	-8.00	90.00	91.05	94.22
8	-9.00	99.74	92.95	97.93

Assay Date	8/2/2005	Test Chemical ID	3	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5				

								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	0.213
	0.265	0.034	0.211
			0.211

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	
M LE, 03-27-06														
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	0.213	0.213	0.019	200	80	4800	0.006	0.006	5.541
Microsomes	0.211	0.211	0.018	200	80	4800	0.006		
Microsomes	0.211	0.211	0.018	200	80	4800	0.006		

Assay Date	8/2/2005	Test Chemical ID	3	# Concentrations tested	8				
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	Lot # 5		

								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	0.213
	0.265	0.034	0.211
			0.211

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	0.522	0.522	0.0424	m, b	0.082	-0.001
125	100	200	0.00013	200	0.0250	0.307	0.307	0.0247	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.140	0.140	0.0109	r^2, se_y	0.998	0.001
25	100	1000	0.00003	200	0.0050	0.068	0.068	0.0049	F, df	1205	3
10	40	1000	0.00001	200	0.0020	0.031	0.031	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							

Regression results are calculated using the function LINEST

r^2= 0.998
m= 0.082
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	0.021	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 100.	037	0.037	0.002	200	1	1	0.000	0.000	0.011
QC 100.	034	0.034	0.002	200	1	1	0.000		
QC 10									
Microsomes	213	0.213	0.017	200	80	4800	0.005	0.005	5.036
Microsomes	0.211	0.211	0.017	200	80	4800	0.005		
Microsomes	0.211	0.211	0.017	200	80	4800	0.005		

Assay Date	8/2/2005	Test Chemical ID	3	# Concentrations tested	8	Microsome type	recombinai	Microsome ID Lot # 5	Technician ID	TD	Replicate # 1

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.036409
Protein Concentration (dilution added to assay, mg/mL):	0.007994

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	6	Microsome type	recombinant	Microsome ID	Lot # 5	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	9175	18350.4	18494.4	36988.8	0.1	155711	23.75	36751	0.0489	1	0.004	15	0.4079
			0.5	2	9319	18638.4											
	2	2	0.5	1	9300	18600.4	18555.1	37110.2	0.1	155711	23.83	36872	0.0491	1	0.004	15	0.4093
			0.5	2	9255	18509.8											
	3	2	0.5	1	8610	17220.8	17332.9	34665.8	0.1	155711	22.26	34428	0.0458	1	0.004	15	0.3821
			0.5	2	8723	17445											
	4	2	0.5	1	8635	17269	17336.7	34673.4	0.1	155711	22.27	34435	0.0458	1	0.004	15	0.3822
			0.5	2	8702	17404.4											
Background control	1	2	0.5	1	66	131.72	117.63	235.26	0.1	155711	0.15	-3	0.0000	1	0.004	15	0.0000
			0.5	2	52	103.54											
	2	2	0.5	1	53	105.64	114.66	229.32	0.1	155711	0.15	-9	0.0000	1	0.004	15	-0.0001
			0.5	2	62	123.68											
	3	2	0.5	1	67	133.9	129.9	259.8	0.1	155711	0.17	22	0.0000	1	0.004	15	0.0002
			0.5	2	63	125.9											
	4	2	0.5	1	53	105.66	113.75	227.5	0.1	155711	0.15	-10	0.0000	1	0.004	15	-0.0001
			0.5	2	61	121.84											
Positive control	1	2	0.5	1	4778	9556.8	9568.2	19136.4	0.1	155711	12.29	18898	0.0252	1	0.004	15	0.2098
			0.5	2	4790	9579.6											
	2	2	0.5	1	4793	9585	9744.3	19488.6	0.1	155711	12.52	19251	0.0256	1	0.004	15	0.2137
			0.5	2	4952	9903.6											
	3	2	0.5	1	4778	9555	9553.8	19107.6	0.1	155711	12.27	18870	0.0251	1	0.004	15	0.2095
			0.5	2	4776	9552.6											
	4	2	0.5	1	4704	9408.4	9446.6	18893.2	0.1	155711	12.13	18655	0.0248	1	0.004	15	0.2071
			0.5	2	4742	9484.8											
Negative Control	1	2	0.5	1	8586	17172.8	17100	34200	0.1	155711	21.96	33962	0.0452	1	0.004	15	0.3770
			0.5	2	8514	17027.2											
	2	2	0.5	1	8704	17407.4	17343.3	34686.6	0.1	155711	22.28	34449	0.0459	1	0.004	15	0.3824
			0.5	2	8640	17279.2											
	3	2	0.5	1	8357	16714.6	16646.1	33292.2	0.1	155711	21.38	33054	0.0440	1	0.004	15	0.3669
			0.5	2	8289	16577.6											
	4	2	0.5	1	7915	15830.6	15961.7	31923.4	0.1	155711	20.50	31685	0.0422	1	0.004	15	0.3517
			0.5	2	8046	16092.8											
3	1-1	2	0.5	1	70	140.8	148.41	296.82	0.1	155711	0.19	59	0.0001	1	0.004	15	0.0007
			0.5	2	78	156.02											
	1-2	2	0.5	1	71	142.78	131.22	262.44	0.1	155711	0.17	24	0.0000	1	0.004	15	0.0003
			0.5	2	60	119.66											
	1-3	2	0.5	1	68	135.38	142.67	285.34	0.1	155711	0.18	47	0.0001	1	0.004	15	0.0005
			0.5	2	75	149.96											
	2-1	2	0.5	1	92	184.48	178.21	356.42	0.1	155711	0.23	118	0.0002	1	0.004	15	0.0013
			0.5	2	86	171.94											
	2-2	2	0.5	1	85	169.28	172.07	344.14	0.1	155711	0.22	106	0.0001	1	0.004	15	0.0012
			0.5	2	87	174.86											
	2-3	2	0.5	1	89	177.7	169.22	338.44	0.1	155711	0.22	100	0.0001	1	0.004	15	0.0011
			0.5	2	80	160.74											
	3-1	2	0.5	1	293	586.24	592.49	1184.98	0.1	155711	0.76	947	0.0013	1	0.004	15	0.0105
			0.5	2	299	598.74											
	3-2	2	0.5	1	316	632.24	625.35	1250.7	0.1	155711	0.80	1013	0.0013	1	0.004	15	0.0112
			0.5	2	309	618.46											
	3-3	2	0.5	1	305	609.22	638.2	1276.4	0.1	155711	0.82	1038	0.0014	1	0.004	15	0.0115
			0.5	2	334	667.18											
	4-1	2	0.5	1	1977	3953.6	3940.1	7880.2	0.1	155711	5.06	7642	0.0102	1	0.004	15	0.0848
			0.5	2	1963	3926.6											
	4-2	2	0.5	1	1938	3875	3855.2	7710.4	0.1	155711	4.95	7472	0.0099	1	0.004	15	0.0829
			0.5	2	1918	3835.4											
	4-3	2	0.5	1	1951	3901.8	3935	7870	0.1	155711	5.05	7632	0.0102	1	0.004	15	0.0847
			0.5	2	1984	3968.2											
	5-1	2	0.5	1	6023	12046.4	12092.7	24185.4	0.1	155711	15.53	23947	0.0319	1	0.004	15	0.2658
			0.5	2	6070	12139											
	5-2	2	0.5	1	6171	12342.4	12316	24632	0.1	155711	15.82	24394	0.0325	1	0.004	15	0.2708
			0.5	2	6145	12289.6											
	5-3	2	0.5	1	6260	12519.2	12328.7	24657.4	0.1	155711	15.84	24419	0.0325	1	0.004	15	0.2711
			0.5	2	6069	12138.2											
	6-1	2	0.5	1	8738	17476.4	17458.2	34916.4	0.1	155711	22.42	34678	0.0462	1	0.004	15	0.3849
			0.5	2	8720	17440											
	6-2	2	0.5	1	8854	17707.8	17802.2	35604.4	0.1	155711	22.87	35366	0.0471	1	0.004	15	0.3926
			0.5	2	8948	17896.6											
	6-3	2	0.5	1	8602	17204.2	17338.6	34677.2	0.1	155711	22.27	34439	0.0458	1	0.004	15	0.3823
			0.5	2	8737	17473											
	7-1	2	0.5	1	8957	17914.2	17804.4	35608.8	0.1	155711	22.87	35371	0.0471	1	0.004	15	0.3926
			0.5	2	8847	17694.6											
	7-2	2	0.5	1	8872	17743.4	17622.8	35245.6	0.1	155711	22.64	35008	0.0466	1	0.004	15	0.3886
			0.5	2	8751	17502.2											
	7-3	2	0.5	1	8760	17519	17714.9	35429.8	0.1	155711	22.75	35192	0.0468	1	0.004	15	0.3906
			0.5	2	8955	17910.8											
	8-1	2	0.5	1	9185	18369.6	18230.4	36460.8	0.1	155711	23.42	36223	0.0482	1	0.004	15	0.4021
			0.5	2	9046	18091.2											
	8-2	2	0.5	1	9015	18030.2	18035.5	36071	0.1	155711	23.17	35833	0.0477	1	0.004	15	0.3977
			0.5	2	9020	18040.8											
	8-3	2	0.5	1	8872	17743.2	17740.1	35480.2	0.1	155711	22.79	35242	0.0469	1	0.004	15	0.3912
			0.5	2	8869	17737											

Assay Date	8/2/2005	Test Chemical ID	3	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4086	0.0010
Full activity	End	0.3822	0.0001
Full activity	Overall	0.3954	0.0153
Background	Beginning	-0.0001	4.6622E-05
Background	End	0.0001	0.000253517
Background	Overall	0.0000	0.000165675
Positive	Beginning	0.2117	0.0028
Positive	End	0.2083	0.0017
Positive	Overall	0.2100	0.0027
Negative	Beginning	0.3797	0.0038
Negative	End	0.3593	0.0107
Negative	Overall	0.3695	0.0135

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
3	1	1	1.00E-04	-4.00	0.0007
3	1	2	1.00E-04	-4.00	0.0003
3	1	3	1.00E-04	-4.00	0.0005
3	2	1	1.00E-05	-5.00	0.0013
3	2	2	1.00E-05	-5.00	0.0012
3	2	3	1.00E-05	-5.00	0.0011
3	3	1	1.00E-06	-6.00	0.0105
3	3	2	1.00E-06	-6.00	0.0112
3	3	3	1.00E-06	-6.00	0.0115
3	4	1	1.00E-07	-7.00	0.0848
3	4	2	1.00E-07	-7.00	0.0829
3	4	3	1.00E-07	-7.00	0.0847
3	5	1	1.00E-08	-8.00	0.2658
3	5	2	1.00E-08	-8.00	0.2708
3	5	3	1.00E-08	-8.00	0.2711
3	6	1	1.00E-09	-9.00	0.3849
3	6	2	1.00E-09	-9.00	0.3926
3	6	3	1.00E-09	-9.00	0.3823
3	7	1	1.00E-10	-10.00	0.3926
3	7	2	1.00E-10	-10.00	0.3886
3	7	3	1.00E-10	-10.00	0.3906
3	8	1	1.00E-11	-11.00	0.4021
3	8	2	1.00E-11	-11.00	0.3977
3	8	3	1.00E-11	-11.00	0.3912

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.17	0.07	0.13
2	-5.00	0.33	0.30	0.28
3	-6.00	2.66	2.84	2.92
4	-7.00	21.45	20.98	21.43
5	-8.00	67.23	68.48	68.55
6	-9.00	97.35	99.28	96.68
7	-10.00	99.30	98.28	98.79
8	-11.00	101.69	100.59	98.94

Assay Date	8/3/2005	Test Chemical ID	3	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinar	Microsome ID	Lot #5

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	recombinant	Microsome ID	Lot #5

								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	0.213
	0.268	0.036	0.212
			0.209

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	
in LE 03-22-06 BQC														
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	0.213	0.213	0.018	200	80	4800	0.006	0.005	5.495
Microsomes	0.212	0.212	0.018	200	80	4800	0.006		
Microsomes	0.209	0.209	0.018	200	80	4800	0.005		

Assay Date	8/3/2005	Test Chemical ID	3	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	Lot #5

								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	0.213
	0.268	0.036	0.212
			0.209

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	
fm, LE 03-27-06 BdT											
250	200	200	0.00025	200	0.0500	0.522	0.522	0.0423	m, b	0.082	-0.001
125	100	200	0.00013	200	0.0250	0.309	0.309	0.0247	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.138	0.138	0.0106	r^2, se_y	0.998	0.001
25	100	1000	0.00003	200	0.0050	0.075	0.075	0.0054	F, df	1322	3
10	40	1000	0.00001	200	0.0020	0.030	0.030	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.017	0.017	0.0006			

Regression results are calculated using the function LINEST

Blank	0.000	r^2=	0.998		
		m=	0.082		
		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	0.267	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	0.213	0.213	0.017	200	80	4800	0.005	0.005		4.991
Microsomes	0.212	0.212	0.017	200	80	4800	0.005			
Microsomes	0.209	0.209	0.016	200	80	4800	0.005			

Assay Date	8/3/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	recombina	Microsome ID	Lot #5	Technician ID	TD	Replicate #	2

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.990789
Protein Concentration (dilution added to assay, mg/mL):	0.007922

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	recombinant	Microsome ID	Lot #5	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	7493	14986	14946	29892	0.1	154437	19.36	29643	0.0413	1	0.004	15	0.3475
			0.5	2	7453	14906											
	2	2	0.5	1	7055	14110	14068	28136	0.1	154437	18.22	27887	0.0388	1	0.004	15	0.3269
			0.5	2	7013	14026											
	3	2	0.5	1	7136	14272	14271	28542	0.1	154437	18.48	28293	0.0394	1	0.004	15	0.3317
			0.5	2	7135	14270											
	4	2	0.5	1	7093	14186	14142	28264	0.1	154437	18.31	28035	0.0391	1	0.004	15	0.3287
			0.5	2	7059	14118											
Background control	1	2	0.5	1	58	116	126	252	0.1	154437	0.16	3	0.0000	1	0.004	15	0.0000
			0.5	2	68	136											
	2	2	0.5	1	68	136	125	250	0.1	154437	0.16	1	0.0000	1	0.004	15	0.0000
			0.5	2	57	114											
	3	2	0.5	1	61	122	126	252	0.1	154437	0.16	3	0.0000	1	0.004	15	0.0000
			0.5	2	65	130											
	4	2	0.5	1	58	116	122	244	0.1	154437	0.16	-6	0.0000	1	0.004	15	-0.0001
			0.5	2	64	128											
Positive control	1	2	0.5	1	4235	8470	8434	16868	0.1	154437	10.92	16619	0.0232	1	0.004	15	0.1948
			0.5	2	4199	8398											
	2	2	0.5	1	4429	8858	8759	17518	0.1	154437	11.34	17269	0.0241	1	0.004	15	0.2024
			0.5	2	4330	8660											
	3	2	0.5	1	4115	8230	8166	16332	0.1	154437	10.58	16083	0.0224	1	0.004	15	0.1885
			0.5	2	4051	8102											
	4	2	0.5	1	3945	7890	7996	15992	0.1	154437	10.36	15743	0.0219	1	0.004	15	0.1846
			0.5	2	4051	8102											
Negative Control	1	2	0.5	1	5971	11942	11855	23710	0.1	154437	15.35	23461	0.0327	1	0.004	15	0.2750
			0.5	2	5884	11768											
	2	2	0.5	1	6912	13824	13895	27790	0.1	154437	17.99	27541	0.0384	1	0.004	15	0.3229
			0.5	2	6983	13966											
	3	2	0.5	1	6712	13424	13448	26896	0.1	154437	17.42	26647	0.0371	1	0.004	15	0.3124
			0.5	2	6736	13472											
	4	2	0.5	1	6675	13350	13377	26754	0.1	154437	17.32	26505	0.0369	1	0.004	15	0.3107
			0.5	2	6702	13404											
3	1-1	2	0.5	1	85	170	170	340	0.1	154437	0.22	91	0.0001	1	0.004	15	0.0011
			0.5	2	85	170											
	1-2	2	0.5	1	91	182	182	364	0.1	154437	0.24	115	0.0002	1	0.004	15	0.0013
			0.5	2	91	182											
	1-3	2	0.5	1	81	162	171	342	0.1	154437	0.22	93	0.0001	1	0.004	15	0.0011
			0.5	2	90	180											
	2-1	2	0.5	1	302	604	606	1212	0.1	154437	0.78	963	0.0013	1	0.004	15	0.0113
			0.5	2	304	608											
	2-2	2	0.5	1	296	592	611	1222	0.1	154437	0.79	973	0.0014	1	0.004	15	0.0114
			0.5	2	315	630											
	2-3	2	0.5	1	300	600	601	1202	0.1	154437	0.78	953	0.0013	1	0.004	15	0.0112
			0.5	2	301	602											
	3-1	2	0.5	1	1840	3680	3692	7384	0.1	154437	4.78	7135	0.0099	1	0.004	15	0.0836
			0.5	2	1852	3704											
	3-2	2	0.5	1	1700	3400	3406	6812	0.1	154437	4.41	6563	0.0091	1	0.004	15	0.0769
			0.5	2	1706	3412											
	3-3	2	0.5	1	1775	3550	3565	7170	0.1	154437	4.64	6921	0.0096	1	0.004	15	0.0811
			0.5	2	1810	3620											
	4-1	2	0.5	1	4058	8116	8093	16186	0.1	154437	10.48	15937	0.0222	1	0.004	15	0.1868
			0.5	2	4035	8070											
	4-2	2	0.5	1	3889	7778	7921	15842	0.1	154437	10.26	15593	0.0217	1	0.004	15	0.1828
			0.5	2	4032	8064											
	4-3	2	0.5	1	4074	8148	8272	16544	0.1	154437	10.71	16295	0.0227	1	0.004	15	0.1910
			0.5	2	4199	8398											
	5-1	2	0.5	1	5445	10890	10828	21656	0.1	154437	14.02	21407	0.0298	1	0.004	15	0.2510
			0.5	2	5383	10766											
	5-2	2	0.5	1	5485	10970	10935	21870	0.1	154437	14.16	21621	0.0301	1	0.004	15	0.2535
			0.5	2	5450	10900											
	5-3	2	0.5	1	5539	11078	11238	22476	0.1	154437	14.56	22227	0.0310	1	0.004	15	0.2606
			0.5	2	5699	11398											
	6-1	2	0.5	1	6860	13720	13899	27396	0.1	154437	17.74	27149	0.0378	1	0.004	15	0.3183
			0.5	2	6939	13878											
	6-2	2	0.5	1	6416	12832	12802	25604	0.1	154437	16.58	25355	0.0353	1	0.004	15	0.2972
			0.5	2	6386	12772											
	6-3	2	0.5	1	6634	13268	13313	26626	0.1	154437	17.24	26377	0.0367	1	0.004	15	0.3092
			0.5	2	6679	13358											
	7-1	2	0.5	1	6835	13670	13574	27148	0.1	154437	17.58	26899	0.0375	1	0.004	15	0.3153
			0.5	2	6739	13478											
	7-2	2	0.5	1	6825	13650	13704	27408	0.1	154437	17.75	27159	0.0378	1	0.004	15	0.3184
			0.5	2	6879	13758											
	7-3	2	0.5	1	4743	9486	9573	19146	0.1	154437	12.40	18897	0.0263	1	0.004	15	0.2215
			0.5	2	4830	9660											
	8-1	2	0.5	1	6998	13996	13916	27832	0.1	154437	18.02	27583	0.0384	1	0.004	15	0.3234
			0.5	2	6918	13836											
	8-2	2	0.5	1	6663	13326	13488	26976	0.1	154437	17.47	25727	0.0372	1	0.004	15	0.3133
			0.5	2	6825	13650											
	8-3	2	0.5	1	7055	14110	14191	28382	0.1	154437	18.38	28133	0.0392	1	0.004	15	0.3298
			0.5	2	7136	14272											

Assay Date	8/3/2005	Test Chemical ID	3	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot #5	Technician ID TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3372	0.0146
Full activity	End	0.3302	0.0021
Full activity	Overall	0.3337	0.0094
Background	Beginning	0.0000	1.65797E-05
Background	End	0.0000	6.63187E-05
Background	Overall	0.0000	4.43848E-05
Positive	Beginning	0.1986	0.0054
Positive	End	0.1866	0.0028
Positive	Overall	0.1926	0.0078
Negative	Beginning	0.2990	0.0338
Negative	End	0.3116	0.0012
Negative	Overall	0.3053	0.0209

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
3	1	1	1.00E-05	-5.00	0.0011
3	1	2	1.00E-05	-5.00	0.0013
3	1	3	1.00E-05	-5.00	0.0011
3	2	1	1.00E-06	-6.00	0.0113
3	2	2	1.00E-06	-6.00	0.0114
3	2	3	1.00E-06	-6.00	0.0112
3	3	1	1.00E-07	-7.00	0.0836
3	3	2	1.00E-07	-7.00	0.0769
3	3	3	1.00E-07	-7.00	0.0811
3	4	1	2.50E-08	-7.60	0.1868
3	4	2	2.50E-08	-7.60	0.1828
3	4	3	2.50E-08	-7.60	0.1910
3	5	1	1.00E-08	-8.00	0.2510
3	5	2	1.00E-08	-8.00	0.2535
3	5	3	1.00E-08	-8.00	0.2606
3	6	1	2.50E-09	-8.60	0.3183
3	6	2	2.50E-09	-8.60	0.2972
3	6	3	2.50E-09	-8.60	0.3092
3	7	1	1.00E-09	-9.00	0.3153
3	7	2	1.00E-09	-9.00	0.3184
3	7	3	1.00E-09	-9.00	0.2215
3	8	1	1.00E-10	-10.00	0.3234
3	8	2	1.00E-10	-10.00	0.3133
3	8	3	1.00E-10	-10.00	0.3298

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	0.32	0.40	0.32
2	-6.00	3.38	3.42	3.35
3	-7.00	25.06	23.06	24.31
4	-7.60	55.99	54.78	57.25
5	-8.00	75.21	75.96	78.09
6	-8.60	95.38	89.08	92.67
7	-9.00	94.50	95.41	66.39
8	-10.00	96.90	93.90	98.84

Assay Date	8/4/2005	Test Chemical ID	3	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5				

								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	0.203
	0.258	0.029	0.204
			0.205

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	mg Protein Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
M CE 03-27-06 BBL											
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			

Regression results are calculated using the function LINEST

Blank	0.000	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	0.203	0.203	0.017	200	80	4800	0.005	0.005	5.022
Microsomes	0.204	0.204	0.017	200	80	4800	0.005		
Microsomes	0.205	0.205	0.017	200	80	4800	0.005		

Assay Date	8/4/2005	Test Chemical ID	3	# Concentrations tested	8					
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	Lot # 5			

								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	0.203
	0.258	0.029	0.204
			0.205

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	
µ, LE 03-22-06 LQK											
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 100.	034	0.034	0.002	200	1	1	0.000	0.000	0.011
QC 100.	029	0.029	0.002	200	1	1	0.000		
QC 10									
Microsomes	203	0.203	0.016	200	80	4800	0.005	0.005	4.969
Microsomes	0.204	0.204	0.017	200	80	4800	0.005		
Microsomes	0.205	0.205	0.017	200	80	4800	0.005		

Assay Date	8/4/2005	Test Chemical ID	3	# Concentrations tested	8	Microsome type	recombinai	Microsome ID Lot # 5	Technician ID	TD	Replicate # 3

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.969165
Protein Concentration (dilution added to assay, mg/mL):	0.007888

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	recombinant	Microsome ID	Lot # 5	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	5724	11447.6	11643.6	23287.2	0.1	152443	15.28	23033	0.0316	1	0.004	15	0.2670
			0.5	2	5920	11839.6											
	2	2	0.5	1	6068	12135.2	12190.4	24380.8	0.1	152443	15.99	24127	0.0331	1	0.004	15	0.2796
			0.5	2	6123	12245.6											
	3	2	0.5	1	5701	11402	11399	22798	0.1	152443	14.96	22544	0.0309	1	0.004	15	0.2613
			0.5	2	5698	11396											
	4	2	0.5	1	5737	11474.6	11488.2	22976.4	0.1	152443	15.07	22722	0.0312	1	0.004	15	0.2634
			0.5	2	5751	11501.8											
Background control	1	2	0.5	1	71	141	138.51	277.02	0.1	152443	0.18	23	0.0000	1	0.004	15	0.0003
			0.5	2	68	136.02											
	2	2	0.5	1	63	125.76	120.4	240.8	0.1	152443	0.16	-13	0.0000	1	0.004	15	-0.0002
			0.5	2	58	115.04											
	3	2	0.5	1	59	118.34	119.81	239.62	0.1	152443	0.16	-14	0.0000	1	0.004	15	-0.0002
			0.5	2	61	121.28											
	4	2	0.5	1	63	126.36	129.15	258.3	0.1	152443	0.17	4	0.0000	1	0.004	15	0.0001
			0.5	2	66	131.94											
Positive control	1	2	0.5	1	3801	7602	7547.5	15095	0.1	152443	9.90	14841	0.0204	1	0.004	15	0.1720
			0.5	2	3747	7493											
	2	2	0.5	1	3882	7763.6	7662.2	15324.4	0.1	152443	10.05	15070	0.0207	1	0.004	15	0.1747
			0.5	2	3780	7560.8											
	3	2	0.5	1	3446	6892.4	6907.5	13815	0.1	152443	9.06	13561	0.0186	1	0.004	15	0.1572
			0.5	2	3461	6922.6											
	4	2	0.5	1	3515	7029.2	6972.7	13945.4	0.1	152443	9.15	13691	0.0188	1	0.004	15	0.1587
			0.5	2	3458	6916.2											
Negative Control	1	2	0.5	1	5342	10683.6	10775.9	21551.8	0.1	152443	14.14	21298	0.0292	1	0.004	15	0.2469
			0.5	2	5434	10868.2											
	2	2	0.5	1	5671	11342.8	11351.8	22703.6	0.1	152443	14.89	22450	0.0308	1	0.004	15	0.2602
			0.5	2	5680	11360.8											
	3	2	0.5	1	5359	10718.6	10733.4	21466.8	0.1	152443	14.08	21213	0.0291	1	0.004	15	0.2459
			0.5	2	5374	10748.2											
	4	2	0.5	1	5145	10289.4	10336.9	20673.8	0.1	152443	13.56	20420	0.0280	1	0.004	15	0.2387
			0.5	2	5192	10384.4											
3	1-1	2	0.5	1	94	187.78	191.6	383.2	0.1	152443	0.25	129	0.0002	1	0.004	15	0.0015
			0.5	2	98	195.42											
	1-2	2	0.5	1	85	169.84	161.47	322.94	0.1	152443	0.21	69	0.0001	1	0.004	15	0.0008
			0.5	2	77	153.1											
	1-3	2	0.5	1	82	163.94	164.28	328.56	0.1	152443	0.22	75	0.0001	1	0.004	15	0.0009
			0.5	2	82	164.62											
	2-1	2	0.5	1	289	577.52	574.26	1148.52	0.1	152443	0.75	895	0.0012	1	0.004	15	0.0104
			0.5	2	286	571											
	2-2	2	0.5	1	290	580.86	571.49	1142.98	0.1	152443	0.75	889	0.0012	1	0.004	15	0.0103
			0.5	2	281	562.12											
	2-3	2	0.5	1	264	528.16	539.74	1079.48	0.1	152443	0.71	826	0.0011	1	0.004	15	0.0096
			0.5	2	276	551.32											
	3-1	2	0.5	1	1601	3202.8	3250.7	6501.4	0.1	152443	4.26	6247	0.0086	1	0.004	15	0.0724
			0.5	2	1649	3298.6											
	3-2	2	0.5	1	1592	3183.6	3219.7	6439.4	0.1	152443	4.22	6185	0.0085	1	0.004	15	0.0717
			0.5	2	1628	3255.8											
	3-3	2	0.5	1	1534	3068.6	3098.9	6197.8	0.1	152443	4.07	5944	0.0082	1	0.004	15	0.0689
			0.5	2	1565	3129.2											
	4-1	2	0.5	1	3456	6912.4	6929.6	13859.2	0.1	152443	9.09	13605	0.0187	1	0.004	15	0.1577
			0.5	2	3473	6946.8											
	4-2	2	0.5	1	3553	7106.4	7157	14314	0.1	152443	9.39	14060	0.0193	1	0.004	15	0.1630
			0.5	2	3604	7207.6											
	4-3	2	0.5	1	3458	6935.6	6949.8	13899.6	0.1	152443	9.12	13646	0.0187	1	0.004	15	0.1582
			0.5	2	3482	6964											
	5-1	2	0.5	1	4487	8973.2	8924.8	17849.6	0.1	152443	11.71	17596	0.0241	1	0.004	15	0.2039
			0.5	2	4438	8876.4											
	5-2	2	0.5	1	4449	8898.8	9004.1	18008.2	0.1	152443	11.81	17754	0.0243	1	0.004	15	0.2058
			0.5	2	4555	9109.4											
	5-3	2	0.5	1	4644	9287.2	9284.6	18569.2	0.1	152443	12.18	18316	0.0251	1	0.004	15	0.2123
			0.5	2	4641	9282											
	6-1	2	0.5	1	5578	11155	11128	22256	0.1	152443	14.60	22002	0.0302	1	0.004	15	0.2550
			0.5	2	5551	11101											
	6-2	2	0.5	1	5404	10808.6	10828.8	21657.6	0.1	152443	14.21	21404	0.0294	1	0.004	15	0.2481
			0.5	2	5425	10849											
	6-3	2	0.5	1	5283	10566	10798.2	21596.4	0.1	152443	14.17	21342	0.0293	1	0.004	15	0.2474
			0.5	2	5515	11030.4											
	7-1	2	0.5	1	5495	10990	10948.4	21896.8	0.1	152443	14.36	21643	0.0297	1	0.004	15	0.2509
			0.5	2	5453	10906.8											
	7-2	2	0.5	1	5767	11533.6	11414.9	22829.8	0.1	152443	14.98	22576	0.0310	1	0.004	15	0.2617
			0.5	2	5648	11296.2											
	7-3	2	0.5	1	5701	11401	11355.6	22711.2	0.1	152443	14.90	22457	0.0308	1	0.004	15	0.2603
			0.5	2	5655	11310.2											
	8-1	2	0.5	1	5672	11343	11224.7	22449.4	0.1	152443	14.73	22195	0.0304	1	0.004	15	0.2573
			0.5	2	5553	11106.4											
	8-2	2	0.5	1	5661	11322.8	11414.6	22829.2	0.1	152443	14.98	22575	0.0310	1	0.004	15	0.2617
			0.5	2	5753	11506.4											
	8-3	2	0.5	1	5756	11511	11587.5	23175	0.1	152443	15.20	22921	0.0314	1	0.004	15	0.2657
			0.5	2	5832	11664											

Assay Date	8/4/2005	Test Chemical ID	3	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.2733	0.0090
Full activity	End	0.2623	0.0015
Full activity	Overall	0.2678	0.0082
Background	Beginning	0.0001	0.000296848
Background	End	-0.0001	0.000153096
Background	Overall	0.0000	0.000204007
Positive	Beginning	0.1733	0.0019
Positive	End	0.1579	0.0011
Positive	Overall	0.1656	0.0090
Negative	Beginning	0.2535	0.0094
Negative	End	0.2413	0.0065
Negative	Overall	0.2474	0.0097

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
3	1	1	1.00E-05	-5.00	0.0015
3	1	2	1.00E-05	-5.00	0.0008
3	1	3	1.00E-05	-5.00	0.0009
3	2	1	1.00E-06	-6.00	0.0104
3	2	2	1.00E-06	-6.00	0.0103
3	2	3	1.00E-06	-6.00	0.0096
3	3	1	1.00E-07	-7.00	0.0724
3	3	2	1.00E-07	-7.00	0.0717
3	3	3	1.00E-07	-7.00	0.0689
3	4	1	2.50E-08	-7.60	0.1577
3	4	2	2.50E-08	-7.60	0.1630
3	4	3	2.50E-08	-7.60	0.1582
3	5	1	1.00E-08	-8.00	0.2039
3	5	2	1.00E-08	-8.00	0.2058
3	5	3	1.00E-08	-8.00	0.2123
3	6	1	2.50E-09	-8.60	0.2550
3	6	2	2.50E-09	-8.60	0.2481
3	6	3	2.50E-09	-8.60	0.2474
3	7	1	1.00E-09	-9.00	0.2509
3	7	2	1.00E-09	-9.00	0.2617
3	7	3	1.00E-09	-9.00	0.2603
3	8	1	1.00E-10	-10.00	0.2573
3	8	2	1.00E-10	-10.00	0.2617
3	8	3	1.00E-10	-10.00	0.2657

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	0.56	0.30	0.32
2	-6.00	3.87	3.85	3.57
3	-7.00	27.04	26.77	25.72
4	-7.60	58.88	60.85	59.06
5	-8.00	76.15	76.84	79.26
6	-8.60	95.22	92.63	92.36
7	-9.00	93.67	97.70	97.19
8	-10.00	96.06	97.70	99.20

Assay Date	9/27/2005	Test Chemical ID	4	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	recombinar	Microsome ID	Lot # 5

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 8

Technician ID TD | Replicate # 1 | Microsome type recombinant | Microsome ID Lot # 5

								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	0.195
	0.231	0.027	0.192
			0.192

Standard concentration (mg/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	
m 3-22-06 BJC					Used								
250	200	200	0.00025		200	0.0500		0.449	0.449	0.0479	m, b	0.110	-0.002
125	100	200	0.00013		200	0.0250		0.269	0.269	0.0281	se_m, se_b	0.006	0.001
50	200	1000	0.00005		200	0.0100		0.118	0.118	0.0114	r^2, se_y	0.990	0.002
25	100	1000	0.00003		200	0.0050		0.059	0.059	0.0049	F, df	396	4
10	40	1000	0.00001		200	0.0020		0.025	0.025	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010		0.013	0.013	-0.0003			
				Blank	0.000	r^2=	0.990						
						m=	0.110						
						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	0.235	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	0.195	0.195	0.020	200	80	4800	0.006	0.006	5.882
Microsomes	0.192	0.192	0.020	200	80	4800	0.006		
Microsomes	0.192	0.192	0.019	200	80	4800	0.006		

Assay Date	9/27/2005	Test Chemical ID	4	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	Lot # 5

								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	0.195
	0.231	0.027	0.192
			0.192

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	Variables	Regression results	
M, LE 03-22-06											
250	200	200	0.00025	200	0.0500	0.449	0.449	0.0416	m, b	0.094	0.000
125	100	200	0.00013	200	0.0250	0.269	0.269	0.0247	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.118	0.118	0.0106	r^2, se_y	0.999	0.000
25	100	1000	0.00003	200	0.0050	0.059	0.059	0.0051	F, df	2109	3
10	40	1000	0.00001	200	0.0020	0.025	0.025	0.0019	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=	0.999		
		m=	0.094		
		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	0.235	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 100.	028	0.028	0.002	200	1	1	0.000	0.000	0.011
QC 100.	027	0.027	0.002	200	1	1	0.000		
QC 10									
Microsomes	195	0.195	0.018	200	80	4800	0.005	0.005	5.274
Microsomes	0.192	0.192	0.018	200	80	4800	0.005		
Microsomes	0.192	0.192	0.017	200	80	4800	0.005		

Assay Date	9/27/2005	Test Chemical ID		# Concentrations tested	4	Microsome type	8	recombinai Microsome ID	Lot # 5	Technician ID	TD	Replicate #	1

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.273597
Protein Concentration (dilution added to assay, mg/mL):	0.008371

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	9/27/2005	Test Chemical ID	4	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	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	4669	9337.6	9432.8	18865.6	0.1	122901	15.35	18303	0.0310	1	0.004	15	0.2472
			0.5	2	4764	9528											
	2	2	0.5	1	4633	9265.2	9355.9	18711.8	0.1	122901	15.23	18149	0.0308	1	0.004	15	0.2451
			0.5	2	4723	9446.6											
	3	2	0.5	1	4919	9838.8	9896.8	19793.6	0.1	122901	16.11	19231	0.0326	1	0.004	15	0.2597
			0.5	2	4977	9954.8											
	4	2	0.5	1	4501	9001	8896.9	17793.8	0.1	122901	14.48	17231	0.0292	1	0.004	15	0.2327
			0.5	2	4396	8792.8											
Background control	1	2	0.5	1	157	313.7	318.37	636.74	0.1	122901	0.52	74	0.0001	1	0.004	15	0.0010
			0.5	2	162	323.04											
	2	2	0.5	1	142	283.44	287.28	574.56	0.1	122901	0.47	12	0.0000	1	0.004	15	0.0002
			0.5	2	146	291.12											
	3	2	0.5	1	148	295	273.43	546.86	0.1	122901	0.44	-16	0.0000	1	0.004	15	-0.0002
			0.5	2	126	251.86											
	4	2	0.5	1	113	226.08	246.95	493.9	0.1	122901	0.40	-69	-0.0001	1	0.004	15	-0.0009
			0.5	2	134	267.82											
Positive control	1	2	0.5	1	2991	5982.8	5883.1	11766.2	0.1	122901	9.57	11203	0.0190	1	0.004	15	0.1513
			0.5	2	2892	5783.4											
	2	2	0.5	1	3018	6035.8	6022.3	12044.6	0.1	122901	9.80	11482	0.0195	1	0.004	15	0.1551
			0.5	2	3004	6008.8											
	3	2	0.5	1	3024	6047.6	6008.9	12017.8	0.1	122901	9.78	11455	0.0194	1	0.004	15	0.1547
			0.5	2	2985	5970											
	4	2	0.5	1	2703	5406.6	5511.4	11022.8	0.1	122901	8.97	10460	0.0177	1	0.004	15	0.1413
			0.5	2	2808	5616.2											
Negative Control	1	2	0.5	1	4699	9397.4	9373.7	18747.4	0.1	122901	15.25	18184	0.0308	1	0.004	15	0.2456
			0.5	2	4675	9350											
	2	2	0.5	1	4533	9065.4	9144.5	18289	0.1	122901	14.88	17726	0.0301	1	0.004	15	0.2394
			0.5	2	4612	9223.6											
	3	2	0.5	1	4722	9443	9395.3	18790.6	0.1	122901	15.29	18228	0.0309	1	0.004	15	0.2462
			0.5	2	4674	9347.6											
	4	2	0.5	1	4760	9519.6	9481.2	18962.4	0.1	122901	15.43	18399	0.0312	1	0.004	15	0.2485
			0.5	2	4721	9442.8											
4	1-1	2	0.5	1	201	401.42	389.44	778.88	0.1	122901	0.63	216	0.0004	1	0.004	15	0.0029
			0.5	2	189	377.46											
	1-2	2	0.5	1	197	393.1	382.86	765.72	0.1	122901	0.62	203	0.0003	1	0.004	15	0.0027
			0.5	2	186	372.62											
	1-3	2	0.5	1	170	340.06	331.05	662.1	0.1	122901	0.54	99	0.0002	1	0.004	15	0.0013
			0.5	2	161	322.04											
	2-1	2	0.5	1	462	923.36	940.66	1881.32	0.1	122901	1.53	1318	0.0022	1	0.004	15	0.0178
			0.5	2	479	957.96											
	2-2	2	0.5	1	328	656.52	670.59	1341.18	0.1	122901	1.09	778	0.0013	1	0.004	15	0.0105
			0.5	2	342	684.66											
	2-3	2	0.5	1	344	687.5	689.7	1379.4	0.1	122901	1.12	816	0.0014	1	0.004	15	0.0110
			0.5	2	346	691.9											
	3-1	2	0.5	1	3923	7846.4	8224	16448	0.1	122901	13.38	15885	0.0269	1	0.004	15	0.2146
			0.5	2	4301	8601.6											
	3-2	2	0.5	1	4132	8263.6	8261.2	16522.4	0.1	122901	13.44	15959	0.0271	1	0.004	15	0.2156
			0.5	2	4129	8258.8											
	3-3	2	0.5	1	4035	8070.2	8140.6	16281.2	0.1	122901	13.25	15718	0.0267	1	0.004	15	0.2123
			0.5	2	4106	8211											
	4-1	2	0.5	1	4981	9781.2	9927	19854	0.1	122901	16.15	19291	0.0327	1	0.004	15	0.2606
			0.5	2	5046	10092.8											
	4-2	2	0.5	1	4704	9408	9398.6	18797.2	0.1	122901	15.29	18234	0.0309	1	0.004	15	0.2463
			0.5	2	4695	9389.2											
	4-3	2	0.5	1	5308	10616.6	10565.4	21130.8	0.1	122901	17.19	20568	0.0349	1	0.004	15	0.2778
			0.5	2	5257	10514.2											
	5-1	2	0.5	1	4927	9853.8	9990.4	19980.8	0.1	122901	16.26	19418	0.0329	1	0.004	15	0.2623
			0.5	2	5064	10127											
	5-2	2	0.5	1	5136	10272.2	10115.1	20230.2	0.1	122901	16.46	19667	0.0334	1	0.004	15	0.2656
			0.5	2	4979	9958											
	5-3	2	0.5	1	4553	9105.4	9076	18152	0.1	122901	14.77	17589	0.0298	1	0.004	15	0.2376
			0.5	2	4523	9046.6											
	6-1	2	0.5	1	4959	9918.8	10077	20154	0.1	122901	16.40	19591	0.0332	1	0.004	15	0.2646
			0.5	2	5118	10235.2											
	6-2	2	0.5	1	4958	9917.4	9964.6	19929.2	0.1	122901	16.22	19366	0.0328	1	0.004	15	0.2616
			0.5	2	5006	10011.8											
	6-3	2	0.5	1	5167	10333.8	10348.1	20696.2	0.1	122901	16.84	20133	0.0341	1	0.004	15	0.2719
			0.5	2	5181	10362.6											
	7-1	2	0.5	1	4892	9783.8	9912.4	19824.8	0.1	122901	16.13	19262	0.0327	1	0.004	15	0.2602
			0.5	2	5021	10041.2											
	7-2	2	0.5	1	5007	10014.8	10040.4	20080.8	0.1	122901	16.34	19518	0.0331	1	0.004	15	0.2636
			0.5	2	5033	10066											
	7-3	2	0.5	1	5013	10025.2	10098.5	20197	0.1	122901	16.43	19634	0.0333	1	0.004	15	0.2652
			0.5	2	5086	10171.8											
	8-1	2	0.5	1	4424	8848.4	8832.6	17665.2	0.1	122901	14.37	17102	0.0290	1	0.004	15	0.2310
			0.5	2	4408	8816.8											
	8-2	2	0.5	1	5172	10344	10445.2	20890.4	0.1	122901	17.00	20327	0.0345	1	0.004	15	0.2746
			0.5	2	5273	10546.4											
	8-3	2	0.5	1	5241	10481.4	10421.1	20842.2	0.1	122901	16.96	20279	0.0344	1	0.004	15	0.2739
			0.5	2	5180	10360.8											

Assay Date	9/27/2005	Test Chemical ID	4	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot #	5	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.2462	0.0015
Full activity	End	0.2462	0.0191
Full activity	Overall	0.2462	0.0111
Background	Beginning	0.0006	0.000593877
Background	End	-0.0006	0.000505817
Background	Overall	0.0000	0.000803132
Positive	Beginning	0.1532	0.0027
Positive	End	0.1480	0.0095
Positive	Overall	0.1506	0.0064
Negative	Beginning	0.2425	0.0044
Negative	End	0.2474	0.0016
Negative	Overall	0.2449	0.0039

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
4	1	1	1.00E-03	-3.00	0.0029
4	1	2	1.00E-03	-3.00	0.0027
4	1	3	1.00E-03	-3.00	0.0013
4	2	1	1.00E-04	-4.00	0.0178
4	2	2	1.00E-04	-4.00	0.0105
4	2	3	1.00E-04	-4.00	0.0110
4	3	1	1.00E-05	-5.00	0.2146
4	3	2	1.00E-05	-5.00	0.2156
4	3	3	1.00E-05	-5.00	0.2123
4	4	1	1.00E-06	-6.00	0.2606
4	4	2	1.00E-06	-6.00	0.2463
4	4	3	1.00E-06	-6.00	0.2778
4	5	1	1.00E-07	-7.00	0.2623
4	5	2	1.00E-07	-7.00	0.2656
4	5	3	1.00E-07	-7.00	0.2376
4	6	1	1.00E-08	-8.00	0.2646
4	6	2	1.00E-08	-8.00	0.2616
4	6	3	1.00E-08	-8.00	0.2719
4	7	1	1.00E-09	-9.00	0.2602
4	7	2	1.00E-09	-9.00	0.2636
4	7	3	1.00E-09	-9.00	0.2652
4	8	1	1.00E-10	-10.00	0.2310
4	8	2	1.00E-10	-10.00	0.2746
4	8	3	1.00E-10	-10.00	0.2739

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.18	1.11	0.54
2	-4.00	7.23	4.27	4.48
3	-5.00	87.15	87.55	86.23
4	-6.00	105.83	100.03	112.84
5	-7.00	106.53	107.89	96.49
6	-8.00	107.48	106.24	110.45
7	-9.00	105.67	107.07	107.71
8	-10.00	93.82	111.52	111.25

Assay Date	9/28/2005	Test Chemical ID	4	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinai Microsome ID		Lot # 5

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	recombinant	Microsome ID	Lot # 5

								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	0.013	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.247	0.027	0.203
	0.250	0.027	0.206
			0.201

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		0.477	0.477	0.0479	m, b	0.104	-0.002
125	100	200	0.00013		200		0.0250		0.285	0.285	0.0279	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200		0.0100		0.131	0.131	0.0118	r^2, se_y	0.989	0.002
25	100	1000	0.00003		200		0.0050		0.067	0.067	0.0051	F, df	367	4
10	40	1000	0.00001		200		0.0020		0.026	0.026	0.0008	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.013	0.013	-0.0005			
				Blank	0.000		r^2=	0.989						
							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	0.247	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	0.203	0.203	0.019	200	80	4800	0.006	0.006	5.803
Microsomes	0.206	0.206	0.020	200	80	4800	0.006		
Microsomes	0.201	0.201	0.019	200	80	4800	0.006		

Assay Date	09/28/2005	Test Chemical ID	4	# Concentrations tested	8						
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	Lot # 5				

								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	0.013	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.247	0.027	0.203
	0.250	0.027	0.206
			0.201

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	0.477	0.477	0.0415	m, b	0.088	-0.001
125	100	200	0.00013	200	0.0250	0.285	0.285	0.0246	se_m, se_b	0.003	0.000
50	200	1000	0.00005	200	0.0100	0.131	0.131	0.0109	r^2, se_y	0.996	0.001
25	100	1000	0.00003	200	0.0050	0.067	0.067	0.0053	F, df	773	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	0.0005			
			Blank	0.000							

Regression results are calculated using the function LINEST

r^2= 0.996
m= 0.088
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	0.247	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	0.203	0.203	0.017	200	80	4800	0.005	0.005	5.200
Microsomes	0.206	0.206	0.018	200	80	4800	0.005		
Microsomes	0.201	0.201	0.017	200	80	4800	0.005		

Assay Date	9/28/2005	Test Chemical ID	4	# Concentrations tested	8	Microsome type	recombina	Microsome ID	Lot # 5	Technician ID TD	Replicate # 2

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.199761
Protein Concentration (dilution added to assay, mg/mL):	0.008254

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	recombinant	Microsome ID	Lot # S	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	6346	12681.6	12684.9	25369.8	0.1	115710	21.93	25304	0.0445	1	0.004	15	0.3591
			0.5	2	6339	12678.2											
	2	2	0.5	1	5984	11967.4	12039.2	24078.4	0.1	115710	20.81	24012	0.0422	1	0.004	15	0.3406
			0.5	2	6056	12111											
	3	2	0.5	1	5966	11971	11926.4	23852.8	0.1	115710	20.61	23787	0.0418	1	0.004	15	0.3376
			0.5	2	5941	11881.8											
	4	2	0.5	1	5455	10910.2	10858.7	21717.4	0.1	115710	18.77	21651	0.0380	1	0.004	15	0.3073
			0.5	2	5404	10807.2											
Background control	1	2	0.5	1	17	33.06	31.4	62.8	0.1	115710	0.05	-3	0.0000	1	0.004	15	0.0000
			0.5	2	15	29.74											
	2	2	0.5	1	18	35.02	35.22	70.44	0.1	115710	0.06	4	0.0000	1	0.004	15	0.0001
			0.5	2	18	35.42											
	3	2	0.5	1	18	37.2	35.01	70.02	0.1	115710	0.06	4	0.0000	1	0.004	15	0.0001
			0.5	2	16	32.82											
	4	2	0.5	1	13	26.56	30.72	61.44	0.1	115710	0.05	-5	0.0000	1	0.004	15	-0.0001
			0.5	2	17	34.88											
Positive control	1	2	0.5	1	3283	6566.2	6416	12832	0.1	115710	11.09	12766	0.0224	1	0.004	15	0.1812
			0.5	2	3133	6265.8											
	2	2	0.5	1	3159	6317.8	6289.1	12578.2	0.1	115710	10.87	12512	0.0220	1	0.004	15	0.1775
			0.5	2	3130	6260.4											
	3	2	0.5	1	3131	6261.6	6222.7	12445.4	0.1	115710	10.76	12379	0.0218	1	0.004	15	0.1757
			0.5	2	3092	6183.8											
	4	2	0.5	1	2716	5431	5407.8	10815.6	0.1	115710	9.35	10749	0.0189	1	0.004	15	0.1526
			0.5	2	2692	5384.6											
Negative Control	1	2	0.5	1	5061	10121.6	10245.5	20491	0.1	115710	17.71	20425	0.0359	1	0.004	15	0.2899
			0.5	2	5185	10369.4											
	2	2	0.5	1	5157	10313	10507.1	21014.2	0.1	115710	18.16	20948	0.0368	1	0.004	15	0.2973
			0.5	2	5351	10701.2											
	3	2	0.5	1	5356	10711	10675.1	21350.2	0.1	115710	18.45	21284	0.0374	1	0.004	15	0.3021
			0.5	2	5320	10639.2											
	4	2	0.5	1	5274	10548.8	10569.4	21138.8	0.1	115710	18.27	21073	0.0370	1	0.004	15	0.2991
			0.5	2	5295	10590											
4	1-1	2	0.5	1	58	115.12	103.07	206.14	0.1	115710	0.18	140	0.0002	1	0.004	15	0.0020
			0.5	2	46	91.02											
	1-2	2	0.5	1	104	207	171.51	343.02	0.1	115710	0.30	277	0.0005	1	0.004	15	0.0039
			0.5	2	68	136.02											
	1-3	2	0.5	1	77	154.88	148.86	297.72	0.1	115710	0.26	232	0.0004	1	0.004	15	0.0033
			0.5	2	71	142.84											
	2-1	2	0.5	1	338	678.02	620.3	1240.6	0.1	115710	1.07	1174	0.0021	1	0.004	15	0.0167
			0.5	2	282	564.58											
	2-2	2	0.5	1	207	414.86	412.45	824.9	0.1	115710	0.71	759	0.0013	1	0.004	15	0.0108
			0.5	2	205	410.04											
	2-3	2	0.5	1	219	438.62	434.62	869.24	0.1	115710	0.75	803	0.0014	1	0.004	15	0.0114
			0.5	2	215	430.62											
	3-1	2	0.5	1	667	1333.36	1300.71	2601.42	0.1	115710	2.25	2535	0.0045	1	0.004	15	0.0360
			0.5	2	634	1268.06											
	3-2	2	0.5	1	606	1212.5	1213.42	2426.84	0.1	115710	2.10	2361	0.0041	1	0.004	15	0.0335
			0.5	2	607	1214.34											
	3-3	2	0.5	1	594	1187.24	1180.08	2360.16	0.1	115710	2.04	2294	0.0040	1	0.004	15	0.0326
			0.5	2	586	1172.92											
	4-1	2	0.5	1	2042	4083	4113	8226	0.1	115710	7.11	8160	0.0143	1	0.004	15	0.1158
			0.5	2	2072	4143											
	4-2	2	0.5	1	1990	3980.2	3927.2	7854.4	0.1	115710	6.79	7786	0.0137	1	0.004	15	0.1105
			0.5	2	1937	3874.2											
	4-3	2	0.5	1	1921	3842.6	3841	7682	0.1	115710	6.64	7616	0.0134	1	0.004	15	0.1081
			0.5	2	1920	3839.4											
	5-1	2	0.5	1	4419	8838	8865.6	17731.2	0.1	115710	15.32	17665	0.0310	1	0.004	15	0.2507
			0.5	2	4447	8893.2											
	5-2	2	0.5	1	4242	8484.2	8550.7	17101.4	0.1	115710	14.78	17035	0.0299	1	0.004	15	0.2418
			0.5	2	4309	8617.2											
	5-3	2	0.5	1	4199	8398.6	8524.1	17048.2	0.1	115710	14.73	16982	0.0298	1	0.004	15	0.2410
			0.5	2	4325	8649.6											
	6-1	2	0.5	1	5851	11702.2	11496.8	22993.6	0.1	115710	19.87	22927	0.0403	1	0.004	15	0.3254
			0.5	2	5646	11291.4											
	6-2	2	0.5	1	5188	10376.6	10542.8	21085.6	0.1	115710	18.22	21019	0.0369	1	0.004	15	0.2983
			0.5	2	5355	10709											
	6-3	2	0.5	1	5558	11115.2	11234.9	22469.8	0.1	115710	19.42	22404	0.0394	1	0.004	15	0.3180
			0.5	2	5677	11354.6											
	7-1	2	0.5	1	5199	10397.6	10397.5	20795	0.1	115710	17.97	20729	0.0364	1	0.004	15	0.2942
			0.5	2	5199	10397.4											
	7-2	2	0.5	1	5832	11664	11871.9	23743.8	0.1	115710	20.52	23678	0.0416	1	0.004	15	0.3360
			0.5	2	6040	12079.8											
	7-3	2	0.5	1	5262	10524.6	10534.6	21069.6	0.1	115710	18.21	21003	0.0369	1	0.004	15	0.2981
			0.5	2	5273	10545											
	8-1	2	0.5	1	5701	11401	11372.7	22745.4	0.1	115710	19.66	22679	0.0398	1	0.004	15	0.3219
			0.5	2	5672	11344.4											
	8-2	2	0.5	1	5108	10216.6	10431.6	20863.2	0.1	115710	18.03	20797	0.0365	1	0.004	15	0.2952
			0.5	2	5323	10646.6											
	8-3	2	0.5	1	5725	11449.8	11392.5	22785	0.1	115710	19.69	22719	0.0399	1	0.004	15	0.3224
			0.5	2	5668	11335.2											

Assay Date	9/28/2005	Test Chemical ID	4	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3500	0.0130
Full activity	End	0.3224	0.0214
Full activity	Overall	0.3362	0.0215
Background	Beginning	0.0000	7.66727E-05
Background	End	0.0000	8.61063E-05
Background	Overall	0.0000	6.6964E-05
Positive	Beginning	0.1794	0.0025
Positive	End	0.1641	0.0164
Positive	Overall	0.1718	0.0130
Negative	Beginning	0.2936	0.0053
Negative	End	0.3006	0.0021
Negative	Overall	0.2971	0.0052

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
4	1	1	1.00E-03	-3.00	0.0020
4	1	2	1.00E-03	-3.00	0.0039
4	1	3	1.00E-03	-3.00	0.0033
4	2	1	1.00E-04	-4.00	0.0167
4	2	2	1.00E-04	-4.00	0.0108
4	2	3	1.00E-04	-4.00	0.0114
4	3	1	5.00E-05	-4.30	0.0360
4	3	2	5.00E-05	-4.30	0.0335
4	3	3	5.00E-05	-4.30	0.0326
4	4	1	2.50E-05	-4.60	0.1158
4	4	2	2.50E-05	-4.60	0.1105
4	4	3	2.50E-05	-4.60	0.1081
4	5	1	1.00E-05	-5.00	0.2507
4	5	2	1.00E-05	-5.00	0.2418
4	5	3	1.00E-05	-5.00	0.2410
4	6	1	1.00E-06	-6.00	0.3254
4	6	2	1.00E-06	-6.00	0.2983
4	6	3	1.00E-06	-6.00	0.3180
4	7	1	1.00E-07	-7.00	0.2942
4	7	2	1.00E-07	-7.00	0.3360
4	7	3	1.00E-07	-7.00	0.2981
4	8	1	1.00E-08	-8.00	0.3219
4	8	2	1.00E-08	-8.00	0.2952
4	8	3	1.00E-08	-8.00	0.3224

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.59	1.17	0.98
2	-4.00	4.96	3.20	3.39
3	-4.30	10.70	9.97	9.68
4	-4.60	34.45	32.88	32.15
5	-5.00	74.57	71.91	71.69
6	-6.00	96.79	88.73	94.58
7	-7.00	87.51	99.95	88.67
8	-8.00	95.74	87.79	95.91

Assay Date	9/29/2005	Test Chemical ID	4	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	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			

Samples:	QC 100	QC 10	Microsomes
	0.245	0.035	0.206
	0.245	0.033	0.209
			0.205

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	
µ LE 03 27.06 ERR														
250	200	200	0.00025		200		0.0500		0.471	0.471	0.0480	m, b	0.107	-0.002
125	100	200	0.00013		200		0.0250		0.280	0.280	0.0277	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200		0.0100		0.131	0.131	0.0118	r^2, se_y	0.990	0.002
25	100	1000	0.00003		200		0.0050		0.068	0.068	0.0051	F, df	409	4
10	40	1000	0.00001		200		0.0020		0.030	0.030	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200		0.0010		0.016	0.016	-0.0005			
				Blank	0.000		r^2=	0.990					Regression results are calculated using the function LINEST	
							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/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	0.206	0.206	0.020	200	80	4800	0.006	0.006	5.943
Microsomes	0.209	0.209	0.020	200	80	4800	0.006		
Microsomes	0.205	0.205	0.020	200	80	4800	0.006		

Assay Date	9/29/2005	Test Chemical ID	4	# Concentrations tested	8						
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	Lot # 5				

								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	0.206
	0.245	0.033	0.209
			0.205

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	0.206	0.206	0.018	200	80	4800	0.005	0.005	5.349
Microsomes	0.209	0.209	0.018	200	80	4800	0.005		
Microsomes	0.205	0.205	0.018	200	80	4800	0.005		

Assay Date	9/29/2005	Test Chemical ID	4	# Concentrations tested	8	Microsome type	recombina	Microsome ID Lot #	5	Technician ID TD	Replicate # 3

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.348606
Protein Concentration (dilution added to assay, mg/mL):	0.00849

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	9/29/2006	Test Chemical ID	4	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	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	6118	12235.6	12254.3	24508.6	0.1	122466	20.01	24362	0.0404	1	0.004	15	0.3173
			0.5	2	6137	12273											
	2	2	0.5	1	6381	12762.8	12842.8	25685.2	0.1	122466	20.97	25539	0.0424	1	0.004	15	0.3326
			0.5	2	6461	12922.4											
	3	2	0.5	1	5292	10584	10568.7	21137.4	0.1	122466	17.26	20991	0.0348	1	0.004	15	0.2734
			0.5	2	5277	10553.4											
	4	2	0.5	1	5282	10564.4	10535.9	21071.8	0.1	122466	17.21	20925	0.0347	1	0.004	15	0.2725
			0.5	2	5254	10507.4											
Background control	1	2	0.5	1	38	75.3	71.77	143.54	0.1	122466	0.12	-3	0.0000	1	0.004	15	0.0000
			0.5	2	34	68.24											
	2	2	0.5	1	29	55.12	59.13	118.26	0.1	122466	0.10	-29	0.0000	1	0.004	15	-0.0004
			0.5	2	32	63.14											
	3	2	0.5	1	17	33.04	40.63	81.26	0.1	122466	0.07	-65	-0.0001	1	0.004	15	-0.0008
			0.5	2	24	48.22											
	4	2	0.5	1	79	157.02	121.26	242.52	0.1	122466	0.20	96	0.0002	1	0.004	15	0.0013
			0.5	2	43	85.5											
Positive control	1	2	0.5	1	3270	6540.8	6575	13150	0.1	122466	10.74	13004	0.0216	1	0.004	15	0.1693
			0.5	2	3305	6609.2											
	2	2	0.5	1	3426	6852.2	6866.6	13733.2	0.1	122466	11.21	13587	0.0225	1	0.004	15	0.1769
			0.5	2	3441	6881											
	3	2	0.5	1	2936	5872.6	5842.3	11684.6	0.1	122466	9.54	11538	0.0191	1	0.004	15	0.1503
			0.5	2	2906	5811.8											
	4	2	0.5	1	2995	5990.6	6044.4	12088.8	0.1	122466	9.87	11942	0.0198	1	0.004	15	0.1555
			0.5	2	3049	6098.2											
Negative Control	1	2	0.5	1	5951	11902.8	11931	23862	0.1	122466	19.48	23716	0.0393	1	0.004	15	0.3088
			0.5	2	5980	11959.2											
	2	2	0.5	1	5868	11735.2	11700.9	23401.8	0.1	122466	19.11	23255	0.0386	1	0.004	15	0.3028
			0.5	2	5833	11666.6											
	3	2	0.5	1	5390	10779.2	10820.7	21641.4	0.1	122466	17.67	21495	0.0356	1	0.004	15	0.2799
			0.5	2	5431	10862.2											
	4	2	0.5	1	5174	10348.8	10339.9	20679.8	0.1	122466	16.89	20533	0.0341	1	0.004	15	0.2674
			0.5	2	5166	10331											
4	1-1	2	0.5	1	83	166.64	160.66	321.32	0.1	122466	0.26	175	0.0003	1	0.004	15	0.0023
			0.5	2	77	154.48											
	1-2	2	0.5	1	59	118.32	109.83	219.66	0.1	122466	0.18	73	0.0001	1	0.004	15	0.0010
			0.5	2	51	101.34											
	1-3	2	0.5	1	75	149.82	119.32	238.64	0.1	122466	0.19	92	0.0002	1	0.004	15	0.0012
			0.5	2	44	88.82											
	2-1	2	0.5	1	219	437.64	404.58	809.16	0.1	122466	0.66	663	0.0011	1	0.004	15	0.0086
			0.5	2	186	371.22											
	2-2	2	0.5	1	201	401.06	386.71	773.42	0.1	122466	0.63	627	0.0010	1	0.004	15	0.0082
			0.5	2	186	372.36											
	2-3	2	0.5	1	228	455.58	446.65	893.3	0.1	122466	0.73	747	0.0012	1	0.004	15	0.0097
			0.5	2	219	437.72											
	3-1	2	0.5	1	536	1071.2	1079.92	2159.84	0.1	122466	1.76	2013	0.0033	1	0.004	15	0.0262
			0.5	2	544	1088.64											
	3-2	2	0.5	1	781	1561.52	1563.21	3126.42	0.1	122466	2.55	2980	0.0049	1	0.004	15	0.0388
			0.5	2	782	1564.9											
	3-3	2	0.5	1	785	1569.3	1554.56	3109.12	0.1	122466	2.54	2963	0.0049	1	0.004	15	0.0386
			0.5	2	770	1539.82											
	4-1	2	0.5	1	2408	4816.4	4856.1	9712.2	0.1	122466	7.93	9566	0.0159	1	0.004	15	0.1246
			0.5	2	2448	4896.8											
	4-2	2	0.5	1	2132	4264.2	4250.7	8501.4	0.1	122466	6.94	8355	0.0139	1	0.004	15	0.1088
			0.5	2	2119	4237.2											
	4-3	2	0.5	1	2139	4277.8	4311.3	8622.6	0.1	122466	7.04	8475	0.0141	1	0.004	15	0.1104
			0.5	2	2172	4344.8											
	5-1	2	0.5	1	4393	8785.4	8848.3	17696.6	0.1	122466	14.45	17550	0.0291	1	0.004	15	0.2285
			0.5	2	4456	8911.2											
	5-2	2	0.5	1	4463	8926.8	8988.7	17977.4	0.1	122466	14.68	17831	0.0296	1	0.004	15	0.2322
			0.5	2	4525	9050.6											
	5-3	2	0.5	1	4282	8564.8	8669.5	17379	0.1	122466	14.19	17233	0.0286	1	0.004	15	0.2244
			0.5	2	4407	8814.4											
	6-1	2	0.5	1	5512	11023.2	11252.7	22505.4	0.1	122466	18.38	22359	0.0371	1	0.004	15	0.2912
			0.5	2	5741	11482.2											
	6-2	2	0.5	1	5320	10640.6	10612.3	21224.6	0.1	122466	17.33	21078	0.0350	1	0.004	15	0.2745
			0.5	2	5292	10584											
	6-3	2	0.5	1	3346	6692.2	6656.5	13313	0.1	122466	10.87	13167	0.0218	1	0.004	15	0.1715
			0.5	2	3310	6620.8											
	7-1	2	0.5	1	5186	10371.8	10591.9	21183.8	0.1	122466	17.30	21037	0.0349	1	0.004	15	0.2740
			0.5	2	5406	10812											
	7-2	2	0.5	1	5279	10558	10490.7	20981.4	0.1	122466	17.13	20835	0.0346	1	0.004	15	0.2713
			0.5	2	5212	10423.4											
	7-3	2	0.5	1	5513	11026.4	10915.2	21830.4	0.1	122466	17.83	21684	0.0360	1	0.004	15	0.2824
			0.5	2	5402	10804											
	8-1	2	0.5	1	5796	11591.6	11814.2	23628.4	0.1	122466	19.29	23482	0.0389	1	0.004	15	0.3058
			0.5	2	6018	12036.8											
	8-2	2	0.5	1	5990	11979.2	11918.7	23837.4	0.1	122466	19.46	23691	0.0393	1	0.004	15	0.3085
			0.5	2	5929	11858.2											
	8-3	2	0.5	1	6112	12224	12157.9	24315.8	0.1	122466	19.86	24169	0.0401	1	0.004	15	0.3147
			0.5	2	6046	12091.8											

Assay Date	9/29/2005	Test Chemical ID	4	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3249	0.0108
Full activity	End	0.2729	0.0006
Full activity	Overall	0.2989	0.0307
Background	Beginning	-0.0002	0.000232784
Background	End	0.0002	0.001484921
Background	Overall	0.0000	0.000898525
Positive	Beginning	0.1731	0.0054
Positive	End	0.1529	0.0037
Positive	Overall	0.1630	0.0123
Negative	Beginning	0.3058	0.0042
Negative	End	0.2737	0.0089
Negative	Overall	0.2897	0.0194

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
4	1	1	1.00E-03	-3.00	0.0023
4	1	2	1.00E-03	-3.00	0.0010
4	1	3	1.00E-03	-3.00	0.0012
4	2	1	1.00E-04	-4.00	0.0086
4	2	2	1.00E-04	-4.00	0.0082
4	2	3	1.00E-04	-4.00	0.0097
4	3	1	5.00E-05	-4.30	0.0262
4	3	2	5.00E-05	-4.30	0.0388
4	3	3	5.00E-05	-4.30	0.0386
4	4	1	2.50E-05	-4.60	0.1246
4	4	2	2.50E-05	-4.60	0.1088
4	4	3	2.50E-05	-4.60	0.1104
4	5	1	1.00E-05	-5.00	0.2285
4	5	2	1.00E-05	-5.00	0.2322
4	5	3	1.00E-05	-5.00	0.2244
4	6	1	1.00E-06	-6.00	0.2912
4	6	2	1.00E-06	-6.00	0.2745
4	6	3	1.00E-06	-6.00	0.1715
4	7	1	1.00E-07	-7.00	0.2740
4	7	2	1.00E-07	-7.00	0.2713
4	7	3	1.00E-07	-7.00	0.2824
4	8	1	1.00E-08	-8.00	0.3058
4	8	2	1.00E-08	-8.00	0.3085
4	8	3	1.00E-08	-8.00	0.3147

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.76	0.32	0.40
2	-4.00	2.89	2.73	3.25
3	-4.30	8.77	12.98	12.91
4	-4.60	41.67	36.40	36.93
5	-5.00	76.46	77.68	75.07
6	-6.00	97.41	91.83	57.36
7	-7.00	91.65	90.77	94.47
8	-8.00	102.30	103.21	105.29

Assay Date	9/30/2005	Test Chemical ID	5	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	recombinar	Microsome ID	BD Gentes

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	8						
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5				
							Protein stock (mg BSA)	Total volume of stock (mL)	100	Protein stock ID	11343-77

Standards:	250	125	50	25	10	5	0
	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	0.217
	0.260	0.036	0.223
			0.216

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	
µ LE, 03-22-06 BDL														
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					Regression results are calculated using the function LINEST	
							m=	0.104						
							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	0.266	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	0.040	0.040	0.002	200	1	1	0.000	0.000	0.008
QC 10	0.036	0.036	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.217	0.217	0.020	200	80	4800	0.006	0.006	6.137
Microsomes	0.223	0.223	0.021	200	80	4800	0.006		
Microsomes	0.216	0.216	0.020	200	80	4800	0.006		

Assay Date	9/30/2005	Test Chemical ID	5	# Concentrations tested	8						
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.481	0.294	0.133	0.070	0.034	0.020	0.000	25	100	11343-77
	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	0.217
	0.260	0.036	0.223
			0.216

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	
M,LE 05-22-06 HRL											
250	200	200	0.00025	200	0.0500	0.487	0.487	0.0432	m, b	0.091	-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	0.132	0.132	0.0108	r^2, se_y	0.996	0.001
25	100	1000	0.00003	200	0.0050	0.074	0.074	0.0055	F, df	841	3
10	40	1000	0.00001	200	0.0020	0.033	0.033	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.019	0.019	0.0004			

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	0.266	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 100	040	0.040	0.002	200	1	1	0.000	0.000	0.011	
QC 100	036	0.036	0.002	200	1	1	0.000			
QC 10										
Microsomes	217	0.217	0.019	200	80	4800	0.006	0.006	5.613	
Microsomes	0.223	0.223	0.019	200	80	4800	0.006			
Microsomes	0.216	0.216	0.018	200	80	4800	0.006			

Assay Date	9/30/2005	Test Chemical ID		# Concentrations tested	5	Microsome type	8	recombinant Microsome ID	BD Gentest	Technician ID	TD	Replicate #	1

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.613128
Protein Concentration (dilution added to assay, mg/mL):	0.00891

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	9/30/2005	Test Chemical ID	5	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5	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	5972	11943.2	12056.1	24116.2	0.1	121231	19.89	24004	0.0396	1	0.004	15	0.2962
			0.5	2	6087	12173											
	2	2	0.5	1	6209	12418.4	12405.4	24810.8	0.1	121231	20.47	24699	0.0407	1	0.004	15	0.3048
			0.5	2	6196	12392.4											
	3	2	0.5	1	6242	12483.2	12472.2	24944.4	0.1	121231	20.58	24832	0.0409	1	0.004	15	0.3064
			0.5	2	6231	12461.2											
	4	2	0.5	1	6083	12168.4	12416.7	24833.4	0.1	121231	20.48	24721	0.0408	1	0.004	15	0.3050
			0.5	2	6334	12667											
Background control	1	2	0.5	1	30	59.44	59.52	119.04	0.1	121231	0.10	7	0.0000	1	0.004	15	0.0001
			0.5	2	30	59.6											
	2	2	0.5	1	39	77.16	78.89	157.78	0.1	121231	0.13	46	0.0001	1	0.004	15	0.0006
			0.5	2	40	80.62											
	3	2	0.5	1	23	46.86	45.43	90.86	0.1	121231	0.07	-21	0.0000	1	0.004	15	-0.0003
			0.5	2	22	44											
	4	2	0.5	1	20	39.54	40.62	81.24	0.1	121231	0.07	-31	-0.0001	1	0.004	15	-0.0004
			0.5	2	21	41.7											
Positive control	1	2	0.5	1	3458	6916	6999.5	13999	0.1	121231	11.55	13887	0.0229	1	0.004	15	0.1713
			0.5	2	3542	7083											
	2	2	0.5	1	3340	6679.6	6706.1	13412.2	0.1	121231	11.06	13300	0.0219	1	0.004	15	0.1641
			0.5	2	3366	6732.6											
	3	2	0.5	1	3153	6306.8	6311.9	12623.8	0.1	121231	10.41	12512	0.0206	1	0.004	15	0.1544
			0.5	2	3159	6317											
	4	2	0.5	1	3295	6589.8	6597.7	13195.4	0.1	121231	10.88	13083	0.0216	1	0.004	15	0.1614
			0.5	2	3303	6605.6											
Negative Control	1	2	0.5	1	5919	11837.8	12077.1	24154.2	0.1	121231	19.92	24042	0.0396	1	0.004	15	0.2967
			0.5	2	6158	12316.4											
	2	2	0.5	1	6151	12301	12284	24568	0.1	121231	20.27	24456	0.0403	1	0.004	15	0.3018
			0.5	2	6134	12267											
	3	2	0.5	1	5844	11687.2	11697.2	23394.4	0.1	121231	19.30	23282	0.0384	1	0.004	15	0.2873
			0.5	2	5854	11707.2											
	4	2	0.5	1	5967	11933.8	11986.6	23973.2	0.1	121231	19.77	23861	0.0393	1	0.004	15	0.2944
			0.5	2	6020	12039.4											
5	1-1	2	0.5	1	6299	12597.8	12584.3	25168.6	0.1	121231	20.76	25056	0.0413	1	0.004	15	0.3092
			0.5	2	6286	12571											
	1-2	2	0.5	1	6279	12557.8	12473.1	24946.2	0.1	121231	20.58	24834	0.0410	1	0.004	15	0.3064
			0.5	2	6194	12388.4											
	1-3	2	0.5	1	6346	12691	12639.4	25278.8	0.1	121231	20.85	25167	0.0415	1	0.004	15	0.3105
			0.5	2	6294	12587.8											
	2-1	2	0.5	1	5674	11348.8	11649.2	23298.4	0.1	121231	19.22	23186	0.0382	1	0.004	15	0.2861
			0.5	2	5975	11949.6											
	2-2	2	0.5	1	5740	11479.4	11730.5	23461	0.1	121231	19.35	23349	0.0385	1	0.004	15	0.2881
			0.5	2	5991	11981.6											
	2-3	2	0.5	1	5911	11822.6	11858.3	23716.6	0.1	121231	19.56	23604	0.0389	1	0.004	15	0.2913
			0.5	2	5947	11894											
	3-1	2	0.5	1	6025	12050	12066.4	24132.8	0.1	121231	19.91	24021	0.0396	1	0.004	15	0.2964
			0.5	2	6041	12082.8											
	3-2	2	0.5	1	5949	11898	11865.7	23731.4	0.1	121231	19.58	23619	0.0389	1	0.004	15	0.2914
			0.5	2	5917	11833.4											
	3-3	2	0.5	1	5952	11904	11853.3	23706.6	0.1	121231	19.55	23594	0.0389	1	0.004	15	0.2911
			0.5	2	5901	11802.6											
	4-1	2	0.5	1	5843	11685.4	11668.4	23336.8	0.1	121231	19.25	23225	0.0383	1	0.004	15	0.2866
			0.5	2	5826	11651.4											
	4-2	2	0.5	1	5853	11706.4	11976.3	23952.6	0.1	121231	19.76	23840	0.0393	1	0.004	15	0.2942
			0.5	2	6123	12246.2											
	4-3	2	0.5	1	6295	12590	12482.5	24965	0.1	121231	20.59	24853	0.0410	1	0.004	15	0.3067
			0.5	2	6188	12375											
	5-1	2	0.5	1	5593	11185	11317.7	22635.4	0.1	121231	18.67	22523	0.0371	1	0.004	15	0.2779
			0.5	2	5725	11450.4											
	5-2	2	0.5	1	5702	11404	11443.1	22886.2	0.1	121231	18.88	22774	0.0376	1	0.004	15	0.2810
			0.5	2	5741	11482.2											
	5-3	2	0.5	1	5901	11802.2	11927.5	23855	0.1	121231	19.68	23743	0.0392	1	0.004	15	0.2930
			0.5	2	6026	12052.8											
	6-1	2	0.5	1	5946	11891.6	12268.8	24537.6	0.1	121231	20.24	24425	0.0403	1	0.004	15	0.3014
			0.5	2	6323	12645											
	6-2	2	0.5	1	5967	11933.2	11818.6	23637.2	0.1	121231	19.50	23525	0.0388	1	0.004	15	0.2903
			0.5	2	5852	11704											
	6-3	2	0.5	1	5989	11978.2	11903.2	23806.4	0.1	121231	19.64	23694	0.0391	1	0.004	15	0.2924
			0.5	2	5914	11828.2											
	7-1	2	0.5	1	5500	11000.8	11196.6	22393.2	0.1	121231	18.47	22281	0.0367	1	0.004	15	0.2749
			0.5	2	5696	11392.4											
	7-2	2	0.5	1	6086	12131.4	12149.3	24298.6	0.1	121231	20.04	24186	0.0399	1	0.004	15	0.2984
			0.5	2	6084	12167.2											
	7-3	2	0.5	1	6056	12111	12163.5	24327	0.1	121231	20.07	24215	0.0399	1	0.004	15	0.2988
			0.5	2	6108	12216											
	8-1	2	0.5	1	6271	12541	12783.8	25567.6	0.1	121231	21.09	25455	0.0420	1	0.004	15	0.3141
			0.5	2	6513	13026.6											
	8-2	2	0.5	1	5893	11785.6	11858.3	23716.6	0.1	121231	19.56	23604	0.0389	1	0.004	15	0.2913
			0.5	2	5966	11931											
	8-3	2	0.5	1	6017	12034.4	12093.2	24186.4	0.1	121231	19.95	24074	0.0397	1	0.004	15	0.2971
			0.5	2	6076	12152											

Assay Date	9/30/2005	Test Chemical ID	5	# Concentrations tested	Microsome 8 type	recombinant	Microsome ID	BD Gentest Lo	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.3005	0.0061
Full activity	End	0.3057	0.0010
Full activity	Overall	0.3031	0.0047
Background	Beginning	0.0003	0.000338006
Background	End	-0.0003	8.39343E-05
Background	Overall	0.0000	0.000423752
Positive	Beginning	0.1677	0.0051
Positive	End	0.1579	0.0050
Positive	Overall	0.1628	0.0070
Negative	Beginning	0.2992	0.0036
Negative	End	0.2908	0.0051
Negative	Overall	0.2950	0.0060

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
5	1	1	1.00E-04	-4.00	0.3092
5	1	2	1.00E-04	-4.00	0.3064
5	1	3	1.00E-04	-4.00	0.3105
5	2	1	1.00E-05	-5.00	0.2861
5	2	2	1.00E-05	-5.00	0.2881
5	2	3	1.00E-05	-5.00	0.2913
5	3	1	1.00E-06	-6.00	0.2964
5	3	2	1.00E-06	-6.00	0.2914
5	3	3	1.00E-06	-6.00	0.2911
5	4	1	2.50E-07	-6.60	0.2866
5	4	2	2.50E-07	-6.60	0.2942
5	4	3	2.50E-07	-6.60	0.3067
5	5	1	1.00E-07	-7.00	0.2779
5	5	2	1.00E-07	-7.00	0.2810
5	5	3	1.00E-07	-7.00	0.2930
5	6	1	1.00E-08	-8.00	0.3014
5	6	2	1.00E-08	-8.00	0.2903
5	6	3	1.00E-08	-8.00	0.2924
5	7	1	1.00E-09	-9.00	0.2749
5	7	2	1.00E-09	-9.00	0.2984
5	7	3	1.00E-09	-9.00	0.2988
5	8	1	1.00E-10	-10.00	0.3141
5	8	2	1.00E-10	-10.00	0.2913
5	8	3	1.00E-10	-10.00	0.2971

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	102.00	101.10	102.45
2	-5.00	94.39	95.05	96.09
3	-6.00	97.79	96.15	96.05
4	-6.60	94.55	97.05	101.18
5	-7.00	91.69	92.71	96.66
6	-8.00	99.44	95.77	96.46
7	-9.00	90.71	98.46	98.58
8	-10.00	103.63	96.09	98.01

Assay Date	10/3/2005	Test Chemical ID	5	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinar	Microsome ID	BD Gentes

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	recombinant	Microsome ID	BD Gentest Lot #5			

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.485	0.281	0.125	0.069	0.027	0.013	0.000	25	100	11343-77
	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	0.212
	0.253	0.030	0.209
			0.209

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	
m LE 03-27-06 ISDC														
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					Regression results are calculated using the function LINEST	
							m=	0.103						
							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	0.249	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	0.212	0.212	0.020	200	80	4800	0.006	0.006	5.988
Microsomes	0.209	0.209	0.020	200	80	4800	0.006		
Microsomes	0.209	0.209	0.020	200	80	4800	0.006		

Assay Date	10/3/2005	Test Chemical ID	5	# Concentrations tested	8						
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5				
								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID	
								25	100	11343-77	

Standards:	250	125	50	25	10	5	0
	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	0.212
	0.253	0.030	0.209
			0.209

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	
µL LE 05-27-06 HBC											
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_{resd}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.014	0.014	0.0006			

Regression results are calculated using the function LINEST

Blank	0.000	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 100.	028	0.028	0.002	200	1	1	0.000	0.000	0.010
QC 100.	030	0.030	0.002	200	1	1	0.000		
QC 10									
Microsomes	212	0.212	0.018	200	80	4800	0.005	0.005	5.437
Microsomes	0.209	0.209	0.018	200	80	4800	0.005		
Microsomes	0.209	0.209	0.018	200	80	4800	0.005		

Assay Date	10/3/2005	Test Chemical ID		5	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest	Technician ID TD	Replicate #	2

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.437198
Protein Concentration (dilution added to assay, mg/mL):	0.00863

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	recombinant Microsome ID	BD Gentest Lot #5	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	5029	10057.4	10069.4	20138.8	0.1	112748	17.86	19891	0.0360	1	0.004	15	0.2762
			0.5	2	5041	10081.4											
	2	2	0.5	1	5202	10404.6	10275.9	20551.8	0.1	112748	18.23	20304	0.0368	1	0.004	15	0.2840
			0.5	2	5074	10147.2											
	3	2	0.5	1	4607	9214.2	9256.6	18513.6	0.1	112748	16.42	18266	0.0331	1	0.004	15	0.2554
			0.5	2	4650	9299.4											
	4	2	0.5	1	5115	10229.6	10212.5	20425	0.1	112748	18.12	20178	0.0365	1	0.004	15	0.2822
			0.5	2	5098	10195.4											
Background control	1	2	0.5	1	102	203.9	202.29	404.58	0.1	112748	0.36	157	0.0003	1	0.004	15	0.0022
			0.5	2	100	200.68											
	2	2	0.5	1	42	83.16	84.99	169.98	0.1	112748	0.15	-77	-0.0001	1	0.004	15	-0.0011
			0.5	2	43	86.8											
	3	2	0.5	1	52	104.44	116.53	233.06	0.1	112748	0.21	-14	0.0000	1	0.004	15	-0.0002
			0.5	2	64	128.62											
	4	2	0.5	1	44	87.64	90.82	181.64	0.1	112748	0.16	-66	-0.0001	1	0.004	15	-0.0009
			0.5	2	47	94											
Posiilve control	1	2	0.5	1	2888	5776.4	5824.1	11648.2	0.1	112748	10.33	11401	0.0206	1	0.004	15	0.1594
			0.5	2	2936	5871.8											
	2	2	0.5	1	2989	5978.6	6041.7	12083.4	0.1	112748	10.72	11836	0.0214	1	0.004	15	0.1655
			0.5	2	3052	6104.8											
	3	2	0.5	1	2770	5539.8	5467.5	10935	0.1	112748	9.70	10688	0.0193	1	0.004	15	0.1495
			0.5	2	2698	5395.2											
	4	2	0.5	1	2912	5824.6	5883.5	11767	0.1	112748	10.44	11520	0.0209	1	0.004	15	0.1611
			0.5	2	2971	5942.4											
Negative Control	1	2	0.5	1	5211	10422.6	10412.2	20824.4	0.1	112748	18.47	20577	0.0373	1	0.004	15	0.2878
			0.5	2	5201	10401.8											
	2	2	0.5	1	4596	9191.8	9118.2	18236.4	0.1	112748	16.17	17989	0.0326	1	0.004	15	0.2516
			0.5	2	4522	9044.6											
	3	2	0.5	1	4688	9376.2	9332	18664	0.1	112748	16.55	18417	0.0333	1	0.004	15	0.2576
			0.5	2	4644	9287.8											
	4	2	0.5	1	5069	10137.8	10135.4	20270.8	0.1	112748	17.98	20023	0.0363	1	0.004	15	0.2800
			0.5	2	5067	10133											
5	1-1	2	0.5	1	5245	10489.2	10591.5	21183	0.1	112748	18.79	20936	0.0379	1	0.004	15	0.2928
			0.5	2	5347	10693.8											
	1-2	2	0.5	1	5414	10828.2	10793	21586	0.1	112748	19.15	21339	0.0386	1	0.004	15	0.2984
			0.5	2	5379	10757.8											
	1-3	2	0.5	1	5218	10436	10421.8	20843.6	0.1	112748	18.49	20596	0.0373	1	0.004	15	0.2880
			0.5	2	5204	10407.6											
	2-1	2	0.5	1	5327	10653.4	10674.4	21348.8	0.1	112748	18.94	21101	0.0382	1	0.004	15	0.2951
			0.5	2	5348	10695.4											
	2-2	2	0.5	1	5365	10729.2	10827.8	21655.6	0.1	112748	19.21	21408	0.0388	1	0.004	15	0.2994
			0.5	2	5463	10926.4											
	2-3	2	0.5	1	5344	10688	10878.8	21757.6	0.1	112748	19.30	21510	0.0389	1	0.004	15	0.3008
			0.5	2	5535	11069.6											
	3-1	2	0.5	1	5630	11259	11153.3	22306.6	0.1	112748	19.78	22059	0.0399	1	0.004	15	0.3085
			0.5	2	5524	11047.6											
	3-2	2	0.5	1	5090	10179	10234.3	20468.6	0.1	112748	18.15	20221	0.0366	1	0.004	15	0.2828
			0.5	2	5145	10289.6											
	3-3	2	0.5	1	5489	10977	11017	22034	0.1	112748	19.54	21787	0.0394	1	0.004	15	0.3047
			0.5	2	5529	11057											
	4-1	2	0.5	1	5636	11072.8	11044.9	22089.8	0.1	112748	19.59	21842	0.0395	1	0.004	15	0.3055
			0.5	2	5509	11017											
	4-2	2	0.5	1	4793	9586	9564.2	19128.4	0.1	112748	16.97	18881	0.0342	1	0.004	15	0.2640
			0.5	2	4771	9542.4											
	4-3	2	0.5	1	5099	10197.6	10199.2	20398.4	0.1	112748	18.09	20151	0.0365	1	0.004	15	0.2818
			0.5	2	5100	10200.8											
	5-1	2	0.5	1	5222	10444.6	10500.7	21001.4	0.1	112748	18.63	20754	0.0376	1	0.004	15	0.2902
			0.5	2	5279	10556.8											
	5-2	2	0.5	1	5340	10680.6	10613.9	21227.8	0.1	112748	18.83	20980	0.0380	1	0.004	15	0.2934
			0.5	2	5274	10547.2											
	5-3	2	0.5	1	5390	10780.4	10784.2	21568.4	0.1	112748	19.13	21321	0.0386	1	0.004	15	0.2982
			0.5	2	5394	10788											
	6-1	2	0.5	1	5083	10165.4	10092.4	20184.8	0.1	112748	17.90	19937	0.0361	1	0.004	15	0.2788
			0.5	2	5010	10019.4											
	6-2	2	0.5	1	5052	10104	10105.8	20211.6	0.1	112748	17.93	19964	0.0361	1	0.004	15	0.2792
			0.5	2	5054	10107.6											
	6-3	2	0.5	1	5186	10372.8	10331	20662	0.1	112748	18.33	20415	0.0370	1	0.004	15	0.2855
			0.5	2	5145	10289.2											
	7-1	2	0.5	1	4968	9935.8	9928.5	19857	0.1	112748	17.61	19610	0.0355	1	0.004	15	0.2742
			0.5	2	4961	9921.2											
	7-2	2	0.5	1	5615	11229	11200.4	22400.8	0.1	112748	19.87	22153	0.0401	1	0.004	15	0.3096
			0.5	2	5586	11171.8											
	7-3	2	0.5	1	4879	9757.6	9700.3	19400.6	0.1	112748	17.21	19153	0.0347	1	0.004	15	0.2679
			0.5	2	4822	9643											
	8-1	2	0.5	1	5266	10531	10531.5	21063	0.1	112748	18.68	20816	0.0377	1	0.004	15	0.2911
			0.5	2	5266	10532											
	8-2	2	0.5	1	5666	11331.2	11236.5	22473	0.1	112748	19.93	22226	0.0402	1	0.004	15	0.3108
			0.5	2	5571	11141.8											
	8-3	2	0.5	1	4969	9937.4	9666	19732	0.1	112748	17.50	19485	0.0353	1	0.004	15	0.2725
			0.5	2	4897	9794.6											

Assay Date	10/3/2005	Test Chemical ID	5	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lo	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.2811	0.0041
Full activity	End	0.2688	0.0189
Full activity	Overall	0.2749	0.0132
Background	Beginning	0.0006	0.002319871
Background	End	-0.0006	0.000508473
Background	Overall	0.0000	0.001515454
Positive	Beginning	0.1625	0.0043
Positive	End	0.1553	0.0082
Positive	Overall	0.1589	0.0068
Negative	Beginning	0.2697	0.0256
Negative	End	0.2688	0.0159
Negative	Overall	0.2692	0.0174

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
5	1	1	1.00E-04	-4.00	0.2928
5	1	2	1.00E-04	-4.00	0.2984
5	1	3	1.00E-04	-4.00	0.2880
5	2	1	1.00E-05	-5.00	0.2951
5	2	2	1.00E-05	-5.00	0.2994
5	2	3	1.00E-05	-5.00	0.3008
5	3	1	1.00E-06	-6.00	0.3085
5	3	2	1.00E-06	-6.00	0.2828
5	3	3	1.00E-06	-6.00	0.3047
5	4	1	2.50E-07	-6.60	0.3055
5	4	2	2.50E-07	-6.60	0.2640
5	4	3	2.50E-07	-6.60	0.2818
5	5	1	1.00E-07	-7.00	0.2902
5	5	2	1.00E-07	-7.00	0.2934
5	5	3	1.00E-07	-7.00	0.2982
5	6	1	1.00E-08	-8.00	0.2788
5	6	2	1.00E-08	-8.00	0.2792
5	6	3	1.00E-08	-8.00	0.2855
5	7	1	1.00E-09	-9.00	0.2742
5	7	2	1.00E-09	-9.00	0.3098
5	7	3	1.00E-09	-9.00	0.2679
5	8	1	1.00E-10	-10.00	0.2911
5	8	2	1.00E-10	-10.00	0.3108
5	8	3	1.00E-10	-10.00	0.2725

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	106.49	108.54	104.76
2	-5.00	107.33	108.89	109.41
3	-6.00	112.20	102.86	110.82
4	-6.60	111.10	96.04	102.50
5	-7.00	105.57	106.72	108.45
6	-8.00	101.41	101.55	103.84
7	-9.00	99.74	112.68	97.42
8	-10.00	105.88	113.05	99.11

Assay Date	10/4/2005	Test Chemical ID	5	# Concentrations tested	8	
Technician ID	TD	Replicate #	3	Microsome type	recombina Microsome ID	Lot #5

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.)
= 0.864 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	recombinant	Microsome ID	Lot #5			

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.469	0.279	0.127	0.066	0.027	0.014	0.000	25	100	11343-77
	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	0.212
	0.237	0.029	0.202
			0.207

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	
M LE 3-22-06 BSL														
250	200	200	0.00025		200		0.0500		0.475	0.475	0.0482	m, b	0.105	-0.002
125	100	200	0.00013		200		0.0250		0.278	0.278	0.0275	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200		0.0100		0.127	0.127	0.0116	r^2, se_y	0.992	0.002
25	100	1000	0.00003		200		0.0050		0.063	0.063	0.0049	F, df	512	4
10	40	1000	0.00001		200		0.0020		0.026	0.026	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									
							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	0.212	0.212	0.021	200	80	4800	0.006	0.006	6.007
Microsomes	0.202	0.202	0.020	200	80	4800	0.006		
Microsomes	0.207	0.207	0.020	200	80	4800	0.006		

Assay Date	10/4/2005	Test Chemical ID	5	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	Lot #5

								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	0.212
	0.237	0.029	0.202
			0.207

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}
W LE, 3-22-06 BOC							
250	200	200	0.00025	200	0.0500	0.475	0.475
125	100	200	0.00013	200	0.0250	0.278	0.278
50	200	1000	0.00005	200	0.0100	0.127	0.127
25	100	1000	0.00003	200	0.0050	0.063	0.063
10	40	1000	0.00001	200	0.0020	0.026	0.026
5	20	1000	0.00001	200	0.0010	0.015	0.015
			Blank	0.000			

r^2= 0.997
m= 0.091
b= -0.001

Curve Output	Variables	Regression results	
0.0426	m, b	0.091	-0.001
0.0246	se_m, se_b	0.003	0.000
0.0109	r^2, se_y	0.997	0.001
0.0051	F, df	1019	3
0.0017	ss_{reg}, ss_{resid}	0.000	0.000
0.0007			

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	0.212	0.212	0.019	200	80	4800	0.006	0.005	5.455
Microsomes	0.202	0.202	0.018	200	80	4800	0.005		
Microsomes	0.207	0.207	0.018	200	80	4800	0.005		

Assay Date	10/4/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	recombina	Microsome ID	Lot #5	Technician ID	TD	Replicate #	3
			5										

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.455479
Protein Concentration (dilution added to assay, mg/mL):	0.008659

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	S	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot #5	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	5826	11652.2	11646.6	23293.2	0.1	113587	20.51	23189	0.0422	1	0.004	15	0.3251
			0.5	2	5821	11641											
	2	2	0.5	1	5388	10775	10720.9	21441.8	0.1	113587	18.88	21338	0.0389	1	0.004	15	0.2991
			0.5	2	5333	10666.8											
	3	2	0.5	1	5229	10458.8	10407.9	20815.8	0.1	113587	18.33	20712	0.0377	1	0.004	15	0.2903
			0.5	2	5179	10357											
	4	2	0.5	1	4850	9700.4	9844.8	19689.6	0.1	113587	17.33	19585	0.0357	1	0.004	15	0.2746
			0.5	2	4995	9989.2											
Background control	1	2	0.5	1	30	60.66	59.58	119.16	0.1	113587	0.10	15	0.0000	1	0.004	15	0.0002
			0.5	2	29	58.3											
	2	2	0.5	1	16	32.34	30.03	60.06	0.1	113587	0.05	-44	-0.0001	1	0.004	15	-0.0006
			0.5	2	14	27.72											
	3	2	0.5	1	34	68.8	57.45	114.9	0.1	113587	0.10	11	0.0000	1	0.004	15	0.0001
			0.5	2	23	46.1											
	4	2	0.5	1	28	57.24	61.54	123.08	0.1	113587	0.11	19	0.0000	1	0.004	15	0.0003
			0.5	2	33	65.84											
Positive control	1	2	0.5	1	3451	6901.6	6764.5	13529	0.1	113587	11.91	13425	0.0244	1	0.004	15	0.1882
			0.5	2	3314	6627.4											
	2	2	0.5	1	3151	6302.4	6260.1	12520.2	0.1	113587	11.02	12416	0.0226	1	0.004	15	0.1741
			0.5	2	3109	6217.8											
	3	2	0.5	1	2937	5874.6	5889.5	11779	0.1	113587	10.37	11675	0.0213	1	0.004	15	0.1637
			0.5	2	2952	5904.4											
	4	2	0.5	1	2973	5946	5939.9	11879.8	0.1	113587	10.46	11776	0.0214	1	0.004	15	0.1651
			0.5	2	2967	5933.8											
Negative Control	1	2	0.5	1	5804	11606.2	11819.6	23639.2	0.1	113587	20.81	23535	0.0429	1	0.004	15	0.3299
			0.5	2	6016	12031											
	2	2	0.5	1	5668	11336.8	11319.6	22639.6	0.1	113587	19.93	22535	0.0410	1	0.004	15	0.3159
			0.5	2	5651	11302.8											
	3	2	0.5	1	5439	10878	10945.3	21890.6	0.1	113587	19.27	21786	0.0397	1	0.004	15	0.3054
			0.5	2	5506	11012.6											
	4	2	0.5	1	5454	10907.8	10977.8	21955.6	0.1	113587	19.33	21851	0.0398	1	0.004	15	0.3063
			0.5	2	5524	11048											
5	1-1	2	0.5	1	5746	11491.6	11606.1	23212.2	0.1	113587	20.44	23108	0.0421	1	0.004	15	0.3239
			0.5	2	5860	11720.6											
	1-2	2	0.5	1	6035	12070.2	12015.5	24031	0.1	113587	21.16	23927	0.0436	1	0.004	15	0.3354
			0.5	2	5980	11960.8											
	1-3	2	0.5	1	5926	11852.2	11799.1	23598.2	0.1	113587	20.78	23494	0.0428	1	0.004	15	0.3293
			0.5	2	5873	11746											
	2-1	2	0.5	1	5775	11550.2	11405	22810	0.1	113587	20.08	22706	0.0413	1	0.004	15	0.3183
			0.5	2	5630	11259.8											
	2-2	2	0.5	1	5803	11606.6	11645.7	23291.4	0.1	113587	20.51	23187	0.0422	1	0.004	15	0.3250
			0.5	2	5842	11684.8											
	2-3	2	0.5	1	5492	10984.4	11066.9	22133.8	0.1	113587	19.49	22030	0.0401	1	0.004	15	0.3088
			0.5	2	5575	11149.4											
	3-1	2	0.5	1	6160	12320.4	12277.1	24554.2	0.1	113587	21.62	24450	0.0445	1	0.004	15	0.3427
			0.5	2	6117	12233.8											
	3-2	2	0.5	1	5847	11694.6	11729.8	23459.6	0.1	113587	20.65	23355	0.0425	1	0.004	15	0.3274
			0.5	2	5883	11765											
	3-3	2	0.5	1	5948	11896.4	12025.1	24050.2	0.1	113587	21.17	23946	0.0436	1	0.004	15	0.3357
			0.5	2	6077	12153.8											
	4-1	2	0.5	1	6083	12166.8	12122.3	24244.6	0.1	113587	21.34	24140	0.0440	1	0.004	15	0.3384
			0.5	2	6039	12077.8											
	4-2	2	0.5	1	5319	10638.6	10561.3	21122.6	0.1	113587	18.60	21018	0.0383	1	0.004	15	0.2946
			0.5	2	5242	10484											
	4-3	2	0.5	1	5891	11782.4	11752	23504	0.1	113587	20.69	23400	0.0426	1	0.004	15	0.3260
			0.5	2	5861	11721.5											
	5-1	2	0.5	1	5261	10521.8	10540.8	21081.6	0.1	113587	18.56	20977	0.0382	1	0.004	15	0.2941
			0.5	2	5280	10560											
	5-2	2	0.5	1	5590	11180.6	11187.6	22375.2	0.1	113587	19.70	22271	0.0406	1	0.004	15	0.3122
			0.5	2	5607	11214.6											
	5-3	2	0.5	1	5724	11447	11546.7	23093.4	0.1	113587	20.33	22989	0.0419	1	0.004	15	0.3223
			0.5	2	5823	11646.4											
	6-1	2	0.5	1	5841	11682.2	11661.9	23323.8	0.1	113587	20.53	23220	0.0423	1	0.004	15	0.3255
			0.5	2	5821	11641.6											
	6-2	2	0.5	1	5555	11109.2	11077.3	22154.6	0.1	113587	19.50	22050	0.0402	1	0.004	15	0.3091
			0.5	2	5523	11045.4											
	6-3	2	0.5	1	5650	11299.2	11183.4	22366.8	0.1	113587	19.69	22263	0.0405	1	0.004	15	0.3121
			0.5	2	5634	11267.6											
	7-1	2	0.5	1	5450	10899.2	10894.8	21789.6	0.1	113587	19.16	21685	0.0395	1	0.004	15	0.3040
			0.5	2	5445	10890.4											
	7-2	2	0.5	1	5889	11778.6	11725	23450	0.1	113587	20.65	23346	0.0425	1	0.004	15	0.3275
			0.5	2	5836	11671.4											
	7-3	2	0.5	1	5835	11670.4	11611.6	23223.2	0.1	113587	20.45	23119	0.0421	1	0.004	15	0.3241
			0.5	2	5776	11552.8											
	8-1	2	0.5	1	5599	11198	11138	22276	0.1	113587	19.61	22172	0.0404	1	0.004	15	0.3108
			0.5	2	5539	11078											
	8-2	2	0.5	1	5376	10752.6	10727.2	21454.4	0.1	113587	18.89	21350	0.0389	1	0.004	15	0.2993
			0.5	2	5351	10701.8											
	8-3	2	0.5	1	5465	10930.4	10885.9	21771.8	0.1	113587	19.17	21668	0.0395	1	0.004	15	0.3037
			0.5	2	5421	10841.4											

Assay Date	10/4/2005	Test Chemical ID	5	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot #5	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3121	0.0184
Full activity	End	0.2824	0.0112
Full activity	Overall	0.2973	0.0211
Background	Beginning	-0.0002	0.000585828
Background	End	0.0002	8.10842E-05
Background	Overall	0.0000	0.000416092
Positive	Beginning	0.1811	0.0100
Positive	End	0.1644	0.0010
Positive	Overall	0.1727	0.0113
Negative	Beginning	0.3229	0.0099
Negative	End	0.3059	0.0006
Negative	Overall	0.3144	0.0114

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
5	1	1	1.00E-04	-4.00	0.3239
5	1	2	1.00E-04	-4.00	0.3354
5	1	3	1.00E-04	-4.00	0.3293
5	2	1	1.00E-05	-5.00	0.3183
5	2	2	1.00E-05	-5.00	0.3250
5	2	3	1.00E-05	-5.00	0.3088
5	3	1	1.00E-06	-6.00	0.3427
5	3	2	1.00E-06	-6.00	0.3274
5	3	3	1.00E-06	-6.00	0.3357
5	4	1	2.50E-07	-6.60	0.3384
5	4	2	2.50E-07	-6.60	0.2946
5	4	3	2.50E-07	-6.60	0.3280
5	5	1	1.00E-07	-7.00	0.2941
5	5	2	1.00E-07	-7.00	0.3122
5	5	3	1.00E-07	-7.00	0.3223
5	6	1	1.00E-08	-8.00	0.3255
5	6	2	1.00E-08	-8.00	0.3091
5	6	3	1.00E-08	-8.00	0.3121
5	7	1	1.00E-09	-9.00	0.3040
5	7	2	1.00E-09	-9.00	0.3273
5	7	3	1.00E-09	-9.00	0.3241
5	8	1	1.00E-10	-10.00	0.3108
5	8	2	1.00E-10	-10.00	0.2993
5	8	3	1.00E-10	-10.00	0.3037

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	108.97	112.83	110.79
2	-5.00	107.07	109.34	103.88
3	-6.00	115.30	110.14	112.92
4	-6.60	113.84	99.12	110.35
5	-7.00	98.92	105.02	108.41
6	-8.00	109.50	103.98	104.98
7	-9.00	102.26	110.09	109.02
8	-10.00	104.55	100.68	102.18

Assay Date	8/5/2005	Test Chemical ID	6	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5				

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.520	0.307	0.137	0.073	0.032	0.021	0.000	25	100	11343-77
	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	0.213
	0.270	0.037	0.214
			0.215

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	
m iE 03-23-06 BDL											
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			

Regression results are calculated using the function LINEST

Blank	0.000		r^2=	0.993								
			m=	0.097								
			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	0.269	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	0.213	0.213	0.019	200	80	4800	0.006	0.006	5.607
Microsomes	0.214	0.214	0.019	200	80	4800	0.006		
Microsomes	0.215	0.215	0.019	200	80	4800	0.006		

Assay Date	8/5/2005	Test Chemical ID	6	# Concentrations tested	8						
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	Lot # 5				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.520	0.307	0.137	0.073	0.032	0.021	0.000	25	100	11343-77
	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	0.213
	0.270	0.037	0.214
			0.215

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	
M LE 03-23-06 BBL											
250	200	200	0.00025	200	0.0500	0.521	0.521	0.0429	m, b	0.084	-0.001
125	100	200	0.00013	200	0.0250	0.305	0.305	0.0248	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.135	0.135	0.0105	r^2, se_y	0.999	0.000
25	100	1000	0.00003	200	0.0050	0.074	0.074	0.0053	F, df	2308	3
10	40	1000	0.00001	200	0.0020	0.032	0.032	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.020	0.020	0.0007			

Regression results are calculated using the function LINEST

Blank	0.000	r^2=	0.999		
		m=	0.084		
		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	0.269	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	0.213	0.213	0.017	200	80	4800	0.005	0.005	5.130
Microsomes	0.214	0.214	0.017	200	80	4800	0.005		
Microsomes	0.215	0.215	0.017	200	80	4800	0.005		

Assay Date	8/5/2005	Test Chemical ID		# Concentrations tested	6	Microsome type	8	recombina Microsome ID	Lot # 5	Technician ID	TD	Replicate #	1

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.129848
Protein Concentration (dilution added to assay, mg/mL):	0.008143

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	8/5/2005	Test Chemical ID	6	# Concentrations tested	6	Microsome type	recombinant	Microsome ID	Lot # 5	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	8404	16808	16937.6	33875.2	0.1	154197	21.97	33643	0.0457	1	0.004	15	0.3743
			0.5	2	8534	17067.2											
	2	2	0.5	1	8292	16584.6	16853.8	33707.6	0.1	154197	21.80	33076	0.0449	1	0.004	15	0.3680
			0.5	2	8362	16723											
	3	2	0.5	1	7109	14218.4	14390.9	28781.8	0.1	154197	18.67	28550	0.0386	1	0.004	15	0.3176
			0.5	2	7282	14563.4											
	4	2	0.5	1	7822	15643.4	15681.4	31362.8	0.1	154197	20.34	31131	0.0423	1	0.004	15	0.3483
			0.5	2	7860	15719.4											
Background control	1	2	0.5	1	63	126.46	118.74	237.48	0.1	154197	0.15	5	0.0000	1	0.004	15	0.0001
			0.5	2	56	111.02											
	2	2	0.5	1	55	109.08	104.51	209.02	0.1	154197	0.14	-23	0.0000	1	0.004	15	-0.0003
			0.5	2	50	99.94											
	3	2	0.5	1	58	115.64	113.31	226.62	0.1	154197	0.15	-5	0.0000	1	0.004	15	-0.0001
			0.5	2	55	110.98											
	4	2	0.5	1	63	126.72	127.47	254.94	0.1	154197	0.17	23	0.0000	1	0.004	15	0.0003
			0.5	2	65	129.22											
Positive control	1	2	0.5	1	3600	7200.2	7245.9	14491.8	0.1	154197	9.40	14260	0.0194	1	0.004	15	0.1586
			0.5	2	3646	7291.6											
	2	2	0.5	1	3857	7714.2	7740.4	15480.8	0.1	154197	10.04	15249	0.0207	1	0.004	15	0.1696
			0.5	2	3883	7766.6											
	3	2	0.5	1	4243	8485.6	8484.7	16969.4	0.1	154197	11.01	16737	0.0227	1	0.004	15	0.1862
			0.5	2	4242	8483.8											
	4	2	0.5	1	4199	8398.4	8396.4	16792.8	0.1	154197	10.89	16561	0.0225	1	0.004	15	0.1842
			0.5	2	4197	8394.4											
Negative Control	1	2	0.5	1	8281	16561.4	16383.5	32767	0.1	154197	21.25	32535	0.0442	1	0.004	15	0.3620
			0.5	2	8103	16205.6											
	2	2	0.5	1	8172	16343	16415.8	32831.6	0.1	154197	21.29	32600	0.0443	1	0.004	15	0.3627
			0.5	2	8244	16488.6											
	3	2	0.5	1	7784	15568.8	15718.5	31437	0.1	154197	20.39	31205	0.0424	1	0.004	15	0.3472
			0.5	2	7934	15868.2											
	4	2	0.5	1	7638	15276.4	15339	30678	0.1	154197	19.90	30446	0.0414	1	0.004	15	0.3387
			0.5	2	7701	15401.6											
6	1-1	2	0.5	1	168	336.3	344.06	688.12	0.1	154197	0.45	456	0.0006	1	0.004	15	0.0051
			0.5	2	176	351.82											
	1-2	2	0.5	1	188	375.68	374.55	749.1	0.1	154197	0.49	517	0.0007	1	0.004	15	0.0058
			0.5	2	187	373.42											
	1-3	2	0.5	1	176	351.06	348.72	697.44	0.1	154197	0.45	485	0.0006	1	0.004	15	0.0052
			0.5	2	173	346.38											
	2-1	2	0.5	1	494	988.14	992.07	1984.14	0.1	154197	1.29	1752	0.0024	1	0.004	15	0.0195
			0.5	2	498	996											
	2-2	2	0.5	1	509	1017.14	999.6	1999.2	0.1	154197	1.30	1767	0.0024	1	0.004	15	0.0197
			0.5	2	491	982.06											
	2-3	2	0.5	1	486	971.06	992.44	1984.88	0.1	154197	1.29	1753	0.0024	1	0.004	15	0.0195
			0.5	2	507	1013.82											
	3-1	2	0.5	1	2829	5657.8	5675.2	11350.4	0.1	154197	7.36	11116	0.0151	1	0.004	15	0.1237
			0.5	2	2846	5692.6											
	3-2	2	0.5	1	2876	5752.6	5732.3	11464.6	0.1	154197	7.44	11233	0.0153	1	0.004	15	0.1250
			0.5	2	2856	5712											
	3-3	2	0.5	1	2662	5323.6	5366.6	10733.2	0.1	154197	6.96	10501	0.0143	1	0.004	15	0.1168
			0.5	2	2705	5409.6											
	4-1	2	0.5	1	6493	12986.6	12990.4	25980.8	0.1	154197	16.85	25749	0.0350	1	0.004	15	0.2865
			0.5	2	6497	12994.2											
	4-2	2	0.5	1	6560	13119.4	13153.7	26307.4	0.1	154197	17.06	26075	0.0354	1	0.004	15	0.2901
			0.5	2	6594	13188											
	4-3	2	0.5	1	6581	13161.8	13062.3	26124.6	0.1	154197	16.94	25893	0.0352	1	0.004	15	0.2881
			0.5	2	6481	12962.8											
	5-1	2	0.5	1	7686	15371.4	15216.3	30432.6	0.1	154197	19.74	30201	0.0410	1	0.004	15	0.3360
			0.5	2	7531	15061.2											
	5-2	2	0.5	1	7779	15557.8	15476.9	30953.8	0.1	154197	20.07	30722	0.0417	1	0.004	15	0.3418
			0.5	2	7698	15396											
	5-3	2	0.5	1	7891	15781	15600.3	31200.6	0.1	154197	20.23	30969	0.0421	1	0.004	15	0.3445
			0.5	2	7710	15419.6											
	6-1	2	0.5	1	8143	16285.2	16167.2	32334.4	0.1	154197	20.97	32102	0.0436	1	0.004	15	0.3571
			0.5	2	8025	16049.2											
	6-2	2	0.5	1	8016	16029.2	16161.6	32323.2	0.1	154197	20.96	32091	0.0436	1	0.004	15	0.3570
			0.5	2	8147	16294											
	6-3	2	0.5	1	7811	15621.2	15520.9	31041.8	0.1	154197	20.13	30810	0.0419	1	0.004	15	0.3428
			0.5	2	7710	15420.6											
	7-1	2	0.5	1	7862	15723.2	15731	31462	0.1	154197	20.40	31230	0.0424	1	0.004	15	0.3474
			0.5	2	7869	15738.8											
	7-2	2	0.5	1	7363	14726.8	14857.3	29714.6	0.1	154197	19.27	29483	0.0401	1	0.004	15	0.3280
			0.5	2	7494	14987.8											
	7-3	2	0.5	1	7846	15692.6	15524.3	31048.6	0.1	154197	20.14	30817	0.0419	1	0.004	15	0.3428
			0.5	2	7678	15356											
	8-1	2	0.5	1	7814	15627.4	15733.1	31466.2	0.1	154197	20.41	31234	0.0424	1	0.004	15	0.3475
			0.5	2	7919	15838.8											
	8-2	2	0.5	1	7890	15779.2	15641.5	31283	0.1	154197	20.29	31051	0.0422	1	0.004	15	0.3455
			0.5	2	7752	15503.8											
	8-3	2	0.5	1	7966	15931	15903.9	31807.8	0.1	154197	20.63	31576	0.0429	1	0.004	15	0.3513
			0.5	2	7938	15876.8											

Assay Date	8/5/2005	Test Chemical ID	6	# Concentrations tested	Microsome 8 type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.3711	0.0045
Full activity	End	0.3320	0.0203
Full activity	Overall	0.3516	0.0256
Background	Beginning	-0.0001	0.000223888
Background	End	0.0001	0.000222787
Background	Overall	0.0000	0.000214317
Positive	Beginning	0.1641	0.0078
Positive	End	0.1852	0.0014
Positive	Overall	0.1747	0.0130
Negative	Beginning	0.3623	0.0005
Negative	End	0.3429	0.0060
Negative	Overall	0.3526	0.0117

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
6	1	1	1.00E-03	-3.00	0.0051
6	1	2	1.00E-03	-3.00	0.0058
6	1	3	1.00E-03	-3.00	0.0052
6	2	1	1.00E-04	-4.00	0.0195
6	2	2	1.00E-04	-4.00	0.0197
6	2	3	1.00E-04	-4.00	0.0195
6	3	1	1.00E-05	-5.00	0.1237
6	3	2	1.00E-05	-5.00	0.1250
6	3	3	1.00E-05	-5.00	0.1168
6	4	1	1.00E-06	-6.00	0.2865
6	4	2	1.00E-06	-6.00	0.2901
6	4	3	1.00E-06	-6.00	0.2881
6	5	1	1.00E-07	-7.00	0.3360
6	5	2	1.00E-07	-7.00	0.3418
6	5	3	1.00E-07	-7.00	0.3445
6	6	1	1.00E-08	-8.00	0.3571
6	6	2	1.00E-08	-8.00	0.3570
6	6	3	1.00E-08	-8.00	0.3428
6	7	1	1.00E-09	-9.00	0.3474
6	7	2	1.00E-09	-9.00	0.3280
6	7	3	1.00E-09	-9.00	0.3428
6	8	1	1.00E-10	-10.00	0.3475
6	8	2	1.00E-10	-10.00	0.3455
6	8	3	1.00E-10	-10.00	0.3513

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.44	1.64	1.47
2	-4.00	5.54	5.59	5.55
3	-5.00	35.18	35.55	33.23
4	-6.00	81.48	82.52	81.94
5	-7.00	95.57	97.22	98.00
6	-8.00	101.59	101.55	97.50
7	-9.00	98.83	93.30	97.52
8	-10.00	98.84	98.26	99.92

Assay Date	8/8/2005	Test Chemical ID	6	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinar	Microsome ID	Lot # 5

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	08/08/2005	Test Chemical ID	6	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	Lot # 5

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	0.212
	0.263	0.034	0.210
			0.206

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	0.520	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	0.212	0.212	0.019	200	80	4800	0.006	0.006	5.566
Microsomes	0.210	0.210	0.019	200	80	4800	0.006		
Microsomes	0.206	0.206	0.018	200	80	4800	0.005		

Assay Date	08/08/2005	Test Chemical ID	6	# Concentrations tested	8					
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	Lot # 5			

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	0.212
	0.263	0.034	0.210
			0.206

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	0.520	0.520	0.0435
125	100	200	0.00013	200	0.0250	0.298	0.298	0.0246
50	200	1000	0.00005	200	0.0100	0.134	0.134	0.0107
25	100	1000	0.00003	200	0.0050	0.072	0.072	0.0055
10	40	1000	0.00001	200	0.0020	0.027	0.027	0.0016
5	20	1000	0.00001	200	0.0010	0.015	0.015	0.0006

Variables	Regression results	
m, b	0.085	-0.001
se_m, se_b	0.003	0.000
r^2, se_y	0.997	0.001
F, df	946	3
ss_{reg}, ss_{resid}	0.000	0.000

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	0.212	0.212	0.017	200	80	4800	0.005	0.005	5.132
Microsomes	0.210	0.210	0.017	200	80	4800	0.005		
Microsomes	0.206	0.206	0.017	200	80	4800	0.005		

Assay Date	8/8/2005	Test Chemical ID	6	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	Technician ID	TD	Replicate #	2

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	41	mL total volume
	10.25	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	615	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.132042
Protein Concentration (dilution added to assay, mg/mL):	0.008345

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	2.50E-06
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-09

Assay Date	8/8/2005	Test Chemical ID	6	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	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	7808	15616.8	15627.4	31254.8	0.1	148900	20.99	30988	0.0445	1	0.004	15	0.3559
			0.5	2	7819	15638											
	2	2	0.5	1	8416	16831.6	16614.1	33228.2	0.1	148900	22.32	32961	0.0474	1	0.004	15	0.3786
			0.5	2	8198	16396.6											
	3	2	0.5	1	7636	15271.6	15187.2	30374.4	0.1	148900	20.40	30108	0.0433	1	0.004	15	0.3458
			0.5	2	7551	15102.8											
	4	2	0.5	1	7644	15288	15392.8	30785.6	0.1	148900	20.68	30519	0.0439	1	0.004	15	0.3505
			0.5	2	7749	15497.6											
Background control	1	2	0.5	1	72	143.04	128.24	256.48	0.1	148900	0.17	-10	0.0000	1	0.004	15	-0.0001
			0.5	2	57	113.44											
	2	2	0.5	1	60	119.46	130.79	261.58	0.1	148900	0.18	-5	0.0000	1	0.004	15	-0.0001
			0.5	2	71	142.12											
	3	2	0.5	1	77	154.16	147.55	295.1	0.1	148900	0.20	28	0.0000	1	0.004	15	0.0003
			0.5	2	70	140.94											
	4	2	0.5	1	67	134	126.89	253.78	0.1	148900	0.17	-13	0.0000	1	0.004	15	-0.0001
			0.5	2	60	119.78											
Positive control	1	2	0.5	1	4015	8029.4	8019.3	16038.6	0.1	148900	10.77	15772	0.0227	1	0.004	15	0.1811
			0.5	2	4005	8009.2											
	2	2	0.5	1	3795	7589.4	7540.5	15081	0.1	148900	10.13	14814	0.0213	1	0.004	15	0.1701
			0.5	2	3746	7491.6											
	3	2	0.5	1	4213	8426.4	8282.4	16564.8	0.1	148900	11.12	16298	0.0234	1	0.004	15	0.1872
			0.5	2	4069	8138.4											
	4	2	0.5	1	4201	8402.6	8347.5	16695	0.1	148900	11.21	16428	0.0236	1	0.004	15	0.1887
			0.5	2	4146	8292.4											
Negative Control	1	2	0.5	1	8022	16043	16165.3	32330.6	0.1	148900	21.71	32064	0.0461	1	0.004	15	0.3683
			0.5	2	8144	16287.6											
	2	2	0.5	1	7992	15984.4	16229.1	32458.2	0.1	148900	21.80	32191	0.0463	1	0.004	15	0.3697
			0.5	2	8237	16473.8											
	3	2	0.5	1	7921	15841.8	15806.9	31613.6	0.1	148900	21.23	31347	0.0451	1	0.004	15	0.3600
			0.5	2	7886	15772											
	4	2	0.5	1	7914	15827.6	15867.6	31735.2	0.1	148900	21.31	31468	0.0452	1	0.004	15	0.3614
			0.5	2	7954	15907.6											
6	1-1	2	0.5	1	206	412.02	390.34	780.68	0.1	148900	0.52	514	0.0007	1	0.004	15	0.0059
			0.5	2	184	368.66											
	1-2	2	0.5	1	182	364.46	360.95	721.9	0.1	148900	0.48	455	0.0007	1	0.004	15	0.0052
			0.5	2	179	357.44											
	1-3	2	0.5	1	176	352.44	355.3	710.6	0.1	148900	0.48	444	0.0006	1	0.004	15	0.0051
			0.5	2	179	358.16											
	2-1	2	0.5	1	512	1024.68	1049.82	2099.64	0.1	148900	1.41	1833	0.0026	1	0.004	15	0.0211
			0.5	2	537	1074.96											
	2-2	2	0.5	1	516	1031.02	1023.24	2046.48	0.1	148900	1.37	1780	0.0026	1	0.004	15	0.0204
			0.5	2	508	1015.46											
	2-3	2	0.5	1	503	1006.52	1022.11	2044.22	0.1	148900	1.37	1777	0.0026	1	0.004	15	0.0204
			0.5	2	519	1037.7											
	3-1	2	0.5	1	2991	5982.4	5929.7	11859.4	0.1	148900	7.96	11593	0.0167	1	0.004	15	0.1331
			0.5	2	2939	5877											
	3-2	2	0.5	1	3011	6021.6	6039	12078	0.1	148900	8.11	11811	0.0170	1	0.004	15	0.1357
			0.5	2	3028	6056.4											
	3-3	2	0.5	1	3024	6048.4	5912.1	11824.2	0.1	148900	7.94	11557	0.0166	1	0.004	15	0.1327
			0.5	2	2888	5775.8											
	4-1	2	0.5	1	5466	10932.4	10913.4	21826.8	0.1	148900	14.66	21560	0.0310	1	0.004	15	0.2476
			0.5	2	5447	10894.4											
	4-2	2	0.5	1	5424	10847.4	10879.1	21758.2	0.1	148900	14.61	21491	0.0309	1	0.004	15	0.2468
			0.5	2	5455	10910.8											
	4-3	2	0.5	1	5394	10787.8	10845.5	21691	0.1	148900	14.57	21424	0.0308	1	0.004	15	0.2461
			0.5	2	5452	10903.2											
	5-1	2	0.5	1	6490	12979	12883.4	25766.8	0.1	148900	17.30	25500	0.0367	1	0.004	15	0.2929
			0.5	2	6394	12787.8											
	5-2	2	0.5	1	6423	12846	12800.8	25601.6	0.1	148900	17.19	25335	0.0364	1	0.004	15	0.2910
			0.5	2	6378	12755.6											
	5-3	2	0.5	1	6723	13445.6	13285.8	26571.6	0.1	148900	17.85	26305	0.0378	1	0.004	15	0.3021
			0.5	2	6563	13126											
	6-1	2	0.5	1	8041	16081.2	15882.9	31765.8	0.1	148900	21.33	31499	0.0453	1	0.004	15	0.3618
			0.5	2	7842	15684.6											
	6-2	2	0.5	1	8125	16250	16306.2	32612.4	0.1	148900	21.90	32346	0.0465	1	0.004	15	0.3715
			0.5	2	8181	16362.4											
	6-3	2	0.5	1	7630	15259.4	15253.7	30507.4	0.1	148900	20.49	30241	0.0435	1	0.004	15	0.3473
			0.5	2	7624	15248											
	7-1	2	0.5	1	8108	16216.6	16142.5	32285	0.1	148900	21.68	32018	0.0460	1	0.004	15	0.3677
			0.5	2	8034	16068.4											
	7-2	2	0.5	1	7725	15450.6	15534	31068	0.1	148900	20.87	30801	0.0443	1	0.004	15	0.3538
			0.5	2	7809	15617.4											
	7-3	2	0.5	1	7899	15798.6	15799.8	31599.6	0.1	148900	21.22	31333	0.0450	1	0.004	15	0.3599
			0.5	2	7901	15801											
	8-1	2	0.5	1	7670	15340	15262	30524	0.1	148900	20.50	30257	0.0435	1	0.004	15	0.3475
			0.5	2	7592	15184											
	8-2	2	0.5	1	7764	15528.8	15583.4	31166.8	0.1	148900	20.93	30900	0.0444	1	0.004	15	0.3549
			0.5	2	7819	15638											
	8-3	2	0.5	1	7712	15424.2	15380.3	30760.6	0.1	148900	20.66	30494	0.0438	1	0.004	15	0.3502
			0.5	2	7668	15336.4											

Assay Date	8/8/2005	Test Chemical ID	6	# Concentrations tested	Microsome 8 type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate # 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3672	0.0160
Full activity	End	0.3482	0.0033
Full activity	Overall	0.3577	0.0145
Background	Beginning	-0.0001	4.14181E-05
Background	End	0.0001	0.000335568
Background	Overall	0.0000	0.000220337
Positive	Beginning	0.1756	0.0078
Positive	End	0.1879	0.0011
Positive	Overall	0.1818	0.0084
Negative	Beginning	0.3690	0.0010
Negative	End	0.3607	0.0010
Negative	Overall	0.3649	0.0048

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
6	1	1	1.00E-03	-3.00	0.0059
6	1	2	1.00E-03	-3.00	0.0052
6	1	3	1.00E-03	-3.00	0.0051
6	2	1	1.00E-04	-4.00	0.0211
6	2	2	1.00E-04	-4.00	0.0204
6	2	3	1.00E-04	-4.00	0.0204
6	3	1	1.00E-05	-5.00	0.1331
6	3	2	1.00E-05	-5.00	0.1357
6	3	3	1.00E-05	-5.00	0.1327
6	4	1	2.50E-06	-5.60	0.2476
6	4	2	2.50E-06	-5.60	0.2468
6	4	3	2.50E-06	-5.60	0.2461
6	5	1	1.00E-06	-6.00	0.2929
6	5	2	1.00E-06	-6.00	0.2910
6	5	3	1.00E-06	-6.00	0.3021
6	6	1	1.00E-07	-7.00	0.3618
6	6	2	1.00E-07	-7.00	0.3715
6	6	3	1.00E-07	-7.00	0.3473
6	7	1	1.00E-08	-8.00	0.3677
6	7	2	1.00E-08	-8.00	0.3538
6	7	3	1.00E-08	-8.00	0.3599
6	8	1	1.00E-09	-9.00	0.3475
6	8	2	1.00E-09	-9.00	0.3549
6	8	3	1.00E-09	-9.00	0.3502

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.65	1.46	1.43
2	-4.00	5.89	5.71	5.71
3	-5.00	37.22	37.92	37.11
4	-5.60	69.23	69.01	68.79
5	-6.00	81.88	81.35	84.46
6	-7.00	101.14	103.86	97.10
7	-8.00	102.81	98.90	100.61
8	-9.00	97.15	99.22	97.91

Assay Date	8/9/2005	Test Chemical ID	6	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.524	0.313	0.136	0.062	0.020	0.009	0.000	25	100	11343-77
	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	0.203
	0.261	0.026	0.194
			0.196

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	
IN; LE 03-22-06 BBC											
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			

Regression results are calculated using the function LINEST

Blank	0.000	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	0.203	0.203	0.018	200	80	4800	0.005	0.005	5.289
Microsomes	0.194	0.194	0.017	200	80	4800	0.005		
Microsomes	0.196	0.196	0.018	200	80	4800	0.005		

Assay Date	8/9/2005	Test Chemical ID		6	# Concentrations tested		8				
Technician ID	TD	Replicate #		3	Microsome type	recombinant	Microsome ID	Lot # 5			
									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	0.203								
	0.261	0.026	0.194								
			0.196								

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	
m, LE05-22-06 bk2											
250	200	200	0.00025	200	0.0500	0.522	0.522	0.0416	m, b	0.079	0.000
125	100	200	0.00013	200	0.0250	0.307	0.307	0.0246	se_m, se_b	0.003	0.000
50	200	1000	0.00005	200	0.0100	0.135	0.135	0.0109	r^2, se_y	0.996	0.001
25	100	1000	0.00003	200	0.0050	0.063	0.063	0.0052	F, df	828	3
10	40	1000	0.00001	200	0.0020	0.017	0.017	0.0016	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.006	0.006	0.0007			

Regression results are calculated using the function LINEST

Blank	0.000	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 100.	028	0.028	0.002	200	1	1	0.000	0.000	0.012
QC 100.	026	0.026	0.002	200	1	1	0.000		
QC 10									
Microsomes	203	0.203	0.016	200	80	4800	0.005	0.005	4.771
Microsomes	0.194	0.194	0.016	200	80	4800	0.005		
Microsomes	0.196	0.196	0.016	200	80	4800	0.005		

Assay Date	8/9/2005	Test Chemical ID		# Concentrations tested	6	Microsome type	8	recombinai Microsome ID	Lot # 5	Technician ID	TD	Replicate #	3

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.770837
Protein Concentration (dilution added to assay, mg/mL):	0.007573

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	2.50E-06
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-09

Assay Date	8/9/2005	Test Chemical ID	6	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	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	8089	16177.2	16198.4	32396.8	0.1	153795	21.06	32139	0.0429	1	0.004	15	0.3781
			0.5	2	8110	16219.6											
	2	2	0.5	1	8151	16301.2	16128	32256	0.1	153795	20.97	31999	0.0428	1	0.004	15	0.3764
			0.5	2	7977	15954.8											
	3	2	0.5	1	7795	15589.6	15820	31640	0.1	153795	20.57	31383	0.0419	1	0.004	15	0.3692
			0.5	2	8025	16050.4											
	4	2	0.5	1	7943	15886.2	15922.8	31845.6	0.1	153795	20.71	31588	0.0422	1	0.004	15	0.3716
			0.5	2	7980	15959.4											
Background control	1	2	0.5	1	63	126.06	124.89	249.78	0.1	153795	0.16	-8	0.0000	1	0.004	15	-0.0001
			0.5	2	62	123.72											
	2	2	0.5	1	70	140.58	142.69	285.38	0.1	153795	0.19	28	0.0000	1	0.004	15	0.0003
			0.5	2	72	144.8											
	3	2	0.5	1	62	123.52	128.57	257.14	0.1	153795	0.17	0	0.0000	1	0.004	15	0.0000
			0.5	2	67	133.62											
	4	2	0.5	1	58	116.08	118.81	237.62	0.1	153795	0.15	-20	0.0000	1	0.004	15	-0.0002
			0.5	2	61	121.54											
Positive control	1	2	0.5	1	4304	8607.6	8606.2	17212.4	0.1	153795	11.19	16955	0.0227	1	0.004	15	0.1995
			0.5	2	4302	8604.8											
	2	2	0.5	1	4039	8077.4	8179.4	16358.8	0.1	153795	10.64	16101	0.0215	1	0.004	15	0.1894
			0.5	2	4141	8281.4											
	3	2	0.5	1	3818	7636.4	7734.5	15469	0.1	153795	10.06	15212	0.0203	1	0.004	15	0.1790
			0.5	2	3916	7832.6											
	4	2	0.5	1	4181	8362.2	8298.9	16597.8	0.1	153795	10.79	16340	0.0218	1	0.004	15	0.1922
			0.5	2	4118	8235.6											
Negative Control	1	2	0.5	1	7757	15513	15647.2	31294.4	0.1	153795	20.35	31037	0.0415	1	0.004	15	0.3651
			0.5	2	7891	15781.4											
	2	2	0.5	1	7992	15983	16115.7	32231.4	0.1	153795	20.96	31974	0.0427	1	0.004	15	0.3762
			0.5	2	8124	16248.4											
	3	2	0.5	1	7852	15703.2	15813.9	31627.8	0.1	153795	20.56	31370	0.0419	1	0.004	15	0.3691
			0.5	2	7962	15924.6											
	4	2	0.5	1	7173	14345.4	14507.2	29014.4	0.1	153795	18.87	28757	0.0384	1	0.004	15	0.3383
			0.5	2	7335	14669											
6	1-1	2	0.5	1	166	331.78	333.59	667.18	0.1	153795	0.43	410	0.0005	1	0.004	15	0.0048
			0.5	2	168	335.4											
	1-2	2	0.5	1	172	344.36	332.07	664.14	0.1	153795	0.43	407	0.0005	1	0.004	15	0.0048
			0.5	2	160	319.78											
	1-3	2	0.5	1	168	335.56	345.06	690.12	0.1	153795	0.45	433	0.0006	1	0.004	15	0.0051
			0.5	2	177	354.56											
	2-1	2	0.5	1	514	1027.32	1031.96	2063.92	0.1	153795	1.34	1806	0.0024	1	0.004	15	0.0213
			0.5	2	518	1036.6											
	2-2	2	0.5	1	521	1042.06	1036.47	2072.94	0.1	153795	1.35	1815	0.0024	1	0.004	15	0.0214
			0.5	2	515	1030.88											
	2-3	2	0.5	1	491	982.6	1011.26	2022.52	0.1	153795	1.32	1765	0.0024	1	0.004	15	0.0208
			0.5	2	520	1039.92											
	3-1	2	0.5	1	2953	5905.4	5868.7	11737.4	0.1	153795	7.63	11480	0.0153	1	0.004	15	0.1351
			0.5	2	2916	5832											
	3-2	2	0.5	1	2849	5697.2	5692.9	11385.8	0.1	153795	7.40	11128	0.0149	1	0.004	15	0.1309
			0.5	2	2844	5688.6											
	3-3	2	0.5	1	2986	5971.2	5993.7	11987.4	0.1	153795	7.79	11730	0.0157	1	0.004	15	0.1380
			0.5	2	3008	6016.2											
	4-1	2	0.5	1	5391	10782.6	10765	21530	0.1	153795	14.00	21273	0.0284	1	0.004	15	0.2503
			0.5	2	5374	10747.4											
	4-2	2	0.5	1	5459	10918.4	10861.2	21722.4	0.1	153795	14.12	21465	0.0287	1	0.004	15	0.2525
			0.5	2	5402	10804											
	4-3	2	0.5	1	5281	10561.6	10687.7	21375.4	0.1	153795	13.90	21118	0.0282	1	0.004	15	0.2484
			0.5	2	5407	10813.8											
	5-1	2	0.5	1	6566	13132.8	13119.5	26239	0.1	153795	17.06	25982	0.0347	1	0.004	15	0.3057
			0.5	2	6553	13106.2											
	5-2	2	0.5	1	6512	13023.4	13010.2	26020.4	0.1	153795	16.92	25763	0.0344	1	0.004	15	0.3031
			0.5	2	6499	12997											
	5-3	2	0.5	1	6463	12925.2	12918	25836	0.1	153795	16.80	25579	0.0342	1	0.004	15	0.3009
			0.5	2	6455	12910.8											
	6-1	2	0.5	1	8085	16169.2	16006.1	32012.2	0.1	153795	20.81	31755	0.0424	1	0.004	15	0.3736
			0.5	2	7922	15843											
	6-2	2	0.5	1	7482	14964	15196.9	30393.8	0.1	153795	19.76	30136	0.0403	1	0.004	15	0.3545
			0.5	2	7715	15429.8											
	6-3	2	0.5	1	7269	14537.6	14766.3	29532.6	0.1	153795	19.20	29275	0.0391	1	0.004	15	0.3444
			0.5	2	7498	14995											
	7-1	2	0.5	1	7891	15782	15711	31422	0.1	153795	20.43	31165	0.0416	1	0.004	15	0.3666
			0.5	2	7820	15640											
	7-2	2	0.5	1	8310	16619.6	16673.3	33346.6	0.1	153795	21.68	33089	0.0442	1	0.004	15	0.3893
			0.5	2	8364	16727											
	7-3	2	0.5	1	7916	15831	15998.9	31997.8	0.1	153795	20.81	31740	0.0424	1	0.004	15	0.3734
			0.5	2	8083	16166.8											
	8-1	2	0.5	1	8490	16980.6	16918.4	33836.8	0.1	153795	22.00	33579	0.0449	1	0.004	15	0.3950
			0.5	2	8428	16856.2											
	8-2	2	0.5	1	7053	14105.4	14176.5	28353	0.1	153795	18.44	28096	0.0375	1	0.004	15	0.3305
			0.5	2	7124	14247.6											
	8-3	2	0.5	1	7895	15789.6	15917.7	31835.4	0.1	153795	20.70	31578	0.0422	1	0.004	15	0.3715
			0.5	2	8023	16045.8											

Assay Date	8/9/2005	Test Chemical ID	6	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3773	0.0012
Full activity	End	0.3704	0.0017
Full activity	Overall	0.3738	0.0041
Background	Beginning	0.0001	0.000296145
Background	End	-0.0001	0.00016238
Background	Overall	0.0000	0.000238427
Positive	Beginning	0.1944	0.0071
Positive	End	0.1856	0.0094
Positive	Overall	0.1900	0.0085
Negative	Beginning	0.3706	0.0078
Negative	End	0.3537	0.0217
Negative	Overall	0.3622	0.0165

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
6	1	1	1.00E-03	-3.00	0.0048
6	1	2	1.00E-03	-3.00	0.0048
6	1	3	1.00E-03	-3.00	0.0051
6	2	1	1.00E-04	-4.00	0.0213
6	2	2	1.00E-04	-4.00	0.0214
6	2	3	1.00E-04	-4.00	0.0208
6	3	1	1.00E-05	-5.00	0.1351
6	3	2	1.00E-05	-5.00	0.1309
6	3	3	1.00E-05	-5.00	0.1380
6	4	1	2.50E-06	-5.60	0.2503
6	4	2	2.50E-06	-5.60	0.2525
6	4	3	2.50E-06	-5.60	0.2484
6	5	1	1.00E-06	-6.00	0.3057
6	5	2	1.00E-06	-6.00	0.3031
6	5	3	1.00E-06	-6.00	0.3009
6	6	1	1.00E-07	-7.00	0.3736
6	6	2	1.00E-07	-7.00	0.3545
6	6	3	1.00E-07	-7.00	0.3444
6	7	1	1.00E-08	-8.00	0.3666
6	7	2	1.00E-08	-8.00	0.3893
6	7	3	1.00E-08	-8.00	0.3734
6	8	1	1.00E-09	-9.00	0.3950
6	8	2	1.00E-09	-9.00	0.3305
6	8	3	1.00E-09	-9.00	0.3715

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.29	1.28	1.36
2	-4.00	5.68	5.71	5.55
3	-5.00	36.13	35.02	36.91
4	-5.60	66.94	67.55	66.46
5	-6.00	81.76	81.07	80.49
6	-7.00	99.93	94.84	92.13
7	-8.00	98.07	104.13	99.88
8	-9.00	105.67	88.41	99.37

Assay Date	8/17/2005	Test Chemical ID	7	# Concentrations tested	8	
Technician ID	TD	Replicate #	1	Microsome type	recombinai Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5		

								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			
	0.508	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			

Samples:	QC 100	QC 10	Microsomes
	0.257	0.029	0.202
	0.255	0.027	0.201
			0.198

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		0.506	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	0.202	0.202	0.018	200	80	4800	0.006	0.005	5.492
Microsomes	0.201	0.201	0.018	200	80	4800	0.006		
Microsomes	0.198	0.198	0.018	200	80	4800	0.005		

Assay Date	8/17/2005	Test Chemical ID	7	# Concentrations tested	8						
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	Lot # 5				

								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			
	0.508	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			

Samples:	QC 100	QC 10	Microsomes
	0.257	0.029	0.202
	0.255	0.027	0.201
			0.198

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	
M, LE 03-22-06 LSC											
250	200	200	0.00025	200	0.0500	0.506	0.506	0.0422	m, b	0.084	0.000
125	100	200	0.00013	200	0.0250	0.299	0.299	0.0248	se_m, se_b	0.001	0.000
50	200	1000	0.00005	200	0.0100	0.126	0.126	0.0103	r^2, se_y	0.999	0.000
25	100	1000	0.00003	200	0.0050	0.064	0.064	0.0052	F, df	4806	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	0.013	0.013	0.0009			

Regression results are calculated using the function LINEST

Blank	0.000		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	0.257	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	0.202	0.202	0.017	200	80	4800	0.005	0.005		4.980
Microsomes	0.201	0.201	0.017	200	80	4800	0.005			
Microsomes	0.198	0.198	0.016	200	80	4800	0.005			

Assay Date	8/17/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	recombina	Microsome ID Lot #	5	Technician ID TD	Replicate # 1

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.980144
Protein Concentration (dilution added to assay, mg/mL):	0.007905

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	8/17/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	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	8350	16700.4	16906.1	33812.2	0.1	157922	21.41	33565	0.0432	1	0.004	15	0.3647
			0.5	2	8556	17111.8											
	2	2	0.5	1	8537	17073.4	16965	33930	0.1	157922	21.49	33682	0.0434	1	0.004	15	0.3660
			0.5	2	8428	16856.6											
	3	2	0.5	1	6096	12192.8	12151.5	24303	0.1	157922	15.39	24055	0.0310	1	0.004	15	0.2614
			0.5	2	6055	12110.2											
	4	2	0.5	1	6357	12714.4	12840.2	25680.4	0.1	157922	16.26	25433	0.0328	1	0.004	15	0.2764
			0.5	2	6483	12966											
Background control	1	2	0.5	1	58	116.2	118.36	236.72	0.1	157922	0.15	-11	0.0000	1	0.004	15	-0.0001
			0.5	2	60	120.52											
	2	2	0.5	1	71	142.54	132.42	264.84	0.1	157922	0.17	17	0.0000	1	0.004	15	0.0002
			0.5	2	61	122.3											
	3	2	0.5	1	63	126.48	119	238	0.1	157922	0.15	-10	0.0000	1	0.004	15	-0.0001
			0.5	2	56	111.52											
	4	2	0.5	1	61	122.44	125.39	250.78	0.1	157922	0.16	3	0.0000	1	0.004	15	0.0000
			0.5	2	64	128.34											
Positive control	1	2	0.5	1	4571	9142.8	9219.2	18438.4	0.1	157922	11.68	18191	0.0234	1	0.004	15	0.1977
			0.5	2	4648	9295.6											
	2	2	0.5	1	4840	9680.6	9646.2	19292.4	0.1	157922	12.22	19045	0.0245	1	0.004	15	0.2070
			0.5	2	4806	9611.8											
	3	2	0.5	1	3218	6436.2	6472.9	12945.8	0.1	157922	8.20	12698	0.0164	1	0.004	15	0.1380
			0.5	2	3255	6509.6											
	4	2	0.5	1	3396	6792.2	6853.4	13706.8	0.1	157922	8.68	13459	0.0173	1	0.004	15	0.1463
			0.5	2	3457	6914.6											
Negative Control	1	2	0.5	1	8136	16272.6	16896.7	33393.4	0.1	157922	21.15	33146	0.0427	1	0.004	15	0.3602
			0.5	2	8560	17120.8											
	2	2	0.5	1	7685	15370	15383.1	30766.2	0.1	157922	19.48	30519	0.0393	1	0.004	15	0.3316
			0.5	2	7698	15396.2											
	3	2	0.5	1	6882	13763.8	13727.2	27454.4	0.1	157922	17.38	27207	0.0351	1	0.004	15	0.2957
			0.5	2	6845	13690.6											
	4	2	0.5	1	6328	12656.8	12733.5	25467	0.1	157922	16.13	25219	0.0325	1	0.004	15	0.2741
			0.5	2	6405	12810.2											
7	1-1	2	0.5	1	88	175.1	163.31	326.62	0.1	157922	0.21	79	0.0001	1	0.004	15	0.0009
			0.5	2	76	151.52											
	1-2	2	0.5	1	59	118.54	125.22	250.44	0.1	157922	0.16	3	0.0000	1	0.004	15	0.0000
			0.5	2	66	131.9											
	1-3	2	0.5	1	74	147.74	142.36	284.72	0.1	157922	0.18	37	0.0000	1	0.004	15	0.0004
			0.5	2	68	136.98											
	2-1	2	0.5	1	73	145.3	144.83	289.66	0.1	157922	0.18	42	0.0001	1	0.004	15	0.0005
			0.5	2	72	144.36											
	2-2	2	0.5	1	72	144.72	140.75	281.5	0.1	157922	0.18	34	0.0000	1	0.004	15	0.0004
			0.5	2	68	136.78											
	2-3	2	0.5	1	64	128.74	127.49	254.98	0.1	157922	0.16	7	0.0000	1	0.004	15	0.0001
			0.5	2	63	126.24											
	3-1	2	0.5	1	55	109.72	105.72	211.44	0.1	157922	0.13	-36	0.0000	1	0.004	15	-0.0004
			0.5	2	51	101.72											
	3-2	2	0.5	1	54	107.94	109.65	219.3	0.1	157922	0.14	-28	0.0000	1	0.004	15	-0.0003
			0.5	2	56	111.36											
	3-3	2	0.5	1	56	111.6	110.81	221.62	0.1	157922	0.14	-26	0.0000	1	0.004	15	-0.0003
			0.5	2	56	110.02											
	4-1	2	0.5	1	64	127.58	132.7	265.4	0.1	157922	0.17	18	0.0000	1	0.004	15	0.0002
			0.5	2	69	137.82											
	4-2	2	0.5	1	78	156.5	155.71	311.42	0.1	157922	0.20	64	0.0001	1	0.004	15	0.0007
			0.5	2	77	154.92											
	4-3	2	0.5	1	87	173.7	204.15	408.3	0.1	157922	0.26	161	0.0002	1	0.004	15	0.0017
			0.5	2	117	234.6											
	5-1	2	0.5	1	190	380.52	366.65	733.3	0.1	157922	0.46	486	0.0006	1	0.004	15	0.0053
			0.5	2	176	352.78											
	5-2	2	0.5	1	166	331.02	329.77	659.54	0.1	157922	0.42	412	0.0005	1	0.004	15	0.0045
			0.5	2	164	328.52											
	5-3	2	0.5	1	158	315.08	324.54	649.08	0.1	157922	0.41	401	0.0005	1	0.004	15	0.0044
			0.5	2	167	333											
	6-1	2	0.5	1	940	1879.56	1922.63	3845.26	0.1	157922	2.43	3598	0.0046	1	0.004	15	0.0391
			0.5	2	963	1965.7											
	6-2	2	0.5	1	995	1990.84	1987.28	3974.56	0.1	157922	2.52	3727	0.0048	1	0.004	15	0.0405
			0.5	2	992	1983.72											
	6-3	2	0.5	1	1017	2034.02	2049.22	4098.44	0.1	157922	2.60	3851	0.0050	1	0.004	15	0.0418
			0.5	2	1032	2064.42											
	7-1	2	0.5	1	3896	7792.8	7828.3	15656.6	0.1	157922	9.91	15409	0.0199	1	0.004	15	0.1674
			0.5	2	3932	7863.8											
	7-2	2	0.5	1	4350	8699.6	8659.4	17318.8	0.1	157922	10.97	17071	0.0220	1	0.004	15	0.1855
			0.5	2	4310	8619.2											
	7-3	2	0.5	1	4263	8525.6	8513.3	17026.6	0.1	157922	10.78	16779	0.0216	1	0.004	15	0.1823
			0.5	2	4251	8501											
	8-1	2	0.5	1	5766	11532	11601	23202	0.1	157922	14.69	22954	0.0296	1	0.004	15	0.2494
			0.5	2	5835	11670											
	8-2	2	0.5	1	6049	12097.4	12024.3	24048.6	0.1	157922	15.23	23801	0.0307	1	0.004	15	0.2586
			0.5	2	5976	11951.2											
	8-3	2	0.5	1	6135	12269.6	12296.8	24593.6	0.1	157922	15.57	24346	0.0314	1	0.004	15	0.2646
			0.5	2	6162	12324											

Assay Date	8/17/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot #	5	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.3654	0.0009
Full activity	End	0.2689	0.0106
Full activity	Overall	0.3171	0.0560
Background	Beginning	0.0000	0.000216078
Background	End	0.0000	9.82033E-05
Background	Overall	0.0000	0.000142777
Positive	Beginning	0.2023	0.0066
Positive	End	0.1421	0.0058
Positive	Overall	0.1722	0.0351
Negative	Beginning	0.3459	0.0202
Negative	End	0.2849	0.0153
Negative	Overall	0.3154	0.0382

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
7	1	1	1.00E-03	-3.00	0.0009
7	1	2	1.00E-03	-3.00	0.0000
7	1	3	1.00E-03	-3.00	0.0004
7	2	1	1.00E-04	-4.00	0.0005
7	2	2	1.00E-04	-4.00	0.0004
7	2	3	1.00E-04	-4.00	0.0001
7	3	1	1.00E-05	-5.00	-0.0004
7	3	2	1.00E-05	-5.00	-0.0003
7	3	3	1.00E-05	-5.00	-0.0003
7	4	1	1.00E-06	-6.00	0.0002
7	4	2	1.00E-06	-6.00	0.0007
7	4	3	1.00E-06	-6.00	0.0017
7	5	1	1.00E-07	-7.00	0.0053
7	5	2	1.00E-07	-7.00	0.0045
7	5	3	1.00E-07	-7.00	0.0044
7	6	1	1.00E-08	-8.00	0.0391
7	6	2	1.00E-08	-8.00	0.0405
7	6	3	1.00E-08	-8.00	0.0418
7	7	1	1.00E-09	-9.00	0.1674
7	7	2	1.00E-09	-9.00	0.1855
7	7	3	1.00E-09	-9.00	0.1823
7	8	1	1.00E-10	-10.00	0.2494
7	8	2	1.00E-10	-10.00	0.2586
7	8	3	1.00E-10	-10.00	0.2646

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	0.27	0.01	0.13
2	-4.00	0.14	0.12	0.03
3	-5.00	-0.12	-0.10	-0.09
4	-6.00	0.06	0.22	0.55
5	-7.00	1.66	1.41	1.38
6	-8.00	12.33	12.77	13.20
7	-9.00	52.80	58.50	57.49
8	-10.00	78.65	81.56	83.42

Assay Date	8/18/2005	Test Chemical ID	7	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.514	0.307	0.129	0.064	0.027	0.009	0.000	25	100	11343-77
	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	0.198
	0.261	0.029	0.202
			0.201

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	
M, LE 03-22-06 LBL														
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		r^2=	0.988					Regression results are calculated using the function LINEST	
							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	0.198	0.198	0.018	200	80	4800	0.005	0.006	5.506
Microsomes	0.202	0.202	0.018	200	80	4800	0.006		
Microsomes	0.201	0.201	0.018	200	80	4800	0.006		

Assay Date	8/18/2005	Test Chemical ID	7	# Concentrations tested	8						
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	Lot # 5				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.514	0.307	0.129	0.064	0.027	0.009	0.000	25	100	11343-77
	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	0.198
	0.261	0.029	0.202
			0.201

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	
µL, LE 03-22-06 BBC											
250	200	200	0.00025	200	0.0500	0.498	0.498	0.0410	m, b	0.083	0.000
125	100	200	0.00013	200	0.0250	0.302	0.302	0.0248	se_m, se_b	0.001	0.000
50	200	1000	0.00005	200	0.0100	0.128	0.128	0.0104	r^2, se_y	0.999	0.000
25	100	1000	0.00003	200	0.0050	0.064	0.064	0.0051	F, df	3683	3
10	40	1000	0.00001	200	0.0020	0.025	0.025	0.0019	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.011	0.011	0.0007			

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	0.262	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 100.	027	0.027	0.002	200	1	1	0.000	0.000	0.011
QC 100.	029	0.029	0.002	200	1	1	0.000		
QC 10									
Microsomes	198	0.198	0.016	200	80	4800	0.005	0.005	4.916
Microsomes	0.202	0.202	0.017	200	80	4800	0.005		
Microsomes	0.201	0.201	0.016	200	80	4800	0.005		

Assay Date	8/18/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	recombinar	Microsome ID	Lot # 5	Technician ID	TD	Replicate #	2

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.916317
Protein Concentration (dilution added to assay, mg/mL):	0.007804

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	2.50E-09
7	1.00E-09
8	1.00E-10

Assay Date	8/18/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	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	6929	13858	14083.1	28166.2	0.1	140696	20.02	27930	0.0423	1	0.004	15	0.3613
			0.5	2	7154	14308.2											
	2	2	0.5	1	6365	12730.2	12697.2	25394.4	0.1	140696	18.05	25158	0.0381	1	0.004	15	0.3254
			0.5	2	6332	12664.2											
	3	2	0.5	1	6041	12082.4	12084.6	24169.2	0.1	140696	17.18	23933	0.0362	1	0.004	15	0.3096
			0.5	2	6043	12086.8											
	4	2	0.5	1	6603	13205.8	13132.8	26265.6	0.1	140696	18.67	26029	0.0394	1	0.004	15	0.3367
			0.5	2	6530	13059.8											
Background control	1	2	0.5	1	68	135.8	127.88	255.76	0.1	140696	0.18	20	0.0000	1	0.004	15	0.0003
			0.5	2	60	119.96											
	2	2	0.5	1	59	118.24	122.64	245.28	0.1	140696	0.17	9	0.0000	1	0.004	15	0.0001
			0.5	2	64	127.04											
	3	2	0.5	1	54	108.52	113.96	227.92	0.1	140696	0.16	-8	0.0000	1	0.004	15	-0.0001
			0.5	2	60	119.4											
	4	2	0.5	1	52	103.02	107.91	215.82	0.1	140696	0.15	-20	0.0000	1	0.004	15	-0.0003
			0.5	2	56	112.8											
Positive control	1	2	0.5	1	3530	7059.8	7161	14322	0.1	140696	10.18	14086	0.0213	1	0.004	15	0.1822
			0.5	2	3631	7262.2											
	2	2	0.5	1	3676	7351	7318.7	14637.4	0.1	140696	10.40	14401	0.0218	1	0.004	15	0.1863
			0.5	2	3643	7286.4											
	3	2	0.5	1	3521	7041.2	7062.9	14125.8	0.1	140696	10.04	13890	0.0210	1	0.004	15	0.1797
			0.5	2	3542	7084.6											
	4	2	0.5	1	3716	7431.4	7406.6	14813.2	0.1	140696	10.53	14577	0.0221	1	0.004	15	0.1886
			0.5	2	3691	7381.8											
Negative Control	1	2	0.5	1	6100	12199.4	12251.5	24503	0.1	140696	17.42	24267	0.0367	1	0.004	15	0.3139
			0.5	2	6152	12303.6											
	2	2	0.5	1	6277	12554	12633.3	25266.6	0.1	140696	17.96	25030	0.0379	1	0.004	15	0.3238
			0.5	2	6356	12712.6											
	3	2	0.5	1	6027	12053.8	12119.8	24239.6	0.1	140696	17.23	24003	0.0363	1	0.004	15	0.3105
			0.5	2	6093	12185.8											
	4	2	0.5	1	6407	12813.8	12850.3	25700.6	0.1	140696	18.27	25464	0.0386	1	0.004	15	0.3294
			0.5	2	6443	12886.8											
7	1-1	2	0.5	1	75	149.32	146.7	293.4	0.1	140696	0.21	57	0.0001	1	0.004	15	0.0007
			0.5	2	72	144.08											
	1-2	2	0.5	1	51	101.48	108.92	217.84	0.1	140696	0.15	-18	0.0000	1	0.004	15	-0.0002
			0.5	2	58	116.36											
	1-3	2	0.5	1	54	107.4	112.16	224.32	0.1	140696	0.16	-12	0.0000	1	0.004	15	-0.0002
			0.5	2	58	116.92											
	2-1	2	0.5	1	72	144.36	140.64	281.28	0.1	140696	0.20	45	0.0001	1	0.004	15	0.0006
			0.5	2	68	136.9											
	2-2	2	0.5	1	65	129.96	119.41	238.82	0.1	140696	0.17	3	0.0000	1	0.004	15	0.0000
			0.5	2	54	108.86											
	2-3	2	0.5	1	52	104.94	112.22	224.44	0.1	140696	0.16	-12	0.0000	1	0.004	15	-0.0002
			0.5	2	60	119.5											
	3-1	2	0.5	1	64	128.3	137.2	274.4	0.1	140696	0.20	38	0.0001	1	0.004	15	0.0005
			0.5	2	73	146.1											
	3-2	2	0.5	1	76	152.6	145.43	290.86	0.1	140696	0.21	55	0.0001	1	0.004	15	0.0007
			0.5	2	69	138.26											
	3-3	2	0.5	1	59	118.78	125.78	251.56	0.1	140696	0.18	15	0.0000	1	0.004	15	0.0002
			0.5	2	66	132.78											
	4-1	2	0.5	1	185	369.62	370.47	740.94	0.1	140696	0.53	505	0.0008	1	0.004	15	0.0065
			0.5	2	186	371.32											
	4-2	2	0.5	1	181	362.26	365.52	731.04	0.1	140696	0.52	495	0.0007	1	0.004	15	0.0064
			0.5	2	184	368.78											
	4-3	2	0.5	1	184	367.34	362.92	725.84	0.1	140696	0.52	490	0.0007	1	0.004	15	0.0063
			0.5	2	179	358.5											
	5-1	2	0.5	1	1185	2369.8	2379	4758	0.1	140696	3.38	4522	0.0068	1	0.004	15	0.0585
			0.5	2	1194	2388.2											
	5-2	2	0.5	1	1187	2374.4	2385.3	4770.6	0.1	140696	3.39	4534	0.0069	1	0.004	15	0.0587
			0.5	2	1198	2396.2											
	5-3	2	0.5	1	1166	2331.2	2347.6	4695.2	0.1	140696	3.34	4459	0.0068	1	0.004	15	0.0577
			0.5	2	1182	2364											
	6-1	2	0.5	1	3353	6706.2	6798.9	13597.8	0.1	140696	9.66	13362	0.0202	1	0.004	15	0.1728
			0.5	2	3446	6891.6											
	6-2	2	0.5	1	3280	6559.6	6682.3	13364.6	0.1	140696	9.50	13128	0.0199	1	0.004	15	0.1698
			0.5	2	3403	6805											
	6-3	2	0.5	1	3379	6757.6	6801.1	13602.2	0.1	140696	9.67	13366	0.0202	1	0.004	15	0.1729
			0.5	2	3422	6844.6											
	7-1	2	0.5	1	5051	10101	10205.3	20410.6	0.1	140696	14.51	20174	0.0305	1	0.004	15	0.2610
			0.5	2	5155	10309.6											
	7-2	2	0.5	1	4965	9929	9864.4	19728.8	0.1	140696	14.02	19493	0.0295	1	0.004	15	0.2521
			0.5	2	4900	9799.8											
	7-3	2	0.5	1	4972	9944	10056.5	20113	0.1	140696	14.30	19877	0.0301	1	0.004	15	0.2571
			0.5	2	5085	10169											
	8-1	2	0.5	1	5872	11743	11790.2	23580.4	0.1	140696	16.76	23344	0.0353	1	0.004	15	0.3020
			0.5	2	5919	11837.4											
	8-2	2	0.5	1	5973	11945	12159.3	24318.6	0.1	140696	17.28	24082	0.0365	1	0.004	15	0.3115
			0.5	2	6187	12373.6											
	8-3	2	0.5	1	5905	11809	11945.1	23890.2	0.1	140696	16.98	23654	0.0358	1	0.004	15	0.3060
			0.5	2	6041	12081.2											

Assay Date	8/18/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3434	0.0254
Full activity	End	0.3231	0.0192
Full activity	Overall	0.3333	0.0217
Background	Beginning	0.0002	9.58576E-05
Background	End	-0.0002	0.000110675
Background	Overall	0.0000	0.000230059
Positive	Beginning	0.1842	0.0029
Positive	End	0.1841	0.0063
Positive	Overall	0.1842	0.0040
Negative	Beginning	0.3188	0.0070
Negative	End	0.3199	0.0134
Negative	Overall	0.3194	0.0087

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
7	1	1	1.00E-04	-4.00	0.0007
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	0.0006
7	2	2	1.00E-05	-5.00	0.0000
7	2	3	1.00E-05	-5.00	-0.0002
7	3	1	1.00E-06	-6.00	0.0005
7	3	2	1.00E-06	-6.00	0.0007
7	3	3	1.00E-06	-6.00	0.0002
7	4	1	1.00E-07	-7.00	0.0065
7	4	2	1.00E-07	-7.00	0.0064
7	4	3	1.00E-07	-7.00	0.0063
7	5	1	1.00E-08	-8.00	0.0585
7	5	2	1.00E-08	-8.00	0.0587
7	5	3	1.00E-08	-8.00	0.0577
7	6	1	2.50E-09	-8.60	0.1728
7	6	2	2.50E-09	-8.60	0.1698
7	6	3	2.50E-09	-8.60	0.1729
7	7	1	1.00E-09	-9.00	0.2610
7	7	2	1.00E-09	-9.00	0.2521
7	7	3	1.00E-09	-9.00	0.2571
7	8	1	1.00E-10	-10.00	0.3020
7	8	2	1.00E-10	-10.00	0.3115
7	8	3	1.00E-10	-10.00	0.3060

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.22	-0.07	-0.05
2	-5.00	0.18	0.01	-0.05
3	-6.00	0.15	0.21	0.06
4	-7.00	1.96	1.92	1.90
5	-8.00	17.55	17.60	17.31
6	-8.60	51.86	50.96	51.88
7	-9.00	78.31	75.66	77.15
8	-10.00	90.61	93.48	91.82

Assay Date	8/19/2005	Test Chemical ID	7	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.501	0.299	0.130	0.065	0.027	0.013	0.000	25	100	11343-77
	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	0.213
	0.253	0.032	0.204
			0.204

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	
MLF, 03-27-06 BSL											
250	200	200	0.00025	200	0.0500	0.502	0.502	0.0479	m, b	0.099	-0.002
125	100	200	0.00013	200	0.0250	0.301	0.301	0.0281	se_m, se_b	0.005	0.001
50	200	1000	0.00005	200	0.0100	0.130	0.130	0.0113	r^2, se_y	0.990	0.002
25	100	1000	0.00003	200	0.0050	0.066	0.066	0.0049	F, df	405	4
10	40	1000	0.00001	200	0.0020	0.027	0.027	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.013	0.013	-0.0003			

Regression results are calculated using the function LINEST

Blank	0.000	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	0.213	0.213	0.019	200	80	4800	0.006	0.006		5.646
Microsomes	0.204	0.204	0.019	200	80	4800	0.006			
Microsomes	0.204	0.204	0.019	200	80	4800	0.006			

Assay Date	8/19/2005	Test Chemical ID	7	# Concentrations tested	8						
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	Lot # 5				

								Protein stock (mg	Total volume of	
Standards:	250	125	50	25	10	5	0	BSA)	stock (mL)	Protein stock ID
	0.501	0.299	0.130	0.065	0.027	0.013	0.000	25	100	11343-77
	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	0.213
	0.253	0.032	0.204
			0.204

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	
MALE 03-22-06 BSL											
250	200	200	0.00025	200	0.0500	0.502	0.502	0.0416	m, b	0.084	0.000
125	100	200	0.00013	200	0.0250	0.301	0.301	0.0248	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.130	0.130	0.0105	r^2, se_y	0.999	0.000
25	100	1000	0.00003	200	0.0050	0.066	0.066	0.0051	F, df	2648	3
10	40	1000	0.00001	200	0.0020	0.027	0.027	0.0019	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=	0.999		
		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 100	031	0.031	0.002	200	1	1	0.000	0.000	0.011
QC 100	032	0.032	0.002	200	1	1	0.000		
QC 10									
Microsomes	213	0.213	0.017	200	80	4800	0.005	0.005	5.078
Microsomes	0.204	0.204	0.017	200	80	4800	0.005		
Microsomes	0.204	0.204	0.017	200	80	4800	0.005		

Assay Date	8/19/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	recombinai	Microsome ID	Lot # 5	Technician ID	TD	Replicate #	3

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.077643
Protein Concentration (dilution added to assay, mg/mL):	0.00806

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	2.50E-09
7	1.00E-09
8	1.00E-10

Assay Date	8/19/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	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	7540	15080.2	15082.8	30165.6	0.1	154267	19.55	29956	0.0410	1	0.004	15	0.3387
			0.5	2	7543	15085.4											
	2	2	0.5	1	7574	15148.8	15135.9	30271.8	0.1	154267	19.62	30063	0.0411	1	0.004	15	0.3399
			0.5	2	7562	15123											
	3	2	0.5	1	7037	14073	14207.2	28414.4	0.1	154267	18.42	28205	0.0386	1	0.004	15	0.3189
			0.5	2	7171	14341.4											
	4	2	0.5	1	6724	13448.6	13432.7	26865.4	0.1	154267	17.41	26656	0.0364	1	0.004	15	0.3014
			0.5	2	6708	13416.8											
Background control	1	2	0.5	1	62	124.68	112.64	225.28	0.1	154267	0.15	16	0.0000	1	0.004	15	0.0002
			0.5	2	50	100.6											
	2	2	0.5	1	57	114.2	102.2	204.4	0.1	154267	0.13	-5	0.0000	1	0.004	15	-0.0001
			0.5	2	45	90.2											
	3	2	0.5	1	46	92.36	99.22	198.44	0.1	154267	0.13	-11	0.0000	1	0.004	15	-0.0001
			0.5	2	53	106.08											
	4	2	0.5	1	55	110.76	104.4	208.8	0.1	154267	0.14	0	0.0000	1	0.004	15	0.0000
			0.5	2	49	98.04											
Positive control	1	2	0.5	1	3880	7759.2	7660.1	15320.2	0.1	154267	9.93	15111	0.0207	1	0.004	15	0.1709
			0.5	2	3781	7561											
	2	2	0.5	1	3927	7854.2	7901.3	15802.6	0.1	154267	10.24	15593	0.0213	1	0.004	15	0.1763
			0.5	2	3974	7948.4											
	3	2	0.5	1	3683	7365.6	7408.5	14817	0.1	154267	9.60	14608	0.0200	1	0.004	15	0.1652
			0.5	2	3726	7451.4											
	4	2	0.5	1	3675	7351.2	7279	14558	0.1	154267	9.44	14349	0.0196	1	0.004	15	0.1622
			0.5	2	3603	7206.8											
Negative Control	1	2	0.5	1	6972	13943.8	13920.4	27840.8	0.1	154267	18.05	27632	0.0378	1	0.004	15	0.3124
			0.5	2	6949	13897											
	2	2	0.5	1	7341	14682	14740.8	29481.6	0.1	154267	19.11	29272	0.0400	1	0.004	15	0.3310
			0.5	2	7400	14799.6											
	3	2	0.5	1	6770	13539.2	13584.6	27169.2	0.1	154267	17.61	26960	0.0369	1	0.004	15	0.3049
			0.5	2	6815	13630											
	4	2	0.5	1	6834	13668.4	13661.6	27323.2	0.1	154267	17.71	27114	0.0371	1	0.004	15	0.3066
			0.5	2	6827	13654.8											
7	1-1	2	0.5	1	50	99.74	102.9	205.8	0.1	154267	0.13	-3	0.0000	1	0.004	15	0.0000
			0.5	2	53	106.06											
	1-2	2	0.5	1	77	153.06	141	282	0.1	154267	0.18	73	0.0001	1	0.004	15	0.0008
			0.5	2	64	128.94											
	1-3	2	0.5	1	52	104.18	107.95	215.9	0.1	154267	0.14	7	0.0000	1	0.004	15	0.0001
			0.5	2	56	111.74											
	2-1	2	0.5	1	59	117.44	127.89	255.78	0.1	154267	0.17	47	0.0001	1	0.004	15	0.0005
			0.5	2	69	138.34											
	2-2	2	0.5	1	42	83.42	94.16	188.32	0.1	154267	0.12	-21	0.0000	1	0.004	15	-0.0002
			0.5	2	52	104.9											
	2-3	2	0.5	1	59	118.02	105.54	211.08	0.1	154267	0.14	2	0.0000	1	0.004	15	0.0000
			0.5	2	47	93.06											
	3-1	2	0.5	1	77	154.72	156.18	312.36	0.1	154267	0.20	103	0.0001	1	0.004	15	0.0012
			0.5	2	79	157.64											
	3-2	2	0.5	1	65	129.78	119.17	238.34	0.1	154267	0.15	29	0.0000	1	0.004	15	0.0003
			0.5	2	54	108.56											
	3-3	2	0.5	1	59	117.5	118.44	236.88	0.1	154267	0.15	28	0.0000	1	0.004	15	0.0003
			0.5	2	60	119.38											
	4-1	2	0.5	1	206	411.5	400.16	800.32	0.1	154267	0.52	591	0.0008	1	0.004	15	0.0067
			0.5	2	194	388.82											
	4-2	2	0.5	1	181	362	364.38	728.76	0.1	154267	0.47	520	0.0007	1	0.004	15	0.0059
			0.5	2	183	366.76											
	4-3	2	0.5	1	192	384.36	392.05	784.1	0.1	154267	0.51	575	0.0008	1	0.004	15	0.0065
			0.5	2	200	399.74											
	5-1	2	0.5	1	1204	2408.2	2419.3	4838.6	0.1	154267	3.14	4629	0.0063	1	0.004	15	0.0523
			0.5	2	1215	2430.4											
	5-2	2	0.5	1	1167	2333.6	2371.2	4742.4	0.1	154267	3.07	4533	0.0062	1	0.004	15	0.0513
			0.5	2	1204	2408.8											
	5-3	2	0.5	1	1225	2450.8	2438.4	4876.8	0.1	154267	3.16	4668	0.0064	1	0.004	15	0.0528
			0.5	2	1213	2426											
	6-1	2	0.5	1	3476	6951.2	7014.7	14029.4	0.1	154267	9.09	13820	0.0189	1	0.004	15	0.1563
			0.5	2	3539	7078.2											
	6-2	2	0.5	1	3365	6729.4	6836.3	13672.6	0.1	154267	8.86	13463	0.0184	1	0.004	15	0.1522
			0.5	2	3472	6943.2											
	6-3	2	0.5	1	3325	6649	6598.1	13196.2	0.1	154267	8.55	12987	0.0178	1	0.004	15	0.1469
			0.5	2	3274	6547.2											
	7-1	2	0.5	1	5389	10778.4	10836.2	21672.4	0.1	154267	14.05	21463	0.0293	1	0.004	15	0.2427
			0.5	2	5447	10894											
	7-2	2	0.5	1	4962	9924.4	9891.2	19782.4	0.1	154267	12.82	19573	0.0268	1	0.004	15	0.2213
			0.5	2	4929	9858											
	7-3	2	0.5	1	5003	10006.4	10295.2	20590.4	0.1	154267	13.35	20381	0.0279	1	0.004	15	0.2305
			0.5	2	5292	10584											
	8-1	2	0.5	1	7087	14174.6	14015.2	28030.4	0.1	154267	18.17	27821	0.0380	1	0.004	15	0.3146
			0.5	2	6928	13855.8											
	8-2	2	0.5	1	7014	14027.6	14140.5	28281	0.1	154267	18.33	28072	0.0384	1	0.004	15	0.3174
			0.5	2	7127	14253.4											
	8-3	2	0.5	1	6992	13983.8	14019.5	28039	0.1	154267	18.18	27830	0.0380	1	0.004	15	0.3147
			0.5	2	7028	14055.2											

Assay Date	8/19/2005	Test Chemical ID	7	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3393	0.0008
Full activity	End	0.3102	0.0124
Full activity	Overall	0.3248	0.0183
Background	Beginning	0.0001	0.000166949
Background	End	-0.0001	8.28347E-05
Background	Overall	0.0000	0.000130166
Positive	Beginning	0.1736	0.0039
Positive	End	0.1637	0.0021
Positive	Overall	0.1687	0.0062
Negative	Beginning	0.3217	0.0131
Negative	End	0.3057	0.0012
Negative	Overall	0.3137	0.0120

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	0.0008
7	1	3	1.00E-04	-4.00	0.0001
7	2	1	1.00E-05	-5.00	0.0005
7	2	2	1.00E-05	-5.00	-0.0002
7	2	3	1.00E-05	-5.00	0.0000
7	3	1	1.00E-06	-6.00	0.0012
7	3	2	1.00E-06	-6.00	0.0003
7	3	3	1.00E-06	-6.00	0.0003
7	4	1	1.00E-07	-7.00	0.0067
7	4	2	1.00E-07	-7.00	0.0059
7	4	3	1.00E-07	-7.00	0.0065
7	5	1	1.00E-08	-8.00	0.0523
7	5	2	1.00E-08	-8.00	0.0513
7	5	3	1.00E-08	-8.00	0.0528
7	6	1	2.50E-09	-8.60	0.1563
7	6	2	2.50E-09	-8.60	0.1522
7	6	3	2.50E-09	-8.60	0.1469
7	7	1	1.00E-09	-9.00	0.2427
7	7	2	1.00E-09	-9.00	0.2213
7	7	3	1.00E-09	-9.00	0.2305
7	8	1	1.00E-10	-10.00	0.3146
7	8	2	1.00E-10	-10.00	0.3174
7	8	3	1.00E-10	-10.00	0.3147

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	-0.01	0.25	0.02
2	-5.00	0.16	-0.07	0.01
3	-6.00	0.36	0.10	0.10
4	-7.00	2.06	1.81	2.00
5	-8.00	16.12	15.78	16.25
6	-8.60	48.12	46.88	45.22
7	-9.00	74.73	68.15	70.96
8	-10.00	96.87	97.74	96.90

Assay Date	8/23/2005	Test Chemical ID	8	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5			
									Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
									25	100	11343-77

Standards:	250	125	50	25	10	5	0
	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	0.196
	0.234	0.027	0.202
			0.197

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	
M LE, 03-22-06 DSL											
250	200	200	0.00025	200	0.0500	0.481	0.481	0.0485	m, b	0.104	-0.002
125	100	200	0.00013	200	0.0250	0.280	0.280	0.0276	se_m, se_b	0.004	0.001
50	200	1000	0.00005	200	0.0100	0.116	0.116	0.0104	r^2, se_y	0.994	0.002
25	100	1000	0.00003	200	0.0050	0.064	0.064	0.0050	F, df	715	4
10	40	1000	0.00001	200	0.0020	0.030	0.030	0.0014	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001	200	0.0010	0.017	0.017	0.0001			
				Blank	0.000						

Regression results are calculated using the function LINEST

r^2= 0.994
m= 0.104
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	0.232	0.232	0.022	200	1	1	0.000	0.000	0.113
QC 100	0.234	0.234	0.023	200	1	1	0.000		
QC 100									
QC 10	0.027	0.027	0.001	200	1	1	0.000	0.000	0.006
QC 10	0.027	0.027	0.001	200	1	1	0.000		
QC 10									
Microsomes	0.196	0.196	0.019	200	80	4800	0.006	0.006	5.687
Microsomes	0.202	0.202	0.019	200	80	4800	0.006		
Microsomes	0.197	0.197	0.019	200	80	4800	0.006		

Assay Date	8/23/2005	Test Chemical ID		8	# Concentrations tested		8					
Technician ID	TD	Replicate #		1	Microsome type		recombinant	Microsome ID	Lot # 5			
									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	0.196									
	0.234	0.027	0.202									
			0.197									

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	0.481	0.481	0.0434	m, b	0.092	-0.001
125	100	200	0.00013	200	0.0250	0.280	0.280	0.0250	se_m, se_b	0.001	0.000
50	200	1000	0.00005	200	0.0100	0.116	0.116	0.0099	r^2, se_y	1.000	0.000
25	100	1000	0.00003	200	0.0050	0.064	0.064	0.0051	F, df	29925	3
10	40	1000	0.00001	200	0.0020	0.030	0.030	0.0020	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.017	0.017	0.0009			
			Blank	0.000							

Regression results are calculated using the function LINEST

r^2= 1.000
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/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 100	027	0.027	0.002	200	1	1	0.000	0.000	0.009
QC 100	027	0.027	0.002	200	1	1	0.000		
QC 10									
Microsomes	196	0.196	0.017	200	80	4800	0.005	0.005	5.231
Microsomes	0.202	0.202	0.018	200	80	4800	0.005		
Microsomes	0.197	0.197	0.017	200	80	4800	0.005		

Assay Date	8/23/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	recombinai	Microsome ID	Lot # 5	Technician ID	TD	Replicate #	1

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.231346
Protein Concentration (dilution added to assay, mg/mL):	0.008304

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	8/23/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	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	7810	15619.6	15751.3	31502.6	0.1	159485	19.75	31267	0.0411	1	0.004	15	0.3300
			0.5	2	7942	15883											
	2	2	0.5	1	7559	15118.8	15044.2	30088.4	0.1	159485	18.87	29853	0.0392	1	0.004	15	0.3151
			0.5	2	7485	14969.6											
	3	2	0.5	1	7019	14038.2	14022.4	28044.8	0.1	159485	17.58	27809	0.0366	1	0.004	15	0.2935
			0.5	2	7003	14006.6											
	4	2	0.5	1	7429	14857	15057	30114	0.1	159485	18.88	29876	0.0393	1	0.004	15	0.3154
			0.5	2	7629	15257											
Background control	1	2	0.5	1	51	101.46	98.44	196.88	0.1	159485	0.12	-39	-0.0001	1	0.004	15	-0.0004
			0.5	2	48	95.42											
	2	2	0.5	1	62	123.8	132.4	264.8	0.1	159485	0.17	29	0.0000	1	0.004	15	0.0003
			0.5	2	71	141											
	3	2	0.5	1	70	139.12	127.87	255.74	0.1	159485	0.16	20	0.0000	1	0.004	15	0.0002
			0.5	2	58	116.62											
	4	2	0.5	1	57	113.4	112.52	225.04	0.1	159485	0.14	-11	0.0000	1	0.004	15	-0.0001
			0.5	2	56	111.64											
Positive control	1	2	0.5	1	4007	8013.4	7959.5	15919	0.1	159485	9.98	15683	0.0205	1	0.004	15	0.1655
			0.5	2	3953	7905.6											
	2	2	0.5	1	4087	8173.6	8234.5	16469	0.1	159485	10.33	16233	0.0213	1	0.004	15	0.1713
			0.5	2	4148	8295.4											
	3	2	0.5	1	3882	7764.8	7793.2	15585.4	0.1	159485	9.77	15351	0.0202	1	0.004	15	0.1620
			0.5	2	3911	7821.6											
	4	2	0.5	1	3955	7910.8	7999.1	15998.2	0.1	159485	10.03	15763	0.0207	1	0.004	15	0.1664
			0.5	2	4044	8087.4											
Negative Control	1	2	0.5	1	7750	15499.6	15669.2	31338.4	0.1	159485	19.65	31103	0.0409	1	0.004	15	0.3283
			0.5	2	7919	15838.8											
	2	2	0.5	1	7945	15890.2	15902.5	31805	0.1	159485	19.94	31569	0.0415	1	0.004	15	0.3332
			0.5	2	7957	15914.8											
	3	2	0.5	1	7364	14728	14671.2	29342.4	0.1	159485	18.40	29107	0.0383	1	0.004	15	0.3072
			0.5	2	7307	14614.4											
	4	2	0.5	1	7371	14742.8	14858.8	29717.6	0.1	159485	18.63	29482	0.0388	1	0.004	15	0.3112
			0.5	2	7487	14974.8											
8	1-1	2	0.5	1	2040	4079	4230.7	8461.4	0.1	159485	5.31	8226	0.0108	1	0.004	15	0.0868
			0.5	2	2191	4382.4											
	1-2	2	0.5	1	1972	3943.2	3953	7906	0.1	159485	4.96	7670	0.0101	1	0.004	15	0.0810
			0.5	2	1981	3962.8											
	1-3	2	0.5	1	2135	4269.6	4253.2	8506.4	0.1	159485	5.33	8271	0.0109	1	0.004	15	0.0873
			0.5	2	2118	4236.8											
	2-1	2	0.5	1	1888	3775.4	3842.1	7684.2	0.1	159485	4.82	7449	0.0098	1	0.004	15	0.0786
			0.5	2	1954	3908.8											
	2-2	2	0.5	1	1966	3931	3937.4	7874.8	0.1	159485	4.94	7639	0.0100	1	0.004	15	0.0806
			0.5	2	1972	3943.8											
	2-3	2	0.5	1	2032	4064.6	4008.5	8017	0.1	159485	5.03	7781	0.0102	1	0.004	15	0.0821
			0.5	2	1976	3952.4											
	3-1	2	0.5	1	3031	6061.2	6079.1	12158.2	0.1	159485	7.62	11923	0.0157	1	0.004	15	0.1258
			0.5	2	3049	6097											
	3-2	2	0.5	1	3718	7436.6	7486.8	14973.6	0.1	159485	9.39	14738	0.0194	1	0.004	15	0.1556
			0.5	2	3769	7537											
	3-3	2	0.5	1	4066	8132.8	8101.1	16202.2	0.1	159485	10.16	15967	0.0210	1	0.004	15	0.1685
			0.5	2	4035	8069.4											
	4-1	2	0.5	1	4038	8075	8122.6	16245.2	0.1	159485	10.19	16010	0.0210	1	0.004	15	0.1690
			0.5	2	4085	8170.2											
	4-2	2	0.5	1	5113	10226.8	10355.5	20711	0.1	159485	12.99	20475	0.0269	1	0.004	15	0.2161
			0.5	2	5242	10484.2											
	4-3	2	0.5	1	5354	10707.6	10804.6	21609.2	0.1	159485	13.55	21374	0.0281	1	0.004	15	0.2256
			0.5	2	5451	10901.6											
	5-1	2	0.5	1	5598	11195.8	11216.1	22432.2	0.1	159485	14.07	22197	0.0292	1	0.004	15	0.2343
			0.5	2	5618	11236.4											
	5-2	2	0.5	1	5653	11306.8	11382.2	22764.4	0.1	159485	14.27	22529	0.0296	1	0.004	15	0.2378
			0.5	2	5729	11457.6											
	5-3	2	0.5	1	5717	11434.4	11463.9	22927.8	0.1	159485	14.38	22692	0.0298	1	0.004	15	0.2395
			0.5	2	5747	11493.4											
	6-1	2	0.5	1	5690	11379.2	11271.7	22543.4	0.1	159485	14.14	22308	0.0293	1	0.004	15	0.2355
			0.5	2	5582	11164.2											
	6-2	2	0.5	1	5223	10446.4	10428.7	20857.4	0.1	159485	13.08	20622	0.0271	1	0.004	15	0.2177
			0.5	2	5206	10411											
	6-3	2	0.5	1	5766	11532.8	11392.8	22785.6	0.1	159485	14.29	22550	0.0296	1	0.004	15	0.2380
			0.5	2	5626	11252.8											
	7-1	2	0.5	1	5341	10681.6	10762.5	21525	0.1	159485	13.50	21289	0.0280	1	0.004	15	0.2247
			0.5	2	5422	10843.4											
	7-2	2	0.5	1	5616	11232.6	11187.9	22375.8	0.1	159485	14.03	22140	0.0291	1	0.004	15	0.2337
			0.5	2	5572	11143.2											
	7-3	2	0.5	1	5707	11413.4	11432.3	22864.6	0.1	159485	14.34	22629	0.0297	1	0.004	15	0.2388
			0.5	2	5726	11451.2											
	8-1	2	0.5	1	5579	11157.8	11085.8	22171.6	0.1	159485	13.90	21936	0.0288	1	0.004	15	0.2315
			0.5	2	5507	11013.8											
	8-2	2	0.5	1	5633	11266.2	11228.8	22457.6	0.1	159485	14.08	22222	0.0292	1	0.004	15	0.2345
			0.5	2	5596	11191.4											
	8-3	2	0.5	1	5623	11245.8	11462	22924	0.1	159485	14.37	22688	0.0298	1	0.004	15	0.2395
			0.5	2	5839	11678.2											

Assay Date	8/23/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.3225	0.0106
Full activity	End	0.3044	0.0154
Full activity	Overall	0.3135	0.0150
Background	Beginning	-0.0001	0.000506905
Background	End	0.0001	0.000229122
Background	Overall	0.0000	0.0003264
Positive	Beginning	0.1684	0.0041
Positive	End	0.1642	0.0031
Positive	Overall	0.1663	0.0038
Negative	Beginning	0.3307	0.0035
Negative	End	0.3092	0.0028
Negative	Overall	0.3200	0.0127

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
8	1	1	1.00E-04	-4.00	0.0868
8	1	2	1.00E-04	-4.00	0.0810
8	1	3	1.00E-04	-4.00	0.0873
8	2	1	1.00E-05	-5.00	0.0786
8	2	2	1.00E-05	-5.00	0.0806
8	2	3	1.00E-05	-5.00	0.0821
8	3	1	1.00E-06	-6.00	0.1258
8	3	2	1.00E-06	-6.00	0.1556
8	3	3	1.00E-06	-6.00	0.1685
8	4	1	2.50E-07	-6.60	0.1690
8	4	2	2.50E-07	-6.60	0.2161
8	4	3	2.50E-07	-6.60	0.2256
8	5	1	1.00E-07	-7.00	0.2343
8	5	2	1.00E-07	-7.00	0.2378
8	5	3	1.00E-07	-7.00	0.2395
8	6	1	1.00E-08	-8.00	0.2355
8	6	2	1.00E-08	-8.00	0.2177
8	6	3	1.00E-08	-8.00	0.2380
8	7	1	1.00E-09	-9.00	0.2247
8	7	2	1.00E-09	-9.00	0.2337
8	7	3	1.00E-09	-9.00	0.2388
8	8	1	1.00E-10	-10.00	0.2315
8	8	2	1.00E-10	-10.00	0.2345
8	8	3	1.00E-10	-10.00	0.2395

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	27.69	25.82	27.85
2	-5.00	25.08	25.72	26.20
3	-6.00	40.14	49.62	53.76
4	-6.60	53.90	68.94	71.96
5	-7.00	74.73	75.85	76.40
6	-8.00	75.11	69.43	75.92
7	-9.00	71.68	74.54	76.19
8	-10.00	73.85	74.82	76.39

Assay Date	8/24/2005	Test Chemical ID	8	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinar	Microsome ID	Lot # 5

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	recombinant	Microsome ID	Lot # 5			
									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	0.199								
	0.244	0.031	0.201								
			0.199								

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	0.475	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						Regression results are calculated using the function LINEST	
					r^2=	0.991					
					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	0.242	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	0.199	0.199	0.019	200	80	4800	0.006	0.006	5.732
Microsomes	0.201	0.201	0.019	200	80	4800	0.006		
Microsomes	0.199	0.199	0.019	200	80	4800	0.006		

Assay Date	8/24/2005	Test Chemical ID		8	# Concentrations tested		8				
Technician ID	TD	Replicate #		2	Microsome type		recombinant	Microsome ID	Lot # 5		
									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	0.199								
	0.244	0.031	0.201								
			0.199								

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	
MILE 03-27-06 BBL											
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							

Regression results are calculated using the function LINEST

r^2= 0.999
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/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 100.	030	0.030	0.002	200	1	1	0.000	0.000	0.010
QC 100.	031	0.031	0.002	200	1	1	0.000		
QC 10									
Microsomes	199	0.199	0.017	200	80	4800	0.005	0.005	5.179
Microsomes	0.201	0.201	0.017	200	80	4800	0.005		
Microsomes	0.199	0.199	0.017	200	80	4800	0.005		

Assay Date	8/24/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	recombinai	Microsome ID Lot #	5	Technician ID	TD	Replicate #	2

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

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	1.00E-07
7	1.00E-08
8	1.00E-09

Protein Concentration (stock microsomes, mg/mL):	5.179305
Protein Concentration (dilution added to assay, mg/mL):	0.008221

Battelle Study No. G608317

B-137

Assay Date	8/24/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	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	7015	14030.4	14032.8	28065.6	0.1	157017	17.87	27820	0.0380	1	0.004	15	0.3083
			0.5	2	7018	14035.2											
	2	2	0.5	1	6611	13222.6	13279.9	26559.8	0.1	157017	16.92	26315	0.0360	1	0.004	15	0.2917
			0.5	2	6669	13337.2											
	3	2	0.5	1	7079	14157.8	14105.1	28210.2	0.1	157017	17.97	27965	0.0382	1	0.004	15	0.3099
			0.5	2	7026	14052.4											
	4	2	0.5	1	6633	13266	13359.9	26719.8	0.1	157017	17.02	26475	0.0362	1	0.004	15	0.2934
			0.5	2	6727	13453.8											
Background control	1	2	0.5	1	64	127.42	128.81	257.62	0.1	157017	0.16	12	0.0000	1	0.004	15	0.0001
			0.5	2	65	130.2											
	2	2	0.5	1	52	104.08	113.5	227	0.1	157017	0.14	-18	0.0000	1	0.004	15	-0.0002
			0.5	2	61	122.92											
	3	2	0.5	1	78	156.22	141.2	282.4	0.1	157017	0.18	37	0.0001	1	0.004	15	0.0004
			0.5	2	63	126.18											
	4	2	0.5	1	53	106.94	106.83	213.66	0.1	157017	0.14	-32	0.0000	1	0.004	15	-0.0003
			0.5	2	53	106.72											
Positive control	1	2	0.5	1	3635	7271.4	7199	14398	0.1	157017	9.17	14153	0.0193	1	0.004	15	0.1569
			0.5	2	3563	7126.6											
	2	2	0.5	1	3766	7531.4	7585.9	15171.8	0.1	157017	9.66	14927	0.0204	1	0.004	15	0.1654
			0.5	2	3820	7640.4											
	3	2	0.5	1	3574	7147.6	7175.1	14350.2	0.1	157017	9.14	14105	0.0193	1	0.004	15	0.1563
			0.5	2	3601	7202.6											
	4	2	0.5	1	3420	6839.4	6785.7	13571.4	0.1	157017	8.64	13326	0.0182	1	0.004	15	0.1477
			0.5	2	3366	6732											
Negative Control	1	2	0.5	1	5737	11474.2	11645.6	23291.2	0.1	157017	14.83	23046	0.0315	1	0.004	15	0.2554
			0.5	2	5909	11817											
	2	2	0.5	1	6827	13654	13535.1	27070.2	0.1	157017	17.24	26825	0.0367	1	0.004	15	0.2973
			0.5	2	6708	13416.2											
	3	2	0.5	1	6529	13058.8	13122.3	26244.6	0.1	157017	16.71	25999	0.0355	1	0.004	15	0.2882
			0.5	2	6593	13185.8											
	4	2	0.5	1	6859	13718.8	13890.7	27781.4	0.1	157017	17.69	27536	0.0376	1	0.004	15	0.3052
			0.5	2	7031	14062.6											
8	1-1	2	0.5	1	1583	3166.6	3163.5	6327	0.1	157017	4.03	6082	0.0083	1	0.004	15	0.0674
			0.5	2	1580	3160.4											
	1-2	2	0.5	1	1546	3092.8	3118.1	6236.2	0.1	157017	3.97	5991	0.0082	1	0.004	15	0.0664
			0.5	2	1572	3143.4											
	1-3	2	0.5	1	1746	3492.4	3565.8	7131.6	0.1	157017	4.54	6886	0.0094	1	0.004	15	0.0763
			0.5	2	1820	3639.2											
	2-1	2	0.5	1	1804	3608	3661.7	7323.4	0.1	157017	4.66	7078	0.0097	1	0.004	15	0.0785
			0.5	2	1858	3715.4											
	2-2	2	0.5	1	1946	3892.2	3949.8	7899.6	0.1	157017	5.03	7654	0.0105	1	0.004	15	0.0848
			0.5	2	2004	4007.4											
	2-3	2	0.5	1	1850	3699.8	3754.2	7508.4	0.1	157017	4.78	7263	0.0099	1	0.004	15	0.0805
			0.5	2	1904	3808.6											
	3-1	2	0.5	1	2681	5362.6	5476.4	10952.8	0.1	157017	6.98	10708	0.0146	1	0.004	15	0.1167
			0.5	2	2795	5590.2											
	3-2	2	0.5	1	2378	4755	4820.9	9641.8	0.1	157017	6.14	9397	0.0128	1	0.004	15	0.1041
			0.5	2	2443	4886.8											
	3-3	2	0.5	1	2870	5740.6	5577.7	11155.4	0.1	157017	7.10	10910	0.0149	1	0.004	15	0.1209
			0.5	2	2707	5414.8											
	4-1	2	0.5	1	3746	7492.6	7599.6	15199.2	0.1	157017	9.68	14954	0.0204	1	0.004	15	0.1657
			0.5	2	3853	7706.6											
	4-2	2	0.5	1	3982	7963.2	8011.3	16022.6	0.1	157017	10.20	15777	0.0216	1	0.004	15	0.1749
			0.5	2	4030	8059.4											
	4-3	2	0.5	1	2202	4404.4	4434.6	8869.2	0.1	157017	5.65	8624	0.0118	1	0.004	15	0.0956
			0.5	2	2232	4464.8											
	5-1	2	0.5	1	3843	7685.4	7701.7	15403.4	0.1	157017	9.81	15158	0.0207	1	0.004	15	0.1680
			0.5	2	3859	7718											
	5-2	2	0.5	1	4412	8824.4	8866.9	17733.8	0.1	157017	11.29	17489	0.0239	1	0.004	15	0.1938
			0.5	2	4455	8909.4											
	5-3	2	0.5	1	4808	9615.2	9540.1	19080.2	0.1	157017	12.15	18835	0.0257	1	0.004	15	0.2088
			0.5	2	4733	9465											
	6-1	2	0.5	1	4899	9798.8	9953	19906	0.1	157017	12.68	19661	0.0269	1	0.004	15	0.2179
			0.5	2	5054	10107.2											
	6-2	2	0.5	1	5363	10725	10534.2	21268.4	0.1	157017	13.55	21023	0.0287	1	0.004	15	0.2330
			0.5	2	5272	10543.4											
	6-3	2	0.5	1	5516	11032.6	11141.3	22282.6	0.1	157017	14.19	22037	0.0301	1	0.004	15	0.2443
			0.5	2	5625	11250											
	7-1	2	0.5	1	6209	12417.4	12563.5	25127	0.1	157017	16.00	24882	0.0340	1	0.004	15	0.2758
			0.5	2	6355	12709.6											
	7-2	2	0.5	1	6269	12538	12574.6	25149.2	0.1	157017	16.02	24904	0.0340	1	0.004	15	0.2760
			0.5	2	6306	12611.2											
	7-3	2	0.5	1	6470	12940.8	12926.5	25853	0.1	157017	16.47	25608	0.0350	1	0.004	15	0.2838
			0.5	2	6456	12912.2											
	8-1	2	0.5	1	5720	11439.2	11778.5	23557	0.1	157017	15.00	23312	0.0319	1	0.004	15	0.2584
			0.5	2	6059	12117.8											
	8-2	2	0.5	1	6265	12529	12646.1	25292.2	0.1	157017	16.11	25047	0.0342	1	0.004	15	0.2776
			0.5	2	6382	12763.2											
	8-3	2	0.5	1	5984	11968	12093.1	24186.2	0.1	157017	15.40	23941	0.0327	1	0.004	15	0.2653
			0.5	2	6109	12218.2											

Assay Date	8/24/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3000	0.0118
Full activity	End	0.3017	0.0117
Full activity	Overall	0.3008	0.0096
Background	Beginning	0.0000	0.000239975
Background	End	0.0000	0.000538729
Background	Overall	0.0000	0.00034246
Positive	Beginning	0.1612	0.0061
Positive	End	0.1520	0.0061
Positive	Overall	0.1566	0.0072
Negative	Beginning	0.2764	0.0296
Negative	End	0.2967	0.0120
Negative	Overall	0.2865	0.0219

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
8	1	1	1.00E-04	-4.00	0.0674
8	1	2	1.00E-04	-4.00	0.0664
8	1	3	1.00E-04	-4.00	0.0763
8	2	1	1.00E-05	-5.00	0.0785
8	2	2	1.00E-05	-5.00	0.0848
8	2	3	1.00E-05	-5.00	0.0805
8	3	1	5.00E-06	-5.30	0.1187
8	3	2	5.00E-06	-5.30	0.1041
8	3	3	5.00E-06	-5.30	0.1209
8	4	1	2.50E-06	-5.60	0.1657
8	4	2	2.50E-06	-5.60	0.1749
8	4	3	2.50E-06	-5.60	0.0956
8	5	1	1.00E-06	-6.00	0.1680
8	5	2	1.00E-06	-6.00	0.1938
8	5	3	1.00E-06	-6.00	0.2088
8	6	1	1.00E-07	-7.00	0.2179
8	6	2	1.00E-07	-7.00	0.2330
8	6	3	1.00E-07	-7.00	0.2443
8	7	1	1.00E-08	-8.00	0.2758
8	7	2	1.00E-08	-8.00	0.2760
8	7	3	1.00E-08	-8.00	0.2838
8	8	1	1.00E-09	-9.00	0.2584
8	8	2	1.00E-09	-9.00	0.2776
8	8	3	1.00E-09	-9.00	0.2653

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	22.41	22.07	25.37
2	-5.00	26.08	28.20	26.76
3	-5.30	39.45	34.62	40.19
4	-5.60	55.09	58.13	31.77
5	-6.00	55.84	64.43	69.39
6	-7.00	72.43	77.45	81.19
7	-8.00	91.67	91.75	94.34
8	-9.00	85.88	92.28	88.20

Assay Date	8/25/2005	Test Chemical ID	8	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinar	Microsome ID	Lot # 5

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		recombinant	Microsome ID	Lot # 5			

								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	0.285	0.121	0.066	0.029	0.015	0.000			

Samples:	QC 100	QC 10	Microsomes
	0.248	0.029	0.199
	0.249	0.029	0.201
			0.205

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	
IN LE3-22-06 BBL														
250	200	200	0.00025		200		0.0500		0.492	0.492	0.0484	m, b	0.102	-0.002
125	100	200	0.00013		200		0.0250		0.286	0.286	0.0274	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200		0.0100		0.125	0.125	0.0110	r^2, se_y	0.994	0.002
25	100	1000	0.00003		200		0.0050		0.066	0.066	0.0050	F, df	646	4
10	40	1000	0.00001		200		0.0020		0.029	0.029	0.0012	ss_{regr}, 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=	0.994						
							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	0.199	0.199	0.019	200	80	4800	0.006	0.006	5.649
Microsomes	0.201	0.201	0.019	200	80	4800	0.006		
Microsomes	0.205	0.205	0.019	200	80	4800	0.006		

Assay Date	8/25/2005	Test Chemical ID	8	# Concentrations tested	8			
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	Lot # 5	

Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.492	0.287	0.127	0.065	0.028	0.016	0.000	25	100	11343-77
	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	0.199
	0.249	0.029	0.201
			0.205

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	0.492	0.492	0.0432
125	100	200	0.00013	200	0.0250	0.286	0.286	0.0248
50	200	1000	0.00005	200	0.0100	0.125	0.125	0.0104
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	0.0019
5	20	1000	0.00001	200	0.0010	0.016	0.016	0.0007
			Blank	0.000				

Variables	Regression results	
m, b	0.089	-0.001
se_m, se_b	0.002	0.000
r^2, se_y	0.999	0.000
F, df	3151	3
ss_{reg}, ss_{resid}	0.000	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 100.	029	0.029	0.002	200	1	1	0.000	0.000	0.010
QC 100.	029	0.029	0.002	200	1	1	0.000		
QC 10									
Microsomes	199	0.199	0.017	200	80	4800	0.005	0.005	5.184
Microsomes	0.201	0.201	0.017	200	80	4800	0.005		
Microsomes	0.205	0.205	0.018	200	80	4800	0.005		

Assay Date	8/25/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	recombinar	Microsome ID	Lot # 5	Technician ID	TD	Replicate #	3

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.18384
Protein Concentration (dilution added to assay, mg/mL):	0.008228

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	1.00E-07
7	1.00E-08
8	1.00E-09

Assay Date	8/25/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot # 5	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	7708	15415	15380.7	30761.4	0.1	156957	19.60	30536	0.0421	1	0.004	15	0.3414
			0.5	2	7673	15346.4											
	2	2	0.5	1	7296	14591.8	14705.3	29410.6	0.1	156957	18.74	29185	0.0403	1	0.004	15	0.3263
			0.5	2	7409	14818.8											
	3	2	0.5	1	7557	15113.6	15292.1	30584.2	0.1	156957	19.49	30359	0.0419	1	0.004	15	0.3394
			0.5	2	7735	15470.6											
	4	2	0.5	1	7941	15882.6	15927.4	31854.8	0.1	156957	20.30	31630	0.0436	1	0.004	15	0.3536
			0.5	2	7986	15972.2											
Background control	1	2	0.5	1	46	91.22	97.57	195.14	0.1	156957	0.12	-30	0.0000	1	0.004	15	-0.0003
			0.5	2	52	103.92											
	2	2	0.5	1	64	128.4	122.15	244.3	0.1	156957	0.16	19	0.0000	1	0.004	15	0.0002
			0.5	2	58	115.9											
	3	2	0.5	1	59	117.76	116.45	232.9	0.1	156957	0.15	8	0.0000	1	0.004	15	0.0001
			0.5	2	58	115.12											
	4	2	0.5	1	54	108.78	114.13	228.26	0.1	156957	0.15	3	0.0000	1	0.004	15	0.0000
			0.5	2	60	119.48											
Positive control	1	2	0.5	1	4101	8201.2	8148.2	16296.4	0.1	156957	10.38	16071	0.0222	1	0.004	15	0.1797
			0.5	2	4048	8095.2											
	2	2	0.5	1	4349	8697.6	8741.2	17482.4	0.1	156957	11.14	17257	0.0238	1	0.004	15	0.1929
			0.5	2	4392	8784.8											
	3	2	0.5	1	4056	8112.6	8122.7	16245.4	0.1	156957	10.35	16020	0.0221	1	0.004	15	0.1791
			0.5	2	4066	8132.8											
	4	2	0.5	1	3804	7607.8	7580.5	15161	0.1	156957	9.66	14936	0.0206	1	0.004	15	0.1670
			0.5	2	3777	7553.2											
Negative Control	1	2	0.5	1	7277	14554.8	14647.2	29294.4	0.1	156957	18.66	29069	0.0401	1	0.004	15	0.3250
			0.5	2	7370	14739.6											
	2	2	0.5	1	7647	15293	15514.5	31029	0.1	156957	19.77	30804	0.0425	1	0.004	15	0.3444
			0.5	2	7868	15736											
	3	2	0.5	1	7232	14463.8	14335.4	28670.8	0.1	156957	18.27	28446	0.0392	1	0.004	15	0.3180
			0.5	2	7104	14207											
	4	2	0.5	1	7045	14090.4	14126.5	28253	0.1	156957	18.00	28028	0.0387	1	0.004	15	0.3133
			0.5	2	7081	14162.6											
8	1-1	2	0.5	1	1763	3526.8	3526.4	7052.8	0.1	156957	4.49	6828	0.0094	1	0.004	15	0.0763
			0.5	2	1763	3526											
	1-2	2	0.5	1	1759	3517.6	3602.1	7204.2	0.1	156957	4.59	6979	0.0096	1	0.004	15	0.0780
			0.5	2	1843	3686.6											
	1-3	2	0.5	1	1526	3051.2	3018.1	6036.2	0.1	156957	3.85	5811	0.0080	1	0.004	15	0.0650
			0.5	2	1493	2985											
	2-1	2	0.5	1	2005	4011	3971.7	7943.4	0.1	156957	5.06	7718	0.0106	1	0.004	15	0.0863
			0.5	2	1966	3932.4											
	2-2	2	0.5	1	1831	3662	3722.1	7444.2	0.1	156957	4.74	7219	0.0100	1	0.004	15	0.0807
			0.5	2	1891	3782.2											
	2-3	2	0.5	1	1983	3965.8	3983.8	7967.6	0.1	156957	5.08	7742	0.0107	1	0.004	15	0.0866
			0.5	2	2001	4001.8											
	3-1	2	0.5	1	2643	5285.6	5212.2	10424.4	0.1	156957	6.64	10199	0.0141	1	0.004	15	0.1140
			0.5	2	2569	5138.8											
	3-2	2	0.5	1	2816	5631.6	5610.3	11220.6	0.1	156957	7.15	10995	0.0152	1	0.004	15	0.1229
			0.5	2	2795	5589											
	3-3	2	0.5	1	3194	6388.6	6388.5	12777	0.1	156957	8.14	12552	0.0173	1	0.004	15	0.1403
			0.5	2	3194	6388.4											
	4-1	2	0.5	1	4447	8894.6	9005.8	18011.6	0.1	156957	11.48	17786	0.0245	1	0.004	15	0.1988
			0.5	2	4559	9117											
	4-2	2	0.5	1	4636	9272.2	9361.7	18723.4	0.1	156957	11.93	18498	0.0255	1	0.004	15	0.2068
			0.5	2	4725	9451.2											
	4-3	2	0.5	1	4141	8281.2	8345.6	16691.2	0.1	156957	10.63	16466	0.0227	1	0.004	15	0.1841
			0.5	2	4205	8410											
	5-1	2	0.5	1	5137	10273.8	10318.1	20636.2	0.1	156957	13.15	20411	0.0282	1	0.004	15	0.2282
			0.5	2	5181	10362.4											
	5-2	2	0.5	1	5193	10386.2	10421.4	20842.8	0.1	156957	13.28	20618	0.0284	1	0.004	15	0.2305
			0.5	2	5228	10456.6											
	5-3	2	0.5	1	5068	10135.8	10466.8	20933.6	0.1	156957	13.34	20708	0.0286	1	0.004	15	0.2315
			0.5	2	5399	10797.8											
	6-1	2	0.5	1	6622	13243.6	13246.1	26492.2	0.1	156957	16.88	26267	0.0362	1	0.004	15	0.2936
			0.5	2	6624	13248.6											
	6-2	2	0.5	1	7050	14100.4	14233.4	28466.8	0.1	156957	18.14	28242	0.0390	1	0.004	15	0.3157
			0.5	2	7183	14366.4											
	6-3	2	0.5	1	6968	13935	14043.2	28086.4	0.1	156957	17.89	27861	0.0384	1	0.004	15	0.3115
			0.5	2	7076	14151.4											
	7-1	2	0.5	1	5958	11915.4	12362.6	24725.2	0.1	156957	15.75	24500	0.0338	1	0.004	15	0.2739
			0.5	2	6405	12809.8											
	7-2	2	0.5	1	7029	14058	14070.4	28140.8	0.1	156957	17.93	27916	0.0385	1	0.004	15	0.3121
			0.5	2	7041	14082.8											
	7-3	2	0.5	1	7326	14652.2	14907.9	29815.8	0.1	156957	19.00	29591	0.0408	1	0.004	15	0.3308
			0.5	2	7582	15163.6											
	8-1	2	0.5	1	7497	14994	15121.8	30243.6	0.1	156957	19.27	30018	0.0414	1	0.004	15	0.3358
			0.5	2	7625	15249.6											
	8-2	2	0.5	1	7395	14790.6	15116.8	30233.6	0.1	156957	19.26	30008	0.0414	1	0.004	15	0.3355
			0.5	2	7722	15443											
	8-3	2	0.5	1	7661	15322.6	15365.1	30730.2	0.1	156957	19.58	30505	0.0421	1	0.004	15	0.3410
			0.5	2	7704	15407.6											

Assay Date	8/25/2005	Test Chemical ID	8	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot # 5	Technician ID TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3338	0.0107
Full activity	End	0.3465	0.0100
Full activity	Overall	0.3401	0.0112
Background	Beginning	-0.0001	0.000388593
Background	End	0.0001	3.66776E-05
Background	Overall	0.0000	0.000236
Positive	Beginning	0.1863	0.0094
Positive	End	0.1730	0.0086
Positive	Overall	0.1797	0.0106
Negative	Beginning	0.3347	0.0137
Negative	End	0.3157	0.0033
Negative	Overall	0.3252	0.0137

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
8	1	1	1.00E-04	-4.00	0.0763
8	1	2	1.00E-04	-4.00	0.0780
8	1	3	1.00E-04	-4.00	0.0650
8	2	1	1.00E-05	-5.00	0.0863
8	2	2	1.00E-05	-5.00	0.0807
8	2	3	1.00E-05	-5.00	0.0866
8	3	1	5.00E-06	-5.30	0.1140
8	3	2	5.00E-06	-5.30	0.1229
8	3	3	5.00E-06	-5.30	0.1403
8	4	1	2.50E-06	-5.60	0.1988
8	4	2	2.50E-06	-5.60	0.2068
8	4	3	2.50E-06	-5.60	0.1841
8	5	1	1.00E-06	-6.00	0.2282
8	5	2	1.00E-06	-6.00	0.2305
8	5	3	1.00E-06	-6.00	0.2315
8	6	1	1.00E-07	-7.00	0.2936
8	6	2	1.00E-07	-7.00	0.3157
8	6	3	1.00E-07	-7.00	0.3115
8	7	1	1.00E-08	-8.00	0.2739
8	7	2	1.00E-08	-8.00	0.3121
8	7	3	1.00E-08	-8.00	0.3308
8	8	1	1.00E-09	-9.00	0.3356
8	8	2	1.00E-09	-9.00	0.3355
8	8	3	1.00E-09	-9.00	0.3410

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	22.44	22.94	19.10
2	-5.00	25.37	23.73	25.45
3	-5.30	33.52	36.14	41.25
4	-5.60	58.45	60.79	54.12
5	-6.00	67.08	67.76	68.06
6	-7.00	86.33	92.82	91.57
7	-8.00	80.52	91.74	97.25
8	-9.00	98.66	98.62	100.25

Assay Date	8/29/2005	Test Chemical ID	9	# Concentrations tested	8	
Technician ID	TD	Replicate #	1	Microsome type	recombinar Microsome ID	Lot #5

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	recombinant	Microsome ID	Lot #5			

								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	0.177
	0.236	0.031	0.175
			0.175

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	
M. LE, 03-27-06 BBL														
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	0.177	0.177	0.017	200	80	4800	0.005	0.005	4.969
Microsomes	0.175	0.175	0.016	200	80	4800	0.005		
Microsomes	0.175	0.175	0.016	200	80	4800	0.005		

Assay Date	8/29/2005	Test Chemical ID	9	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	Lot #5

								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	0.177
	0.236	0.031	0.175
			0.175

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	0.477	0.477	0.0433	m, b	0.093	-0.001
125	100	200	0.00013	200	0.0250	0.277	0.277	0.0247	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.125	0.125	0.0107	r^2, se_y	0.998	0.001
25	100	1000	0.00003	200	0.0050	0.066	0.066	0.0052	F, df	1530	3
10	40	1000	0.00001	200	0.0020	0.029	0.029	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.017	0.017	0.0006			

Regression results are calculated using the function LINEST

			r^2=	0.998
Blank	0.000		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	0.237	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	0.177	0.177	0.015	200	80	4800	0.005	0.005	4.594
Microsomes	0.175	0.175	0.015	200	80	4800	0.005		
Microsomes	0.175	0.175	0.015	200	80	4800	0.005		

Assay Date	8/29/2005	Test Chemical ID		# Concentrations tested	9	Microsome type	8	recombina Microsome ID	Lot #5	Technician ID TD	Replicate #	1

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.593615
Protein Concentration (dilution added to assay, mg/mL):	0.007291

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	8/29/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	Lot #5	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	4624	9247.2	9311.8	18623.6	0.1	148250	12.56	18402	0.0267	1	0.004	15	0.2438
			0.5	2	4688	9376.4											
	2	2	0.5	1	4849	9698.8	9794.8	19589.6	0.1	148250	13.21	19368	0.0281	1	0.004	15	0.2568
			0.5	2	4945	9890.8											
	3	2	0.5	1	4689	9377.6	9377.7	18755.4	0.1	148250	12.65	18534	0.0269	1	0.004	15	0.2455
			0.5	2	4689	9377.8											
	4	2	0.5	1	4425	8849.8	8971.7	17943.4	0.1	148250	12.10	17722	0.0257	1	0.004	15	0.2348
			0.5	2	4547	9093.6											
Background control	1	2	0.5	1	57	114.82	110.36	220.72	0.1	148250	0.15	-1	0.0000	1	0.004	15	0.0000
			0.5	2	53	106.1											
	2	2	0.5	1	54	107.84	114.55	229.1	0.1	148250	0.15	6	0.0000	1	0.004	15	0.0001
			0.5	2	61	121.26											
	3	2	0.5	1	53	106.46	107.73	215.46	0.1	148250	0.15	-6	0.0000	1	0.004	15	-0.0001
			0.5	2	55	109											
	4	2	0.5	1	49	98.48	110.56	221.12	0.1	148250	0.15	0	0.0000	1	0.004	15	0.0000
			0.5	2	61	122.64											
Positive control	1	2	0.5	1	2329	4657	4669.5	9339	0.1	148250	5.30	9117	0.0132	1	0.004	15	0.1208
			0.5	2	2341	4682											
	2	2	0.5	1	2444	4886.6	4881.2	9762.4	0.1	148250	5.59	9641	0.0138	1	0.004	15	0.1264
			0.5	2	2437	4873.8											
	3	2	0.5	1	2774	5547.5	5548.5	11097	0.1	148250	7.49	10875	0.0158	1	0.004	15	0.1441
			0.5	2	2775	5549.2											
	4	2	0.5	1	2688	5376.6	5327.1	10654.2	0.1	148250	7.19	10433	0.0151	1	0.004	15	0.1382
			0.5	2	2639	5277.6											
Negative Control	1	2	0.5	1	4968	9936	10049.3	20098.6	0.1	148250	13.56	19877	0.0288	1	0.004	15	0.2633
			0.5	2	5081	10162.6											
	2	2	0.5	1	5122	10244.2	10254.8	20509.6	0.1	148250	13.83	20285	0.0294	1	0.004	15	0.2688
			0.5	2	5133	10265.4											
	3	2	0.5	1	4941	9881	9811	19622	0.1	148250	13.24	19400	0.0281	1	0.004	15	0.2570
			0.5	2	4871	9741											
	4	2	0.5	1	4442	8884.8	8850.6	17701.2	0.1	148250	11.94	17480	0.0253	1	0.004	15	0.2316
			0.5	2	4408	8816.4											
9	1-1	2	0.5	1	148	285.68	296.39	592.78	0.1	148250	0.40	371	0.0005	1	0.004	15	0.0049
			0.5	2	149	297.1											
	1-2	2	0.5	1	157	313.74	320.99	641.98	0.1	148250	0.43	420	0.0006	1	0.004	15	0.0056
			0.5	2	164	328.24											
	1-3	2	0.5	1	130	259.24	277.56	555.12	0.1	148250	0.37	334	0.0005	1	0.004	15	0.0044
			0.5	2	148	295.88											
	2-1	2	0.5	1	754	1507.4	1508.19	3016.38	0.1	148250	2.03	2795	0.0040	1	0.004	15	0.0370
			0.5	2	754	1508.96											
	2-2	2	0.5	1	821	1641.24	1622.18	3244.36	0.1	148250	2.19	3023	0.0044	1	0.004	15	0.0400
			0.5	2	802	1603.12											
	2-3	2	0.5	1	791	1581.86	1578.79	3157.58	0.1	148250	2.13	2936	0.0043	1	0.004	15	0.0389
			0.5	2	788	1575.72											
	3-1	2	0.5	1	2819	5638.2	5590.3	11180.6	0.1	148250	7.54	10959	0.0159	1	0.004	15	0.1452
			0.5	2	2771	5542.4											
	3-2	2	0.5	1	2923	5846.6	5810.3	11620.6	0.1	148250	7.84	11399	0.0165	1	0.004	15	0.1510
			0.5	2	2887	5773.8											
	3-3	2	0.5	1	2868	5735.8	5764.9	11529.8	0.1	148250	7.78	11308	0.0164	1	0.004	15	0.1488
			0.5	2	2897	5794											
	4-1	2	0.5	1	4827	9654.2	9656.6	19313.2	0.1	148250	13.03	19092	0.0277	1	0.004	15	0.2529
			0.5	2	4830	9659											
	4-2	2	0.5	1	4870	9740	9794.1	19588.2	0.1	148250	13.21	19367	0.0281	1	0.004	15	0.2566
			0.5	2	4924	9848.2											
	4-3	2	0.5	1	4649	9297.8	9377.2	18754.4	0.1	148250	12.65	18533	0.0269	1	0.004	15	0.2455
			0.5	2	4728	9456.6											
	5-1	2	0.5	1	4450	8900	9012.3	18024.6	0.1	148250	12.16	17803	0.0258	1	0.004	15	0.2359
			0.5	2	4562	9124.6											
	5-2	2	0.5	1	4352	8703.6	8545.8	17091.6	0.1	148250	11.53	16870	0.0244	1	0.004	15	0.2235
			0.5	2	4194	8388											
	5-3	2	0.5	1	4672	9343.2	9348.3	18696.6	0.1	148250	12.61	18475	0.0268	1	0.004	15	0.2448
			0.5	2	4677	9353.4											
	6-1	2	0.5	1	3616	7232.8	7337.8	14675.6	0.1	148250	9.90	14454	0.0209	1	0.004	15	0.1915
			0.5	2	3721	7442.8											
	6-2	2	0.5	1	4591	9182.6	9198.2	18396.4	0.1	148250	12.41	18175	0.0263	1	0.004	15	0.2408
			0.5	2	4607	9213.8											
	6-3	2	0.5	1	4813	9625	9534.1	19068.2	0.1	148250	12.86	18847	0.0273	1	0.004	15	0.2497
			0.5	2	4722	9443.2											
	7-1	2	0.5	1	4750	9499.4	9635.9	19271.8	0.1	148250	13.00	19050	0.0276	1	0.004	15	0.2524
			0.5	2	4886	9772.4											
	7-2	2	0.5	1	4984	9967	10051.4	20102.8	0.1	148250	13.56	19881	0.0288	1	0.004	15	0.2634
			0.5	2	5068	10135.8											
	7-3	2	0.5	1	4347	8694.6	8780.1	17560.2	0.1	148250	11.84	17339	0.0251	1	0.004	15	0.2297
			0.5	2	4433	8865.6											
	8-1	2	0.5	1	4465	8930	9010.5	18021	0.1	148250	12.15	17799	0.0258	1	0.004	15	0.2358
			0.5	2	4546	9091											
	8-2	2	0.5	1	4448	8896.2	8896.6	17793.2	0.1	148250	12.00	17572	0.0255	1	0.004	15	0.2328
			0.5	2	4449	8897											
	8-3	2	0.5	1	4326	8652.2	8394.5	16789	0.1	148250	11.32	16567	0.0240	1	0.004	15	0.2195
			0.5	2	4068	8136.8											

Assay Date	8/29/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	recombinant	Microsome ID Lot #5	Technician ID TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.2502	0.0090
Full activity	End	0.2402	0.0076
Full activity	Overall	0.2452	0.0090
Background	Beginning	0.0000	7.85019E-05
Background	End	0.0000	5.30215E-05
Background	Overall	0.0000	7.45329E-05
Positive	Beginning	0.1236	0.0040
Positive	End	0.1411	0.0041
Positive	Overall	0.1324	0.0107
Negative	Beginning	0.2661	0.0039
Negative	End	0.2443	0.0180
Negative	Overall	0.2552	0.0165

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
9	1	1	1.00E-03	-3.00	0.0049
9	1	2	1.00E-03	-3.00	0.0056
9	1	3	1.00E-03	-3.00	0.0044
9	2	1	1.00E-04	-4.00	0.0370
9	2	2	1.00E-04	-4.00	0.0400
9	2	3	1.00E-04	-4.00	0.0389
9	3	1	1.00E-05	-5.00	0.1452
9	3	2	1.00E-05	-5.00	0.1510
9	3	3	1.00E-05	-5.00	0.1498
9	4	1	1.00E-06	-6.00	0.2529
9	4	2	1.00E-06	-6.00	0.2566
9	4	3	1.00E-06	-6.00	0.2455
9	5	1	1.00E-07	-7.00	0.2359
9	5	2	1.00E-07	-7.00	0.2235
9	5	3	1.00E-07	-7.00	0.2448
9	6	1	1.00E-08	-8.00	0.1915
9	6	2	1.00E-08	-8.00	0.2408
9	6	3	1.00E-08	-8.00	0.2497
9	7	1	1.00E-09	-9.00	0.2524
9	7	2	1.00E-09	-9.00	0.2634
9	7	3	1.00E-09	-9.00	0.2297
9	8	1	1.00E-10	-10.00	0.2358
9	8	2	1.00E-10	-10.00	0.2328
9	8	3	1.00E-10	-10.00	0.2195

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.01	2.27	1.80
2	-4.00	15.10	16.33	15.86
3	-5.00	59.22	61.59	61.10
4	-6.00	103.16	104.65	100.14
5	-7.00	96.20	91.16	99.83
6	-8.00	78.10	98.21	101.84
7	-9.00	102.94	107.43	93.69
8	-10.00	96.18	94.95	89.52

Assay Date	8/30/2005	Test Chemical ID	9	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinar	Microsome ID	BD Gentes

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	08/30/2005	Test Chemical ID	9	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5

								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	0.204
	0.247	0.034	0.200
			0.204

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		0.493	0.493	0.0487	m, b	0.103	-0.002
125	100	200	0.00013		200	0.0250		0.281	0.281	0.0270	se_m, se_b	0.004	0.001
50	200	1000	0.00005		200	0.0100		0.127	0.127	0.0111	r^2, se_y	0.995	0.001
25	100	1000	0.00003		200	0.0050		0.069	0.069	0.0051	F, df	837	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		0.018	0.018	0.0000			
				Blank	0.000	r^2=	0.995						
						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	0.250	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	0.204	0.204	0.019	200	80	4800	0.006	0.006	5.657
Microsomes	0.200	0.200	0.019	200	80	4800	0.006		
Microsomes	0.204	0.204	0.019	200	80	4800	0.006		

Assay Date	8/30/2005	Test Chemical ID	9	# Concentrations tested	8						
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5				
								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	0.204								
	0.247	0.034	0.200								
			0.204								

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	
M, LEO 3-22-06	BBE										
250	200	200	0.00025	200	0.0500	0.493	0.493	0.0440	m, b	0.091	-0.001
125	100	200	0.00013	200	0.0250	0.281	0.281	0.0247	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.127	0.127	0.0106	r^2, se_y	0.998	0.000
25	100	1000	0.00003	200	0.0050	0.069	0.069	0.0053	F, df	1706	3
10	40	1000	0.00001	200	0.0020	0.031	0.031	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
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

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 100.	032	0.032	0.002	200	1	1	0.000	0.000	0.010
QC 100.	034	0.034	0.002	200	1	1	0.000		
QC 10									
Microsomes	204	0.204	0.018	200	80	4800	0.005	0.005	5.248
Microsomes	0.200	0.200	0.017	200	80	4800	0.005		
Microsomes	0.204	0.204	0.018	200	80	4800	0.005		

Assay Date	8/30/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest	Technician ID	TD	Replicate #	2

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.248474
Protein Concentration (dilution added to assay, mg/mL):	0.008331

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	5.00E-05
4	1.00E-05
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-09

Assay Date	8/30/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5	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	6000	12000.2	12333.3	24666.6	0.1	150764	16.36	24347	0.0351	1	0.004	15	0.2807
			0.5	2	6333	12666.4											
	2	2	0.5	1	6203	12406.4	12422	24844	0.1	150764	16.48	24525	0.0353	1	0.004	15	0.2827
			0.5	2	6219	12437.6											
	3	2	0.5	1	5801	11602.6	11607.5	23215	0.1	150764	15.40	22896	0.0330	1	0.004	15	0.2640
			0.5	2	5806	11612.4											
	4	2	0.5	1	5483	10966.8	10954.8	21909.6	0.1	150764	14.53	21590	0.0311	1	0.004	15	0.2489
			0.5	2	5471	10942.8											
Background control	1	2	0.5	1	100	200.78	194.49	388.98	0.1	150764	0.26	70	0.0001	1	0.004	15	0.0008
			0.5	2	94	188.2											
	2	2	0.5	1	68	135.66	130.05	260.1	0.1	150764	0.17	-59	-0.0001	1	0.004	15	-0.0007
			0.5	2	62	124.44											
	3	2	0.5	1	93	185.38	195	390	0.1	150764	0.26	71	0.0001	1	0.004	15	0.0008
			0.5	2	102	204.62											
	4	2	0.5	1	55	110.24	119.19	238.38	0.1	150764	0.16	-81	-0.0001	1	0.004	15	-0.0009
			0.5	2	64	128.14											
Positive control	1	2	0.5	1	3236	6472.6	6428.1	12856.2	0.1	150764	8.53	12537	0.0181	1	0.004	15	0.1445
			0.5	2	3192	6383.6											
	2	2	0.5	1	3184	6367.4	6304.7	12609.4	0.1	150764	8.36	12290	0.0177	1	0.004	15	0.1417
			0.5	2	3121	6242											
	3	2	0.5	1	3057	6113.8	6096.5	12193	0.1	150764	8.09	11874	0.0171	1	0.004	15	0.1369
			0.5	2	3040	6079.2											
	4	2	0.5	1	3127	6254	6292.8	12585.6	0.1	150764	8.35	12266	0.0177	1	0.004	15	0.1414
			0.5	2	3166	6331.6											
Negative Control	1	2	0.5	1	4335	8670.6	8696.1	17392.2	0.1	150764	11.54	17073	0.0246	1	0.004	15	0.1968
			0.5	2	4361	8721.6											
	2	2	0.5	1	5739	11478.4	11555	23110	0.1	150764	15.33	22791	0.0328	1	0.004	15	0.2628
			0.5	2	5816	11631.6											
	3	2	0.5	1	5602	11204.4	11189.8	22379.6	0.1	150764	14.84	22060	0.0318	1	0.004	15	0.2543
			0.5	2	5588	11175.2											
	4	2	0.5	1	5116	10231.4	10224.3	20448.6	0.1	150764	13.56	20129	0.0290	1	0.004	15	0.2321
			0.5	2	5109	10217.2											
9	1-1	2	0.5	1	190	380.82	391.53	783.06	0.1	150764	0.52	464	0.0007	1	0.004	15	0.0053
			0.5	2	201	402.24											
	1-2	2	0.5	1	201	402.94	405.02	810.04	0.1	150764	0.54	491	0.0007	1	0.004	15	0.0057
			0.5	2	204	407.1											
	1-3	2	0.5	1	225	450.38	441.71	883.42	0.1	150764	0.59	564	0.0008	1	0.004	15	0.0065
			0.5	2	217	433.04											
	2-1	2	0.5	1	1049	2098.24	2112.98	4225.96	0.1	150764	2.80	3907	0.0056	1	0.004	15	0.0450
			0.5	2	1064	2127.72											
	2-2	2	0.5	1	1070	2140.24	2138.86	4277.72	0.1	150764	2.84	3958	0.0057	1	0.004	15	0.0456
			0.5	2	1069	2137.48											
	2-3	2	0.5	1	1035	2070.48	2055.24	4110.48	0.1	150764	2.73	3791	0.0055	1	0.004	15	0.0437
			0.5	2	1020	2040											
	3-1	2	0.5	1	1429	2858.8	2861.9	5723.8	0.1	150764	3.80	5404	0.0078	1	0.004	15	0.0623
			0.5	2	1433	2865											
	3-2	2	0.5	1	1497	2994.2	3007.4	6014.8	0.1	150764	3.99	5695	0.0082	1	0.004	15	0.0657
			0.5	2	1510	3020.6											
	3-3	2	0.5	1	1531	3062.8	3092.6	6185.2	0.1	150764	4.10	5866	0.0085	1	0.004	15	0.0676
			0.5	2	1561	3122.4											
	4-1	2	0.5	1	3299	6597.2	6613.7	13227.4	0.1	150764	8.77	12908	0.0186	1	0.004	15	0.1488
			0.5	2	3315	6630.2											
	4-2	2	0.5	1	3307	6613.4	6712.5	13425	0.1	150764	8.90	13106	0.0189	1	0.004	15	0.1511
			0.5	2	3406	6811.6											
	4-3	2	0.5	1	3421	6841.4	6843.5	13687	0.1	150764	9.08	13368	0.0193	1	0.004	15	0.1541
			0.5	2	3423	6845.6											
	5-1	2	0.5	1	5264	10528	10542.2	21084.4	0.1	150764	13.99	20765	0.0299	1	0.004	15	0.2394
			0.5	2	5278	10556.4											
	5-2	2	0.5	1	5367	10733	10772.7	21545.4	0.1	150764	14.29	21226	0.0306	1	0.004	15	0.2447
			0.5	2	5406	10812.4											
	5-3	2	0.5	1	5426	10852.2	10838.8	21677.6	0.1	150764	14.38	21358	0.0308	1	0.004	15	0.2462
			0.5	2	5413	10825.4											
	6-1	2	0.5	1	5442	10884.4	10932	21864	0.1	150764	14.50	21545	0.0310	1	0.004	15	0.2484
			0.5	2	5490	10979.6											
	6-2	2	0.5	1	5174	10347.6	10231.2	20462.4	0.1	150764	13.57	20143	0.0290	1	0.004	15	0.2322
			0.5	2	5057	10114.8											
	6-3	2	0.5	1	5523	11046.4	11048.1	22096.2	0.1	150764	14.66	21777	0.0314	1	0.004	15	0.2511
			0.5	2	5525	11049.8											
	7-1	2	0.5	1	5523	11045	11082.8	22165.6	0.1	150764	14.70	21846	0.0315	1	0.004	15	0.2519
			0.5	2	5560	11120.6											
	7-2	2	0.5	1	5940	11879.2	11842.1	23684.2	0.1	150764	15.71	23365	0.0337	1	0.004	15	0.2694
			0.5	2	5903	11805											
	7-3	2	0.5	1	5887	11773.4	11881.9	23763.8	0.1	150764	15.76	23444	0.0338	1	0.004	15	0.2703
			0.5	2	5995	11990.4											
	8-1	2	0.5	1	5439	10877	10942.3	21884.6	0.1	150764	14.52	21565	0.0311	1	0.004	15	0.2486
			0.5	2	5504	11007.6											
	8-2	2	0.5	1	5511	11021.8	11134.9	22269.8	0.1	150764	14.77	21950	0.0316	1	0.004	15	0.2531
			0.5	2	5624	11248											
	8-3	2	0.5	1	5808	11615.2	11554.3	23108.6	0.1	150764	15.33	22789	0.0328	1	0.004	15	0.2627
			0.5	2	5747	11493.4											

Assay Date	8/30/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lo	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.2817	0.0014
Full activity	End	0.2564	0.0106
Full activity	Overall	0.2691	0.0159
Background	Beginning	0.0001	0.001050676
Background	End	-0.0001	0.001236061
Background	Overall	0.0000	0.00093915
Positive	Beginning	0.1431	0.0020
Positive	End	0.1392	0.0032
Positive	Overall	0.1411	0.0032
Negative	Beginning	0.2298	0.0466
Negative	End	0.2432	0.0157
Negative	Overall	0.2365	0.0294

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
9	1	1	1.00E-03	-3.00	0.0053
9	1	2	1.00E-03	-3.00	0.0057
9	1	3	1.00E-03	-3.00	0.0065
9	2	1	1.00E-04	-4.00	0.0450
9	2	2	1.00E-04	-4.00	0.0456
9	2	3	1.00E-04	-4.00	0.0437
9	3	1	5.00E-05	-4.30	0.0623
9	3	2	5.00E-05	-4.30	0.0657
9	3	3	5.00E-05	-4.30	0.0676
9	4	1	1.00E-05	-5.00	0.1488
9	4	2	1.00E-05	-5.00	0.1511
9	4	3	1.00E-05	-5.00	0.1541
9	5	1	1.00E-06	-6.00	0.2394
9	5	2	1.00E-06	-6.00	0.2447
9	5	3	1.00E-06	-6.00	0.2462
9	6	1	1.00E-07	-7.00	0.2484
9	6	2	1.00E-07	-7.00	0.2322
9	6	3	1.00E-07	-7.00	0.2511
9	7	1	1.00E-08	-8.00	0.2519
9	7	2	1.00E-08	-8.00	0.2694
9	7	3	1.00E-08	-8.00	0.2703
9	8	1	1.00E-09	-9.00	0.2486
9	8	2	1.00E-09	-9.00	0.2531
9	8	3	1.00E-09	-9.00	0.2627

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	1.99	2.10	2.42
2	-4.00	16.74	16.96	16.24
3	-4.30	23.16	24.40	25.13
4	-5.00	55.31	56.15	57.27
5	-6.00	88.97	90.94	91.51
6	-7.00	92.31	86.30	93.30
7	-8.00	93.60	100.11	100.45
8	-9.00	92.40	94.05	97.64

Assay Date	8/31/2005	Test Chemical ID	9	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinar	Microsome ID	BD Gentes

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	08/31/2005	Test Chemical ID	9	# Concentrations tested	8						
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5				
Standards:	250	125	50	25	10	5	0	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID	
	0.485	0.279	0.129	0.063	0.023	0.013	0.000	25	100	11343-77	
	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	0.203								
	0.247	0.025	0.199								
			0.212								

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	0.488	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								

Regression results are calculated using the function LINEST

r^2= 0.992
m= 0.101
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	0.243	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	0.203	0.203	0.019	200	80	4800	0.006	0.006		5.823
Microsomes	0.199	0.199	0.019	200	80	4800	0.006			
Microsomes	0.212	0.212	0.020	200	80	4800	0.006			

Assay Date	8/31/2005	Test Chemical ID	9	# Concentrations tested	8					
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5			
							Protein stock (mg BSA)	Total volume of stock (mL)	100	Protein stock ID 11343-77

Standards:	250	125	50	25	10	5	0
	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	0.203
	0.247	0.025	0.199
			0.212

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
M, LE 03-22-06 B&L								
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 100.	027	0.027	0.002	200	1	1	0.000	0.000	0.010
QC 100.	025	0.025	0.002	200	1	1	0.000		
QC 10									
Microsomes	203	0.203	0.017	200	80	4800	0.005	0.005	5.286
Microsomes	0.199	0.199	0.017	200	80	4800	0.005		
Microsomes	0.212	0.212	0.018	200	80	4800	0.005		

Assay Date	8/31/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	recombinar	Microsome ID	BD Gentes	Technician ID	TD	Replicate #	3

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	5.00E-05
4	1.00E-05
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-09

Protein Concentration (stock microsomes, mg/mL):	5.286258
Protein Concentration (dilution added to assay, mg/mL):	0.008391

Assay Date	8/31/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5	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	6766	13531	13690	27380	0.1	155113	17.65	27146	0.0349	1	0.004	15	0.2776
			0.5	2	6925	13849											
	2	2	0.5	1	6774	13548.8	13568.3	27136.6	0.1	155113	17.49	26903	0.0346	1	0.004	15	0.2751
			0.5	2	6794	13587.8											
	3	2	0.5	1	5849	11697	11730.7	23461.4	0.1	155113	15.13	23227	0.0299	1	0.004	15	0.2375
			0.5	2	5882	11764.4											
	4	2	0.5	1	6036	12072.6	12145.7	24291.4	0.1	155113	15.66	24057	0.0310	1	0.004	15	0.2460
			0.5	2	6109	12218.8											
Background control	1	2	0.5	1	61	121.66	112.59	225.18	0.1	155113	0.15	-9	0.0000	1	0.004	15	-0.0001
			0.5	2	52	103.52											
	2	2	0.5	1	58	116.2	117.68	235.36	0.1	155113	0.15	1	0.0000	1	0.004	15	0.0000
			0.5	2	60	119.16											
	3	2	0.5	1	60	119.32	125.86	251.72	0.1	155113	0.16	18	0.0000	1	0.004	15	0.0002
			0.5	2	66	132.4											
	4	2	0.5	1	55	110.42	111.72	223.44	0.1	155113	0.14	-10	0.0000	1	0.004	15	-0.0001
			0.5	2	57	113.02											
Positive control	1	2	0.5	1	3290	6579.8	6590	13180	0.1	155113	8.50	12946	0.0167	1	0.004	15	0.1324
			0.5	2	3300	6600.2											
	2	2	0.5	1	3165	6330.2	6333.8	12667.6	0.1	155113	8.17	12434	0.0160	1	0.004	15	0.1271
			0.5	2	3169	6337.4											
	3	2	0.5	1	3114	6228.4	6288	12576	0.1	155113	8.11	12342	0.0159	1	0.004	15	0.1262
			0.5	2	3174	6347.6											
	4	2	0.5	1	2875	5749.2	5785.7	11571.4	0.1	155113	7.46	11337	0.0146	1	0.004	15	0.1159
			0.5	2	2911	5822.2											
Negative Control	1	2	0.5	1	5711	11422.4	11514.9	23029.8	0.1	155113	14.85	22796	0.0293	1	0.004	15	0.2331
			0.5	2	5804	11607.4											
	2	2	0.5	1	5865	11730.6	11568.4	23136.8	0.1	155113	14.92	22903	0.0295	1	0.004	15	0.2342
			0.5	2	5703	11406.2											
	3	2	0.5	1	5813	11625.6	11649.6	23299.2	0.1	155113	15.02	23065	0.0297	1	0.004	15	0.2359
			0.5	2	5837	11673.6											
	4	2	0.5	1	5141	10281.2	10328.2	20656.4	0.1	155113	13.32	20422	0.0263	1	0.004	15	0.2088
			0.5	2	5186	10375.2											
9	1-1	2	0.5	1	252	504.38	495.17	990.34	0.1	155113	0.64	756	0.0010	1	0.004	15	0.0077
			0.5	2	243	485.96											
	1-2	2	0.5	1	262	523.84	521.71	1043.42	0.1	155113	0.67	809	0.0010	1	0.004	15	0.0083
			0.5	2	260	519.58											
	1-3	2	0.5	1	208	416.74	417.33	834.66	0.1	155113	0.54	601	0.0008	1	0.004	15	0.0061
			0.5	2	209	417.92											
	2-1	2	0.5	1	1073	2146.44	2204.42	4408.84	0.1	155113	2.84	4175	0.0054	1	0.004	15	0.0427
			0.5	2	1131	2262.4											
	2-2	2	0.5	1	1142	2283.8	2330.9	4661.8	0.1	155113	3.01	4428	0.0057	1	0.004	15	0.0453
			0.5	2	1189	2378											
	2-3	2	0.5	1	1174	2348.8	2352.1	4704.2	0.1	155113	3.03	4470	0.0058	1	0.004	15	0.0457
			0.5	2	1178	2355.4											
	3-1	2	0.5	1	1295	2590.6	2652	5304	0.1	155113	3.42	5070	0.0065	1	0.004	15	0.0518
			0.5	2	1357	2713.4											
	3-2	2	0.5	1	1441	2882.4	2910.1	5820.2	0.1	155113	3.75	5586	0.0072	1	0.004	15	0.0571
			0.5	2	1469	2937.8											
	3-3	2	0.5	1	1503	3006.6	3025.7	6051.4	0.1	155113	3.90	5817	0.0075	1	0.004	15	0.0595
			0.5	2	1522	3044.8											
	4-1	2	0.5	1	4305	8609.4	8663.9	17327.8	0.1	155113	11.17	17094	0.0220	1	0.004	15	0.1748
			0.5	2	4359	8718.4											
	4-2	2	0.5	1	4064	8168.8	8142.7	16285.4	0.1	155113	10.50	16051	0.0207	1	0.004	15	0.1641
			0.5	2	4056	8116.6											
	4-3	2	0.5	1	4742	9484.8	9487.7	18975.4	0.1	155113	12.23	18741	0.0241	1	0.004	15	0.1917
			0.5	2	4745	9490.6											
	5-1	2	0.5	1	4736	9471.2	9563.2	19126.4	0.1	155113	12.33	18892	0.0243	1	0.004	15	0.1932
			0.5	2	4828	9655.2											
	5-2	2	0.5	1	5405	10809.6	10933.8	21867.6	0.1	155113	14.10	21634	0.0278	1	0.004	15	0.2212
			0.5	2	5529	11058											
	5-3	2	0.5	1	6012	12024.8	12005.9	24011.8	0.1	155113	15.48	23778	0.0306	1	0.004	15	0.2432
			0.5	2	5994	11987											
	6-1	2	0.5	1	5601	11201.8	11019.6	22039.2	0.1	155113	14.21	21805	0.0281	1	0.004	15	0.2230
			0.5	2	5419	10837.4											
	6-2	2	0.5	1	5421	10841.6	10689.4	21378.8	0.1	155113	13.78	21145	0.0272	1	0.004	15	0.2162
			0.5	2	5269	10537.2											
	6-3	2	0.5	1	5804	11607	11585.2	23170.4	0.1	155113	14.94	22936	0.0295	1	0.004	15	0.2345
			0.5	2	5782	11563.4											
	7-1	2	0.5	1	5922	11844	11749.9	23499.8	0.1	155113	15.15	23266	0.0299	1	0.004	15	0.2379
			0.5	2	5828	11655.8											
	7-2	2	0.5	1	6035	12070.2	12001.3	24002.6	0.1	155113	15.47	23769	0.0306	1	0.004	15	0.2431
			0.5	2	5966	11932.4											
	7-3	2	0.5	1	5843	11685.8	11758	23516	0.1	155113	15.16	23282	0.0300	1	0.004	15	0.2381
			0.5	2	5915	11830.2											
	8-1	2	0.5	1	5890	11780.8	12027.3	24054.6	0.1	155113	15.51	23821	0.0307	1	0.004	15	0.2436
			0.5	2	6137	12273.8											
	8-2	2	0.5	1	5606	11211.2	11261.2	22522.4	0.1	155113	14.52	22288	0.0287	1	0.004	15	0.2279
			0.5	2	5656	11311.2											
	8-3	2	0.5	1	5864	11727.6	11822.4	23644.8	0.1	155113	15.24	23411	0.0301	1	0.004	15	0.2394
			0.5	2	5959	11917.2											

Assay Date	8/31/2005	Test Chemical ID	9	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lo	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.2764	0.0018
Full activity	End	0.2418	0.0060
Full activity	Overall	0.2591	0.0203
Background	Beginning	0.0000	7.36108E-05
Background	End	0.0000	0.00020449
Background	Overall	0.0000	0.000132694
Positive	Beginning	0.1298	0.0037
Positive	End	0.1211	0.0073
Positive	Overall	0.1254	0.0069
Negative	Beginning	0.2337	0.0008
Negative	End	0.2224	0.0191
Negative	Overall	0.2280	0.0128

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
9	1	1	1.00E-03	-3.00	0.0077
9	1	2	1.00E-03	-3.00	0.0083
9	1	3	1.00E-03	-3.00	0.0061
9	2	1	1.00E-04	-4.00	0.0427
9	2	2	1.00E-04	-4.00	0.0453
9	2	3	1.00E-04	-4.00	0.0457
9	3	1	5.00E-05	-4.30	0.0518
9	3	2	5.00E-05	-4.30	0.0571
9	3	3	5.00E-05	-4.30	0.0595
9	4	1	1.00E-05	-5.00	0.1748
9	4	2	1.00E-05	-5.00	0.1641
9	4	3	1.00E-05	-5.00	0.1917
9	5	1	1.00E-06	-6.00	0.1932
9	5	2	1.00E-06	-6.00	0.2212
9	5	3	1.00E-06	-6.00	0.2432
9	6	1	1.00E-07	-7.00	0.2230
9	6	2	1.00E-07	-7.00	0.2162
9	6	3	1.00E-07	-7.00	0.2345
9	7	1	1.00E-08	-8.00	0.2379
9	7	2	1.00E-08	-8.00	0.2431
9	7	3	1.00E-08	-8.00	0.2381
9	8	1	1.00E-09	-9.00	0.2436
9	8	2	1.00E-09	-9.00	0.2279
9	8	3	1.00E-09	-9.00	0.2394

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.99	3.20	2.37
2	-4.00	16.48	17.48	17.65
3	-4.30	20.01	22.05	22.96
4	-5.00	67.48	63.36	73.98
5	-6.00	74.58	85.40	93.86
6	-7.00	86.07	83.47	90.54
7	-8.00	91.84	93.82	91.90
8	-9.00	94.03	87.98	92.41

Assay Date	9/6/2005	Test Chemical ID	10	# Concentrations tested	8		
Technician ID	TD	Replicate #	1	Microsome type	recombinar	Microsome ID	BD Gentes

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	09/06/2005	Test Chemical ID	10	# Concentrations tested	8						
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5				
								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID	
Standards:	250	125	50	25	10	5	0	25	100	11343-77	
	0.475	0.283	0.130	0.069	0.028	0.017	0.000				
	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	0.206								
	0.249	0.032	0.207								
			0.207								

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	0.479	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								

Regression results are calculated using the function LINEST

r^2= 0.991
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	0.248	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	0.206	0.206	0.020	200	80	4800	0.006	0.006	5.873
Microsomes	0.207	0.207	0.020	200	80	4800	0.006		
Microsomes	0.207	0.207	0.020	200	80	4800	0.006		

Assay Date	9/6/2005	Test Chemical ID	10	# Concentrations tested	8						
Technician ID	TD	Replicate #	1	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5				
							Protein stock (mg BSA)	Total volume of stock (mL)		Protein stock ID	
Standards:	250	125	50	25	10	5	0	25	100	11343-77	
	0.475	0.283	0.130	0.069	0.028	0.017	0.000				
	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	0.206								
	0.249	0.032	0.207								
			0.207								

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	0.479	0.479	0.0421	m, b	0.090	-0.001
125	100	200	0.00013	200	0.0250	0.285	0.285	0.0247	se_m, se_b	0.002	0.000
50	200	1000	0.00005	200	0.0100	0.129	0.129	0.0107	r^2, se_y	0.998	0.001
25	100	1000	0.00003	200	0.0050	0.068	0.068	0.0052	F, df	1381	3
10	40	1000	0.00001	200	0.0020	0.029	0.029	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
5	20	1000	0.00001	200	0.0010	0.017	0.017	0.0007			
										Regression results are calculated using the function LINEST	
		Blank	0.000		r^2=	0.998					
					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/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 100.	031	0.031	0.002	200	1	1	0.000	0.000	0.010
QC 100.	032	0.032	0.002	200	1	1	0.000		
QC 10									
Microsomes	206	0.206	0.018	200	80	4800	0.005	0.005	5.302
Microsomes	0.207	0.207	0.018	200	80	4800	0.005		
Microsomes	0.207	0.207	0.018	200	80	4800	0.005		

Assay Date	9/6/2005	Test Chemical ID		# Concentrations tested	10	Microsome type	8	recombinai Microsome ID	BD Gentes	Technician ID	TD	Replicate #	1

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.302074
Protein Concentration (dilution added to assay, mg/mL):	0.008416

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	9/6/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5	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	5815	11630	11720.5	23441	0.1	150764	15.55	23230	0.0317	1	0.004	15	0.2515
			0.5	2	5906	11811											
	2	2	0.5	1	5861	11722.2	11719.8	23439.6	0.1	150764	15.55	23229	0.0317	1	0.004	15	0.2515
			0.5	2	5859	11717.4											
	3	2	0.5	1	5288	10575.8	10473.9	20947.8	0.1	150764	13.89	20737	0.0283	1	0.004	15	0.2245
			0.5	2	5186	10372											
	4	2	0.5	1	5250	10499.8	10398	20796	0.1	150764	13.79	20585	0.0281	1	0.004	15	0.2229
			0.5	2	5148	10296.2											
Background control	1	2	0.5	1	57	113.1	105.09	210.18	0.1	150764	0.14	0	0.0000	1	0.004	15	0.0000
			0.5	2	49	97.08											
	2	2	0.5	1	56	112.28	102.25	204.5	0.1	150764	0.14	-6	0.0000	1	0.004	15	-0.0001
			0.5	2	46	92.22											
	3	2	0.5	1	54	107.3	104.02	208.04	0.1	150764	0.14	-3	0.0000	1	0.004	15	0.0000
			0.5	2	50	100.74											
	4	2	0.5	1	61	121.22	109.91	219.82	0.1	150764	0.15	9	0.0000	1	0.004	15	0.0001
			0.5	2	49	98.6											
Positive control	1	2	0.5	1	3017	6034.4	6270.5	12541	0.1	150764	8.32	12330	0.0169	1	0.004	15	0.1335
			0.5	2	3253	6506.6											
	2	2	0.5	1	3139	6278.8	6216	12432	0.1	150764	8.25	12221	0.0167	1	0.004	15	0.1323
			0.5	2	3077	6153.2											
	3	2	0.5	1	2879	5757.4	5779.6	11559.2	0.1	150764	7.67	11349	0.0155	1	0.004	15	0.1229
			0.5	2	2901	5801.8											
	4	2	0.5	1	2788	5575	5604.6	11209.2	0.1	150764	7.43	10999	0.0150	1	0.004	15	0.1191
			0.5	2	2817	5634.2											
Negative Control	1	2	0.5	1	6312	12624.6	12527.8	25055.6	0.1	150764	16.62	24845	0.0340	1	0.004	15	0.2690
			0.5	2	6216	12431											
	2	2	0.5	1	6031	12061.2	12044.7	24089.4	0.1	150764	15.98	23879	0.0326	1	0.004	15	0.2585
			0.5	2	6014	12028.2											
	3	2	0.5	1	5546	11091.4	11057.4	22114.8	0.1	150764	14.67	21904	0.0299	1	0.004	15	0.2371
			0.5	2	5512	11023.4											
	4	2	0.5	1	5670	11339.4	11343.2	22686.4	0.1	150764	15.05	22476	0.0307	1	0.004	15	0.2433
			0.5	2	5674	11347											
10	1-1	2	0.5	1	5052	10103.4	10078.8	20157.6	0.1	150764	13.37	19947	0.0273	1	0.004	15	0.2159
			0.5	2	5027	10054.2											
	1-2	2	0.5	1	5217	10434	10425.7	20851.4	0.1	150764	13.83	20641	0.0282	1	0.004	15	0.2235
			0.5	2	5209	10417.4											
	1-3	2	0.5	1	5252	10503.6	10569.8	21139.6	0.1	150764	14.02	20929	0.0286	1	0.004	15	0.2266
			0.5	2	5318	10636											
	2-1	2	0.5	1	5523	11045.8	11169.2	22338.4	0.1	150764	14.82	22128	0.0302	1	0.004	15	0.2396
			0.5	2	5646	11292.6											
	2-2	2	0.5	1	6121	12242	12269.8	24539.6	0.1	150764	16.28	24329	0.0333	1	0.004	15	0.2634
			0.5	2	6149	12297.6											
	2-3	2	0.5	1	5648	11295.2	11367.6	22735.2	0.1	150764	15.08	22525	0.0308	1	0.004	15	0.2439
			0.5	2	5720	11440											
	3-1	2	0.5	1	6031	12062.6	12100.1	24200.2	0.1	150764	16.05	23990	0.0328	1	0.004	15	0.2597
			0.5	2	6069	12137.6											
	3-2	2	0.5	1	5874	11747.4	11752.8	23505.6	0.1	150764	15.59	23295	0.0318	1	0.004	15	0.2522
			0.5	2	5879	11758.2											
	3-3	2	0.5	1	5744	11488.6	11597.7	23195.4	0.1	150764	15.39	22985	0.0314	1	0.004	15	0.2488
			0.5	2	5853	11706.8											
	4-1	2	0.5	1	5629	11257	11347.3	22694.6	0.1	150764	15.05	22484	0.0307	1	0.004	15	0.2434
			0.5	2	5719	11437.6											
	4-2	2	0.5	1	6035	12070.4	12124.7	24249.4	0.1	150764	16.08	24039	0.0329	1	0.004	15	0.2602
			0.5	2	6090	12179											
	4-3	2	0.5	1	6146	12292.2	12297.1	24594.2	0.1	150764	16.31	24384	0.0333	1	0.004	15	0.2640
			0.5	2	6151	12302											
	5-1	2	0.5	1	6226	12455.2	12380.9	24761.8	0.1	150764	16.42	24551	0.0336	1	0.004	15	0.2658
			0.5	2	6153	12306.6											
	5-2	2	0.5	1	5867	11733.5	11768.4	23536.8	0.1	150764	15.61	23326	0.0319	1	0.004	15	0.2525
			0.5	2	5902	11803.2											
	5-3	2	0.5	1	6178	12356.8	12408.3	24816.6	0.1	150764	16.46	24606	0.0336	1	0.004	15	0.2664
			0.5	2	6230	12459.8											
	6-1	2	0.5	1	6075	12149.4	12253.1	24506.2	0.1	150764	16.25	24296	0.0332	1	0.004	15	0.2630
			0.5	2	6178	12356.8											
	6-2	2	0.5	1	5831	11661	11581.3	23162.6	0.1	150764	15.36	22952	0.0314	1	0.004	15	0.2485
			0.5	2	5751	11501.6											
	6-3	2	0.5	1	5859	11717	11752.7	23505.4	0.1	150764	15.59	23295	0.0318	1	0.004	15	0.2522
			0.5	2	5894	11788.4											
	7-1	2	0.5	1	5735	11469.4	11580	23160	0.1	150764	15.36	22949	0.0314	1	0.004	15	0.2485
			0.5	2	5845	11690.6											
	7-2	2	0.5	1	5865	11730	11810.5	23621	0.1	150764	15.67	23410	0.0320	1	0.004	15	0.2534
			0.5	2	5946	11891											
	7-3	2	0.5	1	5893	11785.6	11767.7	23535.4	0.1	150764	15.61	23325	0.0319	1	0.004	15	0.2525
			0.5	2	5875	11749.8											
	8-1	2	0.5	1	5842	11684.8	11760.9	23521.8	0.1	150764	15.60	23311	0.0319	1	0.004	15	0.2524
			0.5	2	5919	11837											
	8-2	2	0.5	1	5618	11236.8	11309.3	22618.6	0.1	150764	15.00	22408	0.0305	1	0.004	15	0.2426
			0.5	2	5691	11381.8											
	8-3	2	0.5	1	5846	11692	11843.7	23687.4	0.1	150764	15.71	23477	0.0321	1	0.004	15	0.2542
			0.5	2	5998	11995.4											

Assay Date	9/6/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lo	Technician ID	TD	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.2515	0.0000
Full activity	End	0.2237	0.0012
Full activity	Overall	0.2376	0.0161
Background	Beginning	0.0000	4.34818E-05
Background	End	0.0000	9.01788E-05
Background	Overall	0.0000	7.09763E-05
Positive	Beginning	0.1329	0.0008
Positive	End	0.1210	0.0027
Positive	Overall	0.1269	0.0071
Negative	Beginning	0.2637	0.0074
Negative	End	0.2402	0.0044
Negative	Overall	0.2520	0.0145

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
10	1	1	1.00E-03	-3.00	0.2159
10	1	2	1.00E-03	-3.00	0.2235
10	1	3	1.00E-03	-3.00	0.2266
10	2	1	1.00E-04	-4.00	0.2396
10	2	2	1.00E-04	-4.00	0.2634
10	2	3	1.00E-04	-4.00	0.2439
10	3	1	1.00E-05	-5.00	0.2597
10	3	2	1.00E-05	-5.00	0.2522
10	3	3	1.00E-05	-5.00	0.2488
10	4	1	1.00E-06	-6.00	0.2434
10	4	2	1.00E-06	-6.00	0.2602
10	4	3	1.00E-06	-6.00	0.2640
10	5	1	1.00E-07	-7.00	0.2658
10	5	2	1.00E-07	-7.00	0.2525
10	5	3	1.00E-07	-7.00	0.2664
10	6	1	1.00E-08	-8.00	0.2630
10	6	2	1.00E-08	-8.00	0.2485
10	6	3	1.00E-08	-8.00	0.2522
10	7	1	1.00E-09	-9.00	0.2485
10	7	2	1.00E-09	-9.00	0.2534
10	7	3	1.00E-09	-9.00	0.2525
10	8	1	1.00E-10	-10.00	0.2524
10	8	2	1.00E-10	-10.00	0.2426
10	8	3	1.00E-10	-10.00	0.2542

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	90.89	94.05	95.37
2	-4.00	100.83	110.86	102.64
3	-5.00	109.31	106.15	104.74
4	-6.00	102.45	109.54	111.11
5	-7.00	111.87	106.29	112.12
6	-8.00	110.71	104.59	106.15
7	-9.00	104.57	106.68	106.29
8	-10.00	106.22	102.11	106.98

Assay Date	9/7/2005	Test Chemical ID	10	# Concentrations tested	8		
Technician ID	TD	Replicate #	2	Microsome type	recombinar	Microsome ID	BD Gentes

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 09/07/2005 | Test Chemical ID 10 | # Concentrations tested 8

Technician ID TD | Replicate # 2 | Microsome type recombinant | Microsome ID BD Gentest Lot #5

								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	0.193
	0.234	0.025	0.195
			0.192

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	0.476	0.476	0.0481	m, b	0.104	-0.001
125	100	200	0.00013		200	0.0250	0.280	0.280	0.0278	se_m, se_b	0.005	0.001
50	200	1000	0.00005		200	0.0100	0.122	0.122	0.0112	r^2, se_y	0.992	0.002
25	100	1000	0.00003		200	0.0050	0.062	0.062	0.0050	F, df	474	4
10	40	1000	0.00001		200	0.0020	0.026	0.026	0.0012	ss_{reg}, ss_{resid}	0.002	0.000
5	20	1000	0.00001		200	0.0010	0.010	0.010	-0.0004			
				Blank	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	0.193	0.193	0.019	200	80	4800	0.006	0.006	5.603
Microsomes	0.195	0.195	0.019	200	80	4800	0.006		
Microsomes	0.192	0.192	0.019	200	80	4800	0.006		

Assay Date	9/7/2005	Test Chemical ID	10	# Concentrations tested	8						
Technician ID	TD	Replicate #	2	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5				
								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID	
								25	100	11343-77	

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

Samples:	QC 100	QC 10	Microsomes
	0.239	0.024	0.193
	0.234	0.025	0.195
			0.192

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

Regression results are calculated using the function LINEST

	Blank	0.000	r^2=	0.998
			m=	0.089
			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	0.239	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 100.	024	0.024	0.002	200	1	1	0.000	0.000	0.009
QC 100.	025	0.025	0.002	200	1	1	0.000		
QC 10									
Microsomes	193	0.193	0.017	200	80	4800	0.005	0.005	5.081
Microsomes	0.195	0.195	0.017	200	80	4800	0.005		
Microsomes	0.192	0.192	0.017	200	80	4800	0.005		

Assay Date	9/7/2005	Test Chemical ID		# Concentrations tested	8	Microsome type	recombinai	Microsome ID BD Gentes	Technician ID TD	Replicate #	2

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.080551
Protein Concentration (dilution added to assay, mg/mL):	0.008064

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	9.00E-04
3	8.00E-04
4	7.50E-04
5	5.00E-04
6	1.00E-04
7	1.00E-05
8	1.00E-06

Assay Date	9/7/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5	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	4072	8144.8	8271	16542	0.1	122775	13.47	16361	0.0260	1	0.004	15	0.2315
			0.5	2	4199	8397.2											
	2	2	0.5	1	3473	6945	7034	14068	0.1	122775	11.46	13887	0.0238	1	0.004	15	0.1965
			0.5	2	3562	7123											
	3	2	0.5	1	2731	5462	5471.2	10942.4	0.1	122775	8.91	10761	0.0184	1	0.004	15	0.1522
			0.5	2	2740	5480.4											
	4	2	0.5	1	3553	7106.6	7100.8	14201.6	0.1	122775	11.57	14020	0.0240	1	0.004	15	0.1983
			0.5	2	3548	7095											
Background control	1	2	0.5	1	50	99.5	100.6	201.2	0.1	122775	0.16	20	0.0000	1	0.004	15	0.0003
			0.5	2	51	101.7											
	2	2	0.5	1	48	95.2	95.99	191.98	0.1	122775	0.16	11	0.0000	1	0.004	15	0.0002
			0.5	2	48	96.78											
	3	2	0.5	1	35	70.16	77.74	155.48	0.1	122775	0.13	-26	0.0000	1	0.004	15	-0.0004
			0.5	2	43	85.32											
	4	2	0.5	1	40	79.14	88.25	176.5	0.1	122775	0.14	-5	0.0000	1	0.004	15	-0.0001
			0.5	2	49	97.36											
Positive control	1	2	0.5	1	2225	4449.4	4449.3	8898.6	0.1	122775	7.25	8717	0.0149	1	0.004	15	0.1233
			0.5	2	2225	4449.2											
	2	2	0.5	1	2307	4614.8	4564.5	9129	0.1	122775	7.44	8948	0.0153	1	0.004	15	0.1266
			0.5	2	2257	4514.2											
	3	2	0.5	1	2023	4046.4	4080.8	8161.6	0.1	122775	6.65	7960	0.0137	1	0.004	15	0.1129
			0.5	2	2058	4115.2											
	4	2	0.5	1	2199	4397.4	4435.5	8871	0.1	122775	7.23	8690	0.0149	1	0.004	15	0.1229
			0.5	2	2237	4473.6											
Negative Control	1	2	0.5	1	3753	7506.8	7530.6	15061.2	0.1	122775	12.27	14880	0.0255	1	0.004	15	0.2105
			0.5	2	3777	7554.4											
	2	2	0.5	1	3585	7170	7143.5	14287	0.1	122775	11.64	14106	0.0241	1	0.004	15	0.1996
			0.5	2	3559	7117											
	3	2	0.5	1	3295	6590	6548	13096	0.1	122775	10.67	12915	0.0221	1	0.004	15	0.1827
			0.5	2	3253	6506											
	4	2	0.5	1	3630	7260.4	7275.6	14551.2	0.1	122775	11.85	14370	0.0246	1	0.004	15	0.2033
			0.5	2	3645	7290.8											
10	1-1	2	0.5	1	3488	6976.4	6966.4	13932.8	0.1	122775	11.35	13752	0.0235	1	0.004	15	0.1945
			0.5	2	3478	6956.4											
	1-2	2	0.5	1	3412	6823.2	6940.4	13880.8	0.1	122775	11.31	13700	0.0234	1	0.004	15	0.1938
			0.5	2	3529	7057.6											
	1-3	2	0.5	1	3186	6372.8	6402.8	12805.6	0.1	122775	10.43	12624	0.0216	1	0.004	15	0.1786
			0.5	2	3216	6432.8											
	2-1	2	0.5	1	3414	6828.6	6828.3	13656.6	0.1	122775	11.12	13475	0.0231	1	0.004	15	0.1906
			0.5	2	3414	6828											
	2-2	2	0.5	1	3261	6521.6	6553.9	13107.8	0.1	122775	10.68	12927	0.0221	1	0.004	15	0.1829
			0.5	2	3293	6586.2											
	2-3	2	0.5	1	3373	6746.4	6735.8	13471.6	0.1	122775	10.97	13290	0.0227	1	0.004	15	0.1880
			0.5	2	3363	6725.2											
	3-1	2	0.5	1	3183	6366	6458.9	12917.8	0.1	122775	10.52	12737	0.0218	1	0.004	15	0.1802
			0.5	2	3276	6551.8											
	3-2	2	0.5	1	3439	6877.8	6869.4	13738.8	0.1	122775	11.19	13558	0.0232	1	0.004	15	0.1918
			0.5	2	3431	6861											
	3-3	2	0.5	1	3262	6523	6473	12946	0.1	122775	10.54	12765	0.0218	1	0.004	15	0.1806
			0.5	2	3212	6423											
	4-1	2	0.5	1	3319	6637.2	6636.4	13272.8	0.1	122775	10.81	13092	0.0224	1	0.004	15	0.1852
			0.5	2	3318	6635.6											
	4-2	2	0.5	1	3396	6791.8	6771.2	13542.4	0.1	122775	11.03	13361	0.0229	1	0.004	15	0.1890
			0.5	2	3375	6750.6											
	4-3	2	0.5	1	3314	6627.4	6662.9	13325.8	0.1	122775	10.85	13145	0.0225	1	0.004	15	0.1860
			0.5	2	3349	6698.4											
	5-1	2	0.5	1	3341	6682.2	6707.7	13415.4	0.1	122775	10.93	13234	0.0226	1	0.004	15	0.1872
			0.5	2	3367	6733.2											
	5-2	2	0.5	1	3503	7005.2	6981.6	13963.2	0.1	122775	11.37	13782	0.0236	1	0.004	15	0.1950
			0.5	2	3479	6958											
	5-3	2	0.5	1	3450	6900.2	6970.9	13941.8	0.1	122775	11.36	13761	0.0235	1	0.004	15	0.1947
			0.5	2	3521	7041.6											
	6-1	2	0.5	1	3744	7488.6	7458	14916	0.1	122775	12.15	14735	0.0252	1	0.004	15	0.2085
			0.5	2	3714	7427.4											
	6-2	2	0.5	1	3771	7542.2	7546	15092	0.1	122775	12.29	14911	0.0255	1	0.004	15	0.2109
			0.5	2	3775	7549.8											
	6-3	2	0.5	1	3735	7469.6	7440.1	14880.2	0.1	122775	12.12	14699	0.0252	1	0.004	15	0.2079
			0.5	2	3705	7410.6											
	7-1	2	0.5	1	3970	7939.6	7980.4	15960.8	0.1	122775	13.00	15780	0.0270	1	0.004	15	0.2232
			0.5	2	4011	8021.2											
	7-2	2	0.5	1	3702	7404.6	7432.7	14865.4	0.1	122775	12.11	14684	0.0251	1	0.004	15	0.2077
			0.5	2	3730	7460.8											
	7-3	2	0.5	1	3723	7445	7485.9	14971.8	0.1	122775	12.19	14791	0.0253	1	0.004	15	0.2092
			0.5	2	3763	7526.8											
	8-1	2	0.5	1	3748	7495	7530.2	15060.4	0.1	122775	12.27	14879	0.0255	1	0.004	15	0.2105
			0.5	2	3783	7565.4											
	8-2	2	0.5	1	3796	7592.4	7550.4	15100.8	0.1	122775	12.30	14920	0.0255	1	0.004	15	0.2111
			0.5	2	3754	7508.4											
	8-3	2	0.5	1	3840	7680.2	7821	15642	0.1	122775	12.74	15461	0.0265	1	0.004	15	0.2187
			0.5	2	3981	7961.8											

Assay Date	9/7/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lo	Technician ID	TD	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.2140	0.0247
Full activity	End	0.1753	0.0326
Full activity	Overall	0.1946	0.0325
Background	Beginning	0.0002	9.22335E-05
Background	End	-0.0002	0.000210276
Background	Overall	0.0000	0.00028292
Positive	Beginning	0.1250	0.0023
Positive	End	0.1179	0.0071
Positive	Overall	0.1214	0.0059
Negative	Beginning	0.2050	0.0077
Negative	End	0.1930	0.0146
Negative	Overall	0.1990	0.0118

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
10	1	1	1.00E-03	-3.00	0.1945
10	1	2	1.00E-03	-3.00	0.1938
10	1	3	1.00E-03	-3.00	0.1786
10	2	1	9.00E-04	-3.05	0.1906
10	2	2	9.00E-04	-3.05	0.1829
10	2	3	9.00E-04	-3.05	0.1880
10	3	1	8.00E-04	-3.10	0.1802
10	3	2	8.00E-04	-3.10	0.1918
10	3	3	8.00E-04	-3.10	0.1806
10	4	1	7.50E-04	-3.12	0.1852
10	4	2	7.50E-04	-3.12	0.1890
10	4	3	7.50E-04	-3.12	0.1860
10	5	1	5.00E-04	-3.30	0.1872
10	5	2	5.00E-04	-3.30	0.1950
10	5	3	5.00E-04	-3.30	0.1947
10	6	1	1.00E-04	-4.00	0.2085
10	6	2	1.00E-04	-4.00	0.2109
10	6	3	1.00E-04	-4.00	0.2079
10	7	1	1.00E-05	-5.00	0.2232
10	7	2	1.00E-05	-5.00	0.2077
10	7	3	1.00E-05	-5.00	0.2092
10	8	1	1.00E-06	-6.00	0.2105
10	8	2	1.00E-06	-6.00	0.2111
10	8	3	1.00E-06	-6.00	0.2187

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	99.96	99.58	91.77
2	-3.05	97.95	93.96	96.61
3	-3.10	92.58	98.55	92.79
4	-3.12	95.16	97.12	95.55
5	-3.30	96.20	100.18	100.02
6	-4.00	107.11	108.38	106.85
7	-5.00	114.70	106.74	107.51
8	-6.00	108.15	108.45	112.38

Assay Date	9/8/2005	Test Chemical ID	10	# Concentrations tested	8		
Technician ID	TD	Replicate #	3	Microsome type	recombinar	Microsome ID	BD Gentes

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	09/08/2005	Test Chemical ID	10	# Concentrations tested	8							
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5					
								Protein stock (mg BSA)	Total volume of stock (mL)		Protein stock ID	
								25		100	11343-77	

Standards:	250	125	50	25	10	5	0
	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
	0.491	0.292	0.135	0.068	0.028	0.014	0.000

Samples:	QC 100	QC 10	Microsomes
	0.246	0.027	0.211
	0.243	0.026	0.213
			0.210

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	0.492	0.492	0.0481	m, b	0.101	-0.002
125	100	200	0.00013		200	0.0250	0.291	0.291	0.0277	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	429	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							

Regression results are calculated using the function LINEST

r^2= 0.991
m= 0.101
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	0.246	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	0.211	0.211	0.020	200	80	4800	0.006	0.006	5.890
Microsomes	0.213	0.213	0.020	200	80	4800	0.006		
Microsomes	0.210	0.210	0.020	200	80	4800	0.006		

Assay Date	9/8/2005	Test Chemical ID	10	# Concentrations tested	8						
Technician ID	TD	Replicate #	3	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5				
							Protein stock (mg BSA)	25	Total volume of stock (mL)	100	Protein stock ID 11343-77

Standards:	250	125	50	25	10	5	0
	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
	0.491	0.292	0.135	0.068	0.028	0.014	0.000

Samples:	QC 100	QC 10	Microsomes
	0.246	0.027	0.211
	0.243	0.026	0.213
			0.210

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	0.492	0.492	0.0420	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 100.	027	0.027	0.002	200	1	1	0.000	0.000	0.008
QC 100.	026	0.026	0.002	200	1	1	0.000		
QC 10									
Microsomes	211	0.211	0.018	200	80	4800	0.005	0.005	5.312
Microsomes	0.213	0.213	0.018	200	80	4800	0.005		
Microsomes	0.210	0.210	0.018	200	80	4800	0.005		

Assay Date	9/8/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest	Technician ID	TD	Replicate #	3

Microsome Dilution Details		
Dilution A	0.08	mL microsome Stock used
	4.8	mL total volume
	60	dilution factor
Dilution B	4	mL microsome Dilution A used
	42	mL total volume
	10.5	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	630	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.312347
Protein Concentration (dilution added to assay, mg/mL):	0.008432

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	9.00E-04
3	8.00E-04
4	7.50E-04
5	5.00E-04
6	1.00E-04
7	1.00E-05
8	1.00E-06

Assay Date	9/8/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lot #5	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	4236	8472	8443	16886	0.1	123249	13.70	16453	0.0275	1	0.004	15	0.2172
			0.5	2	4207	8414											
	2	2	0.5	1	4365	8730	8731	17462	0.1	123249	14.17	17029	0.0284	1	0.004	15	0.2248
			0.5	2	4366	8732											
	3	2	0.5	1	4131	8262	8422	16844	0.1	123249	13.67	16411	0.0274	1	0.004	15	0.2167
			0.5	2	4291	8582											
	4	2	0.5	1	4203	8406	8443	16886	0.1	123249	13.70	16453	0.0275	1	0.004	15	0.2172
			0.5	2	4240	8480											
Background control	1	2	0.5	1	76	152	626	1252	0.1	123249	1.02	819	0.0014	1	0.004	15	0.0108
			0.5	2	550	1100											
	2	2	0.5	1	54	108	101	202	0.1	123249	0.16	-231	-0.0004	1	0.004	15	-0.0030
			0.5	2	47	94											
	3	2	0.5	1	31	62	67	134	0.1	123249	0.11	-299	-0.0005	1	0.004	15	-0.0039
			0.5	2	36	72											
	4	2	0.5	1	39	78	72	144	0.1	123249	0.12	-289	-0.0005	1	0.004	15	-0.0038
			0.5	2	33	66											
Positive control	1	2	0.5	1	2754	5508	5496	10992	0.1	123249	8.92	10559	0.0176	1	0.004	15	0.1394
			0.5	2	2742	5484											
	2	2	0.5	1	2851	5702	5684	11368	0.1	123249	9.22	10935	0.0183	1	0.004	15	0.1444
			0.5	2	2833	5666											
	3	2	0.5	1	2414	4828	4903	9806	0.1	123249	7.96	9373	0.0157	1	0.004	15	0.1237
			0.5	2	2489	4978											
	4	2	0.5	1	2435	4870	4880	9760	0.1	123249	7.92	9327	0.0156	1	0.004	15	0.1231
			0.5	2	2445	4890											
Negative Control	1	2	0.5	1	4340	8680	8748	17496	0.1	123249	14.20	17063	0.0285	1	0.004	15	0.2253
			0.5	2	4408	8816											
	2	2	0.5	1	4132	8264	8324	16648	0.1	123249	13.51	16215	0.0271	1	0.004	15	0.2141
			0.5	2	4192	8384											
	3	2	0.5	1	3789	7578	7703	15406	0.1	123249	12.50	14973	0.0250	1	0.004	15	0.1977
			0.5	2	3914	7828											
	4	2	0.5	1	4331	8662	8758	17516	0.1	123249	14.21	17083	0.0285	1	0.004	15	0.2255
			0.5	2	4427	8854											
10	1-1	2	0.5	1	3542	7084	7063	14126	0.1	123249	11.46	13693	0.0229	1	0.004	15	0.1808
			0.5	2	3521	7042											
	1-2	2	0.5	1	3742	7484	7493	14986	0.1	123249	12.16	14553	0.0243	1	0.004	15	0.1921
			0.5	2	3751	7502											
	1-3	2	0.5	1	3731	7462	7422	14844	0.1	123249	12.04	14411	0.0241	1	0.004	15	0.1903
			0.5	2	3691	7382											
	2-1	2	0.5	1	3684	7368	7334	14668	0.1	123249	11.90	14235	0.0238	1	0.004	15	0.1879
			0.5	2	3650	7300											
	2-2	2	0.5	1	3613	7226	7244	14488	0.1	123249	11.76	14055	0.0235	1	0.004	15	0.1856
			0.5	2	3631	7262											
	2-3	2	0.5	1	3592	7184	7254	14508	0.1	123249	11.77	14075	0.0235	1	0.004	15	0.1858
			0.5	2	3662	7324											
	3-1	2	0.5	1	3345	6690	6739	13478	0.1	123249	10.94	13045	0.0216	1	0.004	15	0.1722
			0.5	2	3394	6788											
	3-2	2	0.5	1	3622	7244	7220	14440	0.1	123249	11.72	14007	0.0234	1	0.004	15	0.1849
			0.5	2	3598	7196											
	3-3	2	0.5	1	3506	7012	7075	14150	0.1	123249	11.48	13717	0.0229	1	0.004	15	0.1811
			0.5	2	3569	7138											
	4-1	2	0.5	1	3592	7184	7161	14322	0.1	123249	11.62	13889	0.0232	1	0.004	15	0.1834
			0.5	2	3569	7138											
	4-2	2	0.5	1	3440	6880	6879	13758	0.1	123249	11.16	13325	0.0223	1	0.004	15	0.1759
			0.5	2	3439	6878											
	4-3	2	0.5	1	3574	7148	7230	14460	0.1	123249	11.73	14027	0.0234	1	0.004	15	0.1852
			0.5	2	3656	7312											
	5-1	2	0.5	1	3468	6936	6968	13936	0.1	123249	11.31	13503	0.0225	1	0.004	15	0.1783
			0.5	2	3500	7000											
	5-2	2	0.5	1	3664	7328	7275	14550	0.1	123249	11.81	14117	0.0236	1	0.004	15	0.1864
			0.5	2	3611	7222											
	5-3	2	0.5	1	3355	6710	6681	13362	0.1	123249	10.84	12929	0.0216	1	0.004	15	0.1707
			0.5	2	3326	6652											
	6-1	2	0.5	1	3967	7934	7914	15828	0.1	123249	12.84	15395	0.0257	1	0.004	15	0.2033
			0.5	2	3947	7894											
	6-2	2	0.5	1	3945	7890	7938	15876	0.1	123249	12.88	15443	0.0258	1	0.004	15	0.2039
			0.5	2	3993	7986											
	6-3	2	0.5	1	3680	7360	7421	14842	0.1	123249	12.04	14409	0.0241	1	0.004	15	0.1902
			0.5	2	3741	7482											
	7-1	2	0.5	1	4482	8964	8959	17918	0.1	123249	14.54	17485	0.0292	1	0.004	15	0.2308
			0.5	2	4477	8954											
	7-2	2	0.5	1	4264	8528	8458	16916	0.1	123249	13.73	16483	0.0275	1	0.004	15	0.2176
			0.5	2	4194	8388											
	7-3	2	0.5	1	4074	8148	8040	16080	0.1	123249	13.05	15647	0.0261	1	0.004	15	0.2066
			0.5	2	3966	7932											
	8-1	2	0.5	1	4400	8800	8752	17504	0.1	123249	14.20	17071	0.0285	1	0.004	15	0.2254
			0.5	2	4352	8704											
	8-2	2	0.5	1	4039	8078	8048	16096	0.1	123249	13.06	15663	0.0262	1	0.004	15	0.2068
			0.5	2	4009	8018											
	8-3	2	0.5	1	3992	7984	8060	16120	0.1	123249	13.08	15687	0.0262	1	0.004	15	0.2071
			0.5	2	4068	8136											

Assay Date	9/8/2005	Test Chemical ID	10	# Concentrations tested	8	Microsome type	recombinant	Microsome ID	BD Gentest Lo	Technician ID	TD	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.2210	0.0054
Full activity	End	0.2169	0.0004
Full activity	Overall	0.2190	0.0039
Background	Beginning	0.0039	0.009802509
Background	End	-0.0039	9.33572E-05
Background	Overall	0.0000	0.007219535
Positive	Beginning	0.1419	0.0035
Positive	End	0.1234	0.0004
Positive	Overall	0.1327	0.0108
Negative	Beginning	0.2197	0.0079
Negative	End	0.2116	0.0197
Negative	Overall	0.2156	0.0131

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
10	1	1	1.00E-03	-3.00	0.1808
10	1	2	1.00E-03	-3.00	0.1921
10	1	3	1.00E-03	-3.00	0.1903
10	2	1	9.00E-04	-3.05	0.1879
10	2	2	9.00E-04	-3.05	0.1856
10	2	3	9.00E-04	-3.05	0.1858
10	3	1	8.00E-04	-3.10	0.1722
10	3	2	8.00E-04	-3.10	0.1849
10	3	3	8.00E-04	-3.10	0.1811
10	4	1	7.50E-04	-3.12	0.1834
10	4	2	7.50E-04	-3.12	0.1759
10	4	3	7.50E-04	-3.12	0.1852
10	5	1	5.00E-04	-3.30	0.1783
10	5	2	5.00E-04	-3.30	0.1864
10	5	3	5.00E-04	-3.30	0.1707
10	6	1	1.00E-04	-4.00	0.2033
10	6	2	1.00E-04	-4.00	0.2039
10	6	3	1.00E-04	-4.00	0.1902
10	7	1	1.00E-05	-5.00	0.2308
10	7	2	1.00E-05	-5.00	0.2176
10	7	3	1.00E-05	-5.00	0.2066
10	8	1	1.00E-06	-6.00	0.2254
10	8	2	1.00E-06	-6.00	0.2068
10	8	3	1.00E-06	-6.00	0.2071

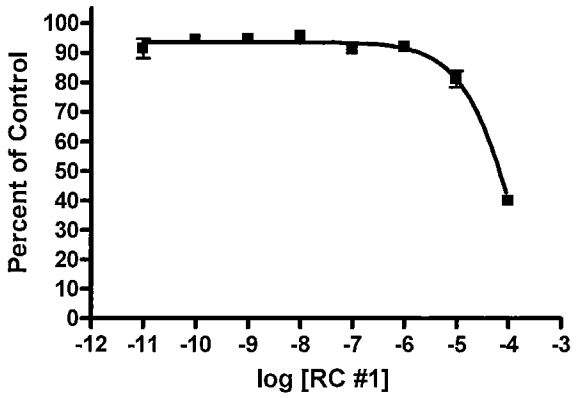
Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	82.56	87.74	86.88
2	-3.05	85.82	84.74	84.86
3	-3.10	78.65	84.45	82.70
4	-3.12	83.74	80.34	84.57
5	-3.30	81.41	85.11	77.95
6	-4.00	92.82	93.11	86.87
7	-5.00	105.42	99.38	94.34
8	-6.00	102.92	94.43	94.58

Appendix C

Prism Output C-1

Recombinant Microsomes Assay 7-20-05

Replicate 1 (Aminoglutethimide)

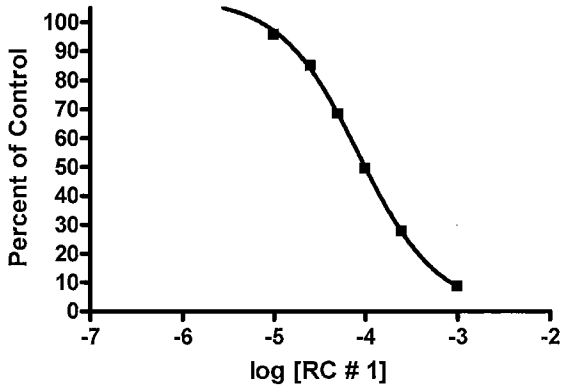


-4.00	41.23	39.23	39.76
-5.00	85.93	81.34	76.21
-6.00	92.61	93.18	90.53
-7.00	93.24	87.68	92.85
-8.00	96.15	95.72	95.05
-9.00	96.97	94.43	92.76
-10.00	95.91	95.47	92.10
-11.00	84.98	96.22	92.97

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-42.74
TOP	93.67
LOGEC50	-3.766
HILLSLOPE	-0.8087
EC50	0.0001712
Std. Error	
BOTTOM	295.2
TOP	0.9012
LOGEC50	2.057
HILLSLOPE	0.5081
95% Confidence Intervals	
BOTTOM	-658.5 to 573.0
TOP	91.79 to 95.55
LOGEC50	-8.057 to 0.5244
HILLSLOPE	-1.869 to 0.2512
EC50	8.766e-009 to 3.345
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9728
Absolute Sum of Squares	204.9
Sy.x	3.201
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant Microsomes Assay 7-21-05

Replicate 2 (Aminoglutethimide)

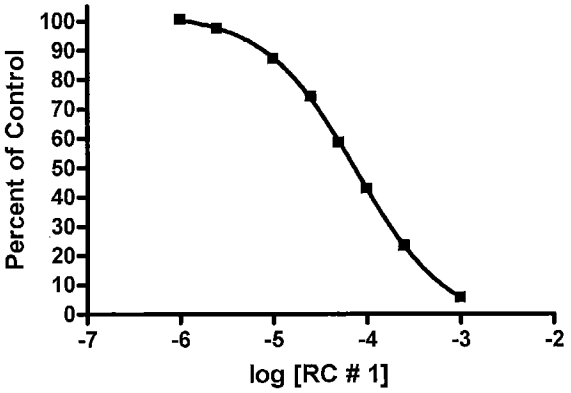


-3.00	8.49	8.75	8.29
-3.60	27.78	27.82	27.30
-4.00	50.69	47.45	49.86
-4.30	68.56	69.01	67.34
-4.60	86.73	84.05	84.00
-5.00	94.45	97.92	94.61
-5.60	109.43	102.17	106.75
-6.00	106.30	107.05	106.86

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.2759
TOP	108.2
LOGEC50	-4.070
HILLSLOPE	-1.009
EC50	8.503e-005
Std. Error	
BOTTOM	2.013
TOP	0.9190
LOGEC50	0.02117
HILLSLOPE	0.04640
95% Confidence Intervals	
BOTTOM	-3.923 to 4.475
TOP	106.3 to 110.1
LOGEC50	-4.115 to -4.026
HILLSLOPE	-1.106 to -0.9126
EC50	7.681e-005 to 9.413e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9979
Absolute Sum of Squares	60.46
Sy.x	1.739
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant Microsomes Assay 7-22-05

Replicate 3 (Aminoglutethimide)

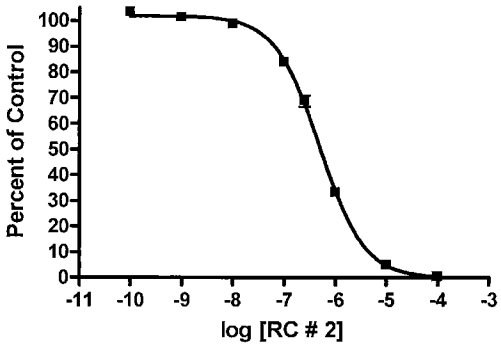


-3.00	5.71	5.81	5.54
-3.60	23.60	22.97	23.16
-4.00	42.65	42.61	43.00
-4.30	58.23	58.81	58.69
-4.60	73.64	75.12	73.78
-5.00	84.36	88.62	88.16
-5.60	98.83	98.51	94.83
-6.00	99.96	99.14	102.47

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-4.090
TOP	102.5
LOGEC50	-4.119
HILLSLOPE	-0.8902
EC50	7.599e-005
Std. Error	
BOTTOM	1.671
TOP	0.8010
LOGEC50	0.01835
HILLSLOPE	0.03348
95% Confidence Intervals	
BOTTOM	-7.577 to -0.6042
TOP	100.8 to 104.2
LOGEC50	-4.158 to -4.081
HILLSLOPE	-0.9600 to -0.8203
EC50	6.958e-005 to 8.299e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9988
Absolute Sum of Squares	31.23
Sy.x	1.250
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-11-05

Replicate 1 (Ketoconazole)

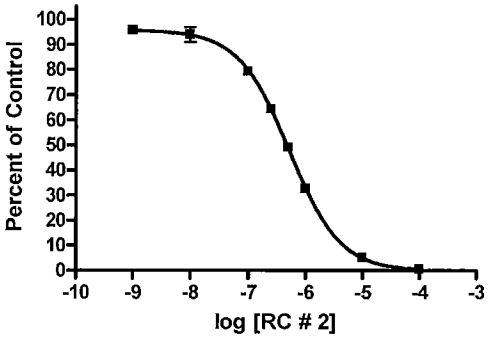


-4.00	0.48	0.37	0.45
-5.00	5.21	4.66	4.75
-6.00	32.69	33.59	33.03
-6.60	67.57	65.72	73.08
-7.00	83.61	83.55	84.54
-8.00	96.91	99.31	99.82
-9.00	102.17	99.47	102.20
-10.00	101.73	104.21	104.06

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.1264
TOP	101.8
LOGEC50	-6.304
HILLSLOPE	-0.9977
EC50	4.969e-007
Std. Error	
BOTTOM	0.9069
TOP	0.6383
LOGEC50	0.01778
HILLSLOPE	0.03745
95% Confidence Intervals	
BOTTOM	-2.018 to 1.765
TOP	100.5 to 103.2
LOGEC50	-6.341 to -6.267
HILLSLOPE	-1.076 to -0.9196
EC50	4.563e-007 to 5.412e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9984
Absolute Sum of Squares	63.01
Sy.x	1.775
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-12-05

Replicate 2 (Ketoconazole)

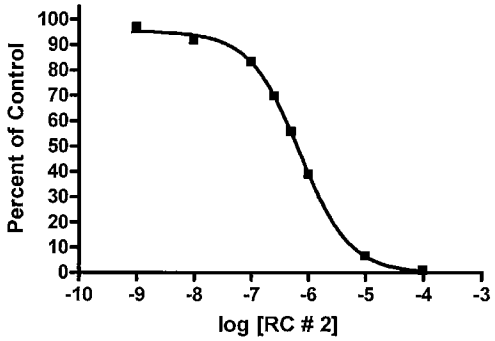


-4.00	0.46	0.49	0.37
-5.00	5.12	5.09	4.89
-6.00	32.55	32.61	32.56
-6.30	49.39	48.93	48.70
-6.60	62.72	65.23	65.23
-7.00	77.17	77.49	83.32
-8.00	88.26	98.13	95.28
-9.00	94.80	93.14	98.55

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.09930
TOP	95.71
LOGEC50	-6.284
HILLSLOPE	-0.9779
EC50	5.196e-007
Std. Error	
BOTTOM	1.146
TOP	1.014
LOGEC50	0.02116
HILLSLOPE	0.05108
95% Confidence Intervals	
BOTTOM	-2.490 to 2.291
TOP	93.59 to 97.82
LOGEC50	-6.328 to -6.240
HILLSLOPE	-1.084 to -0.8714
EC50	4.694e-007 to 5.752e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9967
Absolute Sum of Squares	97.15
Sy.x	2.204
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-15-05

Replicate 3 (Ketoconazole)

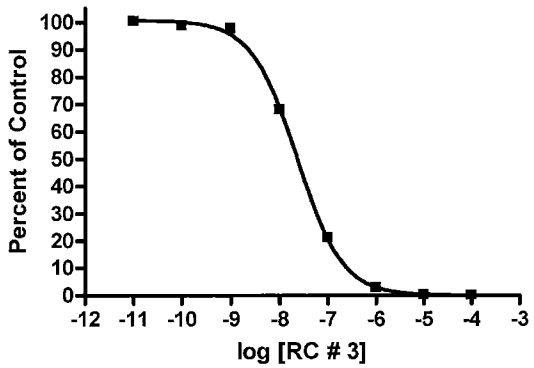


-4.00	0.63	0.80	0.52
-5.00	6.31	6.35	6.27
-6.00	39.33	39.40	37.57
-6.30	54.31	54.60	58.04
-6.60	68.43	69.41	71.21
-7.00	82.22	80.59	86.39
-8.00	90.00	91.05	94.22
-9.00	99.74	92.95	97.93

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.1560
TOP	95.25
LOGEC50	-6.157
HILLSLOPE	-0.9826
EC50	6.972e-007
Std. Error	
BOTTOM	1.172
TOP	0.9456
LOGEC50	0.02177
HILLSLOPE	0.05159
95% Confidence Intervals	
BOTTOM	-2.601 to 2.289
TOP	93.27 to 97.22
LOGEC50	-6.202 to -6.111
HILLSLOPE	-1.090 to -0.8750
EC50	6.280e-007 to 7.741e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9969
Absolute Sum of Squares	90.21
Sy.x	2.124
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-02-05

Replicate 1 (Prochloraz)

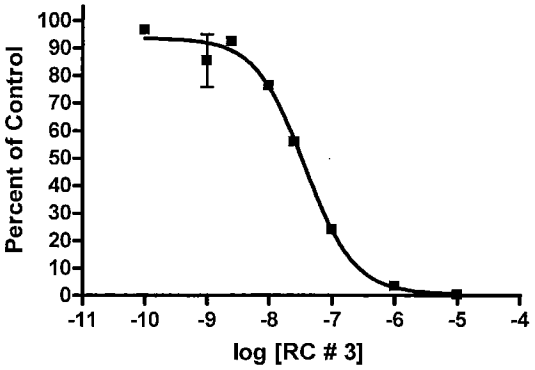


-4.00	0.17	0.07	0.13
-5.00	0.33	0.30	0.28
-6.00	2.66	2.84	2.92
-7.00	21.45	20.98	21.43
-8.00	67.23	68.48	68.55
-9.00	97.35	99.28	96.68
-10.00	99.30	98.28	98.79
-11.00	101.69	100.59	98.94

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.06698
TOP	100.8
LOGEC50	-7.637
HILLSLOPE	-0.9271
EC50	2.307e-008
Std. Error	
BOTTOM	0.4862
TOP	0.5067
LOGEC50	0.01572
HILLSLOPE	0.02568
95% Confidence Intervals	
BOTTOM	-0.9473 to 1.081
TOP	99.70 to 101.8
LOGEC50	-7.670 to -7.604
HILLSLOPE	-0.9807 to -0.8735
EC50	2.139e-008 to 2.488e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9993
Absolute Sum of Squares	33.46
Sy.x	1.293
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-03-05

Replicate 2 (Prochloraz)

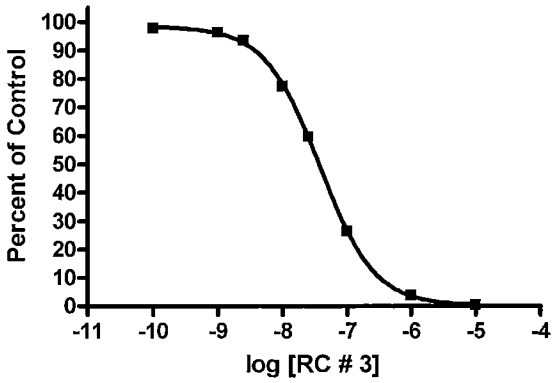


-5.00	0.32	0.40	0.32
-6.00	3.38	3.42	3.35
-7.00	25.06	23.06	24.31
-7.60	55.99	54.78	57.25
-8.00	75.21	75.96	78.09
-8.60	95.38	89.08	92.67
-9.00	94.50	95.41	66.39
-10.00	96.90	93.90	98.84

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.5819
TOP	93.60
LOGEC50	-7.432
HILLSLOPE	-1.101
EC50	3.697e-008
Std. Error	
BOTTOM	2.908
TOP	2.614
LOGEC50	0.06147
HILLSLOPE	0.1579
95% Confidence Intervals	
BOTTOM	-5.484 to 6.648
TOP	88.15 to 99.06
LOGEC50	-7.560 to -7.304
HILLSLOPE	-1.431 to -0.7720
EC50	2.752e-008 to 4.967e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9771
Absolute Sum of Squares	778.1
Sy.x	6.237
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-04-05

Replicate 3 (Prochloraz)

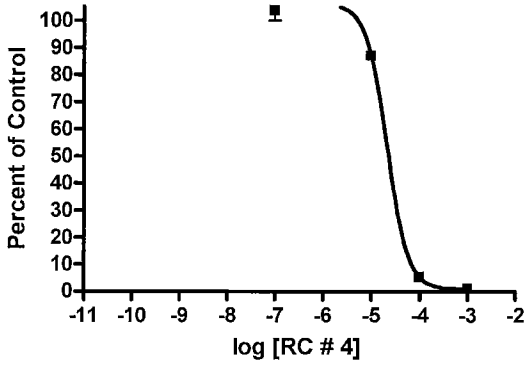


-5.00	0.56	0.30	0.32
-6.00	3.87	3.85	3.57
-7.00	27.04	26.77	25.72
-7.60	58.88	60.85	59.06
-8.00	76.15	76.84	79.26
-8.60	95.22	92.63	92.36
-9.00	93.67	97.70	97.19
-10.00	96.06	97.70	99.20

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.2406
TOP	98.41
LOGEC50	-7.426
HILLSLOPE	-1.021
EC50	3.749e-008
Std. Error	
BOTTOM	0.6303
TOP	0.5727
LOGEC50	0.01301
HILLSLOPE	0.02955
95% Confidence Intervals	
BOTTOM	-1.074 to 1.555
TOP	97.21 to 99.60
LOGEC50	-7.453 to -7.399
HILLSLOPE	-1.083 to -0.9596
EC50	3.522e-008 to 3.991e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9991
Absolute Sum of Squares	33.78
Sy.x	1.300
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (09-27-2005)

Replicate 1 (4-Nonylphenol)

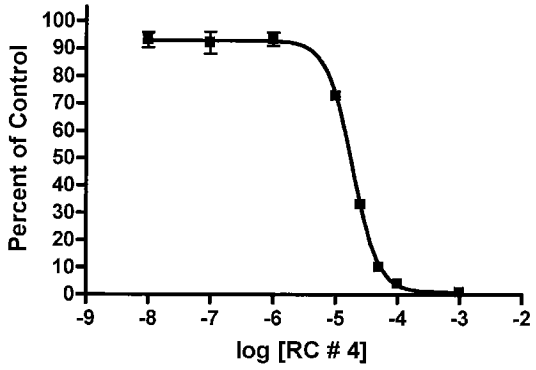


-3.00	1.18	1.11	0.54
-4.00	7.23	4.27	4.48
-5.00	87.15	87.55	86.23
-6.00	105.83	100.03	112.84
-7.00	106.53	107.89	96.49
-8.00	107.48	106.24	110.45
-9.00	105.67	107.07	107.71
-10.00	93.82	111.52	111.25

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.9143
TOP	106.1
LOGEC50	-4.675
HILLSLOPE	-2.014
EC50	2.111e-005
Std. Error	
BOTTOM	2.686
TOP	1.184
LOGEC50	0.06991
HILLSLOPE	0.3821
95% Confidence Intervals	
BOTTOM	-4.689 to 6.517
TOP	103.6 to 108.6
LOGEC50	-4.821 to -4.530
HILLSLOPE	-2.811 to -1.217
EC50	1.509e-005 to 2.954e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9910
Absolute Sum of Squares	416.3
Sy.x	4.562
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (09-28-2005)

Replicate 2 (4-Nonylphenol)

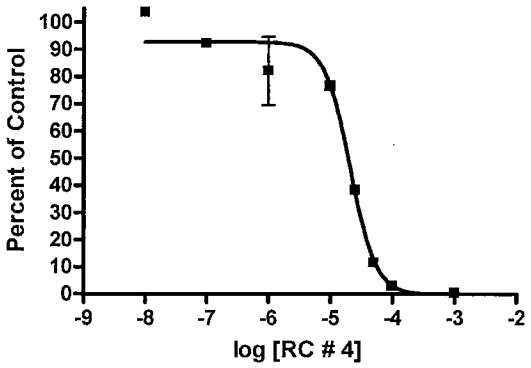


-3.00	0.59	1.17	0.98
-4.00	4.96	3.20	3.39
-4.30	10.70	9.97	9.68
-4.60	34.45	32.88	32.15
-5.00	74.57	71.91	71.69
-6.00	96.79	88.73	94.58
-7.00	87.51	99.95	88.67
-8.00	95.74	87.79	95.91

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.8241
TOP	92.88
LOGEC50	-4.732
HILLSLOPE	-2.096
EC50	1.852e-005
Std. Error	
BOTTOM	1.403
TOP	1.035
LOGEC50	0.01731
HILLSLOPE	0.1438
95% Confidence Intervals	
BOTTOM	-2.103 to 3.751
TOP	90.72 to 95.03
LOGEC50	-4.769 to -4.696
HILLSLOPE	-2.396 to -1.796
EC50	1.704e-005 to 2.012e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9949
Absolute Sum of Squares	190.4
Sy.x	3.085
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (09-29-2005)

Replicate 3 (4-Nonylphenol)

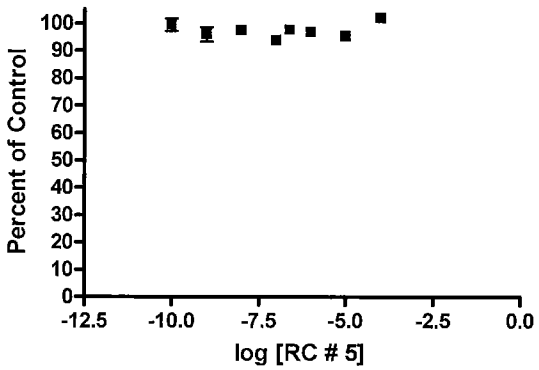


-3.00	0.76	0.32	0.40
-4.00	2.89	2.73	3.25
-4.30	8.77	12.98	12.91
-4.60	41.67	36.40	36.93
-5.00	76.46	77.68	75.07
-6.00	97.41	91.83	57.36
-7.00	91.65	90.77	94.47
-8.00	102.30	103.21	105.29

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.1897
TOP	92.73
LOGEC50	-4.678
HILLSLOPE	-2.112
EC50	2.099e-005
Std. Error	
BOTTOM	4.287
TOP	3.048
LOGEC50	0.04987
HILLSLOPE	0.4305
95% Confidence Intervals	
BOTTOM	-9.132 to 8.752
TOP	86.37 to 99.09
LOGEC50	-4.782 to -4.574
HILLSLOPE	-3.010 to -1.214
EC50	1.652e-005 to 2.668e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9572
Absolute Sum of Squares	1670
Sy.x	9.138
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (09-30-2005)

Replicate 1 (Dibenz[a,h]anthracene)

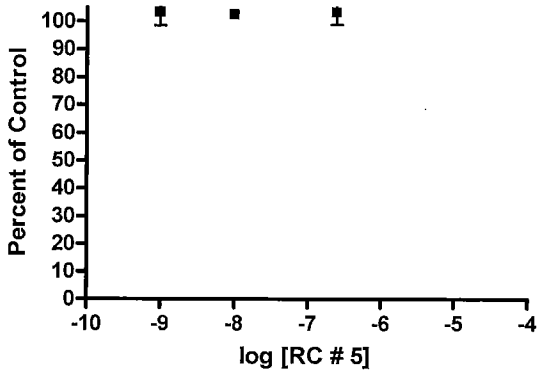


-4.00	102.00	101.10	102.45
-5.00	94.39	95.05	96.09
-6.00	97.79	96.15	96.05
-6.60	94.55	97.05	101.18
-7.00	91.69	92.71	96.66
-8.00	99.44	95.77	96.46
-9.00	90.71	98.46	98.58
-10.00	103.63	96.09	98.01

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	

Recombinant microsomes (10-03-2005)

Replicate 2 (Dibenz[a,h]anthracene)

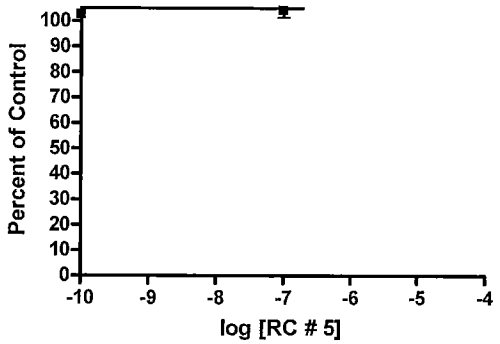


-4.00	106.49	108.54	104.76
-5.00	107.33	108.89	109.41
-6.00	112.20	102.86	110.82
-6.60	111.10	96.04	102.50
-7.00	105.57	106.72	108.45
-8.00	101.41	101.55	103.84
-9.00	99.74	112.68	97.42
-10.00	105.88	113.05	99.11

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	

Recombinant microsomes (10-04-2005)

Replicate 3 (Dibenz[a,h]anthracene)

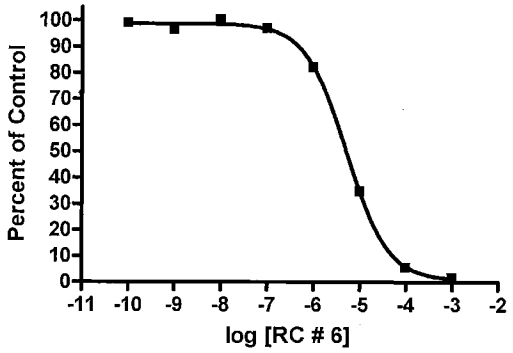


-4.00	108.97	112.83	110.79
-5.00	107.07	109.34	103.88
-6.00	115.30	110.14	112.92
-6.60	113.84	99.12	110.35
-7.00	98.92	105.02	108.41
-8.00	109.50	103.98	104.98
-9.00	102.26	110.09	109.02
-10.00	104.55	100.68	102.18

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	105.0
TOP	110.1
LOGEC50	-6.604
HILLSLOPE	20.96
EC50	2.492e-007
Std. Error	
BOTTOM	1.409
TOP	1.409
LOGEC50	18631
HILLSLOPE	1.104e+008
95% Confidence Intervals	
BOTTOM	102.0 to 107.9
TOP	107.2 to 113.1
LOGEC50	-38870 to 38857
HILLSLOPE	-230200000 to 2.302e+008
EC50	
Goodness of Fit	
Degrees of Freedom	20
R^2	0.2794
Absolute Sum of Squares	357.2
Sy.x	4.226
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-05-05

Replicate 1 (Fenarimol)

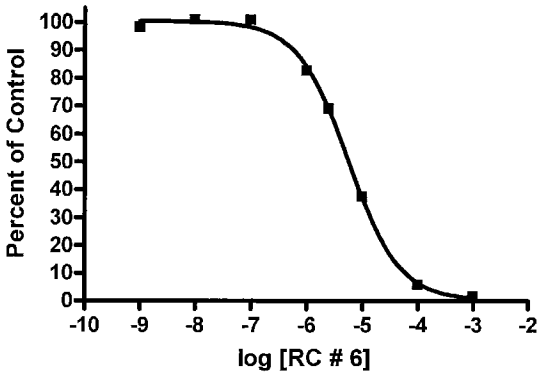


-3.00	1.44	1.64	1.47
-4.00	5.54	5.59	5.55
-5.00	35.18	35.55	33.23
-6.00	81.48	82.52	81.94
-7.00	95.57	97.22	98.00
-8.00	101.59	101.55	97.50
-9.00	98.83	93.30	97.52
-10.00	98.84	98.26	99.92

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.7102
TOP	98.71
LOGEC50	-5.287
HILLSLOPE	-0.9738
EC50	5.165e-006
Std. Error	
BOTTOM	0.9017
TOP	0.5373
LOGEC50	0.02144
HILLSLOPE	0.03955
95% Confidence Intervals	
BOTTOM	-1.171 to 2.591
TOP	97.59 to 99.83
LOGEC50	-5.332 to -5.242
HILLSLOPE	-1.056 to -0.8913
EC50	4.660e-006 to 5.725e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9985
Absolute Sum of Squares	59.83
Sy.x	1.730
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-08-05

Replicate 2 (Fenarimol)

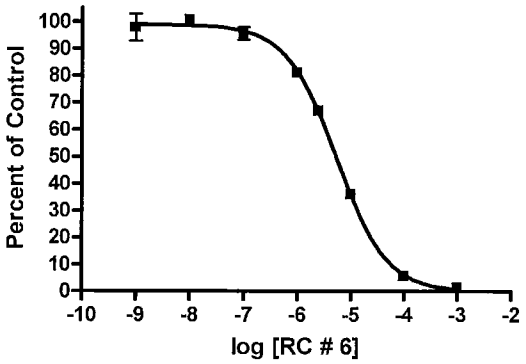


-3.00	1.65	1.46	1.43
-4.00	5.89	5.71	5.71
-5.00	37.22	37.92	37.11
-5.60	69.23	69.01	68.79
-6.00	81.88	81.35	84.46
-7.00	101.14	103.86	97.10
-8.00	102.81	98.90	100.61
-9.00	97.15	99.22	97.91

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.3027
TOP	100.4
LOGEC50	-5.250
HILLSLOPE	-0.9460
EC50	5.626e-006
Std. Error	
BOTTOM	1.110
TOP	0.7436
LOGEC50	0.02250
HILLSLOPE	0.04279
95% Confidence Intervals	
BOTTOM	-2.013 to 2.618
TOP	98.83 to 101.9
LOGEC50	-5.297 to -5.203
HILLSLOPE	-1.035 to -0.8567
EC50	5.049e-006 to 6.268e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9977
Absolute Sum of Squares	83.85
Sy.x	2.048
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes 08-09-05

Replicate 3 (Fenarimol)

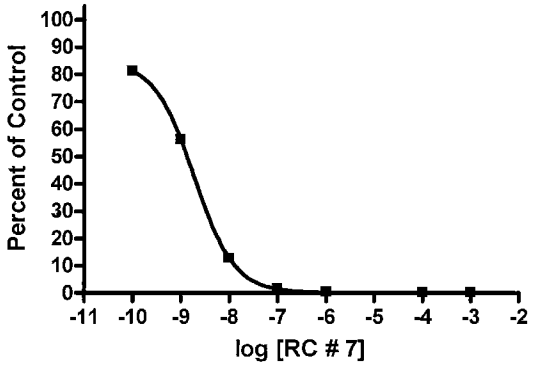


-3.00	1.29	1.28	1.36
-4.00	5.68	5.71	5.55
-5.00	36.13	35.02	36.91
-5.60	66.94	67.55	66.46
-6.00	81.76	81.07	80.49
-7.00	99.93	94.84	92.13
-8.00	98.07	104.13	99.88
-9.00	105.67	88.41	99.37

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.05869
TOP	98.94
LOGEC50	-5.263
HILLSLOPE	-0.9191
EC50	5.454e-006
Std. Error	
BOTTOM	1.868
TOP	1.245
LOGEC50	0.03860
HILLSLOPE	0.07001
95% Confidence Intervals	
BOTTOM	-3.955 to 3.837
TOP	96.34 to 101.5
LOGEC50	-5.344 to -5.183
HILLSLOPE	-1.065 to -0.7730
EC50	4.531e-006 to 6.566e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9936
Absolute Sum of Squares	229.6
Sy.x	3.388
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-17-2005)

Replicate 1 (Econazole)

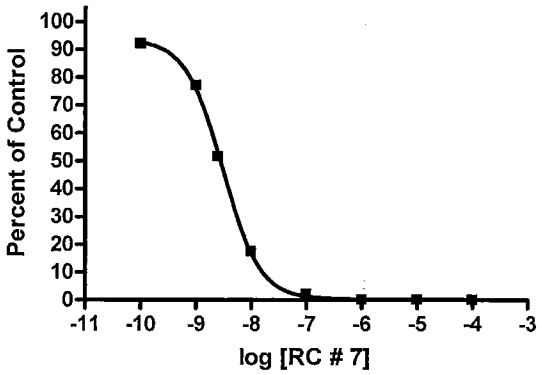


-3.00	0.27	0.01	0.13
-4.00	0.14	0.12	0.03
-5.00	-0.12	-0.10	-0.09
-6.00	0.06	0.22	0.55
-7.00	1.66	1.41	1.38
-8.00	12.33	12.77	13.20
-9.00	52.80	58.50	57.49
-10.00	78.65	81.56	83.42

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.08361
TOP	84.94
LOGEC50	-8.721
HILLSLOPE	-1.046
EC50	1.901e-009
Std. Error	
BOTTOM	0.3373
TOP	1.060
LOGEC50	0.01999
HILLSLOPE	0.03953
95% Confidence Intervals	
BOTTOM	-0.6201 to 0.7873
TOP	82.73 to 87.15
LOGEC50	-8.763 to -8.679
HILLSLOPE	-1.129 to -0.9638
EC50	1.727e-009 to 2.093e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9985
Absolute Sum of Squares	30.82
Sy.x	1.241
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-18-2005)

Replicate 2 (Econazole)

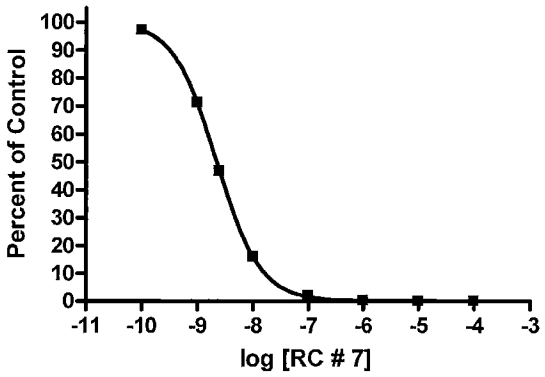


-4.00	0.22	-0.07	-0.05
-5.00	0.18	0.01	-0.05
-6.00	0.15	0.21	0.06
-7.00	1.96	1.92	1.90
-8.00	17.55	17.60	17.31
-8.60	51.86	50.96	51.88
-9.00	78.31	75.66	77.15
-10.00	90.61	93.48	91.82

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.3425
TOP	93.54
LOGEC50	-8.516
HILLSLOPE	-1.297
EC50	3.049e-009
Std. Error	
BOTTOM	0.3082
TOP	0.6593
LOGEC50	0.009402
HILLSLOPE	0.03138
95% Confidence Intervals	
BOTTOM	-0.3004 to 0.9855
TOP	92.17 to 94.92
LOGEC50	-8.535 to -8.496
HILLSLOPE	-1.362 to -1.231
EC50	2.915e-009 to 3.190e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9993
Absolute Sum of Squares	21.43
Sy.x	1.035
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-19-2005)

Replicate 3 (Econazole)

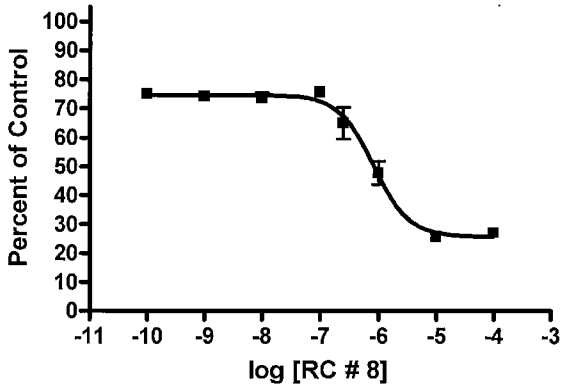


-4.00	-0.01	0.25	0.02
-5.00	0.16	-0.07	0.01
-6.00	0.36	0.10	0.10
-7.00	2.06	1.81	2.00
-8.00	16.12	15.78	16.25
-8.60	48.12	46.88	45.22
-9.00	74.73	68.15	70.96
-10.00	96.87	97.74	96.90

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.1713
TOP	100.3
LOGEC50	-8.652
HILLSLOPE	-1.112
EC50	2.226e-009
Std. Error	
BOTTOM	0.3541
TOP	0.8884
LOGEC50	0.01166
HILLSLOPE	0.03149
95% Confidence Intervals	
BOTTOM	-0.5673 to 0.9099
TOP	98.44 to 102.1
LOGEC50	-8.677 to -8.628
HILLSLOPE	-1.178 to -1.046
EC50	2.105e-009 to 2.355e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9991
Absolute Sum of Squares	27.39
Sy.x	1.170
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-23-2005)

Replicate 1 (Chrysin)

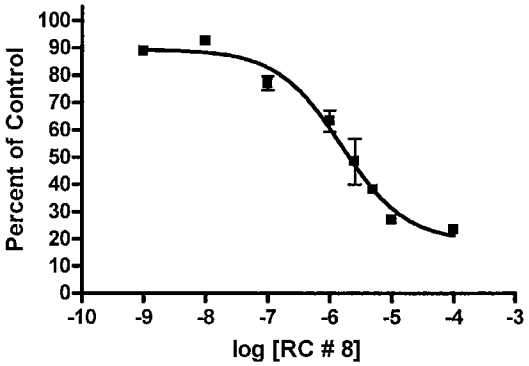


-4.00	27.69	25.82	27.85
-5.00	25.08	25.72	26.20
-6.00	40.14	49.62	53.76
-6.60	53.90	68.94	71.96
-7.00	74.73	75.85	76.40
-8.00	75.11	69.43	75.92
-9.00	71.68	74.54	76.19
-10.00	73.85	74.82	76.39

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	25.83
TOP	74.73
LOGEC50	-6.081
HILLSLOPE	-1.426
EC50	8.299e-007
Std. Error	
BOTTOM	2.033
TOP	1.443
LOGEC50	0.06977
HILLSLOPE	0.3208
95% Confidence Intervals	
BOTTOM	21.59 to 30.07
TOP	71.72 to 77.74
LOGEC50	-6.227 to -5.935
HILLSLOPE	-2.095 to -0.7572
EC50	5.935e-007 to 1.160e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9610
Absolute Sum of Squares	393.2
Sy.x	4.434
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-24-2005)

Replicate 2 (Chrysin)

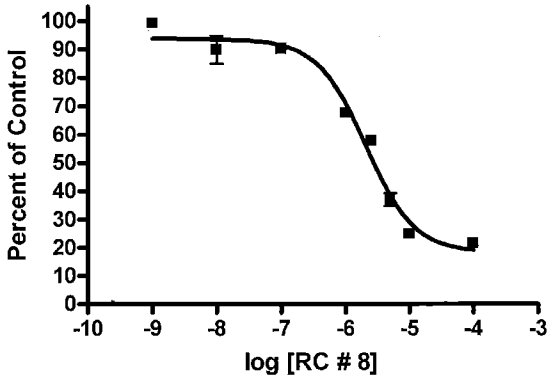


-4.00	22.41	22.07	25.37
-5.00	26.08	28.20	26.76
-5.30	39.45	34.62	40.19
-5.60	55.09	58.13	31.77
-6.00	55.84	64.43	69.39
-7.00	72.43	77.45	81.19
-8.00	91.67	91.75	94.34
-9.00	85.88	92.28	88.20

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	19.03
TOP	89.36
LOGEC50	-5.814
HILLSLOPE	-0.8334
EC50	1.536e-006
Std. Error	
BOTTOM	4.980
TOP	2.890
LOGEC50	0.1152
HILLSLOPE	0.1911
95% Confidence Intervals	
BOTTOM	8.638 to 29.42
TOP	83.34 to 95.39
LOGEC50	-6.054 to -5.573
HILLSLOPE	-1.232 to -0.4347
EC50	8.829e-007 to 2.670e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9472
Absolute Sum of Squares	852.7
Sy.x	6.530
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-25-2005)

Replicate 3 (Chrysin)

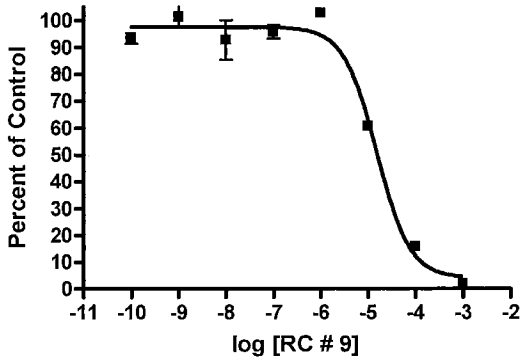


-4.00	22.44	22.94	19.10
-5.00	25.37	23.73	25.45
-5.30	33.52	36.14	41.25
-5.60	58.45	60.79	54.12
-6.00	67.08	67.76	68.06
-7.00	86.33	92.82	91.57
-8.00	80.52	91.74	97.25
-9.00	98.66	98.62	100.25

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	18.31
TOP	93.75
LOGEC50	-5.685
HILLSLOPE	-1.145
EC50	2.066e-006
Std. Error	
BOTTOM	3.429
TOP	1.975
LOGEC50	0.06139
HILLSLOPE	0.1804
95% Confidence Intervals	
BOTTOM	11.16 to 25.46
TOP	89.63 to 97.87
LOGEC50	-5.813 to -5.557
HILLSLOPE	-1.521 to -0.7685
EC50	1.538e-006 to 2.774e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9712
Absolute Sum of Squares	581.4
Sy.x	5.392
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-29-2005)

Replicate 1 (Dicofol)

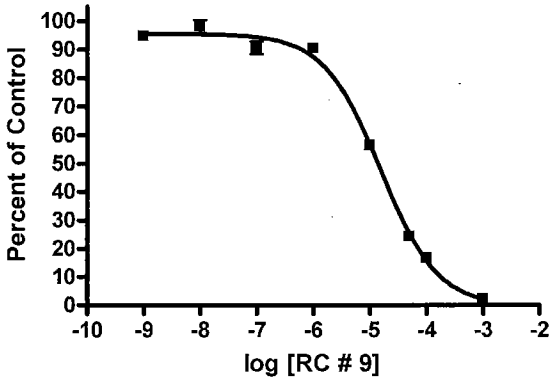


-3.00	2.01	2.27	1.80
-4.00	15.10	16.33	15.86
-5.00	59.22	61.59	61.10
-6.00	103.16	104.65	100.14
-7.00	96.20	91.16	99.83
-8.00	78.10	98.21	101.84
-9.00	102.94	107.43	93.69
-10.00	96.18	94.95	89.52

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	4.122
TOP	97.57
LOGEC50	-4.817
HILLSLOPE	-1.246
EC50	1.525e-005
Std. Error	
BOTTOM	4.327
TOP	1.887
LOGEC50	0.08487
HILLSLOPE	0.3011
95% Confidence Intervals	
BOTTOM	-4.904 to 13.15
TOP	93.64 to 101.5
LOGEC50	-4.994 to -4.640
HILLSLOPE	-1.874 to -0.6178
EC50	1.014e-005 to 2.292e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9729
Absolute Sum of Squares	941.3
Sy.x	6.860
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (08-30-2005)

Replicate 2 (Dicofol)

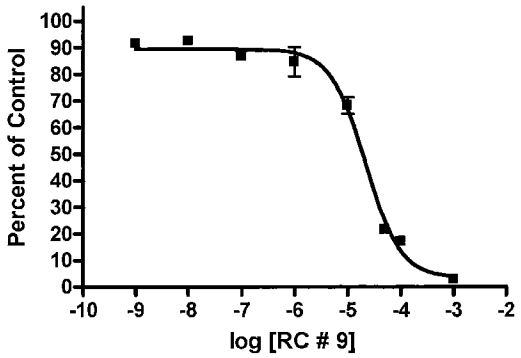


-3.00	1.99	2.10	2.42
-4.00	16.74	16.96	16.24
-4.30	23.16	24.40	25.13
-5.00	55.31	56.15	57.27
-6.00	88.97	90.94	91.51
-7.00	92.31	86.30	93.30
-8.00	93.60	100.11	100.45
-9.00	92.40	94.05	97.64

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.3419
TOP	95.54
LOGEC50	-4.808
HILLSLOPE	-0.8659
EC50	1.557e-005
Std. Error	
BOTTOM	0.5575
TOP	1.115
LOGEC50	0.02623
HILLSLOPE	0.04279
95% Confidence Intervals	
BOTTOM	-1.505 to 0.8210
TOP	93.22 to 97.87
LOGEC50	-4.862 to -4.753
HILLSLOPE	-0.9552 to -0.7766
EC50	1.373e-005 to 1.766e-005
Goodness of Fit	
Degrees of Freedom	20
R^2 (unweighted)	0.9946
Weighted Sum of Squares (1/Y)	2.349
Absolute Sum of Squares	180.4
Sy.x	3.004
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24

Recombinant microsomes (08-31-2005)

Replicate 3 (Dicofol)

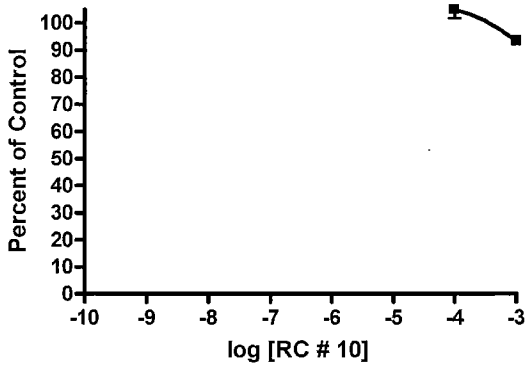


-3.00	2.99	3.20	2.37
-4.00	16.48	17.48	17.65
-4.30	20.01	22.05	22.96
-5.00	67.48	63.36	73.98
-6.00	74.58	85.40	93.86
-7.00	86.07	83.47	90.54
-8.00	91.84	93.82	91.90
-9.00	94.03	87.98	92.41

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	3.439
TOP	89.40
LOGEC50	-4.662
HILLSLOPE	-1.321
EC50	2.179e-005
Std. Error	
BOTTOM	2.945
TOP	1.467
LOGEC50	0.05258
HILLSLOPE	0.1554
95% Confidence Intervals	
BOTTOM	-2.705 to 9.582
TOP	86.34 to 92.46
LOGEC50	-4.771 to -4.552
HILLSLOPE	-1.645 to -0.9972
EC50	1.693e-005 to 2.806e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9843
Absolute Sum of Squares	475.7
Sy.x	4.877
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (09-06-2005)

Replicate 1 (Atrazine)

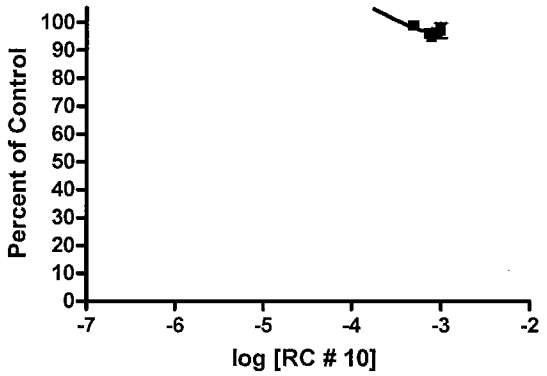


-3.00	90.89	94.05	95.37
-4.00	100.83	110.86	102.64
-5.00	109.31	106.15	104.74
-6.00	102.45	109.54	111.11
-7.00	111.87	106.29	112.12
-8.00	110.71	104.59	106.15
-9.00	104.57	106.68	106.29
-10.00	106.22	102.11	106.98

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	81.45
TOP	107.1
LOGEC50	-3.056
HILLSLOPE	-1.048
EC50	0.0008797
Std. Error	
BOTTOM	240.7
TOP	0.9018
LOGEC50	8.262
HILLSLOPE	4.487
95% Confidence Intervals	
BOTTOM	-420.7 to 583.6
TOP	105.3 to 109.0
LOGEC50	-20.29 to 14.18
HILLSLOPE	-10.41 to 8.312
EC50	5.134e-021 to 1.507e+014
Goodness of Fit	
Degrees of Freedom	20
R^2	0.6820
Absolute Sum of Squares	224.2
Sy.x	3.348
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (09-07-2005)

Replicate 2 (Atrazine)

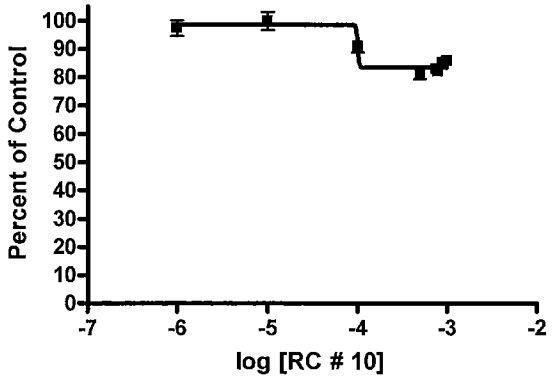


-3.00	99.96	99.58	91.77
-3.05	97.95	93.96	96.61
-3.10	92.58	98.55	92.79
-3.12	95.16	97.12	95.55
-3.30	96.20	100.18	100.02
-4.00	107.11	108.38	106.85
-5.00	114.70	106.74	107.51
-6.00	108.15	108.45	112.38

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	94.66
TOP	109.6
LOGEC50	-3.578
HILLSLOPE	-1.899
EC50	0.0002642
Std. Error	
BOTTOM	3.057
TOP	1.152
LOGEC50	0.2127
HILLSLOPE	1.024
95% Confidence Intervals	
BOTTOM	88.28 to 101.0
TOP	107.2 to 112.0
LOGEC50	-4.022 to -3.134
HILLSLOPE	-4.036 to 0.2379
EC50	9.509e-005 to 0.0007340
Goodness of Fit	
Degrees of Freedom	20
R^2	0.8523
Absolute Sum of Squares	153.4
Sy.x	2.770
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Recombinant microsomes (09-08-2005)

Replicate 3 (Atrazine)



-3.00	82.56	87.74	86.88
-3.05	85.82	84.74	84.86
-3.10	78.65	84.45	82.70
-3.12	83.74	80.34	84.57
-3.30	81.41	85.11	77.95
-4.00	92.82	93.11	86.87
-5.00	105.42	99.38	94.34
-6.00	102.92	94.43	94.58

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	83.44
TOP	98.51
LOGEC50	-4.000
HILLSLOPE	-57.36
EC50	9.998e-005
Std. Error	
BOTTOM	0.9224
TOP	1.458
LOGEC50	351046
HILLSLOPE	2.455e+011
95% Confidence Intervals	
BOTTOM	81.51 to 85.36
TOP	95.47 to 101.6
LOGEC50	-732300 to 732277
HILLSLOPE	-512100000000 to 5.121e+011
EC50	
Goodness of Fit	
Degrees of Freedom	20
R^2	0.7968
Absolute Sum of Squares	255.2
Sy.x	3.572
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

RECOMBINANT AROMATASE VALIDATION STUDY

CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES

INTRALABORATORY STATISTICAL ANALYSIS OF BATTELLE DATA

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-17, TASK 4

June 9, 2006

Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
ENDOCRINE DISRUPTOR SCREENING PROGRAM
WASHINGTON, D.C.

Prepared by

BATTELLE
505 King Avenue
Columbus, Ohio 43201

Recombinant Aromatase Validation Study

Conduct Multiple Chemical Studies with Recombinant Microsomes

Intralaboratory Statistical Analysis of Battelle Data

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-17, TASK 4

Ying-Liang Chou, Author — Date

Paul I. Feder, Reviewer — Date

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	Inspection Date	Date Reported to Battelle Task Leader/ Battelle Management
Audit study file	6/7/2006	6/7/2006
Audit draft report	6/7/2006	6/7/2006

Quality Assurance Unit **Date**

Hillary Flory 6-9-06

This report discusses the methods and results of the intralaboratory statistical analysis on the data collected at Battelle with the recombinant aromatase assay for WA 4-17 Task 4: Conduct Multiple Chemical Studies with Recombinant Microsomes.

Summary and Conclusions

There were ten test substances analyzed in this task: 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). Recombinant microsomes were prepared by one supplier. Battelle analyzed these ten chemicals 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 associated with 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. For test substance V all responses were above 90 percent of control. For test substance X all but two responses were above 80 percent of control. The other two responses were at 78.65 and 77.95 percent of control.
2. For Chemical VIII (chrysin) the low threshold of responses was in the range of 18

percent to 26 percent.

3. Among the four parameters, the top parameter had the most replicate-to-replicate variation. The other three parameters had replicate-to-replicate variation that was small or close to zero.
4. The overall standard errors of the mean of the weighted average estimates of the $\log_{10}IC_{50}$ and the slope were close to zero. For Chemical IV (4-nonylphenol) – slope, Chemical VIII (chrysin) – $\log_{10}IC_{50}$ and slope, Chemical IX (dicofol) – slope the standard errors were several times larger than for the other chemicals.
5. For the full enzyme activity, positive, and negative controls 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, the control averages did not differ significantly across chemicals and the portion effects (i.e. end minus beginning) did not differ significantly across chemicals.
7. 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).
8. For positive controls the majority of variation was from replicate-to-replicate within chemicals (Figures 13a and 13b).
9. There is some ambiguity in the $\log_{10}IC_{50}$ estimate. The "$\log_{10}IC_{50}$" concentration in fact corresponds to the (B+T)/2 percentile, which for some chemicals (e.g. chrysin) deviate somewhat from 50 percent. Such variation in percent inhibition can inflate the replicate-to-replicate variation among the $\log_{10}IC_{50}$ estimates. An alternative (nonstandard) parameterization of the model might be adopted in which the "$\log_{10}IC_{50}$" concentration in corresponds to the 50 percentile, irrespective of the top and bottom thresholds.

Introduction and Background

In Task 4 of the Recombinant Aromatase Validation Study, each laboratory carried out multiple independent replicates of the recombinant aromatase assay with ten test substances according to a common test protocol. Recombinant microsomes were prepared by one supplier. 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.

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 percent within each replicate.

Nominally for an inhibitor, the percent of control activity values vary between approximately 0 percent near the high inhibition concentrations and approximately 100 percent near the low inhibition concentrations, but this may vary with the inhibitor. Chemicals I (aminoglutethimide) and VIII (chrysin) depart 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.

Some potentially outlying responses were identified in the percent of control activity responses inhibitor concentrations data, in that they differed from surrounding data values more than typically:

- Chemical I – Replicate 1 (assay date 7-20-05), level 8, Percent of Control value 84.98
- Chemical II – Replicate 2 (assay date 8-12-05), level 7, Percent of Control value 98.13
- Chemical III – Replicate 2 (assay date 8-3-05), level 7, Percent of Control value 66.39
- Chemical IV – Replicate 2 (assay date 9-28-05), level 7, Percent of Control value 99.95; replicate 3 (assay date 9-29-05), level 6, Percent of Control values 97.41, 57.36
- Chemical VI – Replicate 3 (assay date 8-9-05), level 8, Percent of Control value 88.41
- Chemical VIII – Replicate 1 (assay date 8-23-05), level 4, Percent of Control value 53.90; replicate 2 (assay date 8-24-05), level 4, Percent of Control value 31.77; replicate 3 (assay date 8-25-05), level 7, Percent of Control value 80.52
- Chemical IX – Replicate 1 (assay date 8-29-05), level 4, Percent of Control values 103.16, 104.65, 100.14; replicate 1, level 6, Percent of Control value 78.10; replicate 2 (assay date 8-30-05), level 6, Percent of Control value 86.30; replicate 3 (assay date 8-31-05), level 5, Percent of Control value 93.86.

One background activity control value was identified as an outlier: Chemical X, replicate 3 (assay date 9-8-05), corrected aromatase activity value 0.01081, percent of control value 4.938.

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.

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)
$\mu = \log_{10}IC_{50}$ (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 percent inhibition to less than 50 percent inhibition. It is also indicated as "I", incomplete, if the concentration response curve decreases to between 80 percent and 50 percent of control. If the concentration response curve inhibition does not fall below 80 percent 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 average 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 percent confidence intervals based on the within replicate standard error and the average across replicates with associated 95 percent 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, and 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 percent (background activity controls), at 100 percent (full enzyme activity controls), or at 100 percent (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, one for Chemicals I, II, III, IV, and V, and the other for Chemicals VI, VII, VIII, IX, and X. Additional plots were prepared displaying the differences of the averages 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. Each plot has a reference line at 0. Again, two separate plots (with the same vertical scale) were generated for Chemicals I to V and for Chemicals VI to X.

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 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 percent 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 90 percent of control for Chemical V (dibenz[a,h]anthracene). All responses were above 77.9 percent of control for 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 of 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 responses reached close to 100 percent of control at the $\log_{10}$ concentration=-6. Above $\log_{10}$ concentration -6, the three replicates were similar to one another.. The parameter estimates and their 95 percent confidence intervals are displayed in Table 1-2 and plotted in Figures 1-3 to 1-6. In replicate 1, the tested log concentration levels did not extend above -4. This resulted in the bottom parameter being very poorly determined, estimated at -42.03 with a very high standard error, as shown in Table 1-1, Table 1-3, and Figure 1-3. An outlying value in replicate 1 ($\log_{10}$ concentration = -11, 84.98 percent of control) inflated the replicate-to-replicate variation and contributed to the large variation of top estimates across replicates and a 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 $\log_{10}IC_{50}$ estimate for replicate 1 was extrapolated beyond the

range of the data, at -3.771 with standard error orders of magnitude greater than those of the other replicates, as seen in Figure 1-5. The slope estimates were close to the average of -1 for replicates 2 and 3. The slope estimate was at -0.8 with a relatively large standard error for replicate 1, as seen in Figure 1-6.

Table 1-3 shows the estimated variance components for the parameter estimates. Among the three replicates, replicate 1 had orders of magnitude greater variability for bottom, $\log_{10}IC_{50}$, and slope. The extremely large individual within replicate variation for the bottom threshold parameter for replicate 1 was due to the responses corresponding to no less than about 50 percent inhibition within the range of the data. The replicate-to-replicate variation was large relative to the within replicate variation for replicates 2 and 3 for the bottom parameter.

For the top threshold, the replicate-to-replicate variation was more than sixty times the individual within replicate variations for replicates 1, 2, and 3. For $\log_{10}IC_{50}$ and slope, replicate 1 had more than two orders of magnitude greater within replicate variation than the other replicates. The within replicate variation for replicates 2 and 3 and the replicate-to-replicate variation were close to zero.

Chemical II (ketoconazole): Response Curve Analysis

A potentially outlying response value in replicate 2 was at log10concnetration = -8.00, with response 98.13 percent of control. This data point was retained in the analysis.

Figures 2-1 and 2-2 show that the responses for replicates 1, 2, and 3 were close to one another except for the top threshold, where replicate 1 was a little higher. Figure 2-3 and Table 2-1 show that the bottom threshold estimates were close to zero for the three replicates. For the top threshold parameter, the estimate was about 100 percent and about 95 percent for replicates 2 and 3, as seen in Table 2-1 and Figure 2-4. The relatively high top threshold estimate in replicate 1 inflated the replicate-to-replicate variation. Figure 2-5 shows that replicates 1 and 2 had same $\log_{10}IC_{50}$ estimates and replicate 3 had higher $\log_{10}IC_{50}$ estimate. Figure 2-6 and Table 2-1 show that slope parameter estimates were close to -1 for all three replicates.

Table 2-3 shows that for the bottom threshold parameter and the slope parameter, the replicate-to-replicate variation was zero. For the top threshold parameter, the majority of the overall variation was from replicate-to-replicate variation. The replicate-to-replicate variation for top was an order of magnitude greater than the within replicate variation. For the $\log_{10}IC_{50}$ and slope parameters, the individual replicate within-replicate variances and the replicate-to-replicate variations were all close to zero.

Chemical III (prochloraz): Response Curve Analysis

Figures 3-1 and 3-2 show that three replicates were in agreement. One outlying response for replicate 2 at the level 7 concentration ($\log_{10}$ concentration=-9, 66.39 percent of control) was kept in the analysis since there was no indication of departure from

protocol test procedures or of clerical errors (Table A-1c). The outlying response resulted in a relatively low top estimate and higher standard errors for all four parameters, as seen in Table 3-1 and Figures 3-3 to 3-6. For the bottom threshold parameter, estimates were close to zero for all three replicates, as seen in Table 3-1 and Figure 3-3. Figure 3-4 shows that the outlying value in replicate 2 and the variation among replicates resulted in a relatively wide 95 percent confidence interval for the overall mean for the top parameter. Table 3-1 and Figure 3-5 show that replicate 2 and 3 had similar $\log_{10}IC_{50}$ estimates, -7.43, and replicate 1 had a lower $\log_{10}IC_{50}$ estimate, -7.64. Figure 3-6 shows that the slope estimates were close to -1 in each replicate.

Table 3-3 shows that the replicate-to-replicate variation was zero for the bottom threshold parameter. Replicate 2's within replicate variation was more than twenty times higher than replicates 1 and replicate 3's within-replicate variation. For the top threshold parameter, the replicate-to-replicate variation contributed the majority of the overall variation. For $\log_{10}IC_{50}$ the within-replicate variation was small compared to the replicate-to-replicate variation. For slope the within-replicate variation and the replicate-to-replicate variations were close to zero.

Chemical IV (4-nonylphenol): Response Curve Analysis

There were outlying response values in replicate 2 ($\log_{10}$ concentration=-7, 99.95 percent of control) and in replicate 3 ($\log_{10}$ concentration=-6, 97.41 percent and 57.36 percent of control). These outlying values resulted in higher standard errors in parameter estimates for replicate 3. Table 4-1, Figure 4-1, and Figure 4-4 show that replicate 1 had a higher top threshold than replicates 2 and 3. Figure 4-3 shows that the bottom estimates were similar among replicates. For $\log_{10}IC_{50}$, the three replicates were similar, as seen in Table 4-1 and Figure 4-5. Figure 4-6 shows that the slope estimates were similar for all three replicates.

Table 4-3 shows that for the bottom threshold parameter, the replicate-to-replicate variation was zero and the within-replicate variances for replicates 1 and was relatively high. For the top threshold parameter, the replicate-to-replicate variation was much larger than the within-replicate variation, primarily due to the relatively high threshold in replicate 1.
For $\log_{10}IC_{50}$, the replicate-to-replicate variation and the individual replicate within-replicate variances for replicates 1, 2, and 3 were close to zero. For slope, the replicate-to-replicate variation for replicates 1 and 3 were relatively large.

Chemical V (dibenz[a,h]anthracene): Response Curve Analysis

This is a non-inhibitor chemical. All responses were above 90 percent of control for all three replicates. Response curve analysis was not performed for this chemical since the 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 displayed in Figures 5-2, 5-3, and 5-4. The individual data values are shown in Table A-1e.

Chemical VI (fenarimol): Response Curve Analysis

Figures 6-1 and 6-2 show that the three replicates were in line with one another. Table 6-1 and Figure 6-3 show that the bottom threshold parameter estimates were nearly the same for all the replicates. Table 6-1 and Figure 6-4 show that the top parameter estimates were close to 100 for each replicate.

The relatively large standard error for the bottom parameter estimate for replicate 3 maybe due to an outlying response value in replicate 3 (log_{10} concentration=-9, 88.41 percent of control), as shown in Figures 6-3 and Table 6-1. Table 6-1 and Figure 6-5 show that, for $log_{10}IC_{50}$, the three replicates were similar. Table 6-1 and Figure 6-6 show that for slope, the estimates were similar for all three replicates.

Table 6-3 shows that for the bottom threshold parameter, the replicate-to-replicate variation was zero. Replicate 3's within-replicate variance was larger than for the other two replicates. For the top parameter, replicate 3's within replicate variation was relatively large and larger than the replicate-to-replicate variation. For $log_{10}IC_{50}$ and slope, the replicate-to-replicate variation and the within-replicate variation for all the replicates were zero or close to zero.

Chemical VII (econazole): Response Curve Analysis

Figures 7-1 and 7-2 show that replicates 2 and 3's responses were in line with one other, but replicate 1 had greater inhibition at the lower concentration levels (i.e. in the upper portion of the response curve). Table 7-1 and Figure 7-3 show that the estimated bottom threshold parameters were similar and were close to zero for each of the three replicates. For the top threshold parameter estimates, Table 7-1 and Figure 7-4 show variation among the replicates. This resulted in a large standard error for the overall mean, as seen in Table 7-1 and Figure 7-4. For the $log_{10}IC_{50}$ and slope estimates Figure 7-5 and Figure 7-6 show that variation among the replicate was larger compared to the within replicate variation. This resulted in relatively large standard errors for the overall means, as seen in Table 7-1 and Figure 7-5, 7-6.

Table 7-3 shows that for the bottom threshold parameter the replicate-to-replicate variation was zero. The within-replicate variances for the bottom threshold parameter were compatible for the three replicates. For the top parameter, the replicate-to-replicate variation contributed the majority of the overall variation. The replicate-to-replicate variation was more than fifty times the individual replicate within-replicate variances. For $log_{10}IC_{50}$ and slope, the individual replicate within-replicate variances for replicates 1, 2 and 3 were all close to zero and were one or two orders of magnitude less than the replicate-to-replicate variation.

Chemical VIII (chrysin): Response Curve Analysis

A potentially outlying response was identified in the percent of control activity responses inhibitor concentrations data within each replicate: $\log_{10}$ concentration=-6.6, 53.90 percent of control for replicate 1; $\log_{10}$ concentration=-5.6, 31.77 percent of control for replicate 2; $\log_{10}$ concentration=-8, 80.52 percent of control for replicate 3. These responses were retained in the analysis.

Figure 8-1 shows that there was variation in the concentration response relations among all three replicates. Table 8-1 and Figure 8-3 show that the bottom thresholds were similar and were above 18 percent for all three replicates. Table 8-1 shows that the top thresholds were relatively low in all the replicates, particularly in replicate 1: 74.73, 89.36, and 93.75 percent, with a weighted mean of 85.83 percent. The variability among the top threshold estimates resulted in large overall variability for the mean top threshold estimate. Table 8-1 and Figure 8-5 show that the $\log_{10}IC_{50}$ estimates varied somewhat among replicates. Table 8-1 and Figure 8-6 show that the slopes were comparable across replicates.

It should be noted that the "$\log_{10}IC_{50}$" concentration in fact corresponds to the (B+T)/2 percentile, which were 50.28 percent, 54.20 percent, and 56.04 percent. The variation in percent inhibition contributed to the replicate-to-replicate variation among the $\log_{10}IC_{50}$ estimates.

Table 8-3 shows that for the bottom threshold parameter, the within-replicate variances were relatively large. The replicate-to-replicate variation was about the same size as the within replicate variation. For the top threshold parameter, the replicate-to-replicate variation was an order of magnitude greater than the within-replicate variation, For $\log_{10}IC_{50}$, the replicate-to-replicate variation was larger than the within-replicate variation. For slope the replicate-to-replicate variation was smaller than the within replicate variation.

Chemical IX (dicofol): Response Curve Analysis

A potentially outlying response value for replicate 1 was at $\log_{10}$ concentration=-8 with response 78.10 percent of control. Other potentially outlying responses were identified in replicate 2 at the $\log_{10}$ concentration=-7 (86.30 percent of control) and in replicate 3 at the $\log_{10}$ concentration=-6 (93.86 percent of control). These responses were retained in the analyses.

Figures 9-1 and 9-2 show that three replicates differed primarily at the tops of the response curves. Table A-1i shows that replicate 1's inhibition responses reached more than 100 percent of control (103.16, 104.65, 100.14) at $\log_{10}$ concentration=-6.00. Table 9-1 and Figure 9-3 show similarity among the bottom threshold estimates across replicates. Figure 9-4 shows variation among the top threshold estimates, particularly replicate 3. For $\log_{10}IC_{50}$, Figure 9-5 shows some variation between replicates 1, 2 and replicate 3. Table 9-1 and Figure 9-6 show that the slope estimates were similar among

replicates.

Table 9-3 shows that for the bottom threshold parameter, the replicate-to-replicate variation was zero. The within-replicate variances were relatively large. For the top threshold parameter the replicate-to-replicate variation was large compared to the within replicate variances. For $\log_{10}IC_{50}$ and slope, the within replicate variation was about the same size as the replicate-to-replicate variation. All were close to zero.

Chemical X (atrazine): Response Curve Analysis

This is a non-inhibitor chemical. All responses were above 90 percent of control for replicates 1 and 2. For replicate 3, only two responses were below 80 percent of control (at 78.65 percent and 77.95 percent). Response curve analysis was not performed for this chemical since the parameters were not estimable. Average 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 displayed in Figures 10-2, 10-3, and 10-4. The individual data values are shown in Table 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 first five chemicals and the last five chemicals. Figures 11a, 12a, 13a, 14a, 15a, 16a, 17a, and 18a are for Chemicals I, II, III, IV, and V. Figures 11b, 12b, 13b, 14b, 15b, 16b, 17b, and 18b are for Chemicals VI, VII, VIII, IX, and X. 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 corresponded to the 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 chemical main effect and the chemical by portion interaction effect were not significant for any of the four controls and are not included in Table 11.

Figures 11a, 11b, 13a, 13b, 14a, and 14b show that for the full enzyme activity controls and the positive and negative controls the majority of control responses were lower at the end of the replicate than at the beginning. This is reflected in Figures 15a, 15b, 17a, 17b, 18a, and 18b, where the majority of the curves fall below zero and in the significant portion effects for the full enzyme activity controls, and positive and negative controls in Table 11 ($p<0.0001$, $p=0.0012$, and $p=0.0008$, respectively).

Figures 11a and 11b and Table 11 show that the average levels and the variability about the average levels were consistent for across all the replicates for the full enzyme activity for eight of the ten test substances: II, III, IV, V, VI, VIII, IX, and X. One full enzyme activity control response at the beginning of replicate 2 for Chemical I was well below the average values, while the other two replicates' values were concentrated around 100 percent. This resulted in a large replicate by portion interaction variation for Chemical I, as seen in Table 11. Full enzyme activity control values at the beginning and at the end of replicate 1 of Chemical VII were far apart and away from 100 percent while the other two replicates' values were closer to 100 percent. This resulted in a large replicate by portion interaction variation for Chemical VII, as seen in Table 11. The repetition effect for the full enzyme activity controls also had relatively large variation. With a few exceptions, the majority of full enzyme activity control values were between 90 and 110 percent of control.

Figures 12a, 12b, 16a, 16b, and Table 11 show that except for replicate 3 of Chemical X, the other nine test substances had very little variability in the background activity controls for both the beginning and the end portions of each replicate. The differences between the beginning and the end were essentially zero for the background activity controls. One background activity control value at the beginning was identified as an outlier for Chemical X (Table A-2j), replicate 3 data (percent of control value 4.94). There were no indications for this value of departures from protocol test procedures or of clerical errors. Therefore, it was included in statistical analyses. Except for replicate 3 of Chemical X, the majority of background control values were close to zero percent of control.

Figures 13a and 13b show that the majority of the positive controls were between 45 percent and 65 percent of control. Table 11 shows that the replicate-to-replicate variation was high for Chemicals I, II, III, IV, and X. Positive control values at the beginning and at the end for replicate 1 of Chemical VII were far apart and away from the average while the other two replicates' values fell around the average. This resulted in relatively high replicate by portion interaction variation for Chemical VII, as seen in Table 11.

For the negative controls, Figures 14a and 14b show that the majority were between 90 percent and 110 percent of control. Table 11 shows that the replicate-to-replicate variations were relatively large for Chemicals I, IV, V, and IX. The replicate by portion interaction variation was relatively large for Chemicals IV, VII, VIII, and IX. The residual (repetition within replicate and portion) also had relatively large variation.

Except for several replicates for some test substances, the test system was consistent across chemicals and replicates within chemicals. However there was consistent decrease in control activity between the beginning and the end of nearly all the test replicates, which was statistically significant for the full enzyme activity, positive, and negative 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. Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates. Percent of Control Activity. Recombinant Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-42.030 (291.500)	93.670 (0.901)	-3.771 (2.044)	1.693E-04 (110.662)	-0.810 (0.509)	C
2	0.280 (1.995)	108.200 (0.913)	-4.071 (0.021)	8.484E-05 (1.050)	-1.009 (0.046)	C
3	-4.086 (1.664)	102.500 (0.799)	-4.120 (0.018)	7.580E-05 (1.043)	-0.890 (0.033)	C
Mean [c]	-2.044 (2.178)	101.457 (4.222)	-4.097 (0.024)	8.007E-05 (1.058)	-0.943 (0.058)	C
Average Values [b]						
1	-42.070 (313.700)	93.670 (0.966)	-3.771 (2.195)	1.694E-04 (156.675)	-0.810 (0.546)	C
2	0.280 (2.034)	108.200 (0.930)	-4.071 (0.021)	8.484E-05 (1.051)	-1.009 (0.047)	C
3	-4.086 (1.021)	102.500 (0.490)	-4.120 (0.011)	7.580E-05 (1.026)	-0.890 (0.020)	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.
Parameter Estimates of the Concentration Response Curves and
Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay**

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	-42.030 (-650.088,566.028)	0.280 (-3.881,4.442)	-4.086 (-7.557,-0.615)	-2.044 (-6.588,2.500)
Top	93.670 (91.790,95.550)	108.200 (106.296,110.104)	102.500 (100.834,104.166)	101.457 (83.256,119.658)
$Log_{10}IC_{50}$	-3.771 (-8.035,0.493)	-4.071 (-4.115,-4.027)	-4.120 (-4.158,-4.082)	-4.097 (-4.148,-4.046)
Slope	-0.810 (-1.872,0.252)	-1.009 (-1.105,-0.913)	-0.890 (-0.960,-0.821)	-0.943 (-1.064,-0.823)

a. Parameter estimates and their associated 95 percent 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 percent 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.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant 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	84972.2500 /df=20	3.98003 /df=20	2.76890 /df=20	6.15497 /df=2 (p=0.324)	4.74521 /df=20.012
Top	0.81198 /df=20	0.83302 /df=20	0.63792 /df=20	52.71462 /df=2 (p=0.162)	17.82515 /df=1.996
$Log_{10}IC_{50}$	4.17794 /df=20	0.00044 /df=20	0.00033 /df=20	0.00081 /df=2 (p=0.316)	0.00060 /df=20.030
Slope	0.25928 /df=20	0.00212 /df=20	0.00111 /df=20	0.00518 /df=2 (p=0.289)	0.00334 /df=20.616

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 $2*((1/K)*\Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)*\Sigma(Si^4/df_i))$, 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. Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates. Percent of Control Activity. Recombinant Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-0.129 (0.902)	101.800 (0.635)	-6.305 (0.018)	4.959E-07 (1.042)	-0.997 (0.037)	C
2	-0.100 (1.145)	95.720 (1.013)	-6.285 (0.021)	5.185E-07 (1.050)	-0.977 (0.051)	C
3	-0.168 (1.172)	95.260 (0.945)	-6.157 (0.022)	6.962E-07 (1.051)	-0.981 (0.051)	C
Mean [c]	-0.131 (0.606)	97.650 (2.136)	-6.250 (0.046)	5.630E-07 (1.112)	-0.987 (0.026)	C
Average Values [b]						
1	-0.129 (1.088)	101.800 (0.766)	-6.305 (0.021)	4.959E-07 (1.050)	-0.997 (0.045)	C
2	-0.100 (0.316)	95.720 (0.280)	-6.285 (0.006)	5.185E-07 (1.014)	-0.977 (0.014)	C
3	-0.168 (1.324)	95.260 (1.068)	-6.157 (0.025)	6.962E-07 (1.058)	-0.981 (0.058)	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.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay**

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	-0.129 (-2.011,1.754)	-0.100 (-2.488,2.288)	-0.168 (-2.612,2.277)	-0.131 (-1.345,1.083)
Top	101.800 (100.475,103.125)	95.720 (93.607,97.833)	95.260 (93.288,97.232)	97.650 (88.691,106.609)
$Log_{10}IC_{50}$	-6.305 (-6.342,-6.268)	-6.285 (-6.329,-6.241)	-6.157 (-6.202,-6.112)	-6.250 (-6.450,-6.049)
Slope	-0.997 (-1.074,-0.919)	-0.977 (-1.083,-0.870)	-0.981 (-1.088,-0.874)	-0.987 (-1.039,-0.936)

a. Parameter estimates and their associated 95 percent 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 percent 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.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant 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	0.81415 /df=20	1.31103 /df=20	1.37358 /df=20	0.00000 /df=2 (p=NA)	0.36777 /df=57.358
Top	0.40323 /df=20	1.02617 /df=20	0.89378 /df=20	12.92325 /df=2 (p=0.169)	4.56411 /df=2.055
$Log_{10}IC_{50}$	0.00031 /df=20	0.00045 /df=20	0.00047 /df=20	0.00599 /df=2 (p=0.175)	0.00214 /df=1.987
Slope	0.00138 /df=20	0.00259 /df=20	0.00264 /df=20	0.00000 /df=2 (p=NA)	0.00067 /df=56.086

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 $2*((1/K)*\Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)*\Sigma(Si^4/df_i))$, 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. Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates. Percent of Control Activity. Recombinant Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	0.067 (0.487)	100.800 (0.507)	-7.637 (0.016)	2.307E-08 (1.037)	-0.927 (0.026)	C
2	0.582 (2.910)	93.620 (2.616)	-7.433 (0.062)	3.687E-08 (1.152)	-1.100 (0.158)	C
3	0.245 (0.627)	98.420 (0.570)	-7.427 (0.013)	3.739E-08 (1.030)	-1.020 (0.029)	C
Mean [c]	0.141 (0.381)	98.340 (1.741)	-7.503 (0.072)	3.137E-08 (1.180)	-0.981 (0.043)	C
Average Values [b]						
1	0.067 (0.925)	100.800 (0.964)	-7.637 (0.030)	2.307E-08 (1.071)	-0.927 (0.049)	C
2	0.582 (3.230)	93.620 (2.904)	-7.433 (0.068)	3.687E-08 (1.170)	-1.100 (0.175)	C
3	0.245 (0.547)	98.420 (0.497)	-7.427 (0.011)	3.739E-08 (1.026)	-1.020 (0.026)	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.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay**

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	0.067 (-0.948,1.082)	0.582 (-5.488,6.652)	0.245 (-1.064,1.553)	0.141 (-0.647,0.930)
Top	100.800 (99.742,101.858)	93.620 (88.163,99.077)	98.420 (97.231,99.609)	98.340 (88.489,108.192)
$Log_{10}IC_{50}$	-7.637 (-7.670,-7.604)	-7.433 (-7.561,-7.305)	-7.427 (-7.454,-7.400)	-7.503 (-7.786,-7.221)
Slope	-0.927 (-0.981,-0.874)	-1.100 (-1.429,-0.771)	-1.020 (-1.081,-0.959)	-0.981 (-1.079,-0.883)

a. Parameter estimates and their associated 95 percent 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 percent 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.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay**

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 2	Replicate 3	Random Replicate (p-value) [d]	Variance of Mean
Bottom	0.23688 /df=20	8.46810 /df=20	0.39363 /df=20	0.00000 /df=2 (p=NA)	0.14535 /df=23.021
Top	0.25725 /df=20	6.84346 /df=20	0.32513 /df=20	7.40976 /df=2 (p=0.253)	3.03147 /df=1.569
$Log_{10}IC_{50}$	0.00025 /df=20	0.00379 /df=20	0.00017 /df=20	0.01422 /df=2 (p=0.170)	0.00515 /df=2.205
Slope	0.00066 /df=20	0.02487 /df=20	0.00086 /df=20	0.00322 /df=2 (p=0.266)	0.00186 /df=8.610

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 $2*((1/K)*\Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)*\Sigma(Si^4/df_i))$, 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.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay**

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	0.918 (2.685)	106.100 (1.184)	-4.676 (0.070)	2.111E-05 (1.175)	-2.014 (0.382)	C
2	0.860 (1.398)	92.880 (1.033)	-4.734 (0.017)	1.846E-05 (1.040)	-2.104 (0.144)	C
3	-0.152 (4.277)	92.740 (3.048)	-4.679 (0.050)	2.092E-05 (1.121)	-2.118 (0.432)	C
Mean [c]	0.793 (1.191)	97.415 (4.517)	-4.721 (0.020)	1.899E-05 (1.047)	-2.095 (0.129)	C
Average Values [b]						
1	0.918 (1.679)	106.100 (0.741)	-4.676 (0.044)	2.111E-05 (1.106)	-2.014 (0.239)	C
2	0.860 (0.610)	92.880 (0.451)	-4.734 (0.008)	1.846E-05 (1.017)	-2.104 (0.063)	C
3	-0.152 (6.122)	92.740 (4.363)	-4.679 (0.071)	2.092E-05 (1.178)	-2.118 (0.619)	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. Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals. Percent of Control Activity. Recombinant Aromatase Assay

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	0.918 (-4.683,6.518)	0.860 (-2.056,3.776)	-0.152 (-9.073,8.770)	0.793 (-1.617,3.202)
Top	106.100 (103.630,108.570)	92.880 (90.725,95.035)	92.740 (86.382,99.098)	97.415 (78.798,116.033)
$Log_{10}IC_{50}$	-4.676 (-4.822,-4.530)	-4.734 (-4.770,-4.698)	-4.679 (-4.783,-4.575)	-4.721 (-4.772,-4.671)
Slope	-2.014 (-2.811,-1.217)	-2.104 (-2.405,-1.803)	-2.118 (-3.019,-1.217)	-2.095 (-2.355,-1.835)

a. Parameter estimates and their associated 95 percent 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 percent 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. Variances Associated with Estimated Parameters of Concentration Response Curves. Percent of Control Activity. Recombinant Aromatase Assay

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 2	Replicate 3	Random Replicate (p-value) [d]	Variance of Mean
Bottom	7.20923 /df=20	1.95440 /df=20	18.29273 /df=20	0.00000 /df=2 (p=NA)	1.41835 /df=38.618
Top	1.40186 /df=20	1.06709 /df=20	9.29030 /df=20	57.51606 /df=2 (p=0.169)	20.40352 /df=2.095
$Log_{10}IC_{50}$	0.00489 /df=20	0.00030 /df=20	0.00247 /df=20	0.00021 /df=2 (p=0.448)	0.00039 /df=5.105
Slope	0.14615 /df=20	0.02085 /df=20	0.18671 /df=20	0.00000 /df=2 (p=NA)	0.01662 /df=44.166

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 $2*((1/K)*\Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)*\Sigma(Si^4/df_i))$, 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. Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates. Percent of Control Activity. Recombinant Aromatase Assay

All data points were above 90 percent 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 individual data values.

**Table 6-1. Reference Chemical VI: Fenarimol.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay**

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	0.711 (0.902)	98.710 (0.537)	-5.287 (0.021)	5.165E-06 (1.051)	-0.974 (0.040)	C
2	0.299 (1.109)	100.400 (0.743)	-5.251 (0.023)	5.616E-06 (1.053)	-0.945 (0.043)	C
3	-0.060 (1.867)	98.950 (1.245)	-5.264 (0.039)	5.444E-06 (1.093)	-0.918 (0.070)	C
Mean [c]	0.472 (0.655)	99.347 (0.608)	-5.269 (0.014)	5.382E-06 (1.034)	-0.954 (0.027)	C
Average Values [b]						
1	0.711 (1.275)	98.710 (0.760)	-5.287 (0.030)	5.165E-06 (1.072)	-0.974 (0.056)	C
2	0.299 (1.809)	100.400 (1.212)	-5.251 (0.037)	5.616E-06 (1.088)	-0.945 (0.070)	C
3	-0.060 (1.315)	98.950 (0.876)	-5.264 (0.027)	5.444E-06 (1.065)	-0.918 (0.049)	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.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	0.711 (-1.170,2.593)	0.299 (-2.015,2.612)	-0.060 (-3.954,3.835)	0.472 (-0.850,1.794)
Top	98.710 (97.589,99.831)	100.400 (98.851,101.949)	98.950 (96.353,101.547)	99.347 (97.505,101.189)
$Log_{10}IC_{50}$	-5.287 (-5.332,-5.242)	-5.251 (-5.298,-5.204)	-5.264 (-5.345,-5.183)	-5.269 (-5.298,-5.240)
Slope	-0.974 (-1.056,-0.891)	-0.945 (-1.034,-0.856)	-0.918 (-1.064,-0.772)	-0.954 (-1.008,-0.900)

a. Parameter estimates and their associated 95 percent 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 percent 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.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay**

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 2	Replicate 3	Random Replicate (p-value) [d]	Variance of Mean
Bottom	0.81360 /df=20	1.22988 /df=20	3.48569 /df=20	0.00000 /df=2 (p=NA)	0.42936 /df=42.684
Top	0.28880 /df=20	0.55160 /df=20	1.55003 /df=20	0.50084 /df=2 (p=0.308)	0.36980 /df=3.290
$Log_{10}IC_{50}$	0.00046 /df=20	0.00051 /df=20	0.00149 /df=20	0.00000 /df=2 (p=NA)	0.00021 /df=44.863
Slope	0.00156 /df=20	0.00182 /df=20	0.00488 /df=20	0.00000 /df=2 (p=NA)	0.00072 /df=46.198

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 $2*((1/K)* \Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \Sigma(Si^4 /df_i))$, 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.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay**

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	0.083 (0.337)	84.940 (1.059)	-8.721 (0.020)	1.901E-09 (1.047)	-1.046 (0.040)	C
2	0.347 (0.316)	93.570 (0.676)	-8.517 (0.010)	3.039E-09 (1.022)	-1.296 (0.032)	C
3	0.177 (0.355)	100.300 (0.891)	-8.654 (0.012)	2.220E-09 (1.027)	-1.112 (0.032)	C
Mean [c]	0.210 (0.193)	92.953 (4.432)	-8.630 (0.060)	2.345E-09 (1.148)	-1.152 (0.075)	C
Average Values [b]						
1	0.083 (0.055)	84.940 (0.172)	-8.721 (0.003)	1.901E-09 (1.007)	-1.046 (0.006)	C
2	0.347 (0.559)	93.570 (1.197)	-8.517 (0.017)	3.039E-09 (1.040)	-1.296 (0.057)	C
3	0.177 (0.130)	100.300 (0.327)	-8.654 (0.004)	2.220E-09 (1.010)	-1.112 (0.012)	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.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	0.083 (-0.620,0.787)	0.347 (-0.311,1.005)	0.177 (-0.564,0.918)	0.210 (-0.177,0.597)
Top	84.940 (82.731,87.149)	93.570 (92.161,94.979)	100.300 (98.441,102.159)	92.953 (73.774,112.131)
$Log_{10}IC_{50}$	-8.721 (-8.763,-8.679)	-8.517 (-8.537,-8.497)	-8.654 (-8.678,-8.630)	-8.630 (-8.888,-8.372)
Slope	-1.046 (-1.128,-0.964)	-1.296 (-1.363,-1.229)	-1.112 (-1.178,-1.046)	-1.152 (-1.474,-0.831)

a. Parameter estimates and their associated 95 percent 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 percent 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.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant 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	0.11370 /df=20	0.09954 /df=20	0.12603 /df=20	0.00000 /df=2 (p=NA)	0.03735 /df=59.456
Top	1.12148 /df=20	0.45630 /df=20	0.79424 /df=20	58.13154 /df=2 (p=0.163)	19.64032 /df=1.988
$Log_{10}IC_{50}$	0.00040 /df=20	0.00009 /df=20	0.00014 /df=20	0.01060 /df=2 (p=0.163)	0.00360 /df=2.001
Slope	0.00156 /df=20	0.00103 /df=20	0.00100 /df=20	0.01558 /df=2 (p=0.177)	0.00559 /df=1.999

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 $2*((1/K)*\Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)*\Sigma(Si^4/df_i))$, 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.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay**

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	25.830 (2.036)	74.730 (1.445)	-6.081 (0.070)	8.295E-07 (1.175)	-1.424 (0.320)	C
2	19.040 (4.971)	89.360 (2.887)	-5.815 (0.115)	1.533E-06 (1.303)	-0.834 (0.191)	C
3	18.330 (3.416)	93.750 (1.969)	-5.686 (0.061)	2.061E-06 (1.151)	-1.147 (0.180)	C
Mean [c]	21.979 (2.809)	85.831 (5.848)	-5.862 (0.123)	1.375E-06 (1.327)	-1.074 (0.148)	C
Average Values [b]						
1	25.820 (1.860)	74.730 (1.321)	-6.081 (0.064)	8.295E-07 (1.159)	-1.423 (0.293)	C
2	19.040 (6.034)	89.360 (3.504)	-5.815 (0.140)	1.532E-06 (1.379)	-0.834 (0.232)	C
3	18.330 (5.877)	93.750 (3.388)	-5.686 (0.105)	2.061E-06 (1.274)	-1.147 (0.310)	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 8-2. Reference Chemical VIII: Chrysin.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay**

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	25.830 (21.583,30.077)	19.040 (8.671,29.409)	18.330 (11.204,25.456)	21.979 (13.073,30.885)
Top	74.730 (71.716,77.744)	89.360 (83.338,95.382)	93.750 (89.643,97.857)	85.831 (61.463,110.199)
$Log_{10}IC_{50}$	-6.081 (-6.227,-5.935)	-5.815 (-6.055,-5.575)	-5.686 (-5.813,-5.559)	-5.862 (-6.338,-5.385)
Slope	-1.424 (-2.092,-0.756)	-0.834 (-1.233,-0.435)	-1.147 (-1.523,-0.771)	-1.074 (-1.648,-0.499)

a. Parameter estimates and their associated 95 percent 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 percent 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.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay**

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 2	Replicate 3	Random Replicate (p-value) [d]	Variance of Mean
Bottom	4.14530 /df=20	24.71084 /df=20	11.66906 /df=20	12.76364 /df=2 (p=0.273)	7.88930 /df=3.019
Top	2.08803 /df=20	8.33477 /df=20	3.87696 /df=20	97.88336 /df=2 (p=0.166)	34.19451 /df=2.069
$Log_{10}IC_{50}$	0.00490 /df=20	0.01323 /df=20	0.00373 /df=20	0.03830 /df=2 (p=0.187)	0.01507 /df=2.244
Slope	0.10259 /df=20	0.03660 /df=20	0.03251 /df=20	0.01915 /df=2 (p=0.394)	0.02197 /df=2.250

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 $2*((1/K)*\Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)*\Sigma(Si^4/df_i))$, 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.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	4.121 (4.327)	97.570 (1.887)	-4.817 (0.085)	1.525E-05 (1.216)	-1.246 (0.301)	C
2	0.833 (2.113)	95.400 (1.006)	-4.817 (0.039)	1.525E-05 (1.095)	-0.915 (0.067)	C
3	3.453 (2.946)	89.400 (1.468)	-4.662 (0.053)	2.177E-05 (1.129)	-1.323 (0.156)	C
Mean [c]	2.049 (1.596)	94.067 (2.397)	-4.762 (0.055)	1.729E-05 (1.134)	-1.113 (0.155)	C
Average Values [b]						
1	4.116 (6.595)	97.580 (2.878)	-4.817 (0.129)	1.525E-05 (1.347)	-1.244 (0.463)	C
2	0.833 (3.472)	95.400 (1.652)	-4.817 (0.065)	1.525E-05 (1.161)	-0.915 (0.110)	C
3	3.457 (4.035)	89.400 (2.012)	-4.662 (0.072)	2.176E-05 (1.180)	-1.323 (0.214)	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.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	4.121 (-4.905,13.147)	0.833 (-3.575,5.241)	3.453 (-2.692,9.598)	2.049 (-1.164,5.263)
Top	97.570 (93.634,101.506)	95.400 (93.302,97.498)	89.400 (86.338,92.462)	94.067 (83.436,104.698)
$Log_{10}IC_{50}$	-4.817 (-4.994,-4.640)	-4.817 (-4.899,-4.735)	-4.662 (-4.772,-4.552)	-4.762 (-4.957,-4.567)
Slope	-1.246 (-1.874,-0.618)	-0.915 (-1.055,-0.775)	-1.323 (-1.648,-0.998)	-1.113 (-1.565,-0.662)

a. Parameter estimates and their associated 95 percent 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 percent 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.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay**

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 2	Replicate 3	Random Replicate (p-value) [d]	Variance of Mean
Bottom	18.72293 /df=20	4.46477 /df=20	8.67892 /df=20	0.00000 /df=2 (p=NA)	2.54707 /df=45.557
Top	3.56077 /df=20	1.01204 /df=20	2.15502 /df=20	15.05419 /df=2 (p=0.196)	5.74478 /df=1.939
$Log_{10}IC_{50}$	0.00720 /df=20	0.00155 /df=20	0.00276 /df=20	0.00565 /df=2 (p=0.252)	0.00298 /df=2.507
Slope	0.09054 /df=20	0.00451 /df=20	0.02427 /df=20	0.04531 /df=2 (p=0.236)	0.02392 /df=3.560

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 $2*((1/K)*\Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)*\Sigma(Si^4/df_i))$, 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.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay

Only two data points were below 80 percent (but above 77.95 percent) 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) Estimate (%) (Std. Error)	p-Value / Degree of Freedom	Variance Components	Chemical										Residual (Repetition)
				I	II	III	IV	V	VI	VII	VIII	IX	X	
Full Enzyme Activity Control	-5.878 (1.234)	<0.0001 /df=35.85	Replicate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.07
			Replicate*Portion	33.59	5.93	0.00	3.95	0.00	0.00	29.20	0.00	0.00	5.28	
Background Activity Control	-0.159 (0.116)	0.1916 /df=15.98	Replicate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23
			Replicate*Portion	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88	
Positive Control	-2.795 (0.669)	0.0012 /df=12.53	Replicate	34.44	11.44	17.61	24.90	5.21	0.00	0.00	0.00	0.43	20.96	3.11
			Replicate*Portion	0.00	2.49	1.43	1.16	0.00	3.47	27.28	0.00	14.16	1.50	
Negative Control	-4.115 (1.049)	0.0008 /df=20.39	Replicate	31.37	0.00	0.00	20.29	17.49	2.21	0.00	0.62	74.20	9.78	18.46
			Replicate*Portion	0.00	0.00	0.00	18.02	0.00	0.00	20.07	19.06	15.75	0.00	

Note: The chemical main effect and the chemical by portion interaction fixed effect were not significant for all four types of control activity data, therefore, the effects were not summarized in this table.

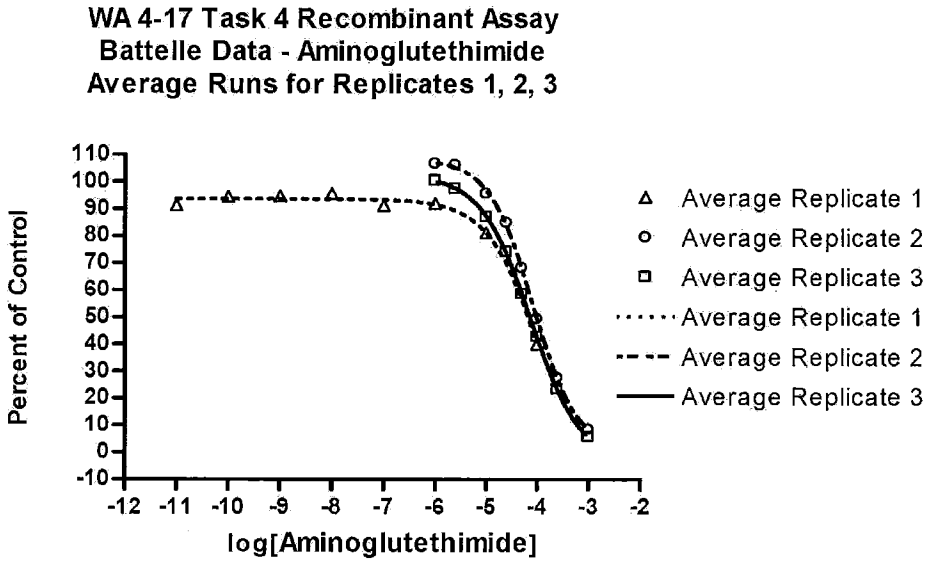


Figure 1-1. **Reference Chemical I: Aminoglutethimide.
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.**

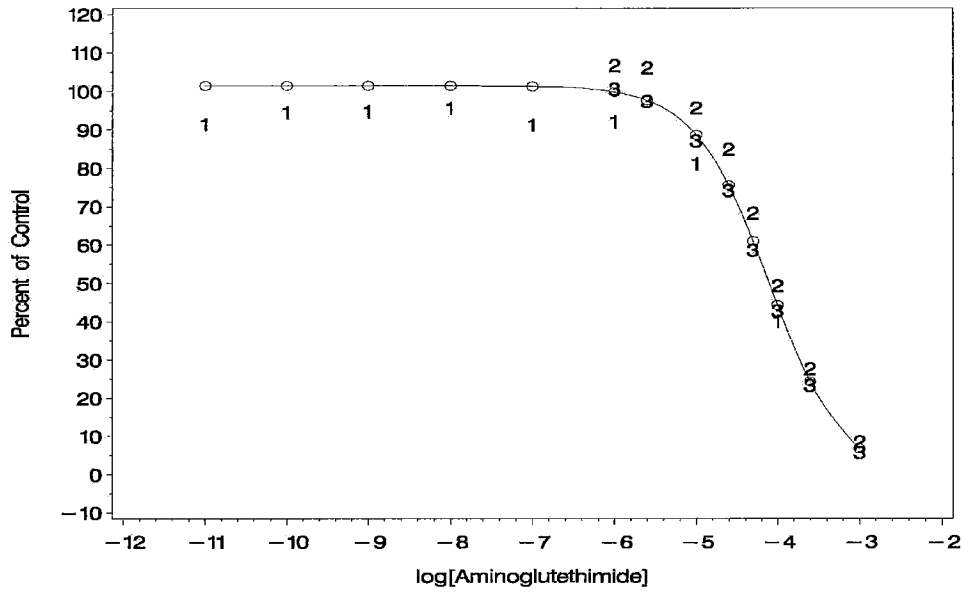


Figure 1-2. **Reference Chemical I: Aminoglutethimide. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

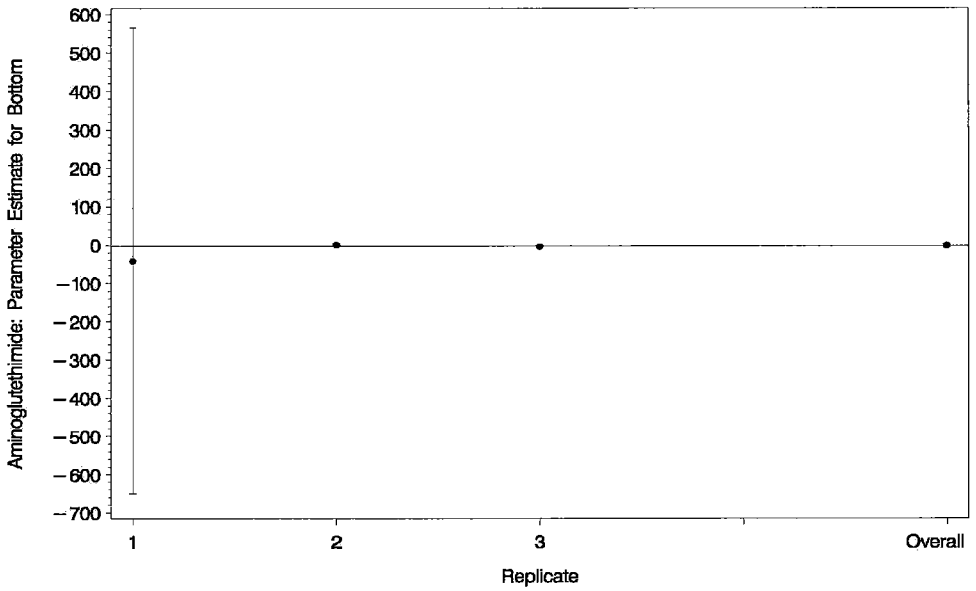


Figure 1-3. **Reference Chemical I: Aminoglutethimide. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

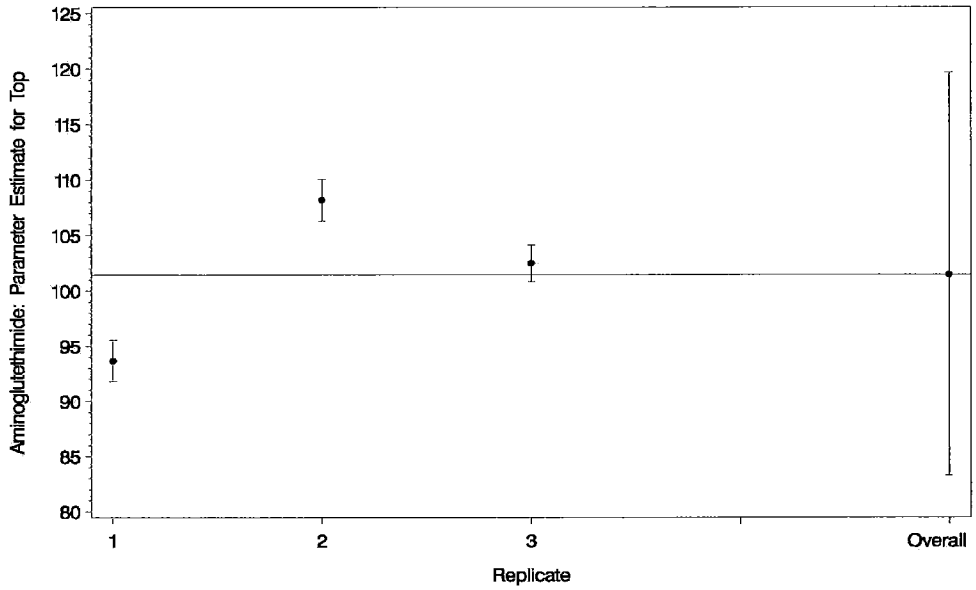


Figure 1-4. **Reference Chemical I: Aminoglutethimide. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

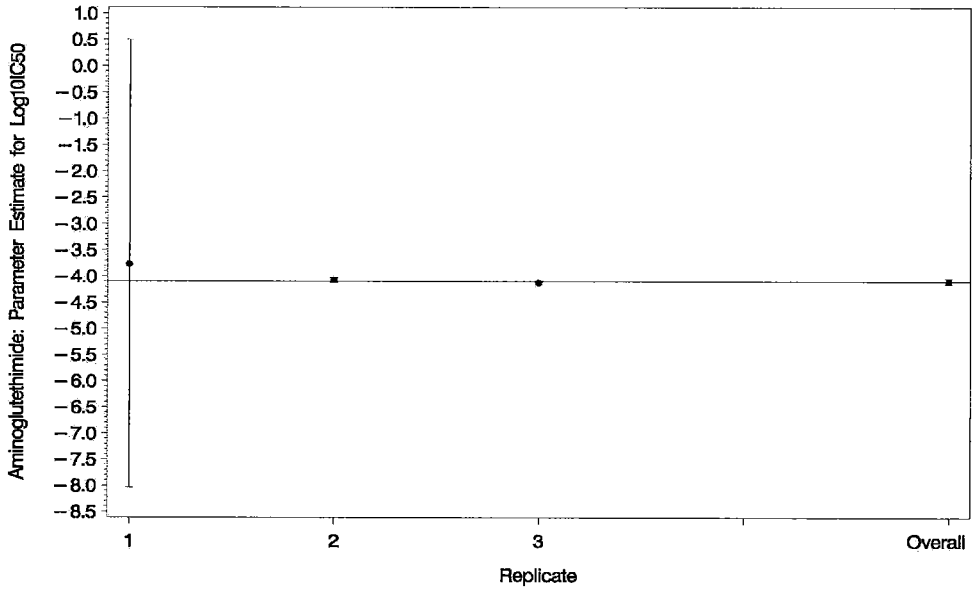


Figure 1-5. Reference Chemical I: Aminoglutethimide. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

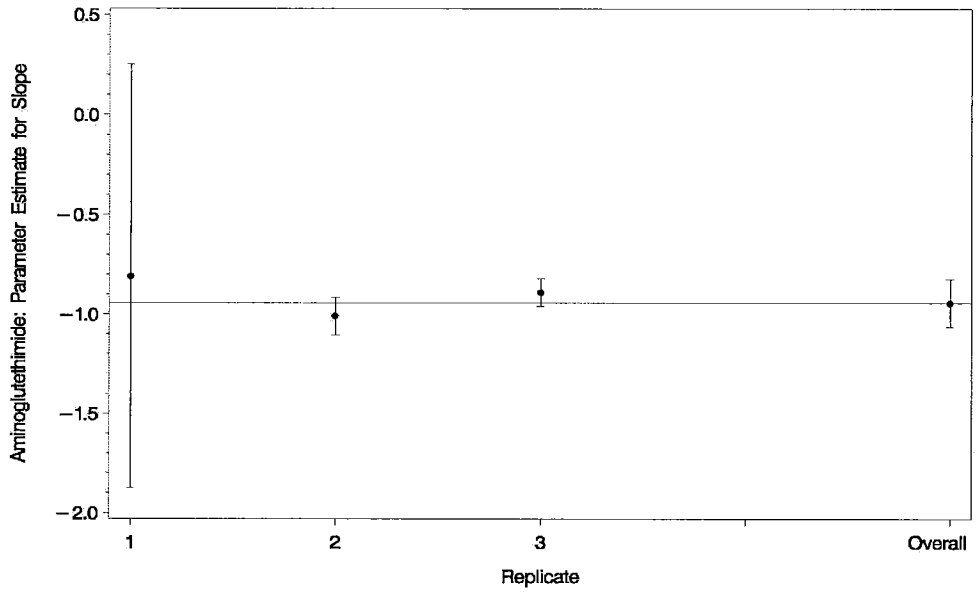


Figure 1-6. Reference Chemical I: Aminoglutethimide. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

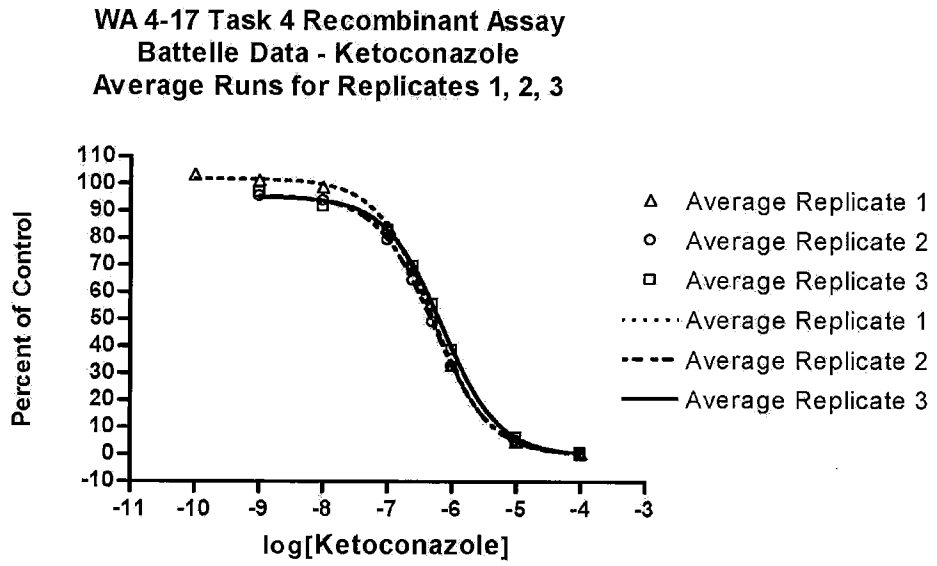


Figure 2-1. **Reference Chemical II: Ketoconazole. Concentration Response Curves and Averages of Repetitions within Each Concentration. Recombinant Aromatase Assay.**

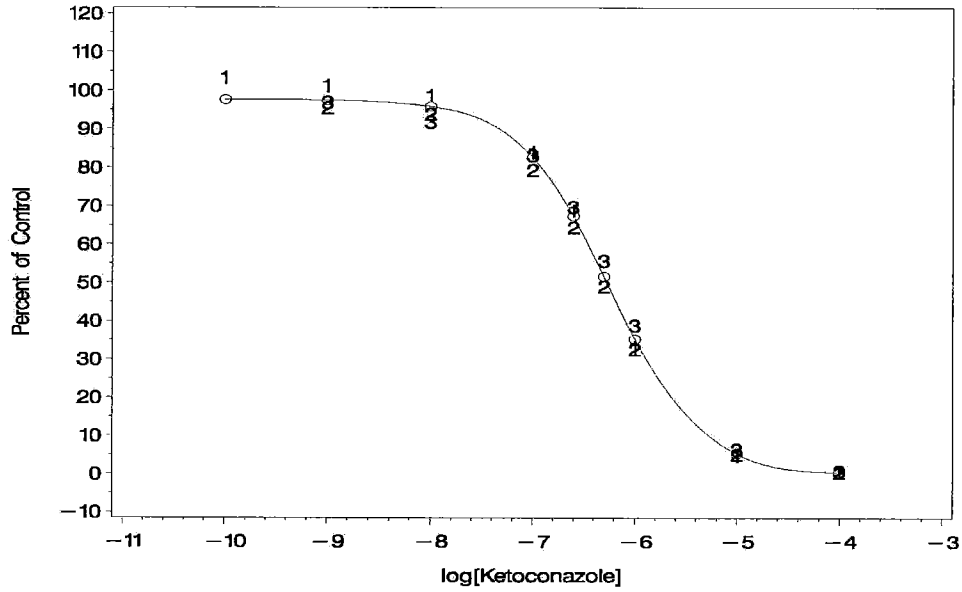


Figure 2-2. **Reference Chemical II: Ketoconazole. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

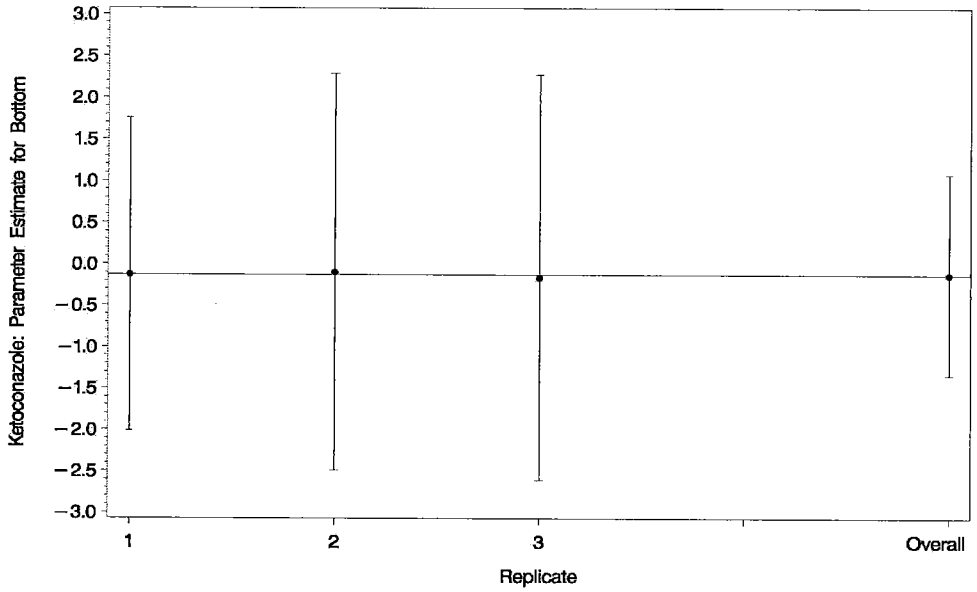


Figure 2-3. **Reference Chemical II: Ketoconazole. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

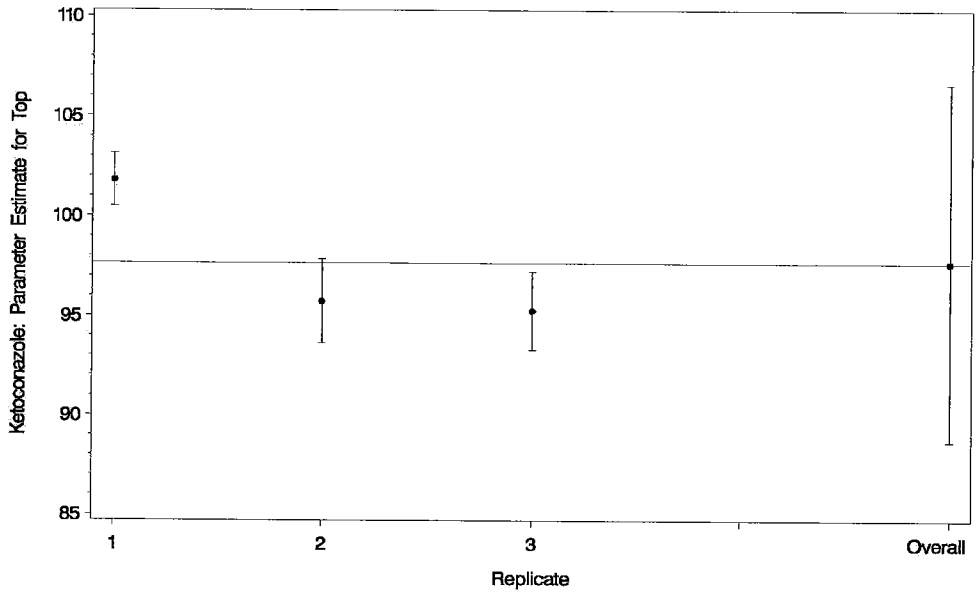


Figure 2-4. **Reference Chemical II: Ketoconazole. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

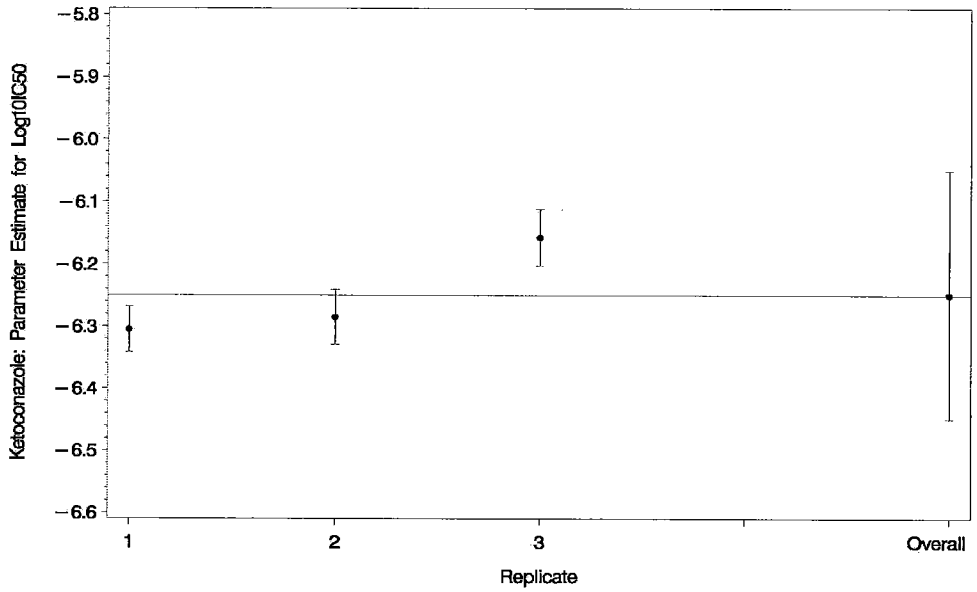


Figure 2-5. Reference Chemical II: Ketoconazole. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

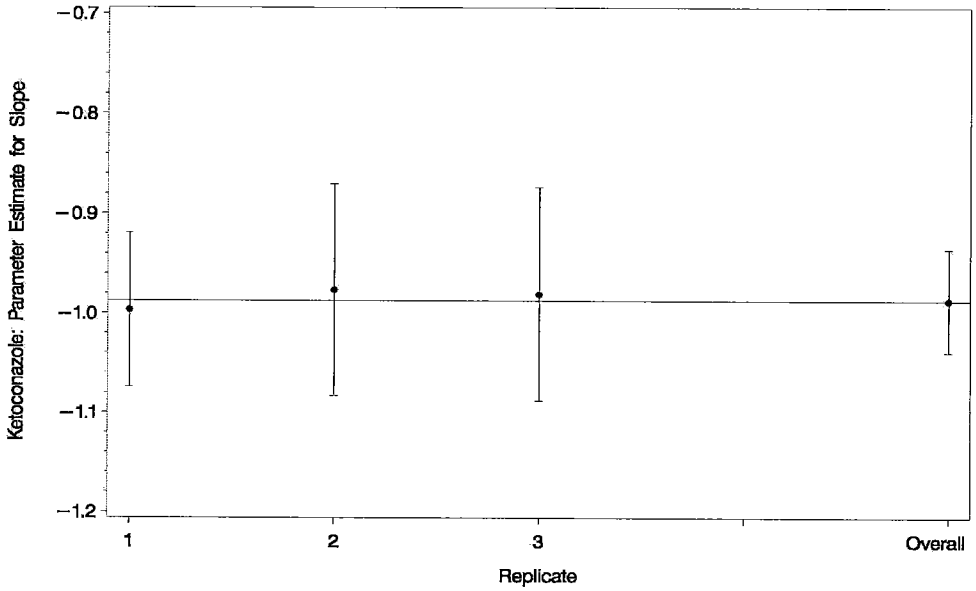


Figure 2-6. Reference Chemical II: Ketoconazole. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

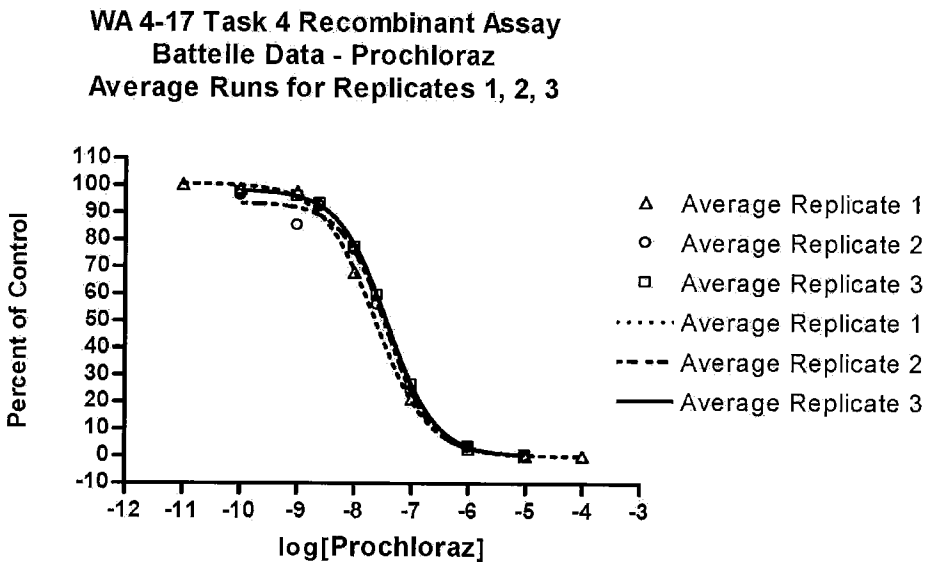


Figure 3-1. **Reference Chemical III: Prochloraz.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

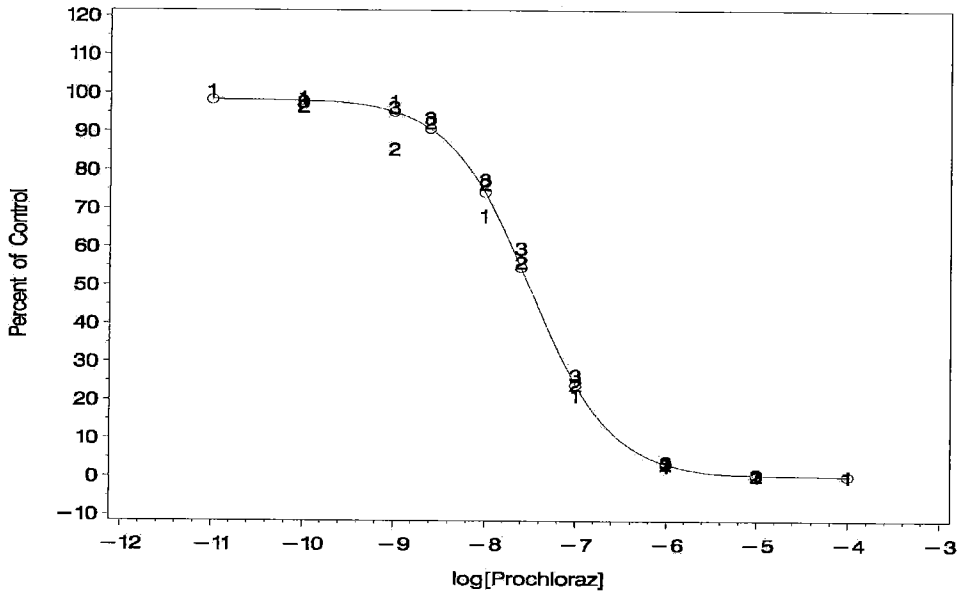


Figure 3-2. **Reference Chemical III: Prochloraz. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

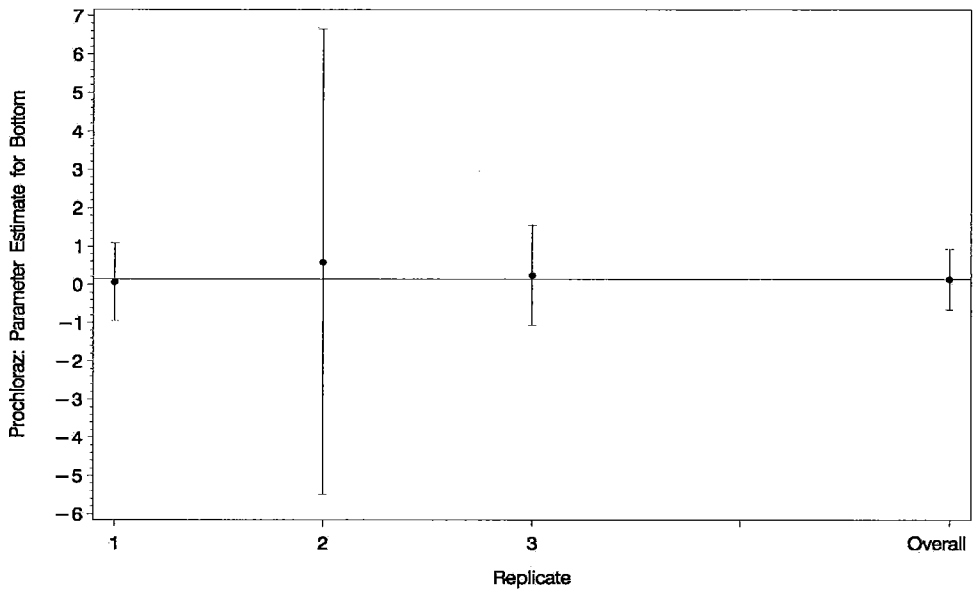


Figure 3-3. Reference Chemical III: Prochloraz. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

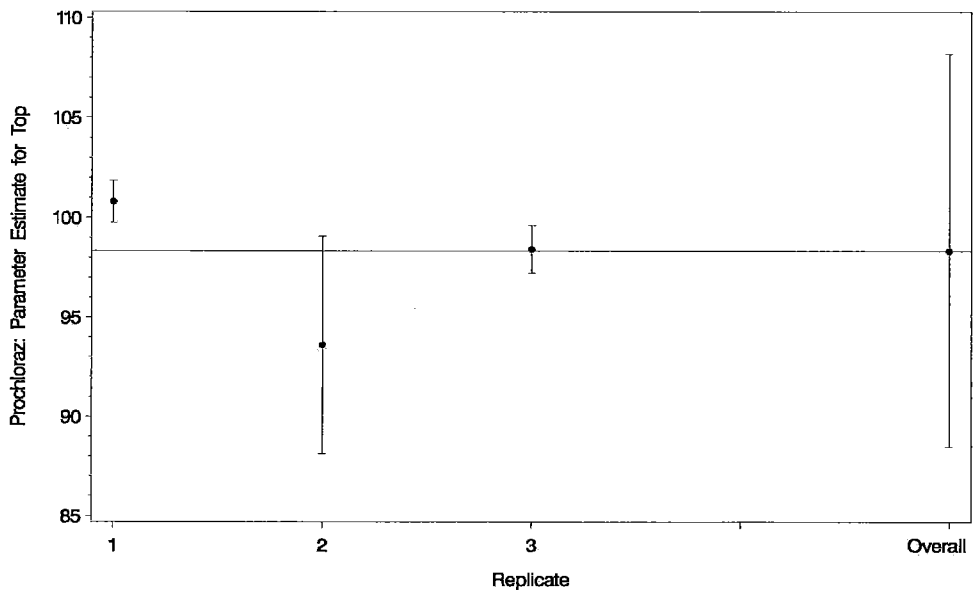


Figure 3-4. **Reference Chemical III: Prochloraz. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

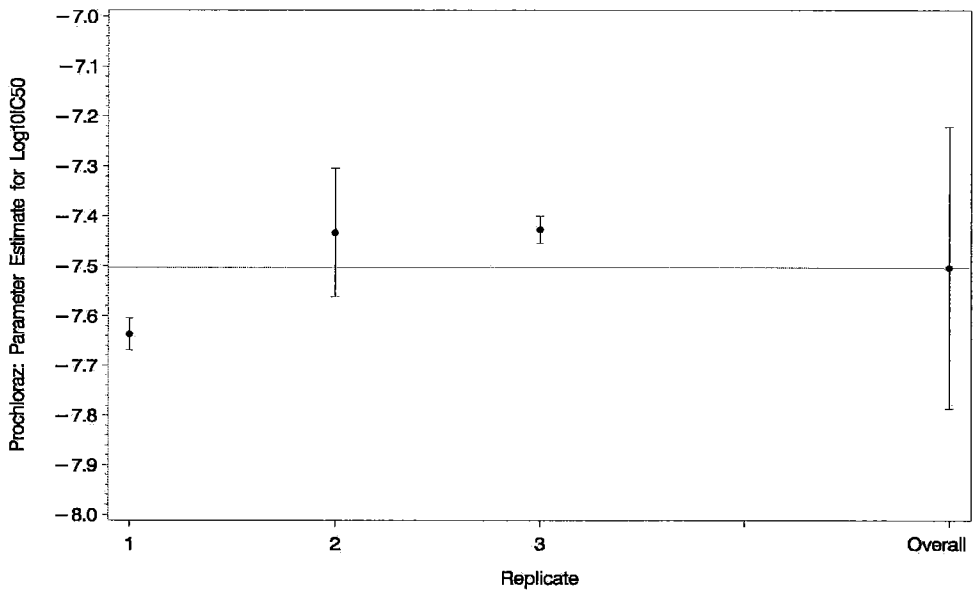


Figure 3-5. Reference Chemical III: Prochloraz. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

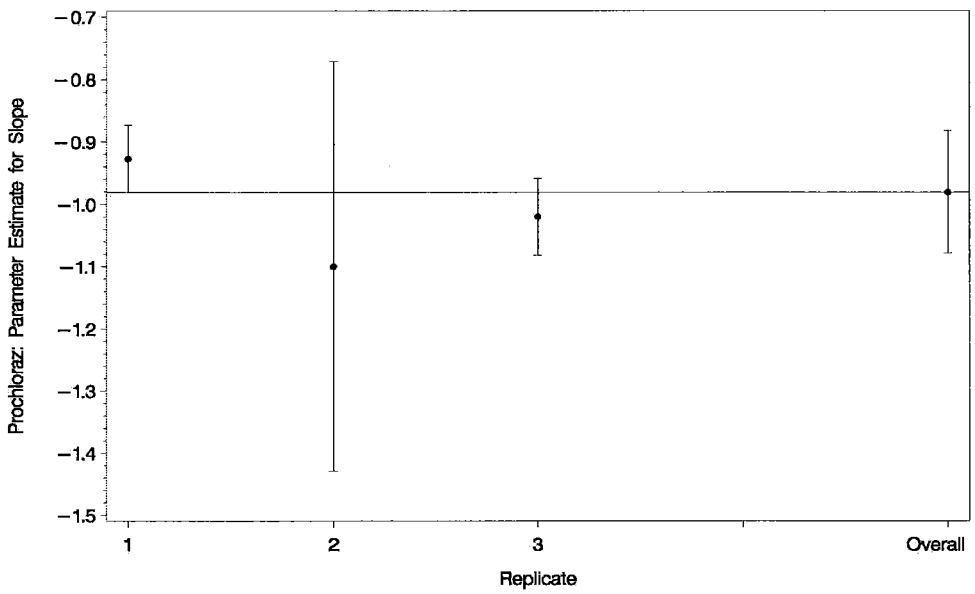


Figure 3-6. Reference Chemical III: Prochloraz. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

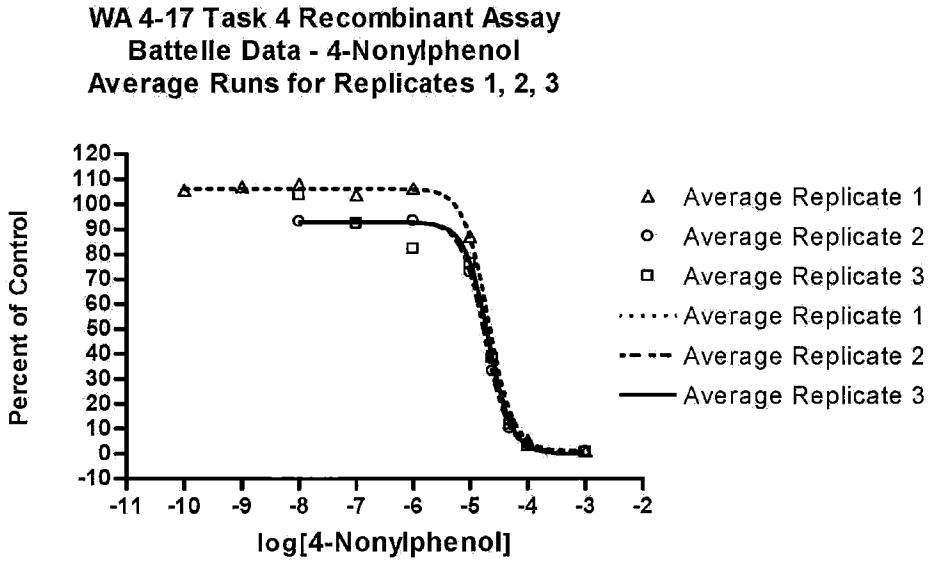


Figure 4-1. **Reference Chemical IV: 4-Nonylphenol. Concentration Response Curves and Averages of Repetitions within Each Concentration. Recombinant Aromatase Assay.**

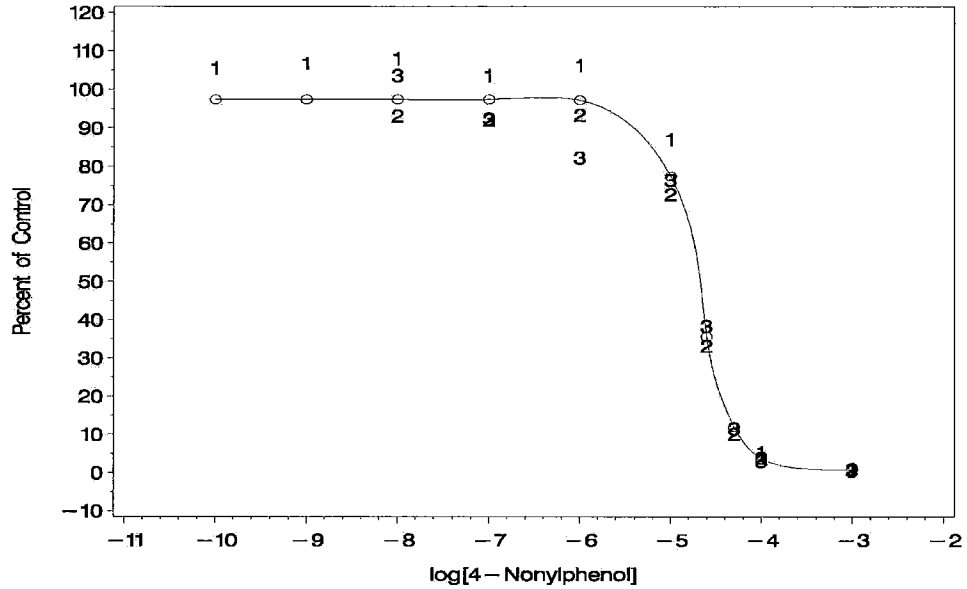


Figure 4-2. **Reference Chemical IV: 4-Nonylphenol. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

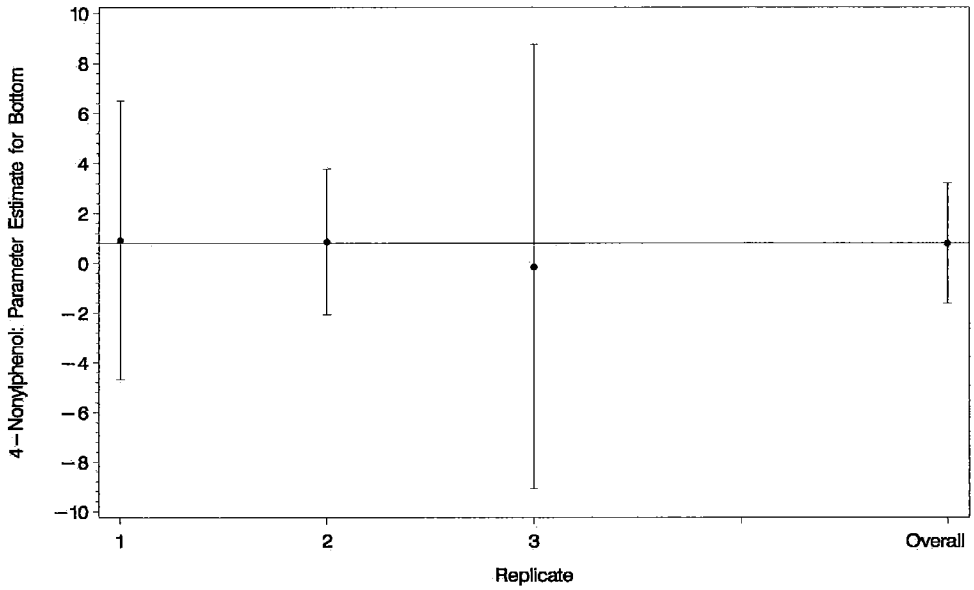


Figure 4-3. **Reference Chemical IV: 4-Nonylphenol. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

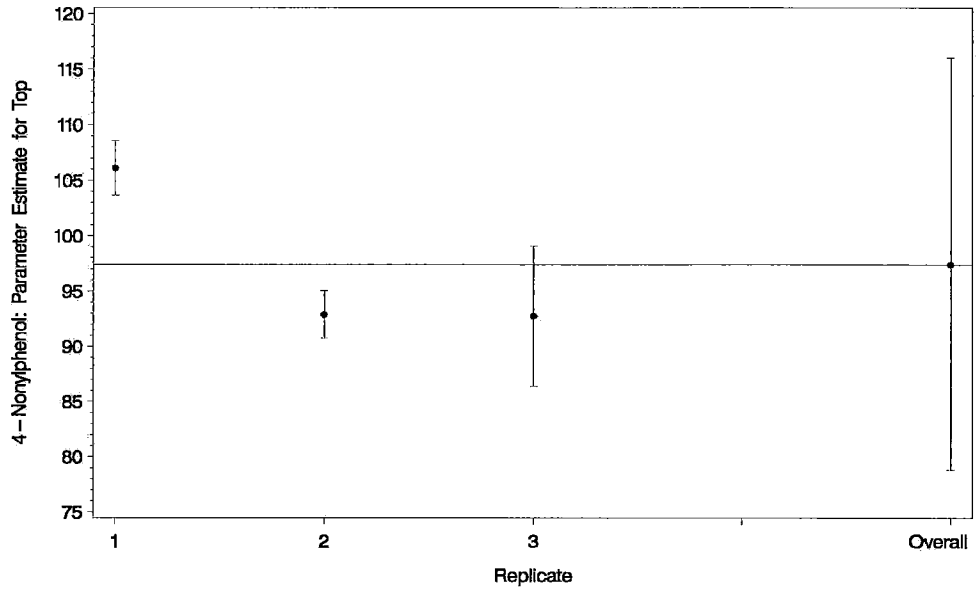


Figure 4-4. Reference Chemical IV: 4-Nonylphenol. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

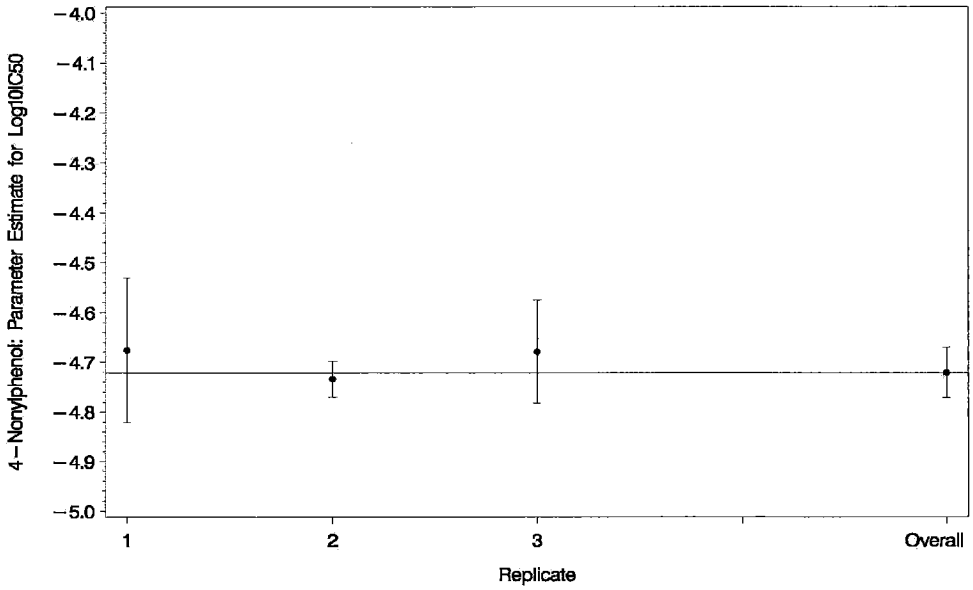


Figure 4-5. Reference Chemical IV: 4-Nonylphenol. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

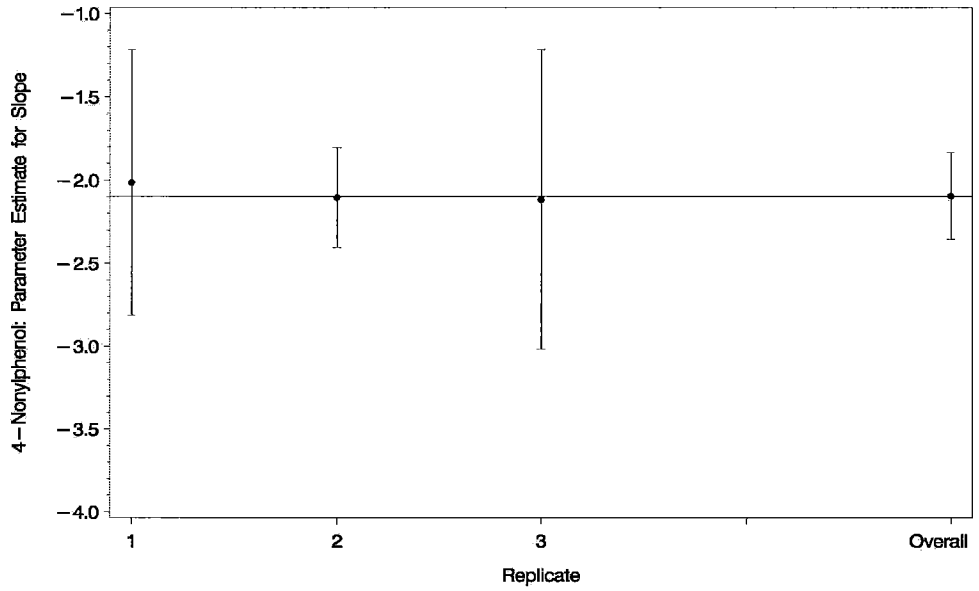


Figure 4-6. Reference Chemical IV: 4-Nonylphenol. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

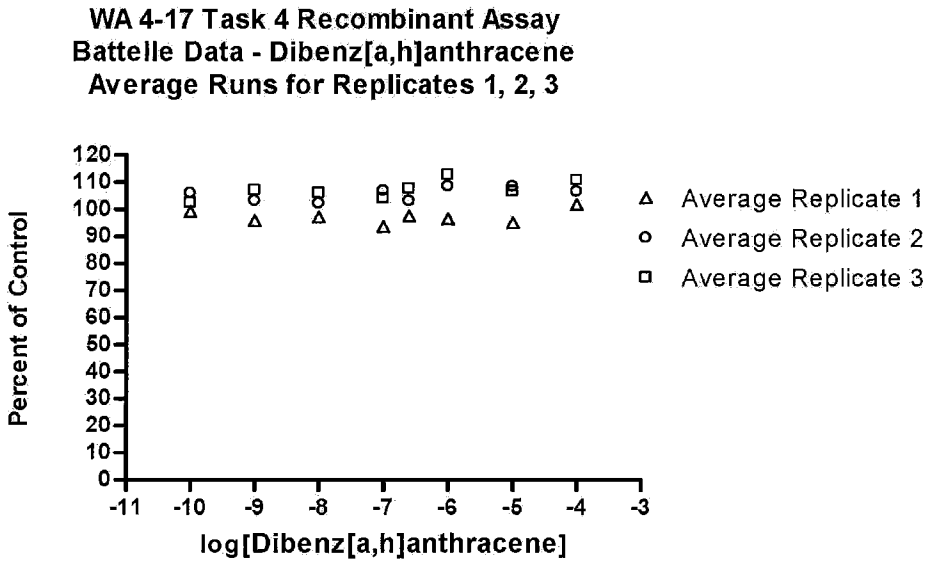


Figure 5-1. **Reference Chemical V: Dibenz[a, h]anthracene.
Scatter Plot for Averages of Repetitions within Concentration for Each Replicate.
Recombinant Aromatase Assay.**

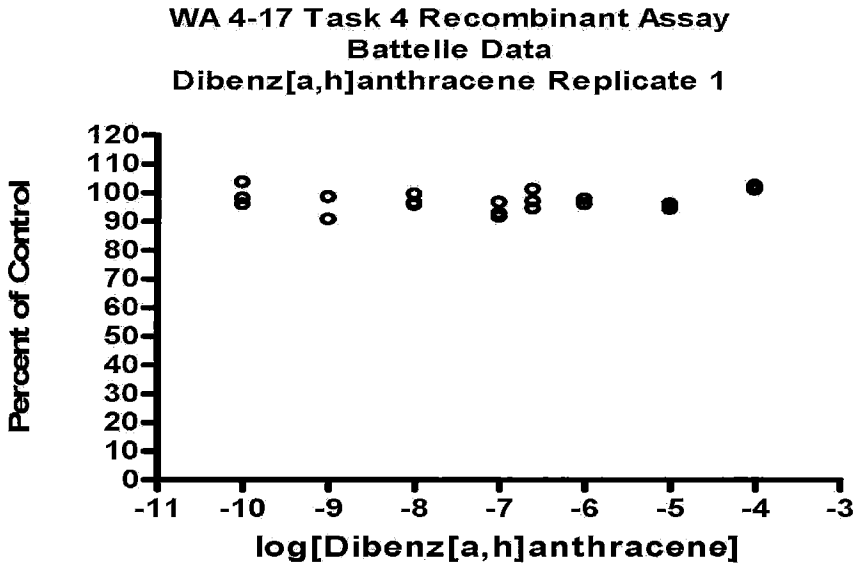


Figure 5-2. **Reference Chemical V: Dibenz[a, h]anthracene. Scatter Plot for Replicate 1 Data. Recombinant Aromatase Assay.**

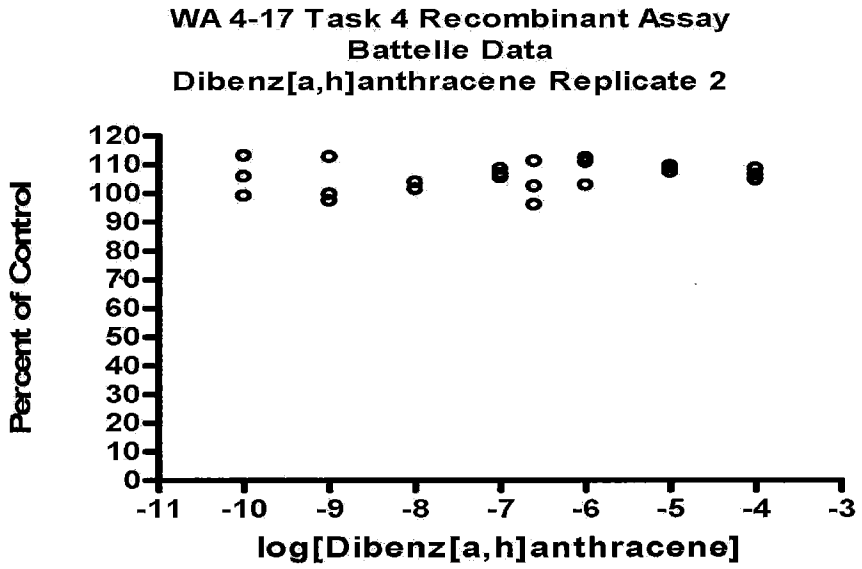


Figure 5-3. **Reference Chemical V: Dibenz[a, h]anthracene. Scatter Plot for Replicate 2 Data. Recombinant Aromatase Assay.**

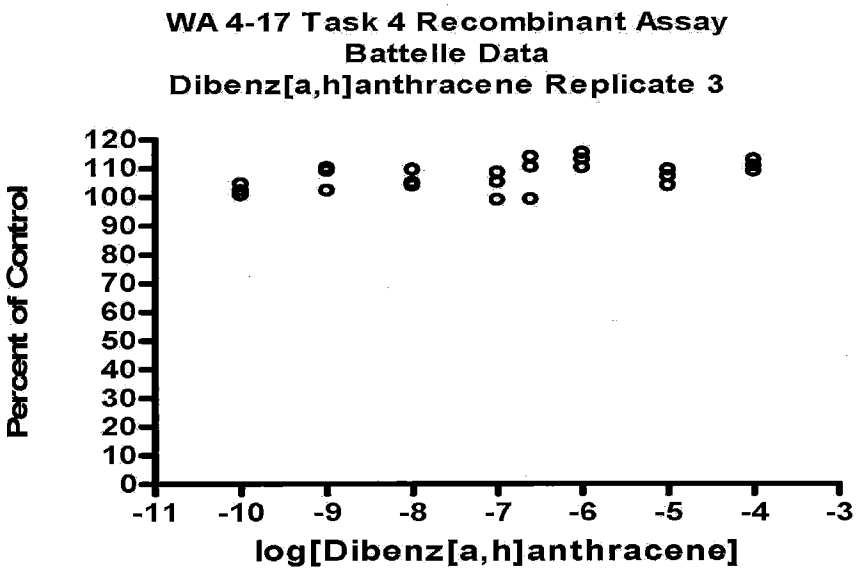


Figure 5-4. **Reference Chemical V: Dibenz[a, h]anthracene. Scatter Plot for Replicate 3 Data. Recombinant Aromatase Assay.**

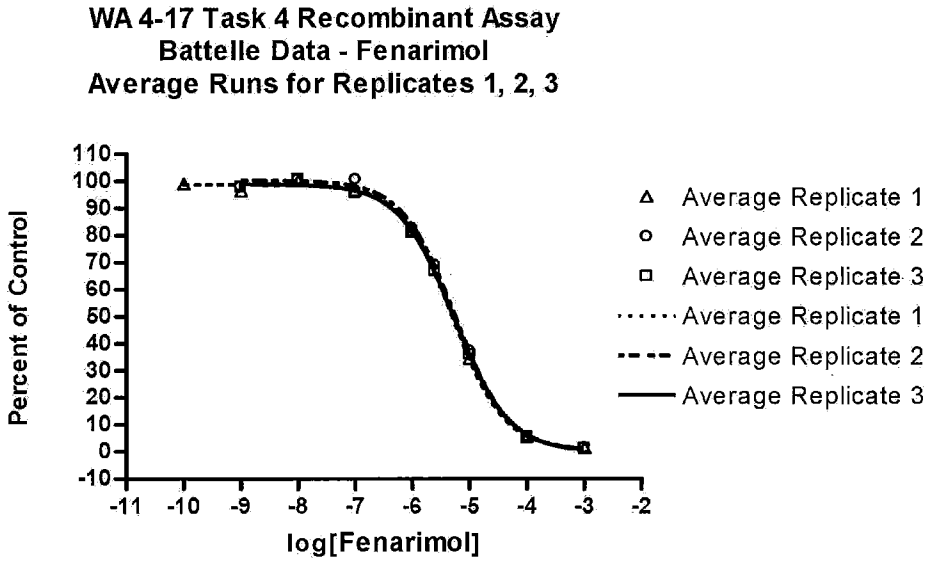


Figure 6-1. **Reference Chemical VI: Fenarimol.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

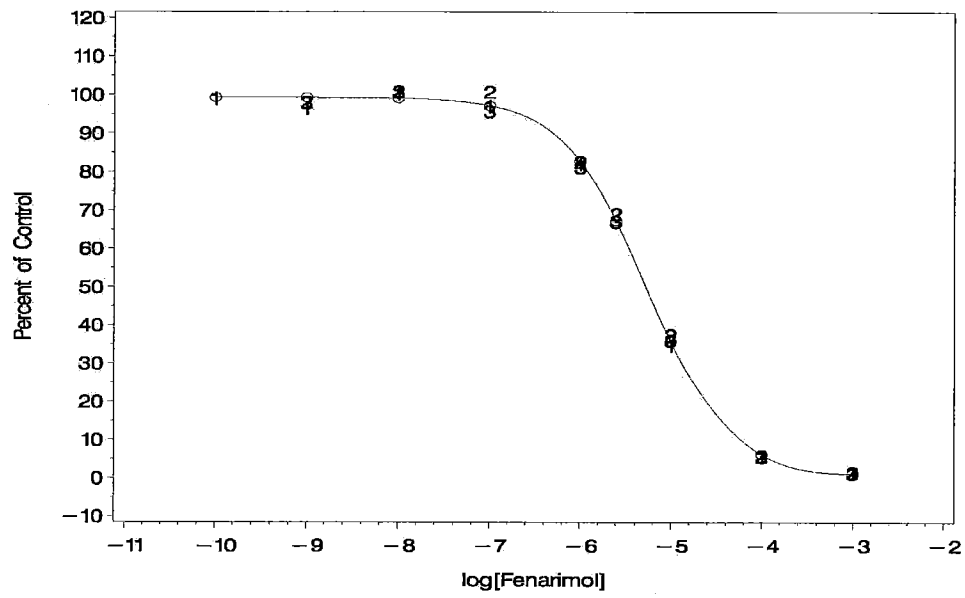


Figure 6-2. Reference Chemical VI: Fenarimol. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

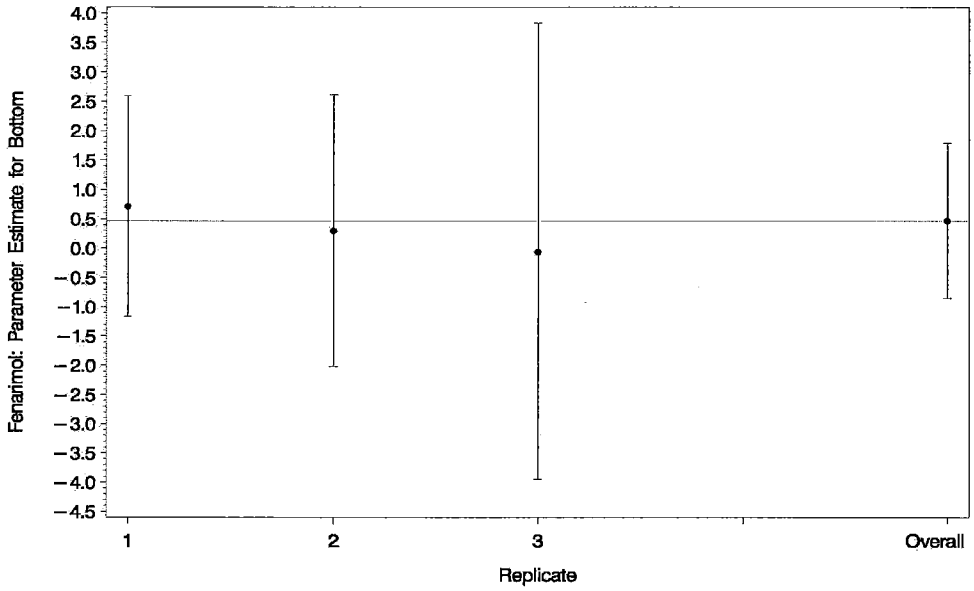


Figure 6-3. Reference Chemical VI: Fenarimol. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

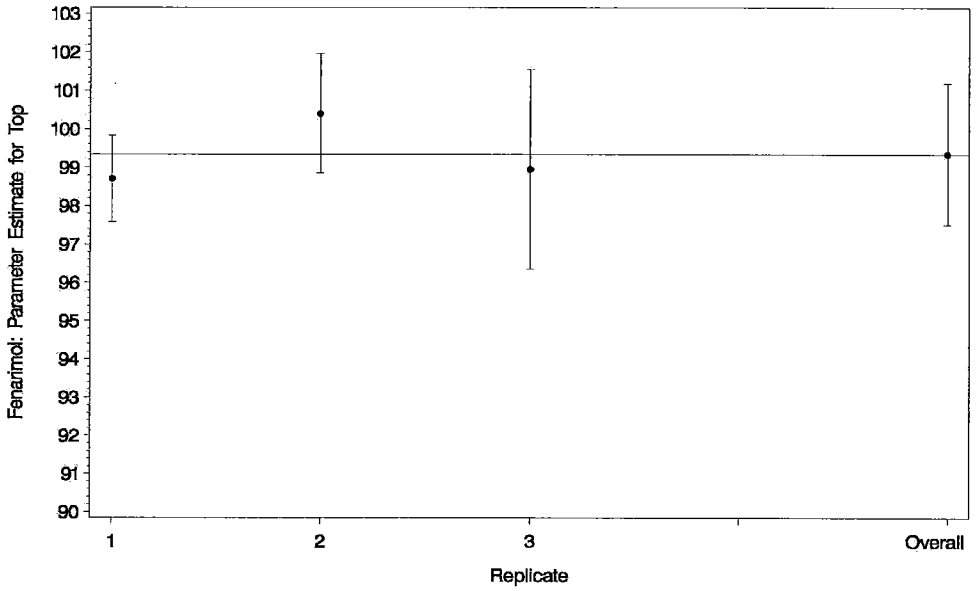


Figure 6-4. Reference Chemical VI: Fenarimol. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

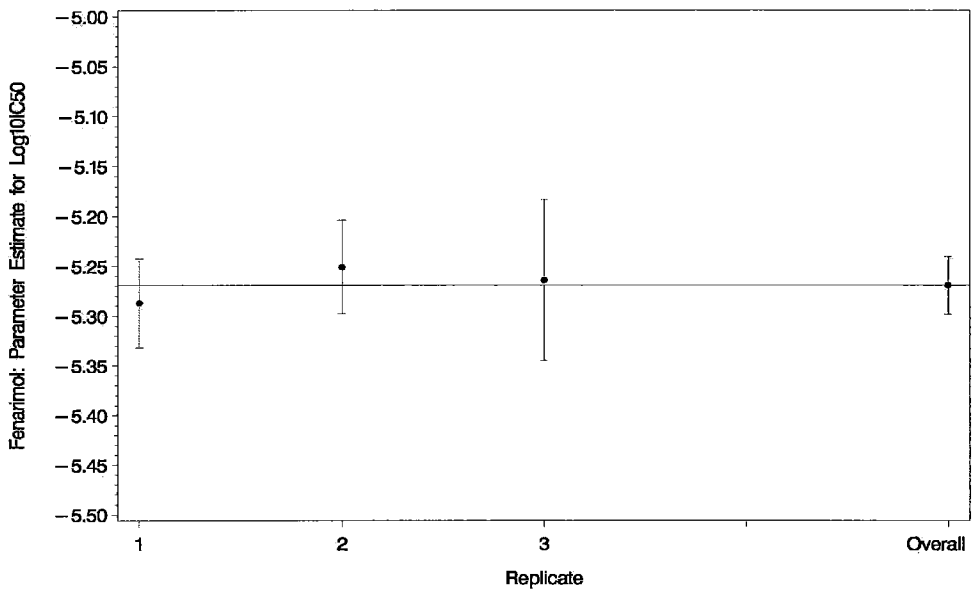


Figure 6-5. Reference Chemical VI: Fenarimol. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

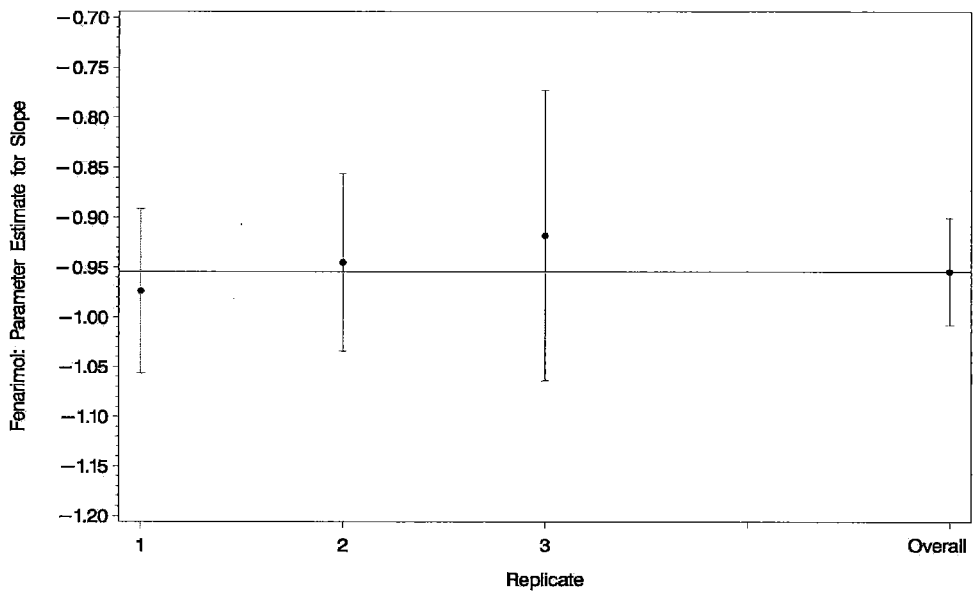


Figure 6-6. **Reference Chemical VI: Fenarimol. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

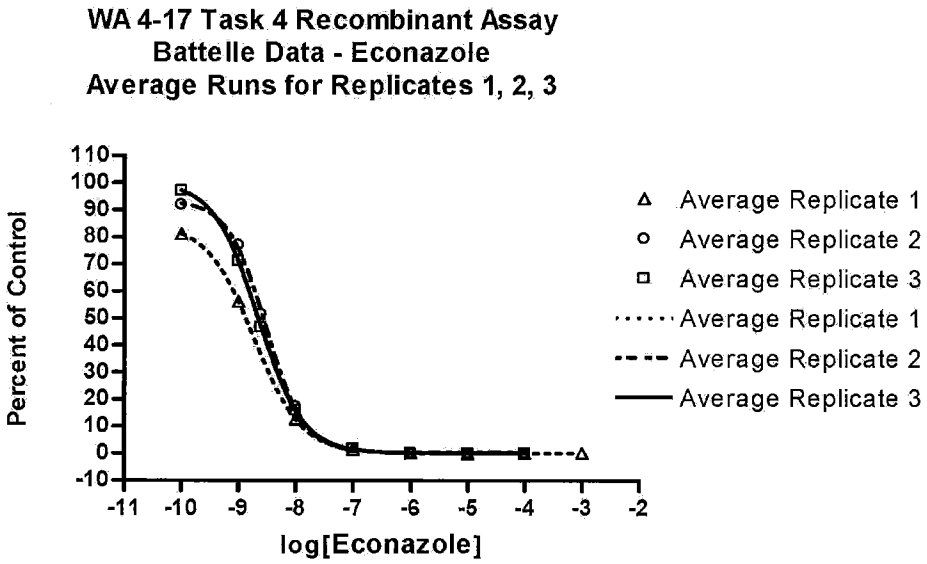


Figure 7-1. **Reference Chemical VII: Econazole. Concentration Response Curves and Averages of Repetitions within Each Concentration. Recombinant Aromatase Assay.**

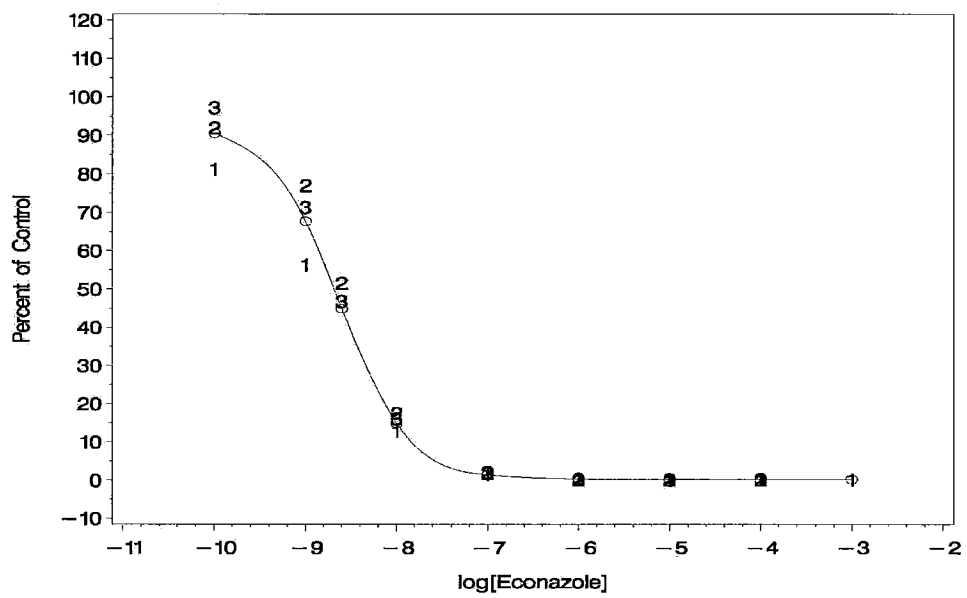


Figure 7-2. **Reference Chemical VII: Econazole.**
Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

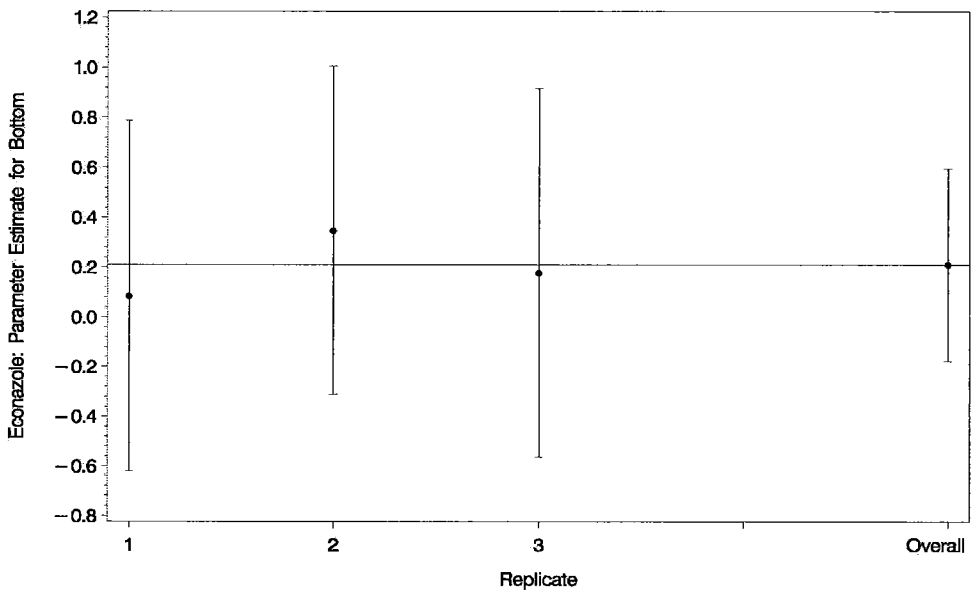


Figure 7-3. Reference Chemical VII: Econazole. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

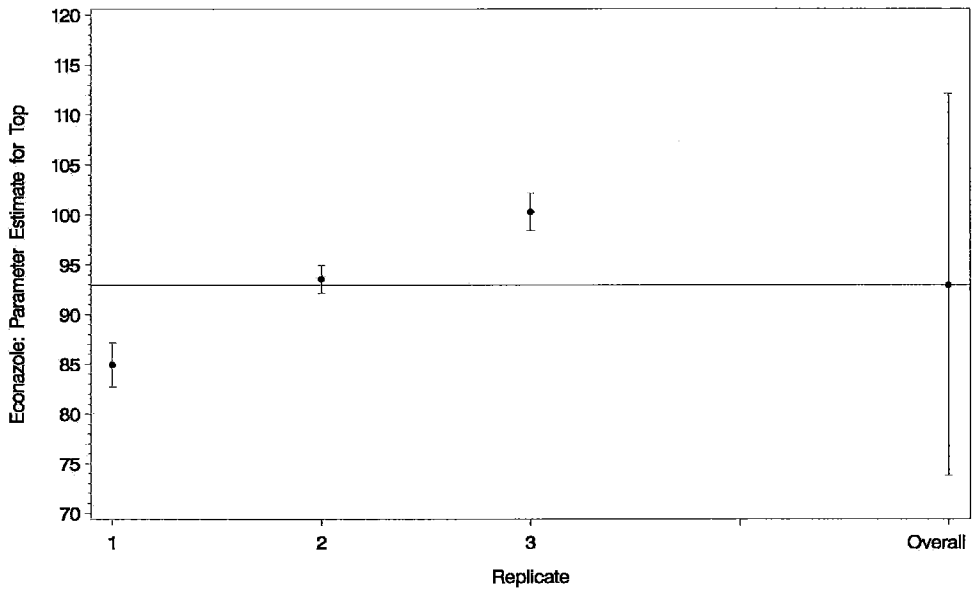


Figure 7-4. **Reference Chemical VII: Econazole. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

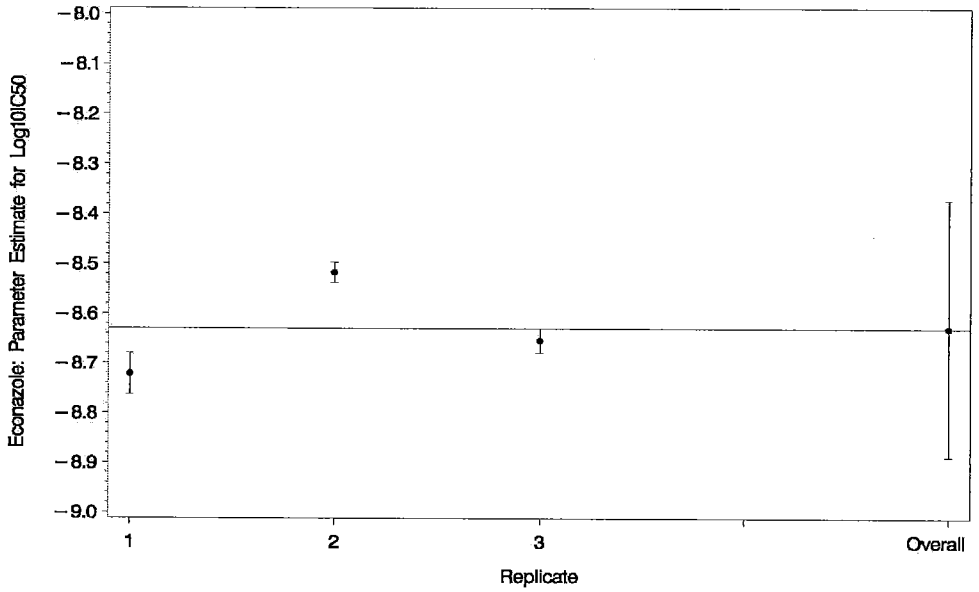


Figure 7-5. **Reference Chemical VII: Econazole. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

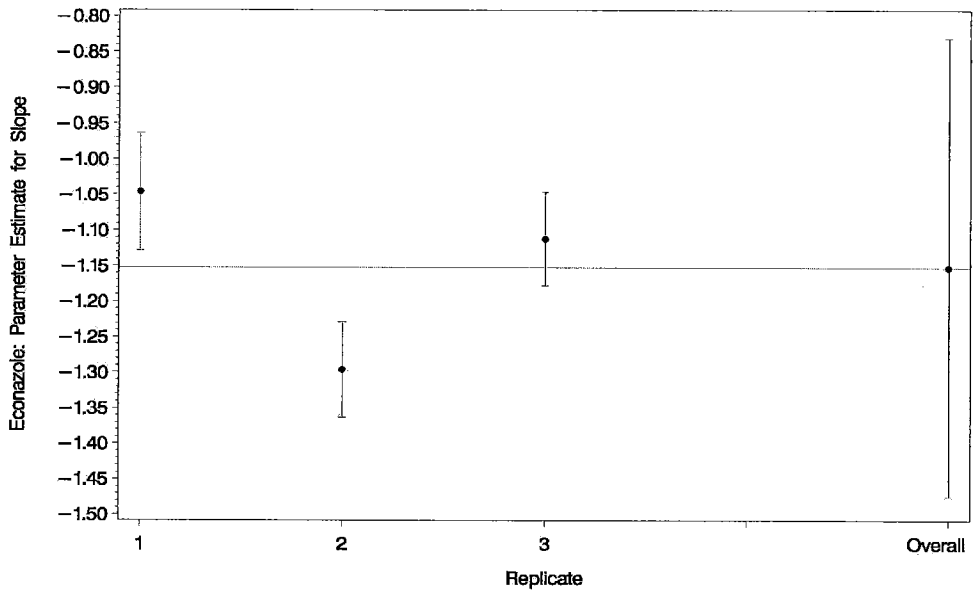


Figure 7-6. Reference Chemical VII: Econazole. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

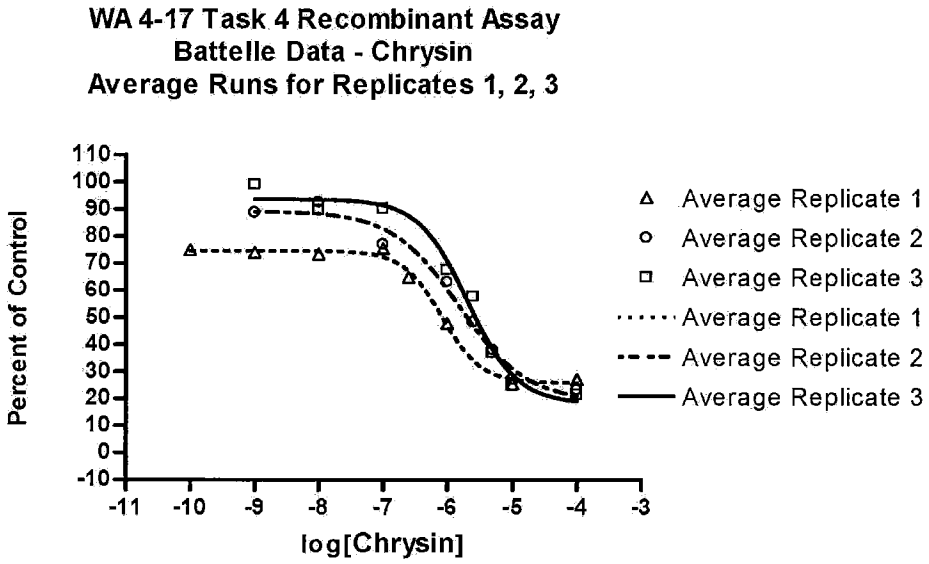


Figure 8-1. **Reference Chemical VIII: Chrysin.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

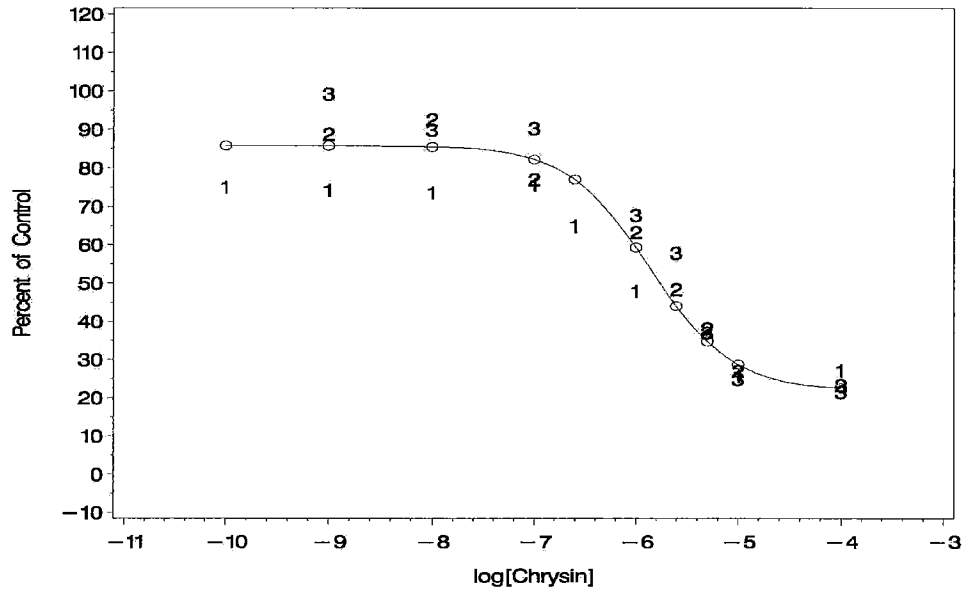


Figure 8-2. **Reference Chemical VIII: Chrysin. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

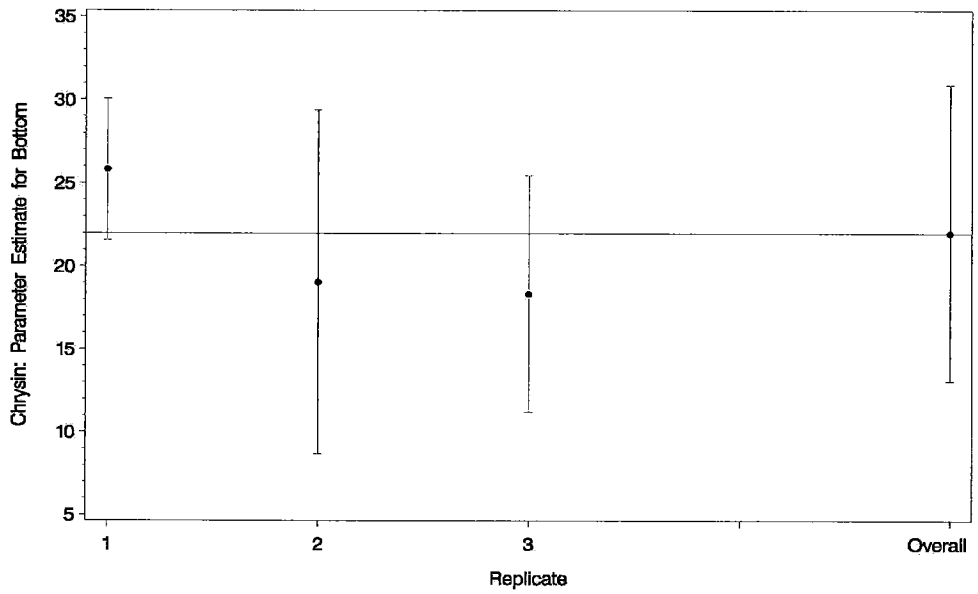


Figure 8-3. **Reference Chemical VIII: Chrysin. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

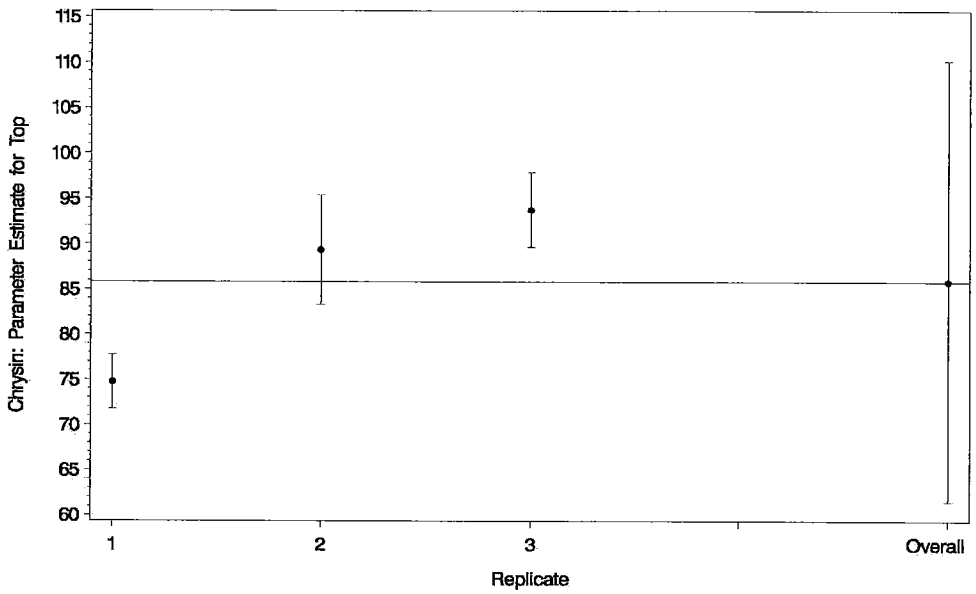


Figure 8-4. **Reference Chemical VIII: Chrysin. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

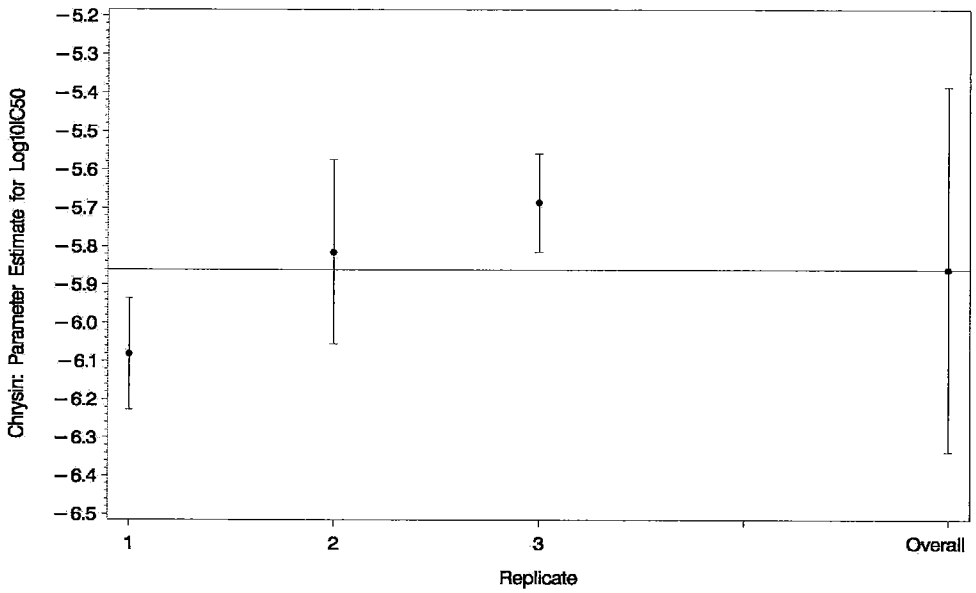


Figure 8-5. Reference Chemical VIII: Chrysin. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

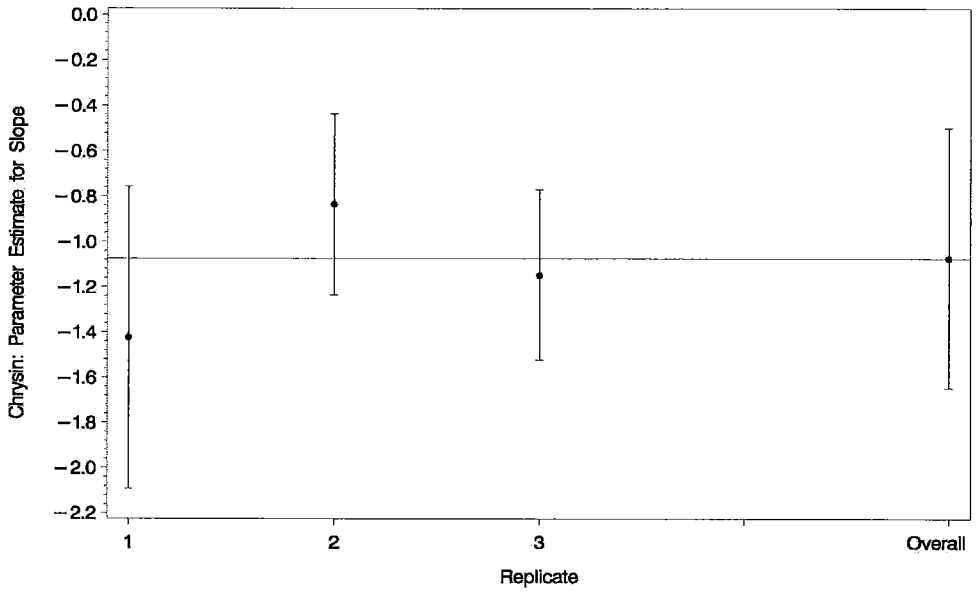


Figure 8-6. **Reference Chemical VIII: Chrysin. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

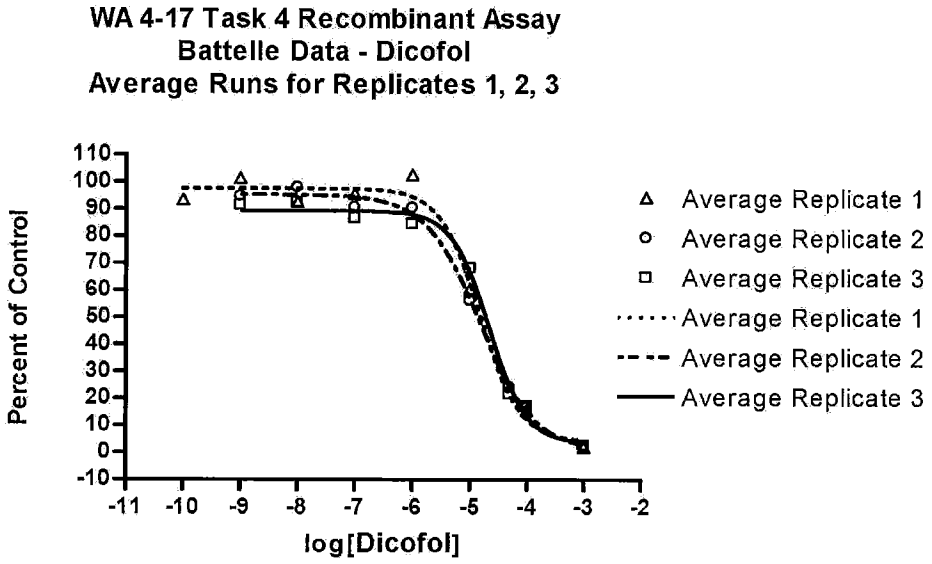


Figure 9-1. **Reference Chemical IX: Dicofol.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

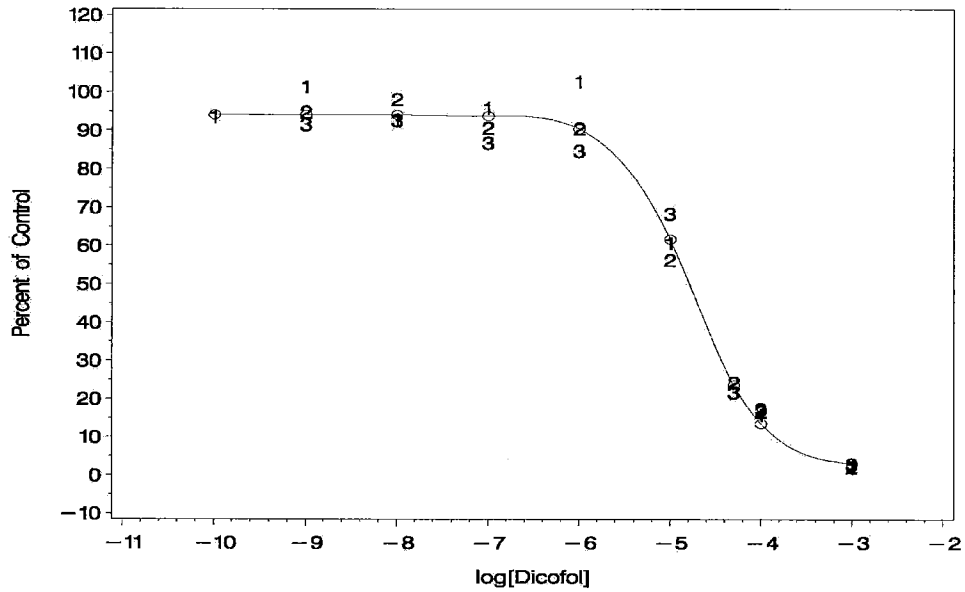


Figure 9-2. **Reference Chemical IX: Dicofol.**
Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

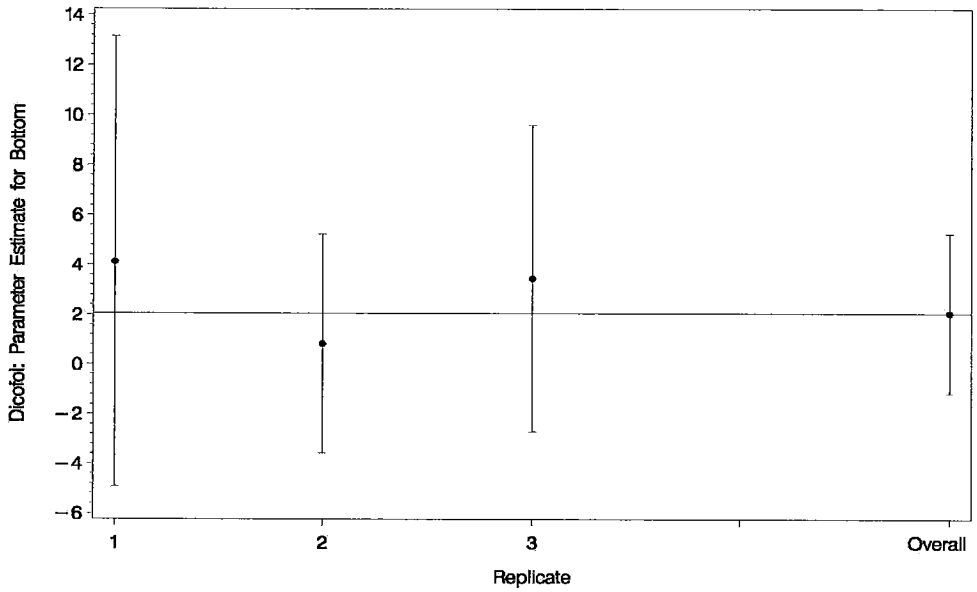


Figure 9-3. **Reference Chemical IX: Dicofol. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

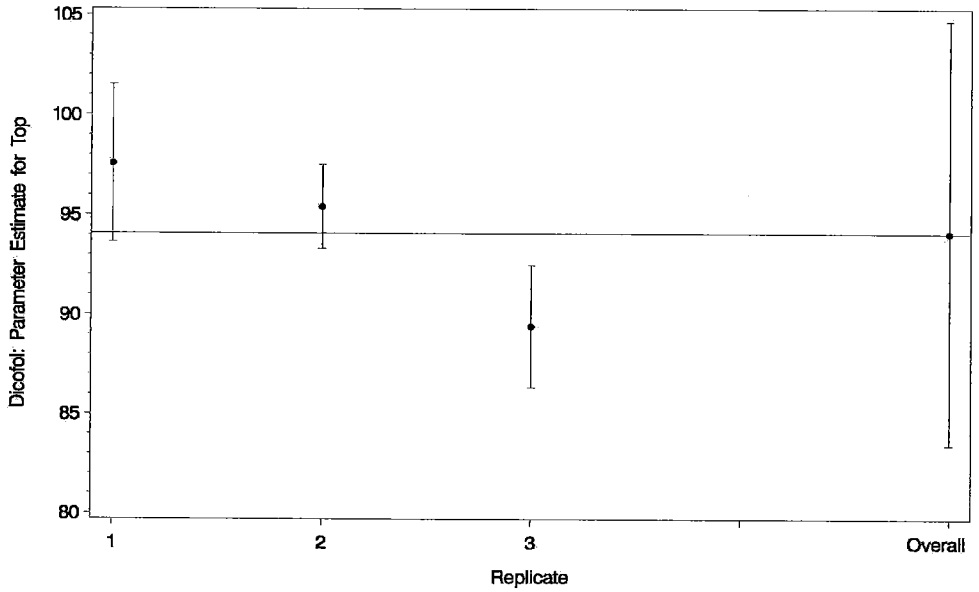


Figure 9-4. **Reference Chemical IX: Dicofol.**
Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

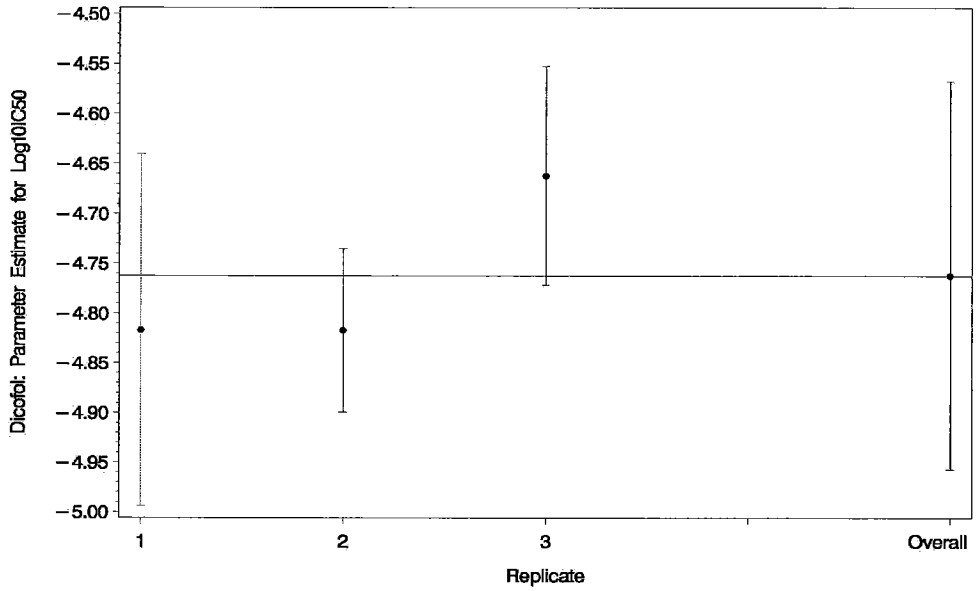


Figure 9-5. Reference Chemical IX: Dicofol. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

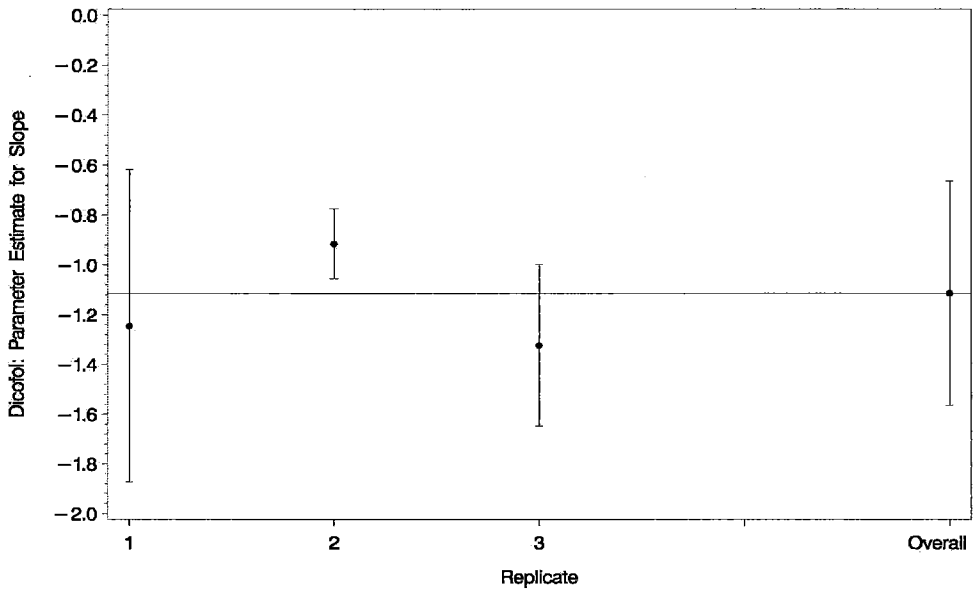


Figure 9-6. Reference Chemical IX: Dicofol. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

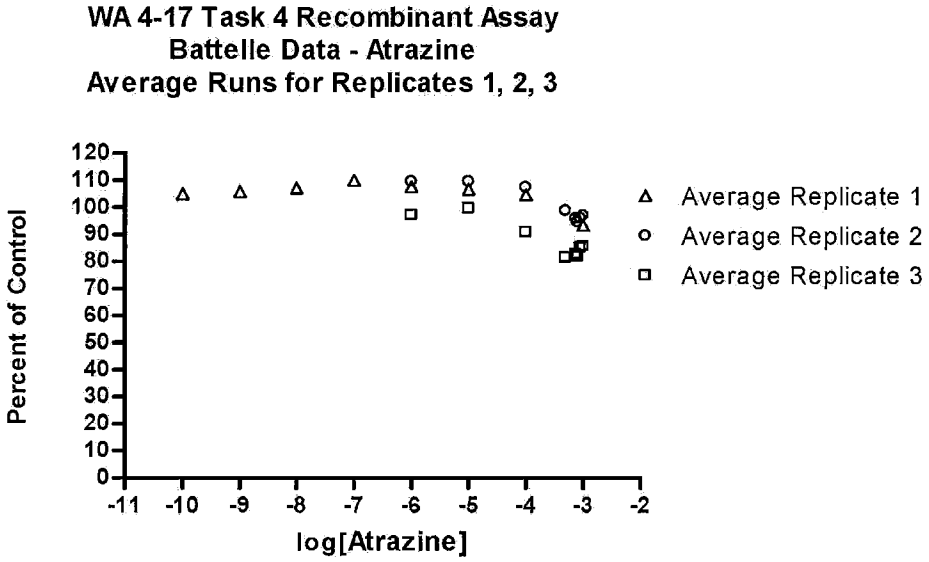


Figure 10-1. Reference Chemical X: Atrazine.
Scatter Plot for Averages of Repetitions within Concentration for Each Replicate.
Recombinant Aromatase Assay.

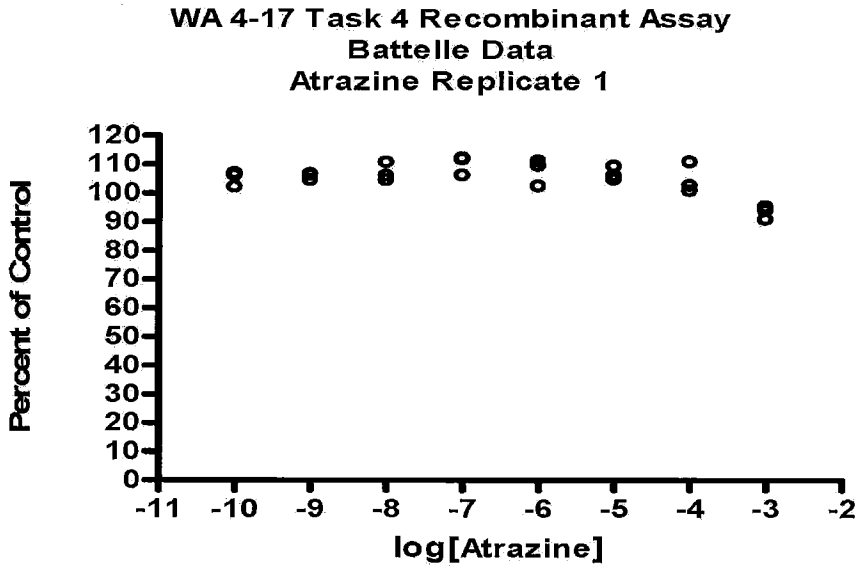


Figure 10-2. Reference Chemical X: Atrazine.
Scatter Plot for Replicate 1 Data. Recombinant Aromatase Assay.

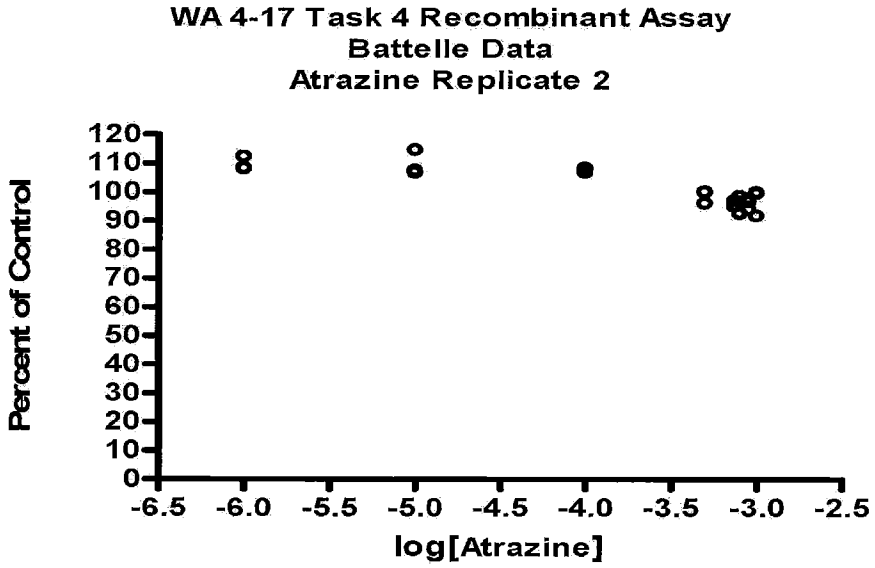


Figure 10-3. Reference Chemical X: Atrazine.
Scatter Plot for Replicate 2 Data. Recombinant Aromatase Assay.

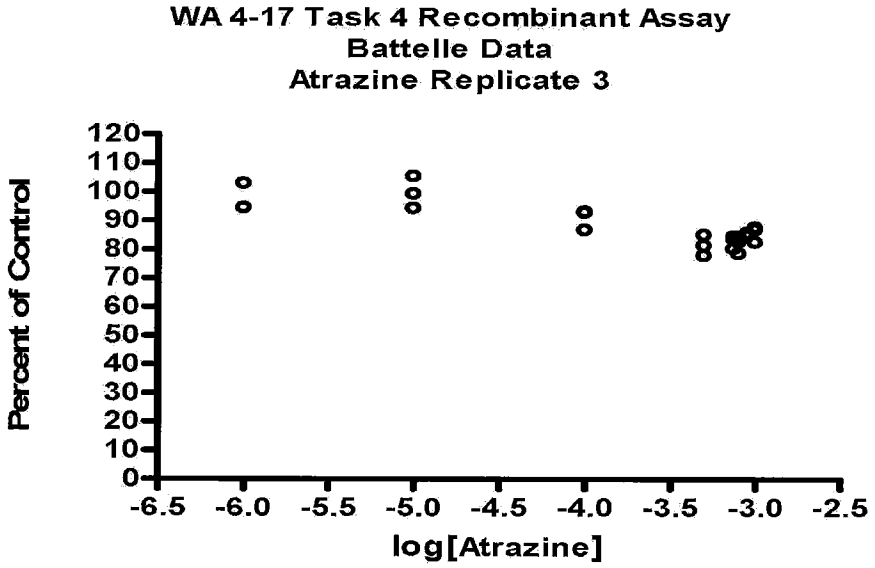


Figure 10-4. Reference Chemical X: Atrazine.
Scatter Plot for Replicate 3 Data. Recombinant Aromatase Assay.

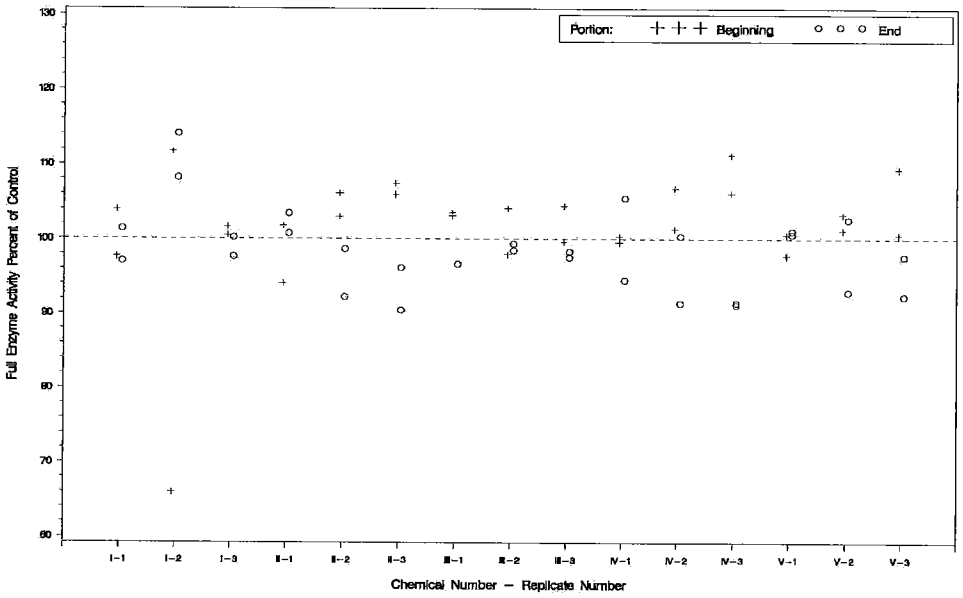


Figure 11a. **Full Enzyme Activity Control Data. Chemicals I to V. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 100 percent.**

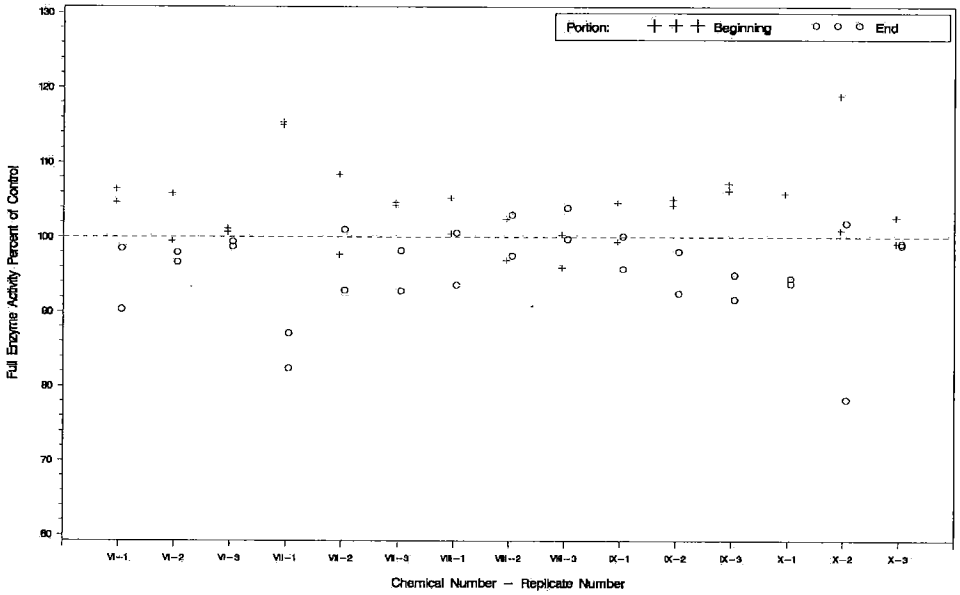


Figure 11b. **Full Enzyme Activity Control Data. Chemicals VI to X. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 100 percent.**

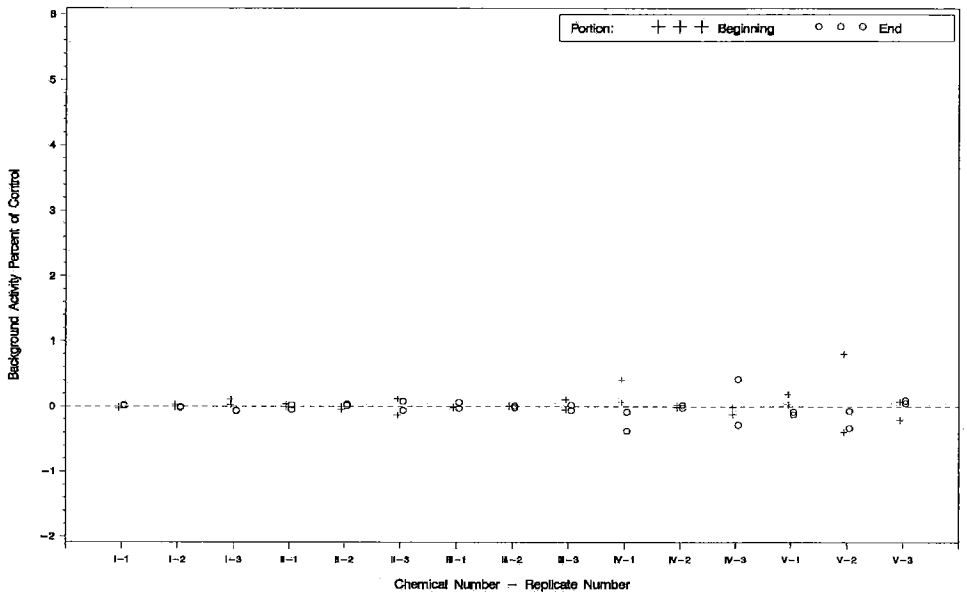


Figure 12a. **Background Activity Control Data. Chemicals I to V. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 0 percent.**

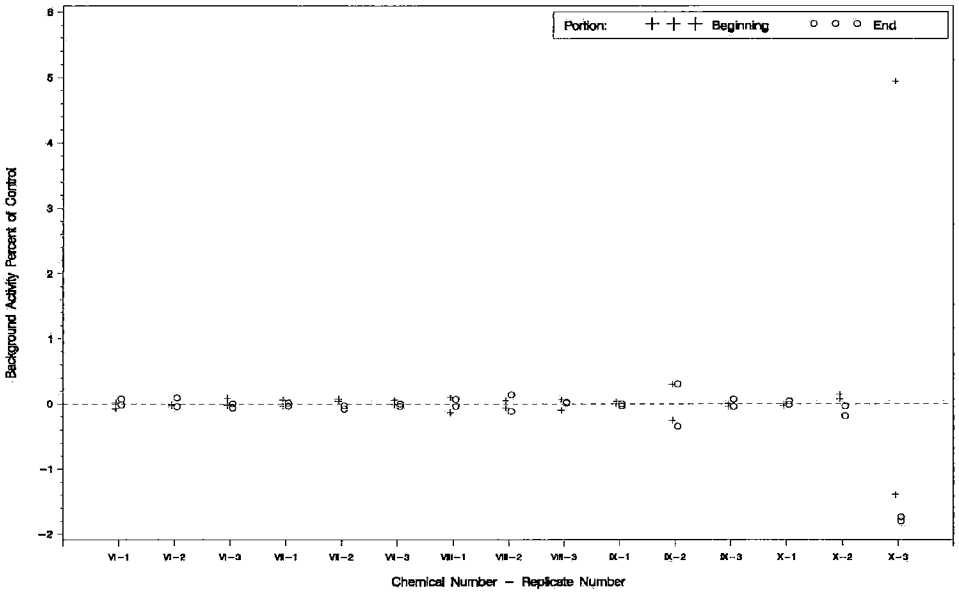


Figure 12b. **Background Activity Control Data. Chemicals VI to X. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 0 percent.**

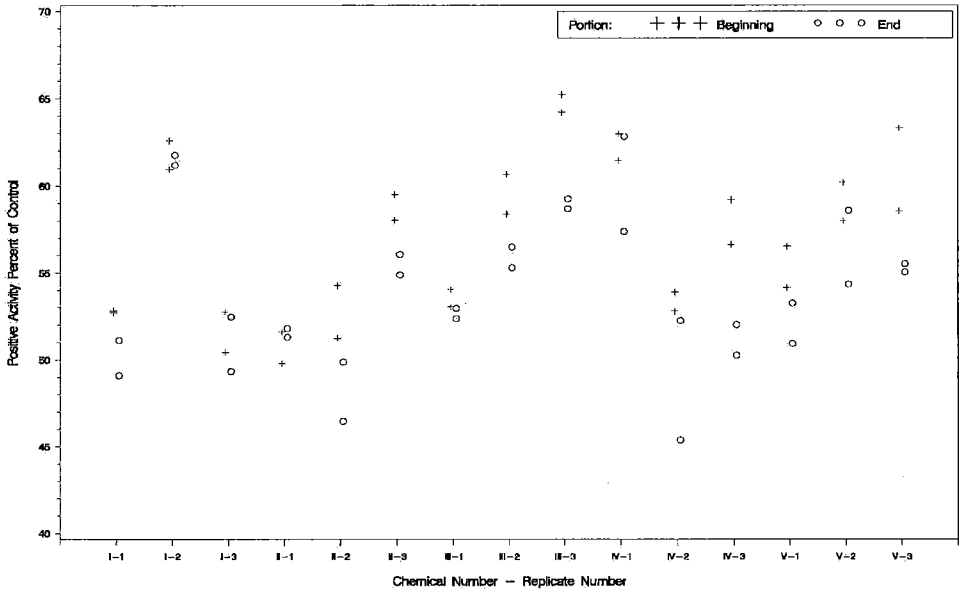


Figure 13a. **Positive Control Data. Chemicals I to V. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay.**

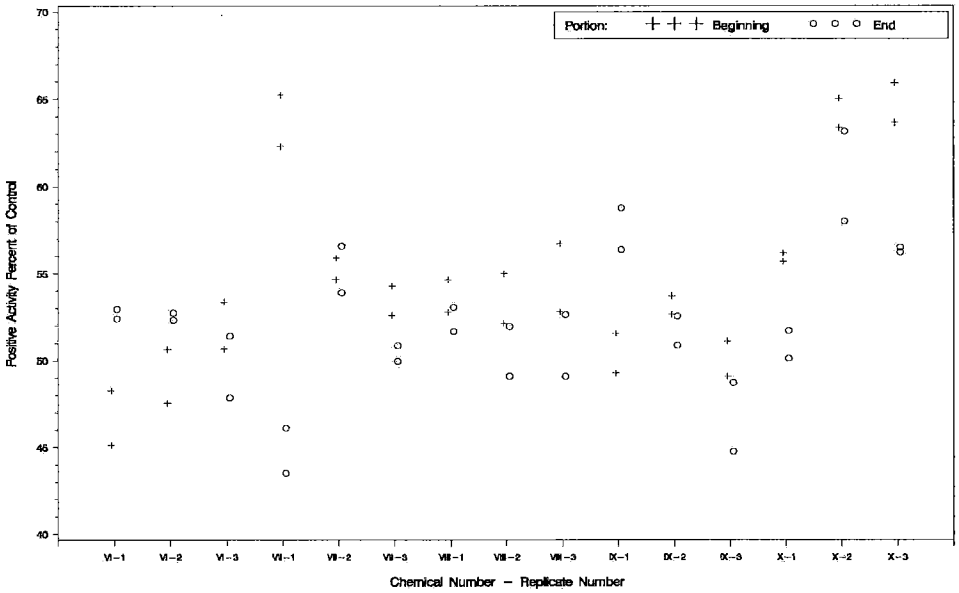


Figure 13b. **Positive Control Data. Chemicals VI to X. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay.**

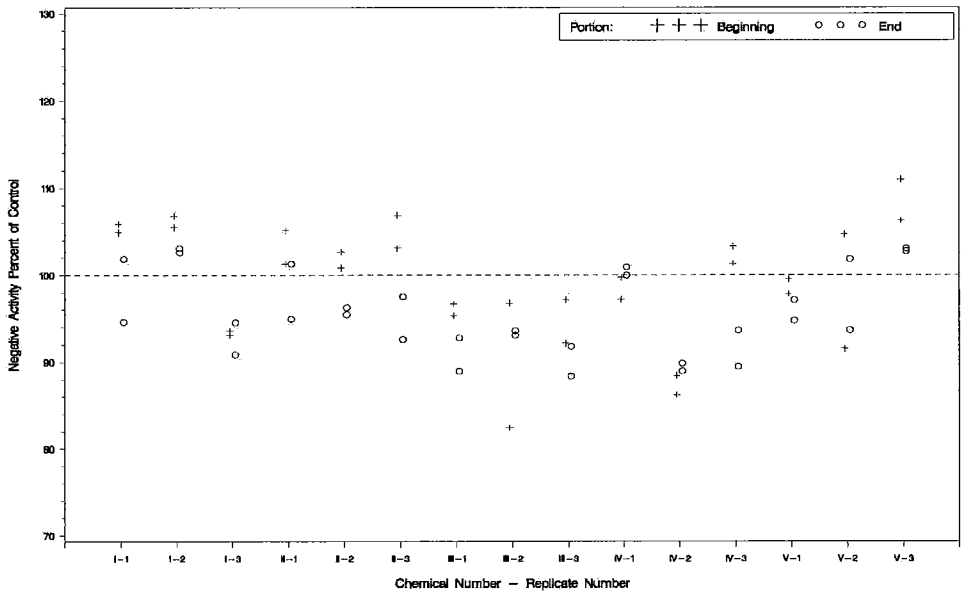


Figure 14a. **Negative Control Data. Chemicals I to V. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 100 percent.**

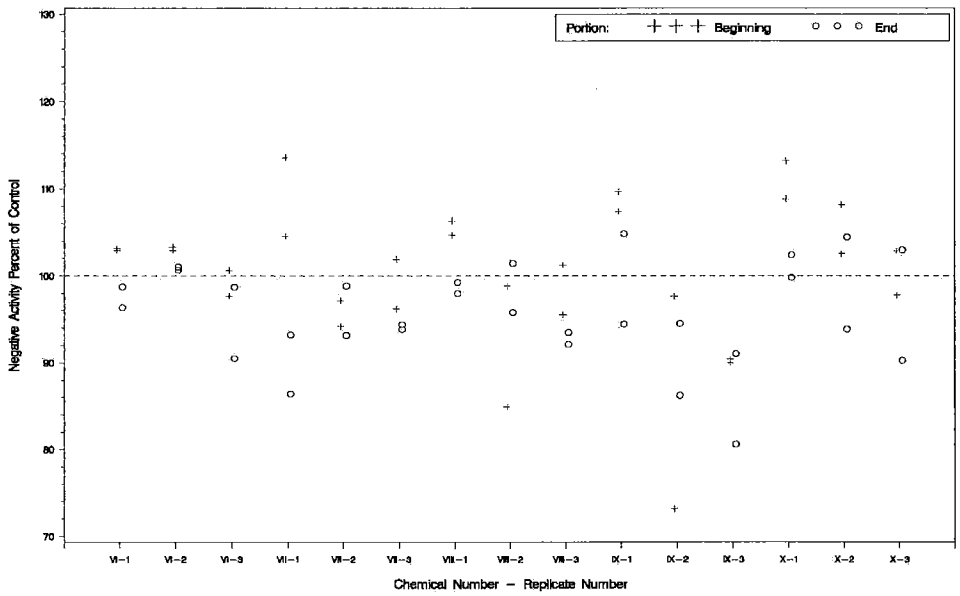


Figure 14b. **Negative Control Data. Chemicals VI to X.**
Percent of Control by Replicate and Portion of Replicate (Beginning or End).
Recombinant Aromatase Assay. Reference Line at 100 percent.

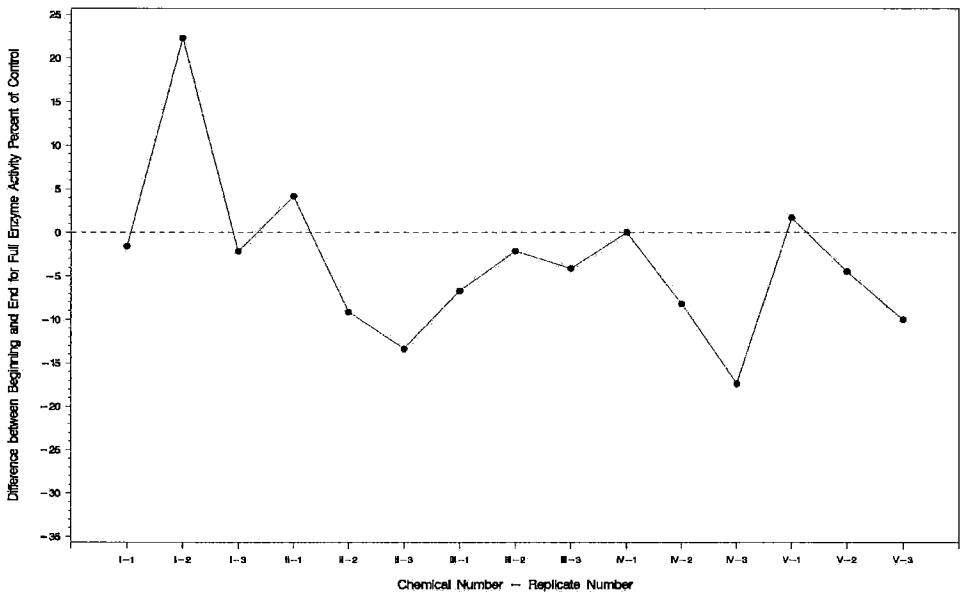


Figure 15a. **Full Enzyme Activity Control Data. Chemicals I to V. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Recombinant Aromatase Assay. Reference Line at 0 percent.**

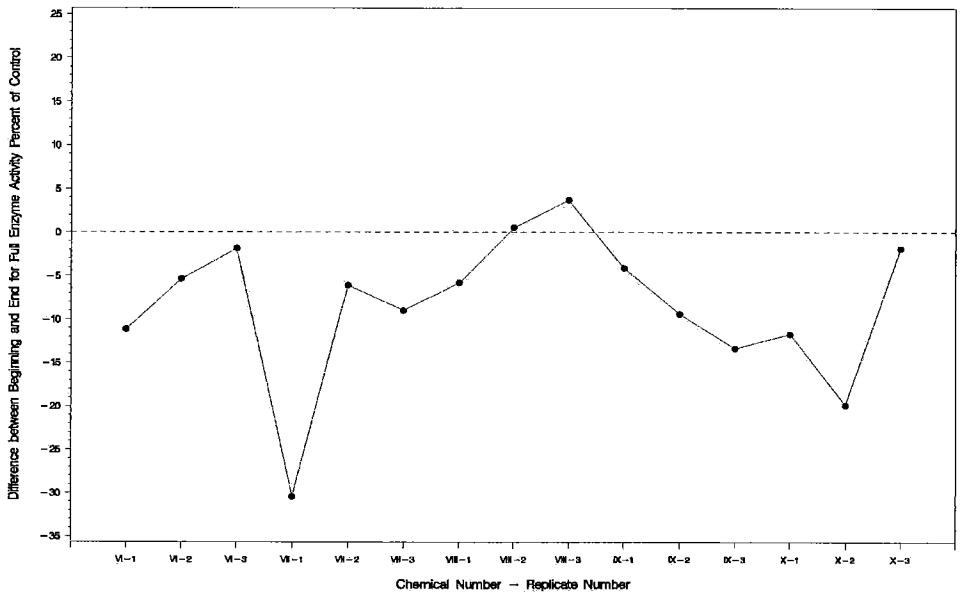


Figure 15b. **Full Enzyme Activity Control Data. Chemicals VI to X. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Recombinant Aromatase Assay. Reference Line at 0 percent.**

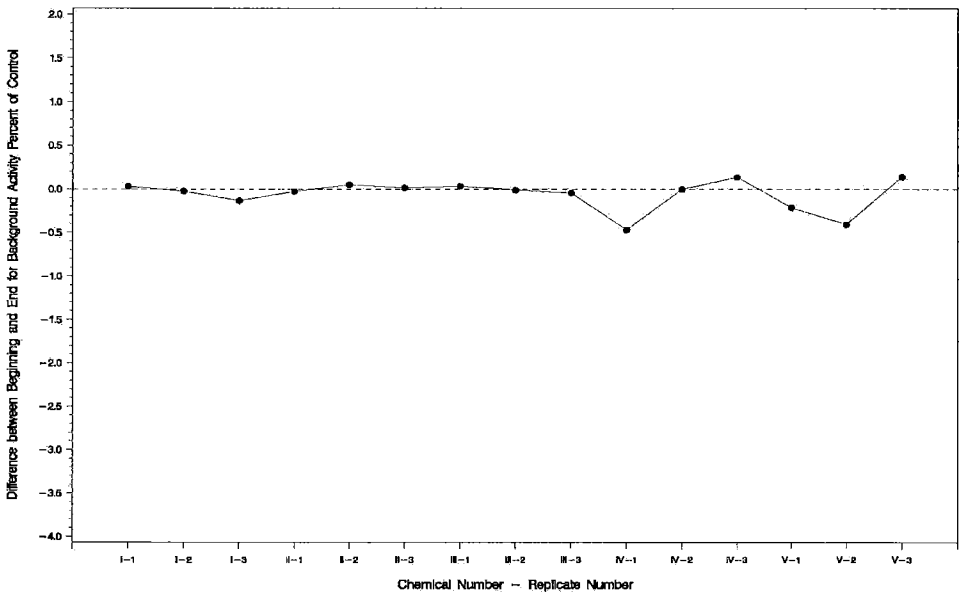


Figure 16a. **Background Activity Control Data. Chemicals I to V. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Recombinant Aromatase Assay. Reference Line at 0 percent.**

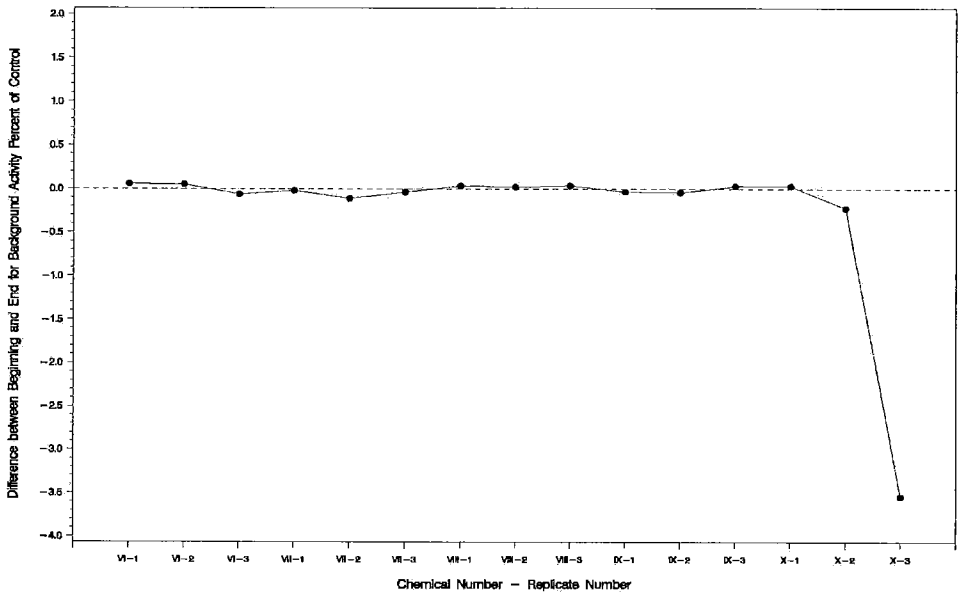


Figure 16b. **Background Activity Control Data. Chemicals VI to X. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Recombinant Aromatase Assay. Reference Line at 0 percent.**

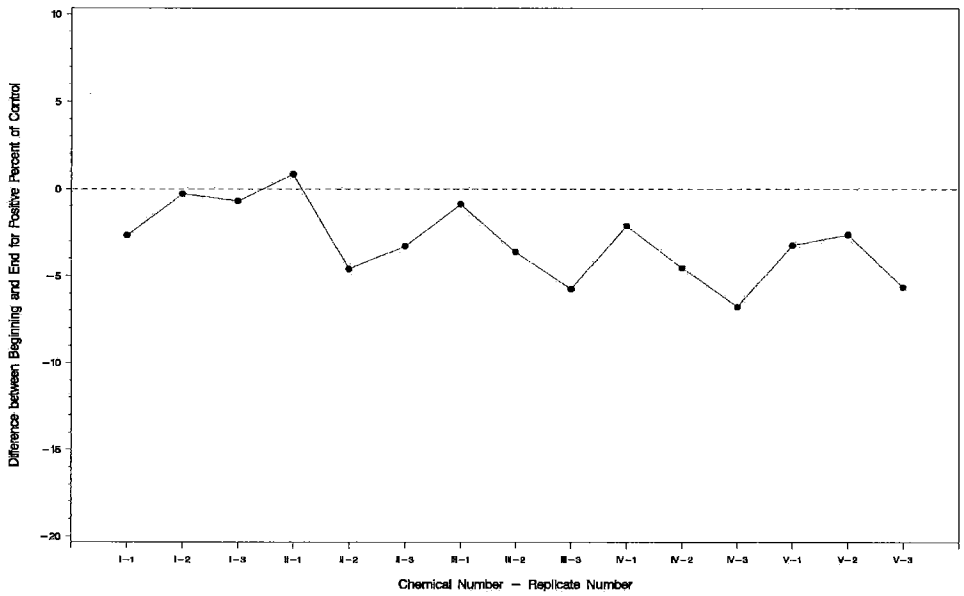


Figure 17a. **Positive Control Data. Chemicals I to V.**
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Recombinant Aromatase Assay. Reference Line at 0 percent.

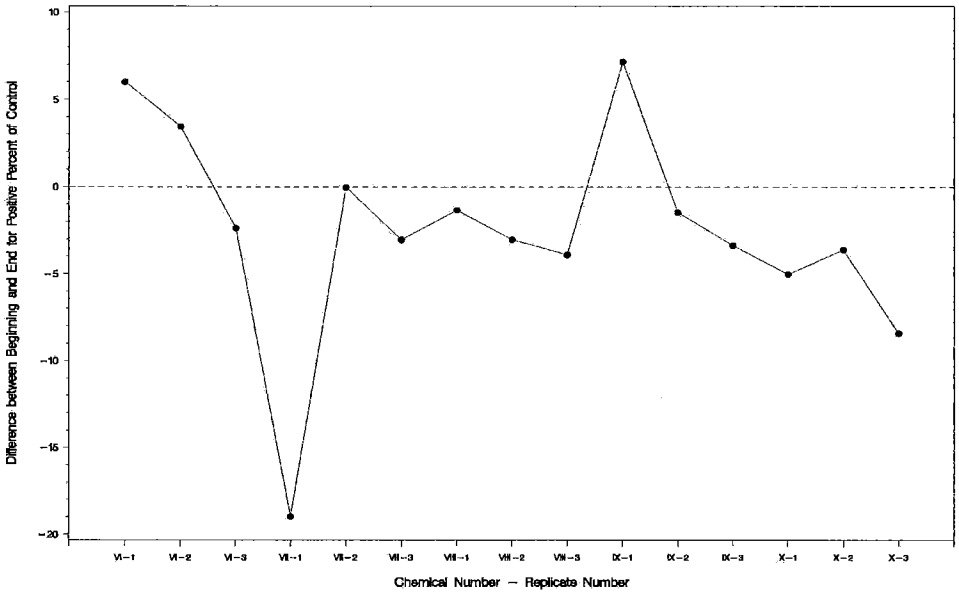


Figure 17b. **Positive Control Data. Chemicals VI to X.**
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Recombinant Aromatase Assay. Reference Line at 0 percent.

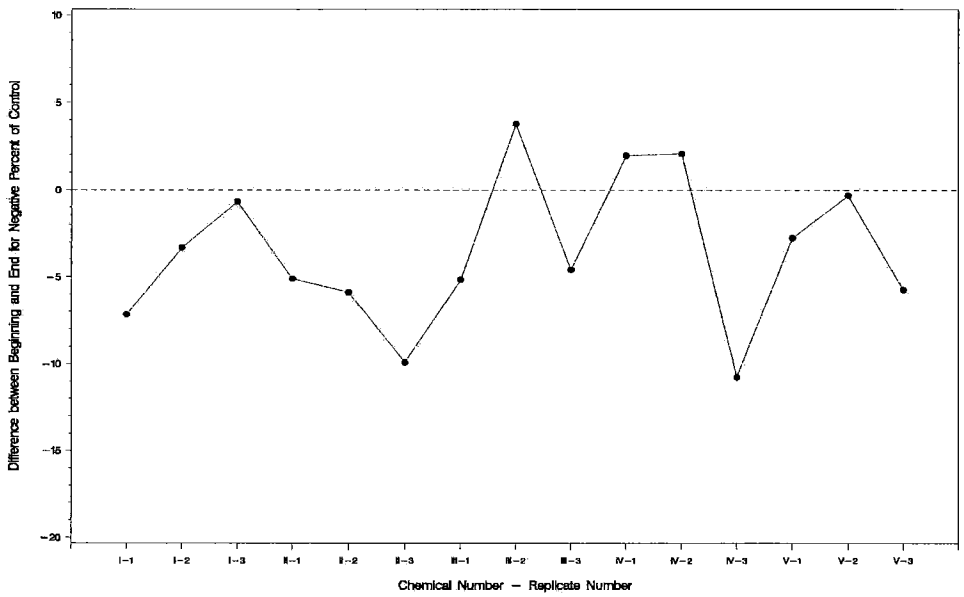


Figure 18a. **Negative Control Data. Chemicals I to V.**
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Recombinant Aromatase Assay. Reference Line at 0 percent.

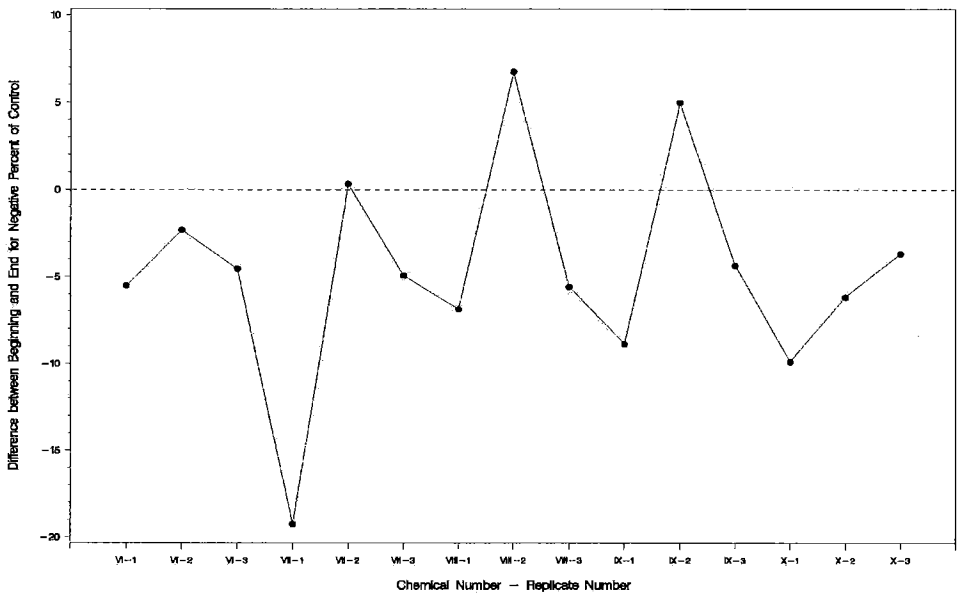


Figure 18b. **Negative Control Data. Chemicals VI to X.**
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Recombinant Aromatase Assay. Reference Line at 0 percent.

Table A-1a. Reference Chemical I: Aminoglutethimide Percent of Control Activity in Recombinant 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	41.230	39.226	39.758
	-5.0000	85.926	81.343	76.208
	-6.0000	92.613	93.185	90.531
	-7.0000	93.245	87.681	92.848
	-8.0000	96.152	95.721	95.051
	-9.0000	96.965	94.427	92.760
	-10.0000	95.911	95.469	92.099
	-11.0000	84.977	96.224	92.966
2	-3.0000	8.495	8.753	8.290
	-3.6021	27.779	27.820	27.296
	-4.0000	50.691	47.451	49.861
	-4.3010	68.559	69.009	67.342
	-4.6021	86.734	84.047	84.005
	-5.0000	94.449	97.921	94.614
	-5.6021	109.427	102.165	106.747
	-6.0000	106.295	107.050	106.861
3	-3.0000	5.715	5.808	5.538
	-3.6021	23.605	22.970	23.158
	-4.0000	42.653	42.613	43.003
	-4.3010	58.233	58.807	58.688
	-4.6021	73.643	75.115	73.775
	-5.0000	84.357	88.619	88.161
	-5.6021	98.830	98.513	94.827
	-6.0000	99.965	99.136	102.467

Table A-1b. Reference Chemical II: Ketoconazole Percent of Control Activity in Recombinant 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	0.477	0.370	0.451
	-5.0000	5.210	4.659	4.747
	-6.0000	32.687	33.588	33.027
	-6.6021	67.567	65.718	73.084
	-7.0000	83.607	83.548	84.541
	-8.0000	96.915	99.310	99.817
	-9.0000	102.171	99.470	102.199
	-10.0000	101.730	104.214	104.063
2	-4.0000	0.460	0.487	0.375
	-5.0000	5.120	5.092	4.895
	-6.0000	32.553	32.613	32.557
	-6.3010	49.387	48.933	48.702
	-6.6021	62.719	65.235	65.227
	-7.0000	77.168	77.490	83.320
	-8.0000	88.256	98.131	95.277
	-9.0000	94.801	93.135	98.546
3	-4.0000	0.625	0.797	0.519
	-5.0000	6.313	6.345	6.272
	-6.0000	39.331	39.405	37.574
	-6.3010	54.311	54.601	58.035
	-6.6021	68.434	69.412	71.212
	-7.0000	82.220	80.591	86.391
	-8.0000	90.003	91.045	94.219
	-9.0000	99.737	92.948	97.929

Table A-1c. Reference Chemical III: Prochloraz
Percent of Control Activity in Recombinant 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	0.165	0.069	0.133
	-5.0000	0.333	0.298	0.282
	-6.0000	2.659	2.843	2.915
	-7.0000	21.454	20.977	21.425
	-8.0000	67.227	68.481	68.552
	-9.0000	97.352	99.284	96.681
	-10.0000	99.296	98.276	98.794
	-11.0000	101.688	100.594	98.935
2	-5.0000	0.318	0.402	0.325
	-6.0000	3.381	3.417	3.346
	-7.0000	25.065	23.055	24.313
	-7.6021	55.988	54.780	57.246
	-8.0000	75.206	75.957	78.086
	-8.6021	95.378	89.076	92.666
	-9.0000	94.500	95.414	66.387
	-10.0000	96.903	93.896	98.835
3	-5.0000	0.559	0.299	0.323
	-6.0000	3.872	3.848	3.573
	-7.0000	27.038	26.769	25.724
	-7.6021	58.880	60.849	59.055
	-8.0000	76.150	76.836	79.264
	-8.6021	95.220	92.630	92.365
	-9.0000	93.665	97.703	97.190
	-10.0000	96.057	97.700	99.197

Table A-1d. Reference Chemical IV: 4-Nonylphenol Percent of Control Activity in Recombinant 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	1.184	1.112	0.544
	-4.0000	7.232	4.269	4.479
	-5.0000	87.145	87.553	86.230
	-6.0000	105.831	100.033	112.835
	-7.0000	106.526	107.894	96.493
	-8.0000	107.476	106.243	110.451
	-9.0000	105.670	107.075	107.712
	-10.0000	93.823	111.516	111.252
2	-3.0000	0.591	1.169	0.977
	-4.0000	4.958	3.203	3.390
	-4.3010	10.702	9.965	9.684
	-4.6021	34.446	32.878	32.150
	-5.0000	74.572	71.914	71.689
	-6.0000	96.787	88.733	94.576
	-7.0000	87.506	99.954	88.665
	-8.0000	95.740	87.794	95.907
3	-3.0000	0.762	0.319	0.402
	-4.0000	2.887	2.732	3.254
	-4.3010	8.772	12.982	12.907
	-4.6021	41.673	36.398	36.926
	-5.0000	76.457	77.680	75.073
	-6.0000	97.406	91.827	57.360
	-7.0000	91.649	90.767	94.466
	-8.0000	102.299	103.209	105.293

Table A-1e. Reference Chemical V: Dibenz[a,h]anthracene Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Dibenze[a,h]anthracene]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	102.005	101.099	102.453
	-5.0000	94.391	95.053	96.093
	-6.0000	97.788	96.154	96.053
	-6.6021	94.547	97.054	101.176
	-7.0000	91.692	92.713	96.657
	-8.0000	99.436	95.770	96.459
	-9.0000	90.706	98.463	98.578
	-10.0000	103.629	96.093	98.006
2	-4.0000	106.489	108.539	104.762
	-5.0000	107.332	108.893	109.412
	-6.0000	112.204	102.855	110.817
	-6.6021	111.101	96.038	102.498
	-7.0000	105.565	106.717	108.449
	-8.0000	101.411	101.548	103.839
	-9.0000	99.744	112.683	97.423
	-10.0000	105.878	113.050	99.108
3	-4.0000	108.970	112.831	110.790
	-5.0000	107.073	109.343	103.884
	-6.0000	115.298	110.136	112.921
	-6.6021	113.838	99.116	110.346
	-7.0000	98.922	105.023	108.409
	-8.0000	109.496	103.982	104.983
	-9.0000	102.261	110.091	109.022
	-10.0000	104.555	100.680	102.177

Table A-1f. Reference Chemical VI: Fenarimol
Percent of Control Activity in Recombinant 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	1.443	1.636	1.473
	-4.0000	5.545	5.592	5.547
	-5.0000	35.185	35.546	33.232
	-6.0000	81.484	82.517	81.939
	-7.0000	95.572	97.221	98.002
	-8.0000	101.590	101.555	97.500
	-9.0000	98.830	93.300	97.521
	-10.0000	98.843	98.263	99.924
2	-3.0000	1.650	1.461	1.425
	-4.0000	5.885	5.715	5.707
	-5.0000	37.223	37.925	37.110
	-5.6021	69.227	69.007	68.791
	-6.0000	81.878	81.347	84.462
	-7.0000	101.140	103.858	97.099
	-8.0000	102.807	98.899	100.606
	-9.0000	97.153	99.217	97.912
3	-3.0000	1.289	1.280	1.361
	-4.0000	5.685	5.713	5.554
	-5.0000	36.126	35.020	36.913
	-5.6021	66.943	67.548	66.456
	-6.0000	81.762	81.074	80.494
	-7.0000	99.930	94.837	92.126
	-8.0000	98.072	104.129	99.884
	-9.0000	105.671	88.414	99.373

Table A-1g. Reference Chemical VII: Econazole
Percent of Control Activity in Recombinant 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	0.271	0.010	0.127
	-4.0000	0.144	0.116	0.025
	-5.0000	-0.124	-0.097	-0.089
	-6.0000	0.061	0.219	0.551
	-7.0000	1.664	1.412	1.376
	-8.0000	12.328	12.771	13.195
	-9.0000	52.800	58.495	57.494
	-10.0000	78.655	81.556	83.423
2	-4.0000	0.222	-0.071	-0.046
	-5.0000	0.175	0.010	-0.046
	-6.0000	0.148	0.212	0.060
	-7.0000	1.959	1.921	1.901
	-8.0000	17.552	17.601	17.308
	-8.6021	51.864	50.959	51.881
	-9.0000	78.309	75.662	77.154
	-10.0000	90.613	93.478	91.815
3	-4.0000	-0.012	0.253	0.023
	-5.0000	0.162	-0.073	0.006
	-6.0000	0.359	0.101	0.096
	-7.0000	2.058	1.809	2.002
	-8.0000	16.119	15.784	16.252
	-8.6021	48.120	46.878	45.219
	-9.0000	74.732	68.152	70.965
	-10.0000	96.870	97.743	96.900

Table A-1h. Reference Chemical VIII: Chrysin
Percent of Control Activity in Recombinant 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	27.695	25.825	27.846
	-5.0000	25.078	25.720	26.198
	-6.0000	40.141	49.620	53.756
	-6.6021	53.901	68.936	71.960
	-7.0000	74.731	75.850	76.400
	-8.0000	75.106	69.429	75.921
	-9.0000	71.677	74.541	76.187
	-10.0000	73.854	74.817	76.387
2	-4.0000	22.406	22.072	25.370
	-5.0000	26.077	28.200	26.758
	-5.3010	39.448	34.618	40.194
	-5.6021	55.092	58.126	31.772
	-6.0000	55.844	64.430	69.390
	-7.0000	72.432	77.452	81.188
	-8.0000	91.667	91.749	94.342
	-9.0000	85.883	92.276	88.201
3	-4.0000	22.439	22.937	19.098
	-5.0000	25.366	23.725	25.445
	-5.3010	33.520	36.136	41.252
	-5.6021	58.455	60.794	54.116
	-6.0000	67.081	67.760	68.058
	-7.0000	86.326	92.816	91.566
	-8.0000	80.519	91.745	97.249
	-9.0000	98.655	98.622	100.255

Table A-1i. Reference Chemical IX: Dicofol
Percent of Control Activity in Recombinant 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	2.006	2.272	1.802
	-4.0000	15.102	16.334	15.865
	-5.0000	59.217	61.595	61.104
	-6.0000	103.162	104.648	100.143
	-7.0000	96.199	91.158	99.830
	-8.0000	78.103	98.208	101.838
	-9.0000	102.938	107.429	93.690
	-10.0000	96.180	94.949	89.523
2	-3.0000	1.987	2.102	2.417
	-4.0000	16.738	16.960	16.243
	-4.3010	23.156	24.403	25.133
	-5.0000	55.306	56.152	57.275
	-6.0000	88.970	90.945	91.511
	-7.0000	92.310	86.305	93.305
	-8.0000	93.602	100.109	100.450
	-9.0000	92.398	94.049	97.643
3	-3.0000	2.986	3.195	2.371
	-4.0000	16.480	17.478	17.646
	-4.3010	20.013	22.051	22.964
	-5.0000	67.476	63.361	73.979
	-6.0000	74.575	85.396	93.860
	-7.0000	86.073	83.466	90.538
	-8.0000	91.839	93.823	91.903
	-9.0000	94.029	87.981	92.411

Table A-1j. Reference Chemical X: Atrazine
Percent of Control Activity in Recombinant 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	90.893	94.055	95.368
	-4.0000	100.831	110.861	102.639
	-5.0000	109.314	106.149	104.736
	-6.0000	102.454	109.539	111.110
	-7.0000	111.874	106.292	112.123
	-8.0000	110.709	104.586	106.148
	-9.0000	104.575	106.675	106.285
	-10.0000	106.223	102.107	106.978
2	-3.0000	99.959	99.581	91.765
	-3.0458	97.951	93.962	96.606
	-3.0969	92.581	98.548	92.786
	-3.1249	95.161	97.121	95.546
	-3.3010	96.198	100.180	100.024
	-4.0000	107.105	108.385	106.845
	-5.0000	114.700	106.738	107.511
	-6.0000	108.155	108.449	112.383
3	-3.0000	82.555	87.740	86.884
	-3.0458	85.823	84.738	84.858
	-3.0969	78.648	84.448	82.700
	-3.1249	83.737	80.336	84.569
	-3.3010	81.410	85.111	77.949
	-4.0000	92.816	93.106	86.872
	-5.0000	105.417	99.376	94.336
	-6.0000	102.921	94.432	94.577

Table A-2a. Reference Chemical I: Aminoglutethimide 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 Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.52233	103.914
		Beginning	0.49081	97.645
		End	0.50969	101.400
		End	0.48777	97.040
	2	Beginning	0.35530	111.736
		Beginning	0.20973	65.957
		End	0.34407	108.207
		End	0.36281	114.100
	3	Beginning	0.35716	100.471
		Beginning	0.36138	101.659
		End	0.34716	97.656
		End	0.35625	100.215
Background Control	1	Beginning	-0.00001	-0.002
		Beginning	-0.00014	-0.027
		End	0.00011	0.022
		End	0.00004	0.008
	2	Beginning	0.00011	0.033
		Beginning	-0.00003	-0.008
		End	-0.00002	-0.006
		End	-0.00006	-0.019
	3	Beginning	0.00038	0.108
		Beginning	0.00009	0.026
		End	-0.00025	-0.069
		End	-0.00023	-0.064
Positive Control	1	Beginning	0.26555	52.830
		Beginning	0.26499	52.719
		End	0.25703	51.135
		End	0.24679	49.097
	2	Beginning	0.19898	62.576
		Beginning	0.19384	60.962
		End	0.19458	61.194
		End	0.19643	61.774
	3	Beginning	0.17928	50.433
		Beginning	0.18757	52.764
		End	0.18654	52.474
		End	0.17540	49.339
Negative Control	1	Beginning	0.52743	104.930
		Beginning	0.53216	105.870
		End	0.47565	94.628
		End	0.51209	101.877
	2	Beginning	0.33554	105.522
		Beginning	0.33964	106.812
		End	0.32781	103.093
		End	0.32632	102.622
	3	Beginning	0.33289	93.643
		Beginning	0.33098	93.105
		End	0.33615	94.561
		End	0.32301	90.865

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 multiplying by 100 percent.

Table A-2b. Reference Chemical II: Ketoconazole
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 Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.39108	101.808
		Beginning	0.36109	94.001
		End	0.39733	103.436
		End	0.38704	100.756
	2	Beginning	0.38358	106.117
		Beginning	0.37232	103.000
		End	0.33342	92.240
		End	0.35657	98.643
	3	Beginning	0.27732	107.428
		Beginning	0.27346	105.931
		End	0.23354	90.468
		End	0.24827	96.173
Background Control	1	Beginning	-0.00005	-0.013
		Beginning	0.00015	0.040
		End	0.00008	0.022
		End	-0.00019	-0.049
	2	Beginning	0.00000	0.001
		Beginning	-0.00018	-0.049
		End	0.00004	0.012
		End	0.00013	0.036
	3	Beginning	-0.00035	-0.134
		Beginning	0.00031	0.120
		End	-0.00016	-0.063
		End	0.00020	0.077
Positive Control	1	Beginning	0.19129	49.798
		Beginning	0.19828	51.617
		End	0.19710	51.310
		End	0.19903	51.812
	2	Beginning	0.19622	54.283
		Beginning	0.18521	51.237
		End	0.16794	46.461
		End	0.18030	49.879
	3	Beginning	0.15362	59.508
		Beginning	0.14983	58.041
		End	0.14474	56.068
		End	0.14171	54.894
Negative Control	1	Beginning	0.40392	105.151
		Beginning	0.38923	101.326
		End	0.36481	94.970
		End	0.38918	101.314
	2	Beginning	0.37114	102.675
		Beginning	0.36450	100.837
		End	0.34803	96.280
		End	0.34517	95.490
	3	Beginning	0.27589	106.873
		Beginning	0.26620	103.120
		End	0.23909	92.618
		End	0.25176	97.526

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 multiplying by 100 percent.

Table A-2c. Reference Chemical III: Prochloraz
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 Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.40793	103.170
		Beginning	0.40928	103.511
		End	0.38215	96.649
		End	0.38223	96.670
	2	Beginning	0.34752	104.140
		Beginning	0.32693	97.971
		End	0.33169	99.397
		End	0.32867	98.491
	3	Beginning	0.26697	99.682
		Beginning	0.27964	104.415
		End	0.26130	97.565
		End	0.26336	98.337
Background Control	1	Beginning	-0.00003	-0.008
		Beginning	-0.00010	-0.024
		End	0.00024	0.061
		End	-0.00012	-0.029
	2	Beginning	0.00003	0.009
		Beginning	0.00001	0.002
		End	0.00003	0.009
		End	-0.00006	-0.019
	3	Beginning	0.00027	0.100
		Beginning	-0.00015	-0.057
		End	-0.00017	-0.062
		End	0.00005	0.019
Positive Control	1	Beginning	0.20977	53.053
		Beginning	0.21368	54.042
		End	0.20945	52.972
		End	0.20707	52.371
	2	Beginning	0.19483	58.384
		Beginning	0.20245	60.668
		End	0.18854	56.501
		End	0.18456	55.307
	3	Beginning	0.17201	64.229
		Beginning	0.17467	65.221
		End	0.15718	58.689
		End	0.15869	59.253
Negative Control	1	Beginning	0.37698	95.341
		Beginning	0.38238	96.707
		End	0.36690	92.793
		End	0.35171	88.950
	2	Beginning	0.27504	82.422
		Beginning	0.32287	96.756
		End	0.31239	93.615
		End	0.31073	93.116
	3	Beginning	0.24685	92.172
		Beginning	0.26020	97.157
		End	0.24587	91.804
		End	0.23668	88.372

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 multiplying by 100 percent.

Table A-2d. Reference Chemical IV: 4-Nonylphenol 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 Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.24721	100.408
		Beginning	0.24514	99.564
		End	0.25975	105.499
		End	0.23274	94.528
	2	Beginning	0.35912	106.819
		Beginning	0.34080	101.367
		End	0.33759	100.415
		End	0.30729	91.400
	3	Beginning	0.31888	106.133
		Beginning	0.33428	111.259
		End	0.27476	91.447
		End	0.27390	91.161
Background Control	1	Beginning	0.00100	0.404
		Beginning	0.00016	0.063
		End	-0.00022	-0.089
		End	-0.00093	-0.379
	2	Beginning	-0.00005	-0.014
		Beginning	0.00006	0.018
		End	0.00005	0.016
		End	-0.00007	-0.020
	3	Beginning	-0.00004	-0.012
		Beginning	-0.00037	-0.123
		End	-0.00085	-0.284
		End	0.00126	0.419
Positive Control	1	Beginning	0.15132	61.461
		Beginning	0.15508	62.988
		End	0.15472	62.841
		End	0.14128	57.382
	2	Beginning	0.18118	53.891
		Beginning	0.17758	52.819
		End	0.17569	52.259
		End	0.15256	45.378
	3	Beginning	0.17021	56.650
		Beginning	0.17784	59.191
		End	0.15103	50.266
		End	0.15632	52.027
Negative Control	1	Beginning	0.24562	99.760
		Beginning	0.23943	97.245
		End	0.24620	99.997
		End	0.24852	100.939
	2	Beginning	0.28988	86.223
		Beginning	0.29731	88.431
		End	0.30208	89.850
		End	0.29908	88.957
	3	Beginning	0.31042	103.316
		Beginning	0.30439	101.312
		End	0.28135	93.642
		End	0.26877	89.453

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 multiplying by 100 percent.

Table A-2e. Reference Chemical V: Dibenz[a,h]anthracene 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 Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	Percent of Control[a]
Full Activity Control	1	Beginning	0.29618	97.720
		Beginning	0.30476	100.548
		End	0.30640	101.092
		End	0.30503	100.640
	2	Beginning	0.27817	101.178
		Beginning	0.28395	103.278
		End	0.25545	92.911
		End	0.28218	102.633
	3	Beginning	0.32238	109.352
		Beginning	0.29664	100.621
		End	0.28793	97.669
		End	0.27228	92.358
Background Control	1	Beginning	0.00008	0.028
		Beginning	0.00056	0.185
		End	-0.00026	-0.087
		End	-0.00038	-0.126
	2	Beginning	0.00220	0.800
		Beginning	-0.00108	-0.393
		End	-0.00020	-0.073
		End	-0.00092	-0.334
	3	Beginning	0.00021	0.070
		Beginning	-0.00062	-0.209
		End	0.00015	0.050
		End	0.00026	0.089
Positive Control	1	Beginning	0.17135	56.533
		Beginning	0.16411	54.144
		End	0.15438	50.935
		End	0.16143	53.262
	2	Beginning	0.15944	57.990
		Beginning	0.16552	60.204
		End	0.14946	54.363
		End	0.16110	58.595
	3	Beginning	0.18663	63.307
		Beginning	0.17261	58.550
		End	0.16230	55.054
		End	0.16371	55.530
Negative Control	1	Beginning	0.29665	97.875
		Beginning	0.30176	99.560
		End	0.28728	94.782
		End	0.29442	97.138
	2	Beginning	0.28776	104.665
		Beginning	0.25157	91.501
		End	0.25755	93.676
		End	0.28002	101.849
	3	Beginning	0.32719	110.983
		Beginning	0.31329	106.270
		End	0.30288	102.737
		End	0.30378	103.044

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 multiplying by 100 percent.

Table A-2f. Reference Chemical VI: Fenarimol
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 Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.37398	106.466
		Beginning	0.36767	104.670
		End	0.31736	90.348
		End	0.34605	98.516
	2	Beginning	0.35590	99.499
		Beginning	0.37857	105.836
		End	0.34579	96.672
		End	0.35051	97.993
	3	Beginning	0.37810	101.140
		Beginning	0.37644	100.697
		End	0.36920	98.758
		End	0.37161	99.405
Background Control	1	Beginning	0.00006	0.017
		Beginning	-0.00026	-0.073
		End	-0.00006	-0.017
		End	0.00025	0.073
	2	Beginning	-0.00012	-0.033
		Beginning	-0.00006	-0.017
		End	0.00033	0.091
		End	-0.00015	-0.042
	3	Beginning	-0.00009	-0.024
		Beginning	0.00033	0.088
		End	0.00000	-0.001
		End	-0.00023	-0.062
Positive Control	1	Beginning	0.15851	45.126
		Beginning	0.16951	48.256
		End	0.18606	52.967
		End	0.18409	52.408
	2	Beginning	0.18114	50.642
		Beginning	0.17014	47.567
		End	0.18718	52.331
		End	0.18868	52.749
	3	Beginning	0.19946	53.356
		Beginning	0.18942	50.670
		End	0.17895	47.869
		End	0.19223	51.422
Negative Control	1	Beginning	0.36166	102.959
		Beginning	0.36238	103.164
		End	0.34688	98.750
		End	0.33844	96.349
	2	Beginning	0.36826	102.954
		Beginning	0.36972	103.363
		End	0.36002	100.652
		End	0.36142	101.042
	3	Beginning	0.36513	97.671
		Beginning	0.37615	100.619
		End	0.36905	98.720
		End	0.33831	90.496

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 multiplying by 100 percent.

Table A-2g. Reference Chemical VII: Econazole
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 Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.36487	115.011
		Beginning	0.36615	115.415
		End	0.26150	82.427
		End	0.27647	87.147
	2	Beginning	0.36129	108.413
		Beginning	0.32543	97.654
		End	0.30958	92.898
		End	0.33670	101.035
	3	Beginning	0.33873	104.305
		Beginning	0.33993	104.674
		End	0.31893	98.207
		End	0.30142	92.814
Background Control	1	Beginning	-0.00012	-0.037
		Beginning	0.00019	0.059
		End	-0.00010	-0.033
		End	0.00003	0.011
	2	Beginning	0.00025	0.076
		Beginning	0.00012	0.035
		End	-0.00011	-0.032
		End	-0.00026	-0.079
	3	Beginning	0.00018	0.056
		Beginning	-0.00005	-0.017
		End	-0.00012	-0.038
		End	0.00000	-0.001
Positive Control	1	Beginning	0.19775	62.332
		Beginning	0.20703	65.258
		End	0.13804	43.511
		End	0.14631	46.119
	2	Beginning	0.18221	54.675
		Beginning	0.18629	55.900
		End	0.17967	53.914
		End	0.18856	56.582
	3	Beginning	0.17087	52.615
		Beginning	0.17632	54.294
		End	0.16518	50.863
		End	0.16225	49.961
Negative Control	1	Beginning	0.36032	113.576
		Beginning	0.33176	104.574
		End	0.29576	93.226
		End	0.27415	86.416
	2	Beginning	0.31390	94.194
		Beginning	0.32378	97.158
		End	0.31049	93.171
		End	0.32939	98.842
	3	Beginning	0.31244	96.210
		Beginning	0.33100	101.923
		End	0.30485	93.872
		End	0.30659	94.408

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 multiplying by 100 percent.

Table A-2h. Reference Chemical VIII: Chrysin
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 Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.33001	105.270
		Beginning	0.31509	100.508
		End	0.29352	93.628
		End	0.31536	100.594
	2	Beginning	0.30835	102.493
		Beginning	0.29166	96.946
		End	0.30995	103.026
		End	0.29343	97.535
	3	Beginning	0.34136	100.357
		Beginning	0.32626	95.918
		End	0.33938	99.775
		End	0.35358	103.951
Background Control	1	Beginning	-0.00041	-0.130
		Beginning	0.00031	0.098
		End	0.00021	0.068
		End	-0.00011	-0.036
	2	Beginning	0.00014	0.046
		Beginning	-0.00020	-0.067
		End	0.00041	0.137
		End	-0.00035	-0.116
	3	Beginning	-0.00034	-0.099
		Beginning	0.00021	0.063
		End	0.00009	0.025
		End	0.00003	0.010
Positive Control	1	Beginning	0.16553	52.803
		Beginning	0.17134	54.654
		End	0.16202	51.683
		End	0.16637	53.069
	2	Beginning	0.15686	52.140
		Beginning	0.16544	54.991
		End	0.15633	51.964
		End	0.14770	49.095
	3	Beginning	0.17966	52.818
		Beginning	0.19292	56.716
		End	0.17909	52.650
		End	0.16697	49.087
Negative Control	1	Beginning	0.32828	104.717
		Beginning	0.33320	106.288
		End	0.30721	97.997
		End	0.31117	99.260
	2	Beginning	0.25543	84.904
		Beginning	0.29731	98.826
		End	0.28816	95.784
		End	0.30520	101.446
	3	Beginning	0.32496	95.536
		Beginning	0.34435	101.237
		End	0.31799	93.486
		End	0.31332	92.113

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 multiplying by 100 percent.

Table A-2i. Reference Chemical IX: Dicofol
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 Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.24379	99.436
		Beginning	0.25659	104.656
		End	0.24554	100.148
		End	0.23478	95.760
	2	Beginning	0.28070	104.318
		Beginning	0.28275	105.078
		End	0.26397	98.098
		End	0.24892	92.505
	3	Beginning	0.27760	107.155
		Beginning	0.27511	106.194
		End	0.23753	91.687
		End	0.24601	94.963
Background Control	1	Beginning	-0.00001	-0.005
		Beginning	0.00010	0.041
		End	-0.00008	-0.033
		End	-0.00001	-0.003
	2	Beginning	0.00080	0.298
		Beginning	-0.00068	-0.254
		End	0.00081	0.303
		End	-0.00093	-0.347
	3	Beginning	-0.00009	-0.035
		Beginning	0.00001	0.006
		End	0.00018	0.070
		End	-0.00011	-0.041
Positive Control	1	Beginning	0.12079	49.266
		Beginning	0.12640	51.554
		End	0.14408	58.766
		End	0.13821	56.373
	2	Beginning	0.14454	53.715
		Beginning	0.14169	52.658
		End	0.13689	50.874
		End	0.14142	52.556
	3	Beginning	0.13239	51.103
		Beginning	0.12715	49.080
		End	0.12621	48.719
		End	0.11594	44.753
Negative Control	1	Beginning	0.26333	107.406
		Beginning	0.26878	109.627
		End	0.25702	104.831
		End	0.23157	94.452
	2	Beginning	0.19684	73.150
		Beginning	0.26276	97.649
		End	0.25434	94.519
		End	0.23207	86.246
	3	Beginning	0.23311	89.983
		Beginning	0.23421	90.406
		End	0.23587	91.047
		End	0.20884	80.615

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 multiplying by 100 percent.

Table A-2j. Reference Chemical X: Atrazine
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 Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.25150	105.855
		Beginning	0.25148	105.849
		End	0.22450	94.494
		End	0.22286	93.802
	2	Beginning	0.23146	118.925
		Beginning	0.19646	100.941
		End	0.15224	78.222
		End	0.19835	101.912
	3	Beginning	0.21722	99.195
		Beginning	0.22483	102.668
		End	0.21667	98.942
		End	0.21722	99.195
Background Control	1	Beginning	0.00000	-0.002
		Beginning	-0.00007	-0.028
		End	-0.00003	-0.012
		End	0.00010	0.042
	2	Beginning	0.00028	0.145
		Beginning	0.00015	0.078
		End	-0.00037	-0.188
		End	-0.00007	-0.035
	3	Beginning	0.01081	4.938
		Beginning	-0.00305	-1.393
		End	-0.00395	-1.803
		End	-0.00382	-1.742
Positive Control	1	Beginning	0.13349	56.186
		Beginning	0.13231	55.690
		End	0.12286	51.713
		End	0.11907	50.118
	2	Beginning	0.12333	63.365
		Beginning	0.12659	65.040
		End	0.11290	58.008
		End	0.12294	63.165
	3	Beginning	0.13941	63.660
		Beginning	0.14437	65.927
		End	0.12375	56.510
		End	0.12314	56.232
Negative Control	1	Beginning	0.26898	113.212
		Beginning	0.25852	108.810
		End	0.23714	99.812
		End	0.24333	102.416
	2	Beginning	0.21051	108.161
		Beginning	0.19956	102.533
		End	0.18271	93.876
		End	0.20329	104.454
	3	Beginning	0.22528	102.873
		Beginning	0.21408	97.760
		End	0.19768	90.272
		End	0.22554	102.993

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 multiplying by 100 percent.