DRAFT FINAL REPORT

PLACENTAL AROMATASE VALIDATION STUDY: Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories (Volume II-Appendices C-G)

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

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Prepared for

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APPENDIX C

IN VITRO TECHNOLOGIES REPORT

ERRATA

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

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

DRAFT FINAL TASK REPORT

PLACENTAL AROMATASE VALIDATION STUDY

WA 4-16 Task 7: Evaluation of the Potential of Reference Chemicals to Inhibit Aromatase Activity in Human Placental Microsomes

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

Sponsor:

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DRAFT FINAL REPORT

Title:	PLACENTAL AROMATASE VALIDATION STUDY WA 4-16 Task 7: Evaluation of the Potential of Reference Chemicals to Inhibit Aromatase Activity in Human Placental Microsomes
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Experimental Dates:	27 July–10 October 2005
Draft Task Report Date	25 May 2006

Author:	Approved:
Signature. Date	Signature. Date

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STATEMENT OF COMPLIANCE

This study was conducted to the standards of U.S. FDA 21 CFR Part 58. Exception: The computer systems at In Vitro Technologies, Inc. are not validated. This study was conducted under my scientific guidance and management.

Aruna Koganti, Ph.D.
Study Director _____ Signature _____ Date

QUALITY ASSURANCE STATEMENT

This study was inspected in accordance with In Vitro Technologies standard operating procedures. Based on audits conducted, the results reported herein accurately reflect the methods used and the data collected for this study. All findings were reported to the Study Director and In Vitro Technologies Management.

Inspection/Audit Dates:	Study Phase Audited:	Date(s) reported to Study Director and Management:
05 July 2005	Protocol review	06 July 2005
27 July 2005	Test article preparation	29 July 2005
28 July 2005	Extraction procedure	29 July 2005
03 August 2005	Compounds 3 and 6: extraction and scintillation counts	04 August 2005
05 August 2005	Compounds 3 and 6: preparation of standards and samples for protein assay	08 August 2005
09 August 2005	Compounds 3 and 6: protein assay, replicate 3	10 August 2005
29 August 2005	Incubation procedures	02 September 2005
15–17, 27 February 2006	Data and report	27 February 2006

_____ _____
Quality Assurance Date

PARTICIPATION

The following principal staff participated in the conduct of this study:

Study Director:	Aruna Koganti, Ph.D.
Scientists:	Eric Balsley, B.Sc.
	Tim Moeller, M.Sc.
Technical Writer:	Blaise Considine, B.A.

DATA RETENTION

In Vitro Technologies will retain all supporting documentation in the In Vitro Technology archives, including raw data and written records, for a period of up to five years following submission of the final report to Battelle Memorial Institute. At the end of this period, Battelle will be notified to determine whether the data (excluding proprietary information) will be transferred, retained, or destroyed.

1.0 Executive Summary

The objective of this study was to evaluate the inhibition of aromatase activity by 10 reference chemicals using human placental microsomes previously prepared and characterized by Battelle and In Vitro Technologies. This study was part of a multi-laboratory effort for the evaluation of the placental aromatase assay. The protocol was specific to the work conducted by In Vitro Technologies.

The study evaluated the inhibition of aromatase activity by 10 reference chemicals. Inhibition by five reference chemicals was evaluated using human placental microsomes prepared by Battelle. Inhibition by the other five reference chemicals was evaluated using human placental microsomes prepared by In Vitro Technologies. Inhibitory potential of each reference chemical was evaluated in three independent replicate experiments. All three replicate experiments for a given reference chemical were conducted by the same technician.

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 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 Disruptor Method Validation Committee (EDMVAC) will provide advice and counsel on the validation assays.

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

Aromatase is a cytochrome P450 enzyme complex responsible for estrogen biosynthesis and converts androgens, such as testosterone and androstenedione, into the estrogens estradiol and estrone. Aromatase is present in the ovary, placenta, uterus, testis, brain, and extraglandular adipose tissues. Two proteins, cytochrome P450arom 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 P450arom and consists of 10 exons, with the exact size of the gene exceeding 70 kilobases. Aromatase is found in breast tissue, and the importance of intratumoral aromatase and local estrogen production is being unraveled. Effective aromatase inhibitors have been developed as therapeutic agents for estrogen-dependent breast cancer

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

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

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

The human placental microsomal aromatase assay was recommended as the *in vitro* aromatase screening assay to be included in the Tier 1 Screening Battery. This assay will detect environmental toxicants that possess the ability to inhibit aromatase activity. Prevalidation studies on recombinant aromatase (WA 2-24) were conducted to optimize the microsomal aromatase assay protocol for human placenta, demonstrate the utility of the microsomal assay to detect known aromatase inhibitors, and compare the performance of a recombinant assay system and the placental microsomal assays. Concerns with this initial work involving high variability in some runs 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 inter-laboratory assay variability estimates to complete the validation of the human placental microsome aromatase assay.

2.2 Task Description and Objectives

The objective of this study was to evaluate the inhibition of aromatase activity by 10 reference chemicals using human placental microsomes previously prepared and characterized by Battelle and In Vitro Technologies. This study is part of a multi-laboratory effort for the evaluation of the placental aromatase assay. This report is specific to the work conducted by In Vitro Technologies.

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

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. It had a reported purity of 100%. The radiolabeled androstenedione ([1β-^{3}H]-androstenedione, [^{3}H]ASDN, lot 3538-496), 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 high-performance liquid chromatography (HPLC) by the lead laboratory.

Since the specific activity of the stock [^{3}H]ASDN was too high for use directly in the assay, a solution containing a mixture of nonradiolabeled and radiolabeled ASDN was prepared such that the final concentration of ASDN in the assay was 100 nM and the amount of tritium added to each incubation was approximately 0.1 µCi. This substrate solution 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 (10 µCi/mL) of the radiolabeled stock in buffer was prepared. A 1 mg/mL solution of ASDN in 95 % ethanol was prepared. Dilutions were prepared in buffer to a final concentration of 1 µg/mL. The 1 µg/mL solution of ASDN (4.5 mL), 800 µL of the [^{3}H]ASDN dilution, and 2.7 mL of buffer were combined to make 8 mL of substrate solution (enough for 80 tubes). The weight of each component added to the substrate solution was recorded. After mixing the solution well, aliquots (approximately 20 µL) were weighed and combined with scintillation cocktail for radiochemical content analysis. 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 0.1 µCi/tube.

3.2 Reference and Control Substances

The Sponsor's Chemical Repository (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. These chemistry activities and results are described in the Sponsor's Chemistry report.

Table 1. Reference and Control Substances

Chemical name	Chemical code	Mfr. Purity	CAS No.	Molecular formula	Molecular weight (g/mol)	Stock Solution ID	Target Stock Formulation Concentration	Vehicle	Storage Conditions
4-Hydroxy androstenedione	270-0049	99%	566-48-3	$C_{19}H_{26}O_3$	302.4	4-ASDN-1	10 mM	95% ethanol	2 to 8°C
Lindane	270-0050	99.6%	58-89-9	$C_6H_6Cl_6$	290.8	3-Lin-1	100 mM	DMSO	2 to 8°C
Aminoglutethimide	270-0052	>99%	125-84-8	$C_{13}H_{16}N_2O_2$	232.3	06016JS	100 mM	DMSO	2 to 8°C
Chrysin	270-0067	98.2%	480-40-0	$C_{15}H_{10}O_4$	254.2	10101DC	10 mM	DMSO	2 to 8°C
Dicofol	270-0070	96.5%	115-32-2	$C_{14}H_9Cl_5O$	370.47	RM00299	100 mM	DMSO	2 to 8°C
Econazole (nitrate)	270-0066	98%	24169-02-6	$C_{18}H_{15}Cl_3N_2O$-HNO_3	444.7	123K1220	100 mM	DMSO	2 to 8°C
Ketoconazole	270-0051	>99%	65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	531.43	121H0524	10 mM	DMSO	2 to 8°C
Atrazine	270-0071	98%	1912-24-9	$C_8H_{14}ClN_5$	215.69	328-137A	100 mM	DMSO	2 to 8°C
Fenarimol	270-0064	99%	60168-88-9	$C_{17}H_{12}Cl_2N_2O$	331.2	325-134C	100 mM	DMSO	Room temperature
4-Nonylphenol	270-0073	>98.5%	84852-15-3	$C_{15}H_{24}O$	220.4	A0192712	100 mM	DMSO	2 to 8°C
Prochloraz	270-0065	99.5%	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	2226X	100 mM	DMSO	Room temperature
Dibenz[*a,h*] anthracene	270-0074	97%	53-70-3	$C_{22}H_{14}$	278.35	11613BC	10 mM	DMSO	2 to 8°C

4-Hydroxy androstenedione (4-OH ASDN; lot 4-ASDN-1) is a known aromatase inhibitor. Positive control stock solutions were prepared and analyzed by Battelle and distributed to the laboratories. The stock was received on 07 July 2005 and stored at 2 to 8°C until use. 4-OH ASDN was formulated in 95% ethanol. The total volume of positive control formulation used in each assay was 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. Dilutions of the stock solution were prepared in ethanol on the day of use such that the target concentration of inhibitor was achieved by the addition of 20 µL of the dilution to a 2 mL assay volume. The final concentration for the positive control was 5×10^{-8} M.

A known aromatase non-inhibitor, lindane, was used as the negative control substance. Battelle provided a stock solution for lindane (lot 3-Lin-1), formulated in dimethyl sulfoxide (DMSO). The stock was received on 07 July 2005 and stored at 2 to 8°C until use. Fresh dilutions of the stock solution were prepared in DMSO on the day of use. Dilutions were prepared such that the concentration of negative control substance, 1×10^{-6} M, was achieved by the addition of 20 µL of the dilution to a 2 mL assay volume.

Reference chemical stocks formulated in DMSO were prepared, analyzed, and distributed by Battelle. The reference chemicals were numbered 1 through 10 by Battelle and these numeric designations were used when the samples were coded prior to distribution to In Vitro Technologies. Analysis of the reference chemical stock solutions occured before the laboratories used the formulations in the assay. The analytical method used to analyze each of the reference chemicals in the stock solutions was gas chromatography with flame ionization detection (4-OH ASDN, aminoglutethimide, lindane, fenarimol, dicofol, atrazine, and dibenz[a,h]anthracene), HPLC with UV-Vis detection (ketoconazole, econazole, chrysin, and prochloraz), and a combination of mass spectrometry and gas chromatography with flame ionization detection (4-nonylphenol). The chemistry procedures and results were given to the laboratories in reports prepared and submitted to the laboratories by the CR.

Fresh dilutions of the reference chemical stock solutions were prepared by serial dilution in the same solvent as the stock solution on the day of use such that the target concentration of reference chemical could be achieved by the addition of 20 µL of the dilution to a 2 mL assay volume. The total volume of reference chemical formulation used in each assay was 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.

Each reference chemical was initially targeted to be tested over the concentration range of 10^{-3} to 10^{-10} M (final concentration), but the range was adjusted due to solubility or based on data from the first replicate as described below.

After completion of the first replicate, the data were reviewed and, if necessary, the concentration of reference chemical used in the second and third replicates was adjusted. In cases where insolubility was observed, the concentrations of the reference chemical were adjusted in the first replicate also. The decision was made by the study director with the following guidelines in mind:

- If insolubility is observed at the high concentration (10^{-3} M), then set the highest concentration 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.

Note: The codes for aminoglutethimide and ketoconazole were inadvertently switched when these chemicals were shipped to In Vitro Technologies. Thus, aminoglutethimide was diluted with the assumption that the stock concentration received was 10 mM and ketoconazole was diluted with the assumption that the stock concentration received was 100 mM. Thus, the actual lowest concentration

assayed for aminoglutethimide was 1 nM and the actual lowest concentration assayed for ketoconazole was 0.01 nM.

Note: A sharp and consistent drop in the aromatase enzyme activity was observed between prochloraz concentrations of 10^{-8} and 10^{-7} M in prochloraz inhibition experiment replicates 2 and 3. The prochloraz concentration range in replicate 4 was changed to include $10^{-8.48}$ M concentration, in order to get a better estimate of the IC_{50} value.

Note: Fenarimol exhibited visible precipitate formation at the concentrations of 10^{-3} and 10^{-4} M. Thus, the highest concentration of fenarimol tested was $10^{-4.48}$.

In Vitro Technologies analyzed five chemicals with the microsomal preparation made at Battelle and five chemicals with the microsomal preparation made at In Vitro Technologies. Each chemical was evaluated in three replicate experiments. The reference chemicals were coded prior to distribution to the assaying technicians in order that the replicates were conducted blind for reference chemical identity. All three replicates for a given reference chemical were conducted by the same technician. Multiple reference chemicals were evaluated by a single technician in a given day. Each replicate for a given reference chemical were conducted entirely independently of the other replicates for that reference chemical. 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 a given reference chemical is described in Table 2.

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

- full enzyme (aromatase) activity controls (substrate, reduced nicotinamide adenine dinucleotide phosphate [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 controls, 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 5×10^{-8} M)
- negative controls (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.

Reference chemical solution (or vehicle) was added to the mixture of propylene glycol, substrate, NADPH and buffer in a volume that did not exceed 20 µL prior to preincubation of that mixture. The volume of buffer used was adjusted so the total incubation volume remained at 2 mL.

Table 2. Study Design – Aromatase Response to Reference Chemicals

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	5×10^{-8}

Sample type	Repetitions (test tubes)	Description	Reference Chemical concentration (M final)
		control chemical (4-OH ASDN) added	
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

3.3 Microsomes

Human placental microsomes previously prepared at Battelle were supplied to In Vitro Technologies. Microsomes previously prepared at In Vitro Technologies were also used. These samples were treated as potentially infectious and appropriate precautions were employed. The microsomes were stored at –70 ± 10°C. The supplier provided the approximate protein content of the microsomes.

On the day of use, microsomes were thawed quickly in a 37 ± 1°C water bath and immediately transferred to an ice bath. The microsomes were rehomogenized by vortexing for about 5 seconds to mix prior to use. The microsomes were diluted in buffer to an approximate protein concentration of 0.025 mg/mL. The addition of 1 mL of that microsome dilution resulted in a final approximate protein concentration of 0.0125 mg/mL in the assay tubes. All microsome samples were kept on ice until they were placed in the water bath just prior to their addition to the aromatase assay. Microsomes were not left on ice for longer than approximately 1 hour before proceeding with the assay. Appropriate documentation of time from thaw to use was documented.

Diluted microsomes were used only on the day of preparation. The thawed or diluted microsomes were never refrozen for later use in the assay.

3.3.1 Microsome type information

Placental microsomes, lot 6-041305, were obtained from Battelle and placental microsomes lot BAA was prepared at In Vitro Technologies for a previous study (WA 4-16, Task 6).

3.4 Other assay components

Chemical	Supplier	Lot Number
NADPH	Sigma	103K7046
Propylene glycol	JT Baker	042343
Sodium phosphate dibasic	JT Baker	A43465
Sodium phosphate monobasic	JT Baker	A28H21
Methylene chloride	Sigma	367-A0070003
95% ethanol	Battelle	04H23QB
Dimethyl sulfoxide	Battelle	2969A24437

3.4.1 NADPH

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

3.4.2 Assay Buffer

Dissolved 13.80 ± 0.55 g NaH_2PO_4 (JT Baker, catalog number 4011-01, 137.99 g/mol) in 1 L distilled, deionized water to prepare 0.1 M NaH_2PO_4. Dissolved 14.20 ± 0.56 g Na_2HPO_4 (JT Baker, catalog number 4062-01, 141.96 g/mol) in 1 L distilled, deionized water to prepare 0.1 M Na_2HPO_4. Combined these solutions to a final pH of 7.4. The assay buffer was stored in the refrigerator (2–8°C) until use.

3.5 Protein Determination

The protein concentration of each microsome preparation prepared in this task was measured by In Vitro Technologies. The protein concentration of the microsome preparation was determined on each day of use of the microsomes in the aromatase assay. A six-point standard curve was prepared, ranging from 5 to 250 µg protein/mL using bovine serum albumin (BSA). Protein was determined by using a DC Protein Assay kit purchased from BioRad (Hercules, CA). Quality control standards (10 and 100 µg/mL BSA), provided by RTI were run in duplicate with each assay. 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 remained at room temperature for at least 15 minutes to allow for color development. Each sample (unknown and standards) was transferred to disposable polystyrene cuvettes and the absorbance (at 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

This procedure was used to measure the aromatase activity in the microsomal preparations. Four types of control samples were included for each replicate. These included:

- full enzyme (aromatase) activity controls (substrate, NADPH, propylene glycol, buffer, vehicle [used for preparation of test substance solutions] and microsomes)
- background activity controls (all components that were in the full aromatase activity controls, except NADPH)
- positive control (all components that were in the full aromatase activity controls, except vehicle, and with the addition of 4-OH ASDN at a single concentration, i.e., 5×10^{-8} M)
- negative control (all components that were in the full aromatase activity controls, except vehicle, and with the addition of lindane at a single concentration, i.e., 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 set.

The assays were performed in 13 × 100 mm test tubes maintained at 37 ± 1°C in a shaking water bath. Each test tube was uniquely identified by 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) were combined in the test tubes (total volume of 1 mL). The final concentrations for the assay components are presented in Table 3. The tubes and the microsomal suspension were placed at 37 ± 1°C in the 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 approximately 5 seconds and placed on ice. The tubes were vortex-mixed an additional 20 to 25 seconds. The tubes were centrifuged using a Jouan CR422 centrifuge with GH-3.8 rotor and a Sorvall RT7 centrifuge with RTH-750 rotor for 10 minutes at a setting of 1,000 rpm. 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 layer was transferred to vials and duplicate aliquots (0.5 mL) were transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Formula 989, Perkin Elmer, 10 mL) was added to each counting vial and shaken to mix the solution. The radiochemical content of each aliquot was determined using liquid scintillation spectrometry (LSS). Radioactivity found in the aqueous fractions represented ^3H_2O formed.

Table 3. Optimized Aromatase Assay Conditions

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

[a] Final concentrations

3.7 Data Analysis

The data analysis described in the following paragraphs addresses all of the experiments of this task. The laboratories were only responsible for performing the data analysis that corresponded to the experiments they were assigned to conduct.

Relevant data were entered into the latest version of the spreadsheet, Aromatase_Master_Version1.4.xls for calculation of aromatase activity and percent of control. The instructions for the spreadsheet are described in Appendix A to the protocol.

Statistical Analyses

Statistical analysis, as described below, was carried out by Battelle. The resulting data were sent to In Vitro Technologies and were included in the final report as Appendix 6.

Concentration Response Fits for the Reference Chemicals

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

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

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
$\mu \equiv \log_{10}IC_{50}$ (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 response curve was fitted by non-weighted least squares nonlinear regression analysis. Model fits were carried out using Prism software (Version 4.02).

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 were (weighted) means across the replicates. The estimated overall standard errors were 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 were 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 are 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}.

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 were plotted versus logarithm of reference chemical concentration. The average concentration response curve across replicates was superimposed on the same plot. The average concentration response curve was the unweighted average of the response curves within each replicate.

Top (T), bottom (B), slope (β) and $\log_{10}IC_{50}$ (μ) were 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 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.

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 were made of the full enzyme activity control, background activity control, and negative and positive control tubes. Half the repetitions were carried out at the beginning of the replicate and half at the end. If the conditions were consistent throughout the replicate test, the control tubes at the beginning should have been equivalent to those at the end.

To assess whether this was the case, the control responses were 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 were necessarily 0% and the average of the four full enzyme activity controls within a replicate were necessarily 100%. 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 were 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 displayed 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 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) (end minus beginning) across reference chemicals and replicates within reference chemicals. Each plot had a reference line of 0.

Three-factor mixed effects analysis of variance models were 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 were

- reference chemical
- portion (beginning or end)

- portion by reference chemical interaction.

The random effects were

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

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

If the daily replicates were in control the portion main effect, the portion by reference chemical interaction, and the portion by replicate within reference chemical interaction should have been nonsignificant. If the portion by reference chemical interaction was significant, the nature of the effect was 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 was significant, the nature of the effect was 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. Simultaneity of inference was adjusted for by using Bonferroni's method.

Statistical Software

Concentration response curves were fitted to the data using the non-linear regression analysis features in the PRISM statistical analysis package, Version 4.02. Supplemental statistical analyses and displays such as summary tables, graphical displays, analysis of variance, and multiple comparisons were carried out using PRISM, the SAS statistical analysis system, Version 9.

Inter-laboratory Statistical Analysis

Battelle carried out "intra-laboratory" statistical analyses based on In Vitro Technologies data, according to the common statistical analysis plan, developed by the Data Coordination Center (Battelle). The Data Coordination Center carried 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.

4.0 Results

4.1 Radiochemical Purity – The measured radiochemical purity of the [^{3}H]ASDN was 97%.

4.2 Stock Formulation Analysis – The analysis of stock solution for the control and reference articles is included in the Battelle Chemistry report.

4.3 Protein Analysis

Experiment ID	Microsome Lot ID	Replicate	Assay Date	Measured Protein stock concentration (mg/mL)	Overall Mean (± sd)	% CV
Incubations for Reference Chemicals Aminoglutethimide and Ketoconazole	BAA	1	27JUL2005	8.803	6.291 (± 1.795)	28.5
	BAA	2	28JUL2005	6.075		
	BAA	3	29JUL2005	5.698		
Incubations for Reference Chemicals Dicofol and Atrazine	BAA	1	08SEP2005	5.186		
	BAA	2	12SEP2005	4.004		
	BAA	3	13SEP2005	5.421		
Incubations for Reference Chemical 4-Nonylphenol	BAA	2	20SEP2005	8.450		
	BAA	3	21SEP2005	8.378		
	BAA	6	10OCT2005	4.602		
Incubations for Reference Chemicals Prochloraz (Rep 2, 3,and 4) and Fenarimol (Rep 1, 2 and 3)	6-041305	1	03AUG2005	16.798	18.581 (± 2.144)	11.5
	6-041305	2	05AUG2005	19.596		
	6-041305	3	08AUG2005	17.757		
	6-041305	4	15AUG2005	18.531		
Incubations for Reference Chemicals Econazole and Chrysin	6-041305	1	29AUG2005	17.857		
	6-041305	2	05SEP2005	14.782		
	6-041305	3	06SEP2005	17.669		
Incubations for Reference Chemical Dibenz[a,h]anthracene	6-041305	1	19SEP2005	22.408		
	6-041305	2	20SEP2005	19.594		
	6-041305	3	21SEP2005	20.822		

4.4 Aromatase Activity (nmol / mg protein / min) – Full Enzyme Activity Control

Experiment ID	Microsome Lot ID	Replicate	FEAC Beginning		FEAC End		Within Replicate Mean (± sd)	% CV	Overall Mean (± sd)	% CV
Incubations for Reference Chemicals Aminoglutethimide and Ketoconazole	BAA	1	0.0343	0.0367	0.0293	0.0310	0.0328 (± 0.0033)	10.1	0.075 (± 0.031)	41.3
	BAA	2	0.0607	0.0518	0.0439	0.0423	0.0497 (± 0.0084)	16.9		
	BAA	3	0.0593	0.0661	0.0564	0.0530	0.0587(± 0.0056)	9.5		
Incubations for Reference Chemicals Dicofol and Atrazine	BAA	1	0.1072	0.1067	0.0829	0.0858	0.0956 (± 0.0131)	13.7		
	BAA	2	0.1323	0.1217	0.1201	0.1191	0.1233 (± 0.0061)	4.9		
	BAA	3	0.1073	0.1165	0.1076	0.1045	0.1090 (± 0.0052)	4.8		
Incubations for Reference Chemical 4-nonylphenol	BAA	2	0.0493	0.0466	0.0452	0.0460	0.0468 (± 0.0018)	3.8		
	BAA	3	0.0583	0.0591	0.0555	0.0524	0.0563 (± 0.0030)	5.3		
	BAA	6	0.1039	0.1045	0.0970	0.0946	0.1000 (± 0.0050)	5.0		
Incubations for Reference Chemicals Prochloraz (Rep 2, 3,and 4) and Fenarimol (Rep 1, 2 and 3)	6-041305	1	0.0540	0.0491	0.0440	0.0490	0.0490 (± 0.0041)	8.4	0.058 (± 0.024)	41.4
	6-041305	2	0.0466	0.0488	0.0421	0.0407	0.0446 (± 0.0038)	8.5		
	6-041305	3	0.0433	0.0433	0.0409	0.0433	0.0427 (± 0.0012)	2.8		
	6-041305	4	0.1081	0.1078	0.1080	0.1096	0.1084 (± 0.0008)	0.7		
Incubations for Reference Chemicals Econazole and Chrysin	6-041305	1	0.0569	0.0538	0.0470	0.0449	0.0506 (± 0.0056)	11.1		
	6-041305	2	0.0620	0.0641	0.0655	0.0642	0.0639 (± 0.0014)	2.2		
	6-041305	3	0.0919	0.0922	0.0910	0.0903	0.0914 (± 0.0009)	1.0		
Incubations for Reference Chemical Dibenz[a,h] anthracene	6-041305	1	0.0232	0.0245	0.0174	0.0193	0.0211 (± 0.0033)	15.7		
	6-041305	2	0.0535	0.0609	0.0527	0.0550	0.0555 (± 0.0037)	6.7		
	6-041305	3	0.0567	0.0555	0.0560	0.0512	0.0548 (± 0.0025)	4.5		

4.5 Percent of Control – Inhibition Experiments

4.5.1 Aminoglutethimide (Lot # BAA)

Reference Chemical	Replicate	Log[Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Aminoglutethimide	1	-3.00	87.10	93.61	59.51	80.07(± 18.10)	22.6
		-3.52	27.71	42.16	29.16	33.01(± 7.96)	24.1
		-4.00	42.05	49.30	34.63	41.99(± 7.34)	17.5
		-5.00	63.90	99.62	94.08	85.87(± 19.22)	22.4
		-6.00	111.55	109.74	112.52	111.27(± 1.41)	1.3
		-7.00	113.39	100.88	103.42	105.90(± 6.61)	6.2
		-8.00	99.67	113.77	106.62	106.69(± 7.05)	6.6
		-9.00	115.45	110.57	123.88	116.63(± 6.73)	5.8
	2	-3.00	2.83	5.52	4.55	4.30(± 1.36)	31.7
		-4.00	40.75	37.58	37.80	38.71(± 1.77)	4.6
		-4.48	59.96	53.86	72.87	62.23(± 9.70)	15.6
		-5.00	87.50	80.80	90.91	86.40(± 5.14)	6.0
		-6.00	103.21	106.03	105.93	105.05(± 1.60)	1.5
		-7.00	103.86	99.13	99.54	100.85(± 2.62)	2.6
		-8.00	96.58	92.65	94.71	94.65(± 1.97)	2.1
		-9.00	106.74	101.78	101.12	103.21(± 3.07)	3.0
	3	-3.00	2.18	1.65	5.29	3.04 (± 1.97)	64.7
		-4.00	40.06	37.39	39.11	38.85(± 1.35)	3.5
		-4.48	64.90	65.29	61.44	63.88(± 2.12)	3.3
		-5.00	80.30	77.60	77.89	78.60(± 1.48)	1.9
		-6.00	105.66	104.42	101.63	103.90(± 2.06)	2.0
		-7.00	111.61	106.39	102.50	106.83(± 4.57)	4.3
		-8.00	102.37	101.62	97.01	100.33(± 2.90)	2.9
		-9.00	98.26	101.32	98.98	99.52(± 1.60)	1.6

Reference Chemical	Log[Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Aminoglutethimide	-3.00	80.07	4.30	3.04	29.14 (± 44.12)	151.4
	-3.52	33.01			33.01 (± NA)	NA
	-4.00	41.99	38.71	38.85	39.85 (± 1.86)	4.7
	-4.48		62.23	63.88	63.05 (± NA)	NA
	-5.00	85.87	86.40	78.60	83.62 (± 4.36)	5.2
	-6.00	111.27	105.05	103.90	106.74 (± 3.96)	3.7
	-7.00	105.90	100.85	106.83	104.53 (± 3.22)	3.1
	-8.00	106.69	94.65	100.33	100.56 (± 6.02)	6.0
	-9.00	116.63	103.21	99.52	106.46 (± 9.01)	8.5

4.5.2 Ketoconazole (Lot # BAA)

Reference chemical	Replicate	Log[Reference chemical]	Percent of Control			Mean ± sd	% CV
			Tube 1	Tube 2	Tube 3		
Ketoconazole	1	-4.00	-1.45	-0.38	2.35	0.17 (± 1.96)	1138.2
		-5.00	3.69	4.51	9.35	5.85 (± 3.06)	52.3
		-6.00	36.81	43.59	27.93	36.11 (± 7.85)	21.7
		-7.00	90.24	94.35	78.78	87.79 (± 8.07)	9.2
		-8.00	99.93	93.45	106.01	99.80 (± 6.28)	6.3
		-9.00	102.55	110.52	107.81	106.96 (± 4.05)	3.8
		-10.00	110.67	112.33	105.23	109.41 (± 3.72)	3.4
		-11.00	108.68	109.50	112.90	110.36 (± 2.23)	2.0
	2	-4.00	0.94	0.02	-0.20	0.25 (± 0.61)	238.9
		-5.00	4.27	2.78	3.97	3.67 (± 0.79)	21.6
		-6.00	26.07	25.18	27.01	26.09 (± 0.92)	3.5
		-7.00	72.77	77.77	77.03	75.86 (± 2.70)	3.6
		-8.00	86.92	92.00	90.27	89.73 (± 2.58)	2.9
		-9.00	95.43	95.70	95.02	95.39 (± 0.34)	0.4
		-10.00	92.69	93.59	95.74	94.00 (± 1.57)	1.7
		-11.00	97.15	91.64	93.17	93.99 (± 2.84)	3.0
	3	-4.00	0.41	0.64	-0.48	0.19 (± 0.59)	309.9
		-5.00	2.94	4.71	3.81	3.82 (± 0.88)	23.1
		-6.00	18.75	24.28	26.93	23.32 (± 4.17)	17.9
		-7.00	77.23	73.51	78.63	76.46 (± 2.65)	3.5
		-8.00	93.89	94.30	96.44	94.88 (± 1.37)	1.4
		-9.00	97.26	99.52	90.09	95.62 (± 4.92)	5.1
		-10.00	102.50	99.52	96.04	99.35 (± 3.23)	3.3
		-11.00	91.89	92.22	91.60	91.90 (± 0.31)	0.3

Reference Chemical	Log[Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Ketoconazole	-4.00	0.17	0.25	0.19	0.21 (± 0.04)	20.8
	-5.00	5.85	3.67	3.82	4.45 (± 1.22)	27.4
	-6.00	36.11	26.09	23.32	28.50 (± 6.73)	23.6
	-7.00	87.79	75.86	76.46	80.04 (± 6.72)	8.4
	-8.00	99.80	89.73	94.88	94.80 (± 5.03)	5.3
	-9.00	106.96	95.39	95.62	99.32 (± 6.62)	6.7
	-10.00	109.41	94.00	99.35	100.92 (± 7.82)	7.8
	-11.00	110.36	93.99	91.90	98.75 (± 10.11)	10.2

4.5.3 Dicofol (Lot # BAA)

Reference Chemical	Replicate	Log[Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Dicofol	1	-4.00	40.90	49.57	55.66	48.71 (± 7.42)	15.2
		-4.48	70.36	66.71	63.66	66.91 (± 3.35)	5.0
		-5.00	68.58	73.41	67.52	69.83 (± 3.14)	4.5
		-6.00	87.96	89.84	86.92	88.24 (± 1.48)	1.7
		-7.00	91.79	90.41	86.07	89.43 (± 2.99)	3.3
		-8.00	89.25	82.66	80.99	84.30 (± 4.37)	5.2
		-9.00	91.72	92.62	94.99	93.11 (± 1.69)	1.8
		-10.00	90.48	90.70	89.43	90.21 (± 0.68)	0.8
	2	-4.00	30.85	67.50	58.22	52.19 (± 19.05)	36.5
		-4.48	70.71	72.09	64.56	69.12 (± 4.01)	5.8
		-5.00	68.17	63.69	61.17	64.35 (± 3.54)	5.5
		-6.00	70.27	97.25	96.30	87.94 (± 15.31)	17.4
		-7.00	104.15	102.72	99.33	102.07 (± 2.47)	2.4
		-8.00	97.75	97.07	91.07	95.30 (± 3.68)	3.9
		-9.00	93.76	89.99	98.14	93.96 (± 4.08)	4.3
		-10.00	102.54	103.11	98.70	101.45 (± 2.40)	2.4
	3	-4.00	25.61	33.49	39.64	32.91 (± 7.03)	21.4
		-4.48	49.88	52.21	43.47	48.52 (± 4.53)	9.3
		-5.00	71.46	73.83	71.44	72.24 (± 1.37)	1.9
		-6.00	89.80	89.78	96.65	92.08 (± 3.96)	4.3
		-7.00	95.82	94.14	96.59	95.51 (± 1.25)	1.3
		-8.00	96.31	92.87	95.90	95.02 (± 1.88)	2.0
		-9.00	98.13	93.14	97.93	96.40 (± 2.83)	2.9
		-10.00	99.92	94.48	97.76	97.39 (± 2.74)	2.8

Reference Chemical	Log[Reference Chemical]	Mean Percent of Control			Overall Mean (±sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Dicofol	-4.00	48.71	52.19	32.91	44.60 (± 10.27)	23.0
	-4.48	66.91	69.12	48.52	61.52 (± 11.31)	18.4
	-5.00	69.83	64.35	72.24	68.81 (± 4.05)	5.9
	-6.00	88.24	87.94	92.08	89.42 (± 2.31)	2.6
	-7.00	89.43	102.07	95.51	95.67 (± 6.32)	6.6
	-8.00	84.30	95.30	95.02	91.54 (± 6.27)	6.9
	-9.00	93.11	93.96	96.40	94.49 (± 1.71)	1.8
	-10.00	90.21	101.45	97.39	96.35 (± 5.70)	5.9

4.5.4 Atrazine (Lot # BAA)

Reference chemical	Replicate	Log[Reference chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Atrazine	1	-4.00	84.23	85.37	78.29	82.63 (± 3.80)	4.6
		-4.48	78.76	87.76	91.02	85.85 (± 6.35)	7.4
		-5.00	93.12	87.12	92.51	90.92 (± 3.30)	3.6
		-6.00	90.33	84.14	86.48	86.98 (± 3.13)	3.6
		-7.00	83.84	83.89	81.43	83.05 (± 1.40)	1.7
		-8.00	88.81	80.93	83.02	84.25 (± 4.08)	4.8
		-9.00	82.19	83.94	82.65	82.93 (± 0.91)	1.1
		-10.00	81.93	82.45	82.79	82.39 (± 0.43)	0.5
	2	-4.00	94.87	90.69	94.51	93.36 (± 2.32)	2.5
		-4.48	91.94	91.26	91.24	91.48 (± 0.40)	0.4
		-5.00	98.47	98.50	97.03	98.00 (± 0.84)	0.9
		-6.00	95.36	99.46	95.66	96.83 (± 2.29)	2.4
		-7.00	96.74	88.05	81.80	88.86 (± 7.50)	8.4
		-8.00	92.49	98.13	73.32	87.98 (± 13.01)	14.8
		-9.00	97.03	95.55	96.50	96.36 (± 0.75)	0.8
		-10.00	94.06	92.26	93.37	93.23 (± 0.91)	1.0
	3	-4.00	90.87	96.09	88.91	91.96 (± 3.71)	4.0
		-4.48	95.41	91.03	90.09	92.18 (± 2.84)	3.1
		-5.00	97.68	97.05	93.92	96.22 (± 2.02)	2.1
		-6.00	93.64	94.62	95.54	94.60 (± 0.95)	1.0
		-7.00	94.45	95.57	97.07	95.70 (± 1.31)	1.4
		-8.00	92.99	98.06	95.43	95.49 (± 2.54)	2.7
		-9.00	92.32	92.75	93.72	92.93 (± 0.72)	0.8
		-10.00	87.62	95.51	99.84	94.32 (± 6.19)	6.6

Reference Chemical	Log[Reference Chemical]	Mean Percent of Control			Overall Mean (±sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Atrazine	-4.00	82.63	93.36	91.96	89.31 (± 5.83)	6.5
	-4.48	85.85	91.48	92.18	89.83 (± 3.47)	3.9
	-5.00	90.92	98.00	96.22	95.04 (± 3.68)	3.9
	-6.00	86.98	96.83	94.60	92.80 (± 5.16)	5.6
	-7.00	83.05	88.86	95.70	89.21 (± 6.33)	7.1
	-8.00	84.25	87.98	95.49	89.24 (± 5.73)	6.4
	-9.00	82.93	96.36	92.93	90.74 (± 6.98)	7.7
	-10.00	82.39	93.23	94.32	89.98 (± 6.60)	7.3

4.5.5 4-nonylphenol (Lot # BAA)

Reference Chemical	Replicate	Log[Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
4-nonylphenol	2	-4.00	18.29	15.24	17.50	17.01 (± 1.58)	9.3
		-4.48	41.28	40.09	45.70	42.36 (± 2.95)	7.0
		-5.00	88.19	91.56	85.31	88.36 (± 3.13)	3.5
		-6.00	98.95	97.56	93.34	96.62 (± 2.92)	3.0
		-7.00	92.64	93.70	87.44	91.26 (± 3.35)	3.7
		-8.00	95.49	100.33	94.44	96.75 (± 3.14)	3.2
		-9.00	93.62	93.80	91.94	93.12 (± 1.02)	1.1
		-10.00	82.40	93.78	76.37	84.18 (± 8.84)	10.5
	3	-4.00	17.67	14.54	13.42	15.21 (± 2.21)	14.5
		-4.48	41.26	38.96	39.43	39.89 (± 1.22)	3.1
		-5.00	81.68	81.90	77.70	80.43 (± 2.37)	2.9
		-6.00	85.37	87.75	92.85	88.66 (± 3.82)	4.3
		-7.00	91.04	88.61	91.92	90.53 (± 1.71)	1.9
		-8.00	87.77	85.53	82.78	85.36 (± 2.50)	2.9
		-9.00	83.35	86.84	87.24	85.81 (± 2.14)	2.5
		-10.00	89.26	87.37	85.68	87.44 (± 1.79)	2.0
	6	-4.00	17.42	13.70	12.82	14.65 (± 2.45)	16.7
		-4.48	43.74	38.90	38.27	40.30 (± 3.00)	7.4
		-5.00	84.21	82.07	80.87	82.38 (± 1.69)	2.1
		-6.00	84.59	89.03	89.04	87.56 (± 2.57)	2.9
		-7.00	92.09	90.81	87.33	90.08 (± 2.46)	2.7
		-8.00	90.08	88.18	86.26	88.17 (± 1.91)	2.2
		-9.00	85.37	86.10	85.38	85.62 (± 0.42)	0.5
		-10.00	83.57	91.16	85.48	86.73 (± 3.95)	4.6

Reference Chemical	Log[Reference Chemical]	Mean Percent of Control			Overall Mean (±sd)	Overall % CV
		Repl 2	Repl 3	Repl 6		
4-nonylphenol	-4.00	17.01	15.21	14.65	15.62 (± 1.24)	7.9
	-4.48	42.36	39.89	40.30	40.85 (± 1.32)	3.2
	-5.00	88.36	80.43	82.38	83.72 (± 4.13)	4.9
	-6.00	96.62	88.66	87.56	90.94 (± 4.95)	5.4
	-7.00	91.26	90.53	90.08	90.62(± 0.60)	0.7
	-8.00	96.75	85.36	88.17	90.10 (± 5.94)	6.6
	-9.00	93.12	85.81	85.62	88.18 (± 4.28)	4.8
	-10.00	84.18	87.44	86.73	86.12 (± 1.71)	2.0

4.5.6 Prochloraz(Lot # 6-041305)

Reference Chemical	Replicate	Log[Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Prochloraz	2	-4.00	1.24	1.18	0.84	1.09 (± 0.21)	19.5
		-5.00	-0.34	0.35	0.04	0.02 (± 0.35)	2170.3
		-6.00	1.24	1.45	1.28	1.32 (± 0.11)	8.5
		-7.00	10.66	10.26	18.23	13.05 (± 4.49)	34.4
		-8.00	64.24	67.08	65.84	65.72 (± 1.42)	2.2
		-8.48	79.45	88.08	81.35	82.96 (± 4.54)	5.5
		-9.00	87.15	86.18	83.27	85.54 (± 2.02)	2.4
		-10.00	105.53	97.95	102.73	102.07 (± 3.83)	3.8
	3	-4.00	1.27	1.64	3.97	2.29 (± 1.46)	63.8
		-5.00	0.79	-0.75	0.18	0.07 (± 0.78)	1087.1
		-6.00	3.43	2.39	2.28	2.70 (± 0.63)	23.5
		-7.00	15.92	17.44	17.49	16.95 (± 0.89)	5.3
		-8.00	66.94	69.24	67.93	68.04 (± 1.16)	1.7
		-8.48	86.19	81.49	79.31	82.33 (± 3.52)	4.3
		-9.00	98.85	99.53	98.08	98.82 (± 0.73)	0.7
		-10.00	102.30	98.89	98.19	99.79 (± 2.20)	2.2
	4	-4.00	0.31	-0.05	-0.18	0.02 (± 0.25)	1048.6
		-5.00	0.37	0.23	0.51	0.37 (± 0.14)	37.8
		-6.00	2.08	2.26	2.23	2.19 (± 0.10)	4.4
		-7.00	17.86	15.34	17.96	17.05 (± 1.49)	8.7
		-7.48	77.92	79.65	77.95	78.51 (± 0.99)	1.3
		-8.00	59.72	60.72	63.47	61.30 (± 1.94)	3.2
		-9.00	78.27	80.69	78.01	78.99 (± 1.48)	1.9
		-10.00	91.12	90.22	80.35	87.23 (± 5.97)	6.8

Reference Chemical	Log[Reference Chemical]	Mean Percent of Control			Overall Mean (±sd)	Overall % CV
		Repl 2	Repl 3	Repl 4		
Prochloraz	-4.00	1.09	2.29	0.02	1.13 (± 1.14)	100.1
	-5.00	0.02	0.07	0.37	0.15 (± 0.19)	125.3
	-6.00	1.32	2.70	2.19	2.07 (± 0.70)	33.6
	-7.00	13.05	16.95	17.05	15.68 (± 2.28)	14.6
	-7.48			78.51	78.51 (± NA)	NA
	-8.00	65.72	68.04	61.30	65.02 (± 3.42)	5.3
	-8.48	82.96	82.33		82.65 (± NA)	NA
	-9.00	85.54	98.82	78.99	87.78 (± 10.10)	11.5
	-10.00	102.07	99.79	87.23	96.36 (± 7.99)	8.3

4.5.7 Fenarimol (Lot # 6-041305)

Reference Chemical	Replicate	Log[Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Fenarimol	1	-4.48	3.91	11.54	40.20	18.55 (± 19.13)	103.1
		-5.00	29.24	28.24	34.78	30.75 (± 3.52)	11.5
		-5.48	51.73	50.61	51.45	51.26 (± 0.58)	1.1
		-6.00	85.14	82.62	78.13	81.96 (± 3.55)	4.3
		-7.00	90.76	91.59	88.97	90.44 (± 1.34)	1.5
		-8.00	98.35	98.23	93.19	96.59 (± 2.95)	3.0
		-9.00	101.21	97.52	97.29	98.67 (± 2.20)	2.2
		-10.00	97.60	94.18	97.89	96.56 (± 2.06)	2.1
	2	-4.48	13.53	12.39	12.68	12.86 (± 0.59)	4.6
		-5.00	30.66	31.35	31.21	31.07 (± 0.36)	1.2
		-5.48	50.09	57.38	53.41	53.63 (± 3.65)	6.8
		-6.00	75.37	71.64	77.77	74.93 (± 3.09)	4.1
		-7.00	91.90	90.28	84.13	88.77 (± 4.10)	4.6
		-8.00	83.79	93.67	92.38	89.95 (± 5.37)	6.0
		-9.00	94.52	92.51	90.70	92.58 (± 1.91)	2.1
		-10.00	93.06	91.94	89.36	91.45 (± 1.90)	2.1
	3	-4.48	12.50	14.69	12.26	13.15 (± 1.34)	10.2
		-5.00	30.61	32.19	35.33	32.71 (± 2.40)	7.3
		-5.48	63.04	62.88	56.53	60.82 (± 3.72)	6.1
		-6.00	87.74	83.69	82.03	84.49 (± 2.94)	3.5
		-7.00	103.54	93.42	95.63	97.53 (± 5.32)	5.5
		-8.00	102.14	101.85	99.28	101.09 (± 1.58)	1.6
		-9.00	100.53	95.71	101.99	99.41 (± 3.29)	3.3
		-10.00	98.67	98.14	99.58	98.80 (± 0.73)	0.7

Reference Chemical	Log[Reference Chemical]	Mean Percent of Control			Overall Mean (±sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Fenarimol	-4.48	18.55	12.86	13.15	14.85 (± 3.20)	21.6
	-5.00	30.75	31.07	32.71	31.51 (± 1.05)	3.3
	-5.48	51.26	53.63	60.82	55.23 (± 4.98)	9.0
	-6.00	81.96	74.93	84.49	80.46 (± 4.95)	6.2
	-7.00	90.44	88.77	97.53	92.25 (± 4.65)	5.0
	-8.00	96.59	89.95	101.09	95.88 (± 5.60)	5.8
	-9.00	98.67	92.58	99.41	96.89 (± 3.75)	3.9
	-10.00	96.56	91.45	98.80	95.60 (± 3.76)	3.9

4.5.8 Econazole (Lot # 6-041305)

Reference Chemical	Replicate	Log[Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Econazole	1	-4.00	-0.43	-0.24	0.79	0.04 (± 0.66)	1595.8
		-4.48	0.08	-0.86	-0.27	-0.35 (± 0.47)	-135.9
		-5.00	-0.51	-0.54	-0.45	-0.50 (± 0.05)	-9.6
		-6.00	-0.46	-0.40	0.01	-0.28 (± 0.26)	-91.6
		-7.00	0.56	1.50	1.19	1.08 (± 0.48)	44.3
		-8.00	11.83	9.69	11.20	10.91 (± 1.10)	10.1
		-9.00	44.84	46.10	33.19	41.38 (± 7.12)	17.2
		-10.00	87.38	79.84	81.39	82.87 (± 3.98)	4.8
	2	-5.00	0.08	-0.67	-0.28	-0.29(± 0.38)	-130.5
		-6.00	0.25	-0.27	-0.46	-0.16 (± 0.37)	-231.1
		-7.00	1.58	1.55	1.04	1.39 (± 0.31)	22.1
		-8.00	10.84	9.48	11.02	10.45 (± 0.84)	8.1
		-8.48	28.07	29.21	30.57	29.28 (± 1.25)	4.3
		-9.00	54.56	55.62	55.30	55.16 (± 0.54)	1.0
		-9.48	69.13	68.32	79.04	72.17 (± 5.97)	8.3
		-10.00	97.30	94.38	94.21	95.30 (± 1.74)	1.8
	3	-5.00	0.65	-0.04	-0.17	0.15 (± 0.44)	299.5
		-6.00	-0.11	-0.04	0.14	0.00 (± 0.13)	-5313.4
		-7.00	0.94	2.49	2.46	1.97 (± 0.89)	45.1
		-8.00	9.98	10.11	12.83	10.97 (± 1.61)	14.7
		-8.48	30.75	25.17	31.10	29.01 (± 3.33)	11.5
		-9.00	56.83	55.42	51.66	54.64 (± 2.67)	4.9
		-9.48	71.33	66.23	80.26	72.61 (± 7.10)	9.8
		-10.00	92.46	95.18	93.42	93.69 (± 1.38)	1.5

Reference Chemical	Log[Reference Chemical]	Mean Percent of Control			Overall Mean (±sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Econazole	-4.00	0.04			0.04 (± NA)	NA
	-4.48	-0.35			-0.35 (± NA)	NA
	-5.00	-0.50	-0.29	0.15	-0.21 (± 0.33)	-154.5
	-6.00	-0.28	-0.16	0.00	-0.15 (± 0.14)	-95.0
	-7.00	1.08	1.39	1.97	1.48 (± 0.45)	30.3
	-8.00	10.91	10.45	10.97	10.77 (± 0.29)	2.7
	-8.48		29.28	29.01	29.15 (± NA)	NA
	-9.00	41.38	55.16	54.64	50.39 (± 7.81)	15.5
	-9.48		72.17	72.61	72.39 (± NA)	NA
	-10.00	82.87	95.30	93.69	90.62 (± 6.76)	7.5

4.5.9 Chrysin (Lot # 6-041305)

Reference Chemical	Replicate	Log[Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Chrysin	1	-4.00	18.80	18.92	15.98	17.90 (± 1.66)	9.3
		-4.48	2.55	6.74	6.62	5.30 (± 2.38)	44.9
		-5.00	18.91	20.93	19.70	19.85 (± 1.02)	5.1
		-6.00	66.24	69.72	58.94	64.96 (± 5.50)	8.5
		-7.00	81.75	79.26	73.99	78.33 (± 3.96)	5.1
		-8.00	87.18	84.22	62.87	78.09 (± 13.26)	17.0
		-9.00	72.33	71.81	69.45	71.19 (± 1.54)	2.2
		-10.00	85.24	88.75	77.30	83.76 (± 5.87)	7.0
	2	-4.48	10.54	7.38	8.14	8.68 (± 1.65)	19.0
		-5.00	20.74	19.74	17.72	19.40 (± 1.54)	7.9
		-5.48	41.46	42.88	41.84	42.06 (± 0.73)	1.7
		-6.00	73.57	70.70	71.84	72.04 (± 1.45)	2.0
		-7.00	88.81	89.95	86.40	88.39 (± 1.81)	2.0
		-8.00	90.77	93.34	92.88	92.33 (± 1.37)	1.5
		-9.00	93.20	92.87	93.07	93.04 (± 0.16)	0.2
		-10.00	96.38	95.71	93.92	95.33 (± 1.27)	1.3
	3	-4.48	11.30	10.15	10.29	10.58 (± 0.63)	5.9
		-5.00	25.00	25.78	28.98	26.59 (± 2.11)	7.9
		-5.48	52.68	52.35	52.09	52.37 (± 0.30)	0.6
		-6.00	78.55	79.41	78.91	78.96 (± 0.43)	0.5
		-7.00	97.47	87.37	89.95	91.60 (± 5.25)	5.7
		-8.00	102.22	103.69	102.23	102.72 (± 0.84)	0.8
		-9.00	103.50	99.94	99.09	100.85 (± 2.34)	2.3
		-10.00	112.20	100.64	94.99	102.61 (± 8.77)	8.6

Reference Chemical	Log[Reference Chemical]	Mean Percent of Control			Overall Mean (±sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Chrysin	-4.00	17.90			17.90 (± NA)	NA
	-4.48	5.30	8.68	10.58	8.19 (± 2.67)	32.6
	-5.00	19.85	19.40	26.59	21.94 (± 4.03)	18.3
	-5.48		42.06	52.37	47.21 (± NA)	NA
	-6.00	64.96	72.04	78.96	71.99 (± 7.00)	9.7
	-7.00	78.33	88.39	91.60	86.10 (± 6.92)	8.0
	-8.00	78.09	92.33	102.72	91.05 (± 12.36)	13.6
	-9.00	71.19	93.04	100.85	88.36(± 15.37)	17.4
	-10.00	83.76	95.33	102.61	93.90 (± 9.50)	10.1

4.5.10 Dibenz[*a,h*]anthrazine (Lot # 6-041305)

Reference Chemical	Replicate	Log[Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Dibenz[*a,h*] anthrazine	1	-4.48	76.51	95.36	91.15	87.67 (± 9.89)	11.3
		-5.00	85.73	86.77	78.92	83.80 (± 4.26)	5.1
		-5.48	87.23	82.60	78.24	82.69 (± 4.50)	5.4
		-6.00	68.41	64.99	91.84	75.08 (± 14.62)	19.5
		-7.00	85.51	88.03	92.46	88.66(± 3.52)	4.0
		-8.00	85.74	85.01	78.16	82.97 (± 4.18)	5.0
		-9.00	79.10	75.25	81.74	78.70 (± 3.26)	4.1
		-10.00	77.42	80.12	80.75	79.43 (± 1.76)	2.2
	2	-4.48	68.76	51.57	95.18	71.83 (± 21.97)	30.6
		-5.00	98.73	97.43	97.30	97.82 (± 0.79)	0.8
		-5.48	96.42	90.46	88.83	91.90 (± 4.00)	4.4
		-6.00	82.64	79.36	68.34	76.78 (± 7.49)	9.8
		-7.00	93.84	94.16	93.00	93.66 (± 0.60)	0.6
		-8.00	88.40	93.36	89.28	90.35 (± 2.65)	2.9
		-9.00	79.73	84.54	79.57	81.28 (± 2.82)	3.5
		-10.00	89.21	87.41	80.27	85.63 (± 4.73)	5.5
	3	-4.48	85.31	92.87	94.41	90.86 (± 4.87)	5.4
		-5.00	96.49	96.75	94.07	95.77 (± 1.48)	1.5
		-5.48	90.00	93.64	87.08	90.24 (± 3.29)	3.6
		-6.00	88.02	95.07	95.60	92.90 (± 4.23)	4.6
		-7.00	95.29	98.68	96.46	96.81 (± 1.72)	1.8
		-8.00	97.89	92.91	93.25	94.68 (± 2.78)	2.9
		-9.00	90.33	93.69	93.59	92.54 (± 1.91)	2.1
		-10.00	99.29	94.19	95.26	96.25 (± 2.69)	2.8

Reference Chemical	Log[Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Dibenz[*a,h*] anthrazine	-4.48	87.67	71.83	90.86	83.46 (± 10.19)	12.2
	-5.00	83.80	97.82	95.77	92.47 (± 7.57)	8.2
	-5.48	82.69	91.90	90.24	88.28 (± 4.91)	5.6
	-6.00	75.08	76.78	92.90	81.59 (± 9.83)	12.1
	-7.00	88.66	93.66	96.81	93.05 (± 4.11)	4.4
	-8.00	82.97	90.35	94.68	89.33 (± 5.92)	6.6
	-9.00	78.70	81.28	92.54	84.17 (± 7.36)	8.7
	-10.00	79.43	85.63	96.25	87.10 (± 8.50)	9.8

4.6 IC_{50}

Reference chemical	Replicate	Log [IC_{50}]	SE log[IC_{50}]	IC_{50}	Slope	SE slope	Status	Overall IC_{50} mean (± sd)	Overall %CV
Amino glutethimide	1	-4.971	38.00	1.07E-05	-5.155	6527	Presumed Inhibitor	4.58E-05 (± 3.071E-05)	67.0
	2	-4.228	0.06845	5.91E-05	-0.9889	0.1367	Inhibitor		
	3	-4.170	0.08325	6.76E-05	-0.7764	0.09117	Inhibitor		
Ketoconazole	1	-6.315	0.06309	4.85E-07	-0.8716	0.09383	Inhibitor	4.09E-07 (± 6.989E-08)	17.1
	2	-6.404	0.02468	3.95E-07	-1.014	0.04492	Inhibitor		
	3	-6.46	0.03977	3.47E-07	-1.097	0.07879	Inhibitor		
Prochloraz	2	-7.73	0.05304	1.86E-08	-1.005	0.09643	Inhibitor	4.08E-08 (± 3.811E-08)	93.3
	3	-7.72	0.03066	1.91E-08	-0.9771	0.05272	Inhibitor		
	4	-7.071	0.8557	8.48E-08	-7.924	91.87	Inhibitor		
4-nonylphenol	2	-4.572	0.03478	2.68E-05	-2.939	0.7787	Inhibitor	2.67E-05 (± 4.005E-07)	1.5
	3	-4.58	0.0211	2.63E-05	-2.34	0.2442	Inhibitor		
	6	-4.567	0.01807	2.71E-05	-2.601	0.2992	Inhibitor		
Dibenz[*a,h*] anthracene	1	-4.901	1.479	1.26E-05	2.133	12.32	No Inhibition	1.82E-05 (± 2.072E-05)	113.8
	2	-4.385	809920	4.12E-05	-16.33	1.12E+08	No Inhibition		
	3	-6.040	137292	9.13E-07	-13.99	4.83E+07	No Inhibition		
Fenarimol	1	-5.531	0.08538	2.95E-06	-1.291	0.2850	Inhibitor	4.24E-06 (± 1.125E-06)	26.5
	2	-5.307	0.07228	4.93E-06	-0.9339	0.09861	Inhibitor		
	3	-5.314	0.04959	4.86E-06	-1.072	0.09480	Inhibitor		
Econazole	1	-9.247	0.1288	5.66E-10	-0.7479	0.09158	Inhibitor	8.18E-10 (± 2.208E-10)	27.0
	2	-9.039	0.07000	9.14E-10	-0.8011	0.06886	Inhibitor		
	3	-9.011	0.07081	9.75E-10	-0.8255	0.07624	Inhibitor		
Chrysin	1	-5.607	0.1347	2.47E-06	-1.599	0.4607	Inhibitor	3.02E-06 (± 8.253E-07)	27.3
	2	-5.582	0.02391	2.62E-06	-1.167	0.0670	Inhibitor		
	3	-5.401	0.09502	3.97E-06	-0.872	0.1198	Inhibitor		
Dicofol	1	-2.83	4.586	1.48E-03	-0.4795	0.2868	Presumed Inhibitor	5.01E-04 (± 8.463E-04)	169.0
	2	-5.402	0.3843	3.97E-06	-0.8759	0.4966	Equivocal		
	3	-4.69	0.111	2.04E-05	-1.038	0.1930	Presumed Inhibitor		
Atrazine	1	NA	NA	NA	NA	NA	No Inhibition	2.87E-05 (NA)	NA
	2	NA	NA	NA	NA	NA	No Inhibition		
	3	-4.543	156017	2.87E-05	-17.34	4.29E+07	No Inhibition		

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

4.7 Statistical Analysis – The statistical analysis was conducted by Battelle and the report is presented in Appendix 6.

5.0 Discussion

Placental microsomes prepared by In Vitro Technologies (Lot BAA) and placental microsomes prepared by Battelle (Lot 6-041305) were used in the evaluation of the inhibitory potential of 10 test chemicals against CYP19 (aromatase). The problems that were encountered while conducting the experiments in general resulted from the technician forgetting to add the substrate or other incubation mixture components.

6.0 Conclusion

Aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, fenarimol, econazole, and chrysin were categorized as inhibitors of aromatase based on the data obtained. Dicofol was categorized as a presumed inhibitor of aromatase activity and dibenz[*a,h*]anthracene and atrazine did not inhibit aromatase activity.

Appendix 1: Copy of In Vitro Technologies Protocol No. 1157

In Vitro Technologies, Inc.
Protocol No. 1157
Version: Final (07 July 2005)

Evaluation of the Potential of Reference Chemicals to Inhibit Aromatase Activity in Human Placental Microsomes (WA 4-16, Task 7)

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Study Director:	Aruna Koganti, Ph.D.
EPA Contract Number:	68-W-01-023 (Battelle Prime Contractor)
In Vitro Technologies Study Number:	270-1157-12
Proposed Experimental Start Date:	18 July 2005
Proposed Experimental End Date:	19 September 2005

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Objectives

The objective of this study is to evaluate the inhibition of aromatase activity by 10 reference chemicals using human placental microsomes previously prepared and characterized by Battelle and In Vitro Technologies. This study is part of a multi-laboratory effort for the evaluation of the placental aromatase assay. This protocol is specific to the work conducted by In Vitro Technologies.

Reference Chemicals

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
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

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Test System Identification

The test system for this study is human placental microsomes previously prepared by Battelle and In Vitro Technologies. The lot or batch number of the microsomes preparation and any other unique identifier assigned by Battelle and In Vitro Technologies will be recorded in study documentation and will be reported in the study report. All tubes used in the incubations will contain unique labels.

Test System Justification

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

Description of Study

The study will evaluate the inhibition of aromatase activity by ten reference chemicals. Inhibition by five reference chemicals will be evaluated using human placental microsomes prepared by Battelle and inhibition by the other five reference chemicals will be evaluated using human placental microsomes prepared by In Vitro Technologies. Inhibitory potential of each reference chemical will be evaluated in three independent replicate experiments. All three replicate experiments for a given reference chemical will be conducted by the same technician.

Test Method

The *in vitro* test method involves combining microsomes, substrate, 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.

Experimental Methods

Materials

Battelle will provide the following materials:

- Placental microsomes
- 4-Androstene-3, 17-dione (ASDN, CAS no. 63-05-8)

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- [1β-^{3}H] Androstenedione ([^{3}H] ASDN, 25.3 Ci/mmol, 1 mCi/ml)
- 4-Hydroxy androstenedione (4-OH ASDN, CAS no. 566-48-3; 302.4 g/mol, Sigma) stock solution (10 mM) in 95% ethanol.
- Lindane (CAS no. 58-89-9; 290.8 g/mol) stock solution (100 mM) in dimethylsulfoxide (DMSO)
- β-Nicotinamide adenine dinucleotide phosphate, reduced form (NADPH, Sigma, catalog no. 1630, molecular weight. 833.4 g/mol)
- 95 % Ethanol
- Dimethyl sulfoxide (DMSO)
- Reference chemical stocks

The following will be prepared at In Vitro Technologies or will be supplied by In Vitro Technologies:

- Placental microsomes
- 0.1 M Sodium phosphate buffer (pH 7.4)
- Glycerol (Sigma, catalog number G7893, 92 g/mol)
- Propylene glycol (JT Baker, catalog number 9402-01, 76.1 g/mol)
- Liquid scintillation cocktail (Formula 989, Perkin Elmer)
- DC Protein Assay kit (BioRad)

The lot numbers and the purity of the materials received and used in this study will be included in the study report.

Positive Control Preparation

4-Hydroxyandrostenedione (4-OH ASDN) is a known aromatase inhibitor. Positive control stock solutions will be prepared and analyzed by Battelle and distributed to In Vitro Technologies. 4-OH ASDN will be formulated in 95 % ethanol. The total volume of positive control formulation used in each assay will 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. Dilutions of the stock solution will be prepared in ethanol on the day of use such that the target concentration of inhibitor can be achieved by the addition of 20 µL of the dilution to a 2 mL assay volume. The final target concentration for the positive control is 5×10^{-8} M.

Negative Control Preparation

A known aromatase non-inhibitor, lindane, will be used as the negative control substance. Battelle will provide a stock solution of lindane, formulated in DMSO. Fresh dilutions of the stock solution will be prepared in DMSO on the day of use. Dilutions will be prepared such that the target concentration of control substance, 1×10^{-6} M, can be achieved by the addition of 20 µL of the dilution to a 2 mL assay volume.

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Reference Chemical Preparation

Reference chemical stocks formulated in buffer, absolute ethanol or DMSO will be prepared, analyzed, and distributed by Battelle. The reference chemicals will be numbered 1 through 10 by Battelle and these numeric designations will be used when the samples are coded prior to distribution to In Vitro Technologies.

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. The total volume of reference chemical formulation used in each assay will 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.

Each reference chemical will initially be tested over the concentration range of 10^{-3} to 10^{-10} M (final concentration) but the range may be adjusted as described in the section "Determination of the Inhibition of Aromatase Activity by Reference Chemicals".

Substrate Preparation

The substrate for the aromatase assay is androstenedione (ASDN). Non-radiolabeled and radiolabeled ASDN will be used. The non-radiolabeled ASDN and the radiolabeled ASDN ([^{3}H]ASDN) will be provided by Battelle. Battelle 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 Battelle in a previous task and was found to be 97%.

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 ASDN will 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 (10 µCi/mL) of the radiolabeled stock in buffer. Prepare a 1 mg/mL solution of ASDN in 95% ethanol and 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 (approximately 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.

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Preparation of Microsomes

Human placental microsomes previously prepared at Battelle will be supplied to In Vitro Technologies. Microsomes previously prepared at In Vitro Technologies will also be used. These samples should be treated as potentially infectious and appropriate precautions must be employed. The microsomes must be stored at -70 ± 10°C. The supplier will provide the approximate protein content of the microsomes.

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

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

Daily Use of Microsomes

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

Aromatase Assay Reagent Preparation

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,

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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 (4 ± 3°C).

NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt, Sigma, catalog number 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 is prepared in assay buffer and 100 µL of the stock is added to the 2 mL assay volume. NADPH must be prepared fresh each day and will be kept on ice.

Assays

Protein Assay

The protein concentration of each microsome preparation prepared in this task will be measured by all participating laboratories. The protein concentration of the microsome preparation will be determined on each day of use of the microsomes in the aromatase assay and at other times as appropriate. A six-point standard curve will be prepared, ranging from 5 to 250 µg protein/mL using bovine serum albumin (BSA). Protein will be determined by using a DC Protein Assay kit purchased from BioRad (Hercules, CA). Quality control standards (10 and 100 µg/mL BSA), provided by RTI will be run in duplicate with each assay. To a 200 µL aliquot of unknown or standard, 100 µL of BioRad DC Protein Kit Reagent A will be added and mixed. Next, 0.8 mL of BioRad DC Protein Kit Reagent B will be added to each standard or unknown and the samples will be vortex mixed. The samples will remain at room temperature for at least 15 minutes 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 (at 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 using the protein standards.

Aromatase Assay

This procedure will be used to measure the aromatase activity in the microsomal preparations. The assays will be performed in 13 × 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 of 1 mL). The final concentrations for the assay components are presented in the table "Optimized Assay Conditions". The tubes and the microsomal suspension will be placed at 37 ± 1°C in the 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 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 approximately 5 seconds and placed on ice. The tubes will be vortex-mixed an additional 20 to 25 seconds. The tubes will be centrifuged using a Jouan CR422 centrifuge with GH-3.8 rotor or equivalent for 10 minutes at a setting of 1,000 rpm. The methylene chloride layer will be removed and discarded; the aqueous layers will be extracted

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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 (Formula 989, Perkin Elmer, 10 mL) will be added to each counting vial and shaken to mix the solution. Analysis of the samples will be performed using liquid scintillation spectrometry (LSS). Radioactivity found in the aqueous fractions represents ^3H_2O formed.

Optimized Aromatase Assay Conditions

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

[a] Final concentrations

Determination of the Inhibition of Aromatase Activity by Reference Chemicals

In Vitro Technologies will analyze five chemicals with microsomal preparation made at Battelle and five chemicals with microsomal preparation made at In Vitro Technologies. Each chemical will be evaluated in three replicate experiments. The reference chemicals must be coded prior to distribution to the assaying technicians in order that the replicates are conducted blind for reference chemical identity. This task will be conducted in three independent replicates. All three replicates for a given reference chemical must be conducted by the same technician. However, the same technician is not required to perform the three replicates for all ten reference chemicals. 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. Each reference chemical will be tested at eight concentrations and there will be three (triplicate) repetitions for each concentration of a given replicate. A single replicate study of a given reference chemical is described below.

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).

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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 Aromatase Assay section 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.

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	3	Complete assay with	1×10^{-4}

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Sample type	Repetitions (test tubes)	Description	Reference Chemical concentration (M final)
Concentration 2		Reference Chemical added	
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

Description of Data Calculations

Relevant data will be 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. The instructions for the spreadsheet are described in Appendix A.

Statistical Analyses

Statistical analysis, as described below, will be carried out by Battelle. The resulting data will be sent to In Vitro Technologies and will be included in the final report.

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 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.

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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%).

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

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

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), $\log_{10}IC_{50}$ (□), 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, $\log_{10}IC_{50}$,

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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}.

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.

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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)
- 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 chemcial effect are both necessarily constrained to be 0.

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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. Simultaneity of inference will be adjusted for by Bonferroni's method.

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.

Interlaboratory Statistical Analysis

Battelle will carry out "intra-laboratory" statistical analyses based on In Vitro Technologies data, according to the 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.

Criteria for Data Acceptance

All data obtained will be reported.

Study Report

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 and log reference 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 reference 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 this protocol.

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Data Retention

In Vitro Technologies will retain all supporting documentation, including raw data and written records, for a period of up to five years following issuance of the final report. At the end of this period, Battelle will be notified to determine whether the data (excluding proprietary information) will be transferred, retained, or destroyed. Study records to be maintained will include:

- 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
- Quality Assurance Project Plan (QAPP) and any amendments
- List of any QAPP deviations

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Protocol Approval

This protocol has been reviewed and approved by the following:

Sponsor Representatives

David P. Houchens, Ph.D. — Signature — 7/7/05 (Date)
Program Manager
Endocrine Disruptor
Screening Program
Battelle Memorial Institute

Jerry D. Johnson, Ph.D. — Signature — 7-7-05 (Date)
Work Assignment Leader
Endocrine Disruptor
Screening Program
Battelle Memorial Institute

Study Director

The study will be conducted to the standards of U.S. FDA 21 CFR Part 58. The study will be conducted under my scientific guidance and management. I have reviewed the procedures outlined in this protocol.

Aruna Koganti, Ph.D. — Signature — 12 July 2005 (Date)
Study Director
In Vitro Technologies

Review

Terri L. Pollock, B.A. — Signature — 7-7-05 (Date)
Quality Assurance Manager
Battelle Memorial Institute

Sharon Isbell — Signature — 12 July 05 / 12 July 2005 (Date)
Director, Quality Systems
In Vitro Technologies

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APPENDIX A

NOTES FOR USE OF THE SPREADSHEET: AROMATASE_MASTER_VERSION1.1.XLS

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Substrate Specific Activity Worksheet

This worksheet calculates:

1. The radiochemical content (DPM/mL) of the substrate solution
2. The new specific activity of the [^{3}H]ASDN in the substrate solution

The first item is based on the results of liquid scintillation counting analysis of weighed aliquots of the substrate solution.

The second item is calculated by:

1. determining the mass of ASDN (both radiolabeled and nonradiolabeled)/g of solution. This calculation uses both the measured mass of nonradiolabeled ASDN used in the solution preparation and also the specific activity of the stock [^{3}H]ASDN.

2. the radiochemical content (mCi/g) of the solution is then divided by the mass of ASDN/g solution to arrive at the new specific activity for [^{3}H]ASDN in the substrate solution.

Data to be input include:

- substrate solution aliquot weights (g) and DPM results
- weight (mg) of ASDN used in original stock and volume (mL) of the original stock
- all dilution factors for the dilution of ASDN stock to the solution that was finally used in substrate preparation.
- weight (g) of ASDN dilution used to prepare substrate solution and total weight (g) of substrate solution, and
- specific activity of the stock [^{3}H]ASDN (μCi/mmol)

Protein Worksheet

This worksheet calculates protein content based on absorbance data of standards and unknown samples obtained when samples are analyzed using a commercially available kit.

Data to be input include the concentration of protein standard stock solution (mg/10 mL), protein stock ID, Sample IDs, absorbance data (in triplicate) for standards and unknowns and appropriate dilution factors.

Absorbance values are corrected for blank absorbance. A calibration curve is prepared by linear regression of the standards data (corrected absorbance vs. mg protein measured). The concentration of protein in the unknowns is calculated based on the standard curve.

Microsomes and Chemical Dilutions Worksheet

This worksheet calculates the concentration of protein in the final microsomes dilution. It also serves as the data input center for the reference chemical concentrations used in the assay.

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Data input include volumes used in the preparation of microsomes dilutions. Also entered is the protein concentration of the stock microsomes. Normally, this value will be determined using the protein worksheet described above.

Reference chemical concentrations are entered in molar units of the final concentrations used in the assay.

Activity Calculation Worksheet

The primary aim of this worksheet is to calculate aromatase activity for each sample in a set based on measured DPM, protein concentration and incubation time.

The function of each section is described below:

Section 1 (Columns A–B)

This section contains fields for sample identification

Section 2 (Columns C–I)

This section calculates the total DPM that remain in the incubation mixture after extraction (this is a measure of the ^3H_2O formed in the reaction).

Data input:

1. Aliquot volume
2. DPM measured for each aliquot of each sample.

Output:

The worksheet calculates the average DPM/mL for each repetition, the average DPM/mL for each sample, and the total DPM contained in the sample (based on the aliquots and total sample volumes)

Section 3: (Columns J–L)

This section calculates the percent turnover of the substrate to product.

Data input: Volume of substrate solution used in each assay tube

Linked Data: Column K links to radiochemical content value for the substrate that is calculated in the substrate specific activity worksheet

Output: Percent conversion to product

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Section 4 (Columns M–N)

This section calculates the nmol ^3H_2O formed

Data input: None

Linked Data: Column N links to specific activity value for the substrate that is calculated in the substrate specific activity worksheet

Calculations: Column M corrects the total DPM in each tube for background DPM determined in negative control tubes

Column N Converts DPM data to nmol using the substrate specific activity

Data output: nmol ^3H_2O formed

Section 5 (Columns O–R)

This section calculates aromatase activity in each tube.

Data input: Volume of diluted microsomes used in assay tube and incubation time

Output: Aromatase activity (nmol/mg protein/min)

Results Summary Worksheet

This worksheet summarizes the results.

Section 1 (Columns A–D, Rows 3–15)

This section summarized control data.

Data input: none

Output: average and SD for control samples for beginning, end and overall portions

Section 2 (Columns A–F, Rows 18–42)

This section summarizes activity values according to inhibitor level and replicate

Data input: None

Output: Log[test inhibitor]

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Section 3 (Columns H–L, Rows 18–28)

This section calculates percent of control values for each reference chemical concentration and replicate and organizes the data in a format suitable for importation into Prism Software.

Data input: None

Output: Percent of control values with data arranged in a format suitable for importation into Prism Software.

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Appendix 2: Copy of Protocol Amendment PAM05-043

PROTOCOL AMENDMENT FORM

IVT Study Number: 270-1157-12 Document Number: PAM-05-043

Date of Sponsor's Verbal Approval: 15 November 2005

Briefly describe the amendment:
The protocol is being amended with regards to the Reference Chemical Formulation and Analysis and with regards to the Concentration Response Fits for the Reference Chemicals. The details of the changes made are attached.

Briefly describe the reason for the amendment:
These changes are made to the protocol per sponsor request.

Approved by: Jerry D Johnson Date: 12-15-05
Sponsor Representative

Approved by: K. Mumtaz Pakkla Date: 16 Dec 2005
Study Director

Effective Date: 04 June 2002

1) The following paragraph is being added to the section entitled "Reference Chemical Preparation" on page 5 of the protocol:

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.

2) The following sentence in section entitled "Concentration Response Fits for the Reference Chemicals" on Page 11 of the protocol

- "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 amended as follows:

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

3) The following sentences under section "Concentration Response Fits for the Reference Chemicals" on Page 11 of the protocol

"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."

will be amended to read as follows:

"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
$\mu \equiv \log_{10} IC_{50}$ (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:

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

Appendix 3: Copies of Protocol Deviations PDV06-021, PDV06-030, and PDV06-031

PROTOCOL DEVIATION FORM

IVT Study Number: 270-1157-~~18~~ EE TM 17APR06 12 Document Number: PDV06-021

Date of Deviation: NA

Scientist (if applicable): TM

Date Sponsor Notified (if applicable): NA

Describe the deviation:

1. Protocol states that Beckman GS-6R centrifuge with GH3.8 rotor would be used in the study, however ~~Juoan~~ * CR422 with GH-38 rotor was used in its place.
2. Protocol states that solutions will be prepared using distilled, deionized water, however the water ~~poliching~~ ** system used only deionizes.
3. The SCADA report for freezer #50509 indicated several excursions from the protocol-defined range.
4. The remaining aqueous solution was not transfered to a vial and stored at -20C.

** polishing EE TM 17APR06

Describe the corrective action:

1. IVT does not possess a Beckman GS-6R centrifuge and the ~~Juoan~~ * Jouan TM 17APR06 EE was substituted in kind and used at the same centrifugal force.
2. The water polishing system performed within the specifications of its SOP documentation and was used in place of distilled, deionized water.
3. This event was addressed in in-process audit for 29JUL2005.
4. A memo was added to the biological folder addressing the change to the protocol.

Assessment:

1. The same centrifugal force was used, therefore there was no impact to the study.
2. The water generated by the water polishing system was of similar quality to distilled, deionized water, therefore there was no impact.
3. The excursions were not of significant duration to affect the microsome storage, therefore there was no impact.
4. The retained samples were utilized, if necessary, within 48 hours of initial storage, and generated expected results, ~~therefore~~ there was no impact.

Form Completed by: _____ (Signature) 05APR06 (Date)

Approved by: K. Anuradha (Study Director) Date: 05 April 2006

K. Anuradha 17 April 2006

Effective Date: 03 February 2006

PROTOCOL DEVIATION FORM

IVT Study Number: 270-1157-12 Document Number: PDV 06-030

Date of Deviation: NA

Scientist (if applicable): TM

Date Sponsor Notified (if applicable): 14NOV2005

Describe the deviation:
Test articles RC-1 and RC-2 were logged into IVT as ketoconazole and aminoglutethimide, respectively, due to a miscommunication from Battelle Chemical Repository. The fact that RC-1 was aminoglutethimide and RC-2, ketoconazole was not realized until after the completion of the experiments. The lowest concentration of ketoconazole used in the experiments when back-calculated was 10(-11) M, which was below the range specified in the protocol.

Describe the corrective action:
None.

Assessment:
The concentration range of the ketoconazole did cover 0-100% inhibition, despite being outside the range specified in the protocol. Therefore the calculated IC50 value was not significantly affected and can be accepted with confidence.

Form Completed by: _____ 18APR06
Signature Date

Approved by: K. Aruna Prabhu Date: 18 Apr 2006
Study Director

PROTOCOL DEVIATION FORM

IVT Study Number: 270-1157-12 Document Number: PDV06-031

Date of Deviation: 15AUG2005

Scientist (if applicable): AK

Date Sponsor Notified (if applicable): NA

Describe the deviation:
The protocol states that concentrations of reference chemicals can be adjusted based on the data from the first replicate experiment. A deviation occurred in that prochloraz concentrations were changed in the 4th replicate, based on the data from 2nd and 3rd replicates.

Describe the corrective action:
None.

Assessment:
The IC50 values were similar for replicates 2 and 3 and replicate 4, therefore the change in concentration did not significantly affect the outcome.

Form Completed by: _____ 18APR06
Signature Date

Approved by: K. Anne Pollica Date: 18 Apr 2006
Study Director

Appendix 4: Excel Spreadsheets for Task 7

Assay Date	7/27/2005	Test Chemical ID	RC#1 AG	# Concentrations tested	8		
Technician ID	TM	Replicate #	1	Microsome type	Placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0195	30963.17	1587855		
2	0.0199	31482.74	1582047		
3	0.0200	32769.37	1638469		
4	0.0197	37171.37	1886872		
5	0.0190	37003.84	1947571		
			Average DPM/g soln		1728563
			SD		174935
			CV		10.12
			µCi/g soln		0.779

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	6.1	6.1		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2449 g
Mass of dilution B used in substrate prep	9.1774 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56494 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00881 µg/g soln.
µg/g soln.

a. µCi/g soln	0.779
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.

= 0.564940 + 0.00881
= 0.573755 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.357 µCi/µg ASDN

862843 dpm/nmol

Assay Date	7/27/2005	Test Chemical ID	RC#1 AG	# Concentrations tested	8				
Technician ID	TM	Replicate #	1	Microsome type	Placental	Microsome ID	BAA		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	2	1	
	0.191	0.101	0.055	0.044	0.026	0.022	0.018			
	0.198	0.104	0.055	0.044	0.027	0.022	0.019			
	0.184	0.101	0.057	0.042	0.026	0.023	0.020			

Samples:	Microsomes	QC 10	QC100
	0.038	0.025	0.096
	0.039	0.026	0.096
	0.042	0.026	0.093

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
0.25	125	1000	0.00025	200	0.0500	0.191	0.172	0.0502
0.125	62.5	1000	0.00013	200	0.0250	0.102	0.083	0.0240
0.05	25	1000	0.00005	200	0.0100	0.056	0.037	0.0103
0.025	25	2000	0.00003	200	0.0050	0.043	0.024	0.0065
0.01	5	1000	0.00001	200	0.0020	0.026	0.007	0.0016
0.005	5	2000	0.00001	200	0.0010	0.022	0.003	0.0004

		Variables	Regression results	
		m, b	0.296	-0.001
		se_m, se_b	0.007	0.001
		r^2, se_y	0.998	0.001
		F, df	1825	4
		ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank	0.019	
	r^2=	0.998
	m=	0.296
	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
Microsomes	0.038	0.019	0.005	200	220	70400	0.008	0.009	8.803
Microsomes	0.039	0.020	0.005	200	220	70400	0.008		
Microsomes	0.042	0.023	0.006	200	220	70400	0.010		
QC 10	0.025	0.006	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.026	0.007	0.002	200	1	1	0.000		
QC 10	0.026	0.007	0.001	200	1	1	0.000		
QC100	0.096	0.077	0.022	200	1	1	0.000	0.000	0.109
QC100	0.096	0.077	0.022	200	1	1	0.000		
QC100	0.093	0.074	0.021	200	1	1	0.000		

Assay Date	7/27/2005	Test Chemical ID	RC#1 AG	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	1

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.803275
Protein Concentration (dilution added to assay, mg/mL):	0.02751

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	3.00E-04
3	1.00E-04
4	1.00E-05
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-09

Assay Date	7/27/2005	Test Chemical ID	RC#1 AG	# Concentrations tested		S Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	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	3331.875	6663.75	6806.575	13613.15	1	1728563	0.79	12195	0.0141	1	0.014	15	0.0343
			0.5	2	3474.7	6949.4			1					1		15	
	2	2	0.5	1	3590.523	7181.046	7243.196	14486.392	1	1728563	0.84	13068	0.0151	1	0.014	15	0.0367
			0.5	2	3652.673	7305.346			1					1		15	
	3	2	0.5	1	2952.294	5904.588	5925.773	11851.546	1	1728563	0.69	10434	0.0121	1	0.014	15	0.0293
			0.5	2	2973.479	5946.958			1					1		15	
	4	2	0.5	1	3218.811	6437.622	6221.704	12443.408	1	1728563	0.72	11025	0.0128	1	0.014	15	0.0310
			0.5	2	3002.893	6005.786			1					1		15	
Background control	1	2	0.5	1	382.4864	764.9728	767.6663	1535.3326	1	1728563	0.09	117	0.0001	1	0.014	15	0.0003
			0.5	2	385.1799	770.3598			1					1		15	
	2	2	0.5	1	508.0612	1016.1224	986.7083	1973.4166	1	1728563	0.11	556	0.0006	1	0.014	15	0.0016
			0.5	2	478.6471	957.2942			1					1		15	
	3	2	0.5	1	165.7884	331.5768	402.6748	805.3496	1	1728563	0.05	-613	-0.0007	1	0.014	15	-0.0017
			0.5	2	236.8864	473.7728			1					1		15	
	4	2	0.5	1	325.9232	651.8464	678.7822	1357.5644	1	1728563	0.08	-60	-0.0001	1	0.014	15	-0.0002
			0.5	2	352.859	705.718			1					1		15	
Positive control	1	2	0.5	1	2146.398	4292.796	4319.931	8639.862	1	1728563	0.50	7222	0.0084	1	0.014	15	0.0203
			0.5	2	2173.533	4347.066			1					1		15	
	2	2	0.5	1	2167.928	4335.856	4209.475	8418.95	1	1728563	0.49	7001	0.0081	1	0.014	15	0.0197
			0.5	2	2041.547	4083.094			1					1		15	
	3	2	0.5	1	1743.452	3486.904	3356.906	6713.812	1	1728563	0.39	5296	0.0061	1	0.014	15	0.0149
			0.5	2	1613.454	3226.908			1					1		15	
	4	2	0.5	1	1815.65	3631.3	3663.396	7326.792	1	1728563	0.42	5909	0.0068	1	0.014	15	0.0166
			0.5	2	1847.746	3695.492			1					1		15	
Negative Control	1	2	0.5	1	3142.261	6284.522	6188.7	12377.4	1	1728563	0.72	10959	0.0127	1	0.014	15	0.0308
			0.5	2	3046.439	6092.878			1					1		15	
	2	2	0.5	1	2744.766	5489.532	5171.26	10342.52	1	1728563	0.60	8925	0.0103	1	0.014	15	0.0251
			0.5	2	2426.494	4852.988			1					1		15	
	3	2	0.5	1	2906.184	5812.368	5659.023	11318.046	1	1728563	0.65	9900	0.0115	1	0.014	15	0.0278
			0.5	2	2752.839	5505.678			1					1		15	
	4	2	0.5	1	2510.892	5021.784	5061.699	10123.398	1	1728563	0.59	8705	0.0101	1	0.014	15	0.0244
			0.5	2	2550.807	5101.614			1					1		15	
RC#1 AG	1-1	2	0.5	1	3067.085	6134.17	5795.682	11591.364	1	1728563	0.67	10173	0.0118	1	0.014	15	0.0286
			0.5	2	2728.597	5457.194			1					1		15	
	1-2	2	0.5	1	3180.25	6360.5	6175.98	12351.96	1	1728563	0.71	10934	0.0127	1	0.014	15	0.0307
			0.5	2	2995.73	5991.46			1					1		15	
	1-3	2	0.5	1	1986.786	3973.572	4184.819	8369.638	1	1728563	0.48	6952	0.0081	1	0.014	15	0.0195
			0.5	2	2198.033	4396.066			1					1		15	
	2-1	2	0.5	1	1171.707	2343.414	2327.253	4654.506	1	1728563	0.27	3237	0.0038	1	0.014	15	0.0091
			0.5	2	1155.546	2311.092			1					1		15	
	2-2	2	0.5	1	1558.372	3116.744	3171.332	6342.664	1	1728563	0.37	4925	0.0057	1	0.014	15	0.0138
			0.5	2	1612.96	3225.92			1					1		15	
	2-3	2	0.5	1	1147.471	2294.942	2411.96	4823.96	1	1728563	0.28	3406	0.0039	1	0.014	15	0.0096
			0.5	2	1264.509	2529.018			1					1		15	
	3-1	2	0.5	1	1603.512	3207.024	3164.547	6329.094	1	1728563	0.37	4911	0.0057	1	0.014	15	0.0138
			0.5	2	1561.035	3122.07			1					1		15	
	3-2	2	0.5	1	1802.29	3604.58	3588.143	7176.286	1	1728563	0.42	5758	0.0067	1	0.014	15	0.0162
			0.5	2	1765.853	3571.706			1					1		15	
	3-3	2	0.5	1	1314.137	2628.274	2731.368	5462.736	1	1728563	0.32	4045	0.0047	1	0.014	15	0.0114
			0.5	2	1417.231	2834.462			1					1		15	
	4-1	2	0.5	1	2176.653	4353.306	4441.216	8882.432	1	1728563	0.51	7465	0.0087	1	0.014	15	0.0210
			0.5	2	2264.563	4529.126			1					1		15	
	4-2	2	0.5	1	3318.459	6636.918	6527.283	13054.566	1	1728563	0.76	11637	0.0135	1	0.014	15	0.0327
			0.5	2	3208.824	6417.648			1					1		15	
	4-3	2	0.5	1	3158.018	6316.036	6203.727	12407.454	1	1728563	0.72	10990	0.0127	1	0.014	15	0.0309
			0.5	2	3045.709	6091.418			1					1		15	
	5-1	2	0.5	1	3630.715	7261.43	7223.97	14447.94	1	1728563	0.84	13030	0.0151	1	0.014	15	0.0366
			0.5	2	3593.255	7186.51			1					1		15	
	5-2	2	0.5	1	3484.248	6968.496	7118.242	14236.484	1	1728563	0.82	12819	0.0149	1	0.014	15	0.0360
			0.5	2	3633.994	7267.988			1					1		15	
	5-3	2	0.5	1	3636.014	7272.028	7280.447	14560.894	1	1728563	0.84	13143	0.0152	1	0.014	15	0.0369
			0.5	2	3644.433	7288.866			1					1		15	
	6-1	2	0.5	1	3582.481	7164.962	7331.458	14662.916	1	1728563	0.85	13245	0.0154	1	0.014	15	0.0372
			0.5	2	3748.977	7497.954			1					1		15	
	6-2	2	0.5	1	3195.454	6390.908	6600.945	13201.89	1	1728563	0.76	11784	0.0137	1	0.014	15	0.0331
			0.5	2	3405.491	6810.982			1					1		15	
	6-3	2	0.5	1	3270.024	6540.048	6748.78	13497.56	1	1728563	0.78	12080	0.0140	1	0.014	15	0.0339
			0.5	2	3478.756	6957.512			1					1		15	
	7-1	2	0.5	1	3232.704	6465.408	6529.993	13059.986	1	1728563	0.76	11642	0.0135	1	0.014	15	0.0327
			0.5	2	3297.289	6594.578			1					1		15	
	7-2	2	0.5	1	3582.481	7164.962	7353.513	14707.026	1	1728563	0.85	13289	0.0154	1	0.014	15	0.0373
			0.5	2	3771.032	7542.064			1					1		15	
	7-3	2	0.5	1	3368.969	6737.938	6936.112	13872.224	1	1728563	0.80	12454	0.0144	1	0.014	15	0.0350
			0.5	2	3567.143	7134.286			1					1		15	
	8-1	2	0.5	1	3777.415	7554.83	7451.708	14903.416	1	1728563	0.86	13486	0.0156	1	0.014	15	0.0379
			0.5	2	3674.293	7348.586			1					1		15	
	8-2	2	0.5	1	3505.659	7011.318	7166.412	14332.824	1	1728563	0.83	12915	0.0150	1	0.014	15	0.0363
			0.5	2	3660.753	7321.506			1					1		15	
	8-3	2	0.5	1	4143.822	8287.644	7943.887	15887.774	1	1728563	0.92	14470	0.0168	1	0.014	15	0.0406
			0.5	2	3800.065	7600.13			1					1		15	

Assay Date	7/27/2005	Test Chemical ID	RC#1 AG	# Concentrations tested		Microsome 8 type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0355	0.0017
Full activity	End	0.0301	0.0012
Full activity	Overall	0.0328	0.0033
Background	Beginning	0.0009	0.000870011
Background	End	-0.0009	0.001096669
Background	Overall	0.0000	0.001357868
Positive	Beginning	0.0200	0.0004
Positive	End	0.0157	0.0012
Positive	Overall	0.0179	0.0026
Negative	Beginning	0.0279	0.0040
Negative	End	0.0261	0.0024
Negative	Overall	0.0270	0.0029

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#1 AG	1	1	1.00E-03	-3.00	0.0286
RC#1 AG	1	2	1.00E-03	-3.00	0.0307
RC#1 AG	1	3	1.00E-03	-3.00	0.0195
RC#1 AG	2	1	3.00E-04	-3.52	0.0091
RC#1 AG	2	2	3.00E-04	-3.52	0.0138
RC#1 AG	2	3	3.00E-04	-3.52	0.0096
RC#1 AG	3	1	1.00E-04	-4.00	0.0138
RC#1 AG	3	2	1.00E-04	-4.00	0.0162
RC#1 AG	3	3	1.00E-04	-4.00	0.0114
RC#1 AG	4	1	1.00E-05	-5.00	0.0210
RC#1 AG	4	2	1.00E-05	-5.00	0.0327
RC#1 AG	4	3	1.00E-05	-5.00	0.0309
RC#1 AG	5	1	1.00E-06	-6.00	0.0366
RC#1 AG	5	2	1.00E-06	-6.00	0.0360
RC#1 AG	5	3	1.00E-06	-6.00	0.0369
RC#1 AG	6	1	1.00E-07	-7.00	0.0372
RC#1 AG	6	2	1.00E-07	-7.00	0.0331
RC#1 AG	6	3	1.00E-07	-7.00	0.0339
RC#1 AG	7	1	1.00E-08	-8.00	0.0327
RC#1 AG	7	2	1.00E-08	-8.00	0.0373
RC#1 AG	7	3	1.00E-08	-8.00	0.0350
RC#1 AG	8	1	1.00E-09	-9.00	0.0379
RC#1 AG	8	2	1.00E-09	-9.00	0.0363
RC#1 AG	8	3	1.00E-09	-9.00	0.0406

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	87.10	93.61	59.51
2	-3.52	27.71	42.16	29.16
3	-4.00	42.05	49.30	34.63
4	-5.00	63.90	99.62	94.08
5	-6.00	111.55	109.74	112.52
6	-7.00	113.39	100.88	103.42
7	-8.00	99.67	113.77	106.62
8	-9.00	115.45	110.57	123.88

Assay Date	7/28/2005	Test Chemical ID	RC-1 AG	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	Placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0203	30654.69	1510083		
2	0.0199	32692.78	1642853		
3	0.0203	33297.46	1640269		
4	0.0202	33107.35	1638978		
5	0.0204	34931	1712304		
			Average DPM/g soln		1628897
			SD		73313
			CV		4.50
			µCi/g soln		0.734

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	17.1	17.1		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2343 g
Mass of dilution B used in substrate prep	9.1771 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.565291 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00831 µg/g soln.
µg/g soln.

a. µCi/g soln	0.734
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.

= 0.565291 + 0.00831
= 0.573597 µg ASDN/g soln.

3) Calculate Solution Specific Activity

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

813317 dpm/nmol

Assay Date	7/28/2005	Test Chemical ID	RC-1 AG		# Concentrations tested		8		
Technician ID	TM	Replicate #	2	Microsome type	Placental	Microsome ID	BAA		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	2	1	
	0.206	0.131	0.060	0.040	0.028	0.023	0.019			
	0.219	0.130	0.057	0.039	0.029	0.022	0.018			
	0.201	0.126	0.058	0.038	0.027	0.022	0.018			

Samples:	Microsomes	QC 10	QC 100
	0.037	0.026	0.102
	0.035	0.028	0.101
	0.034	0.033	0.109

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	
0.25	125	1000	0.00025	200	0.0500	0.209	0.190	0.0484	m, b	0.258	-0.001
0.125	62.5	1000	0.00013	200	0.0250	0.129	0.111	0.0279	se_m, se_b	0.010	0.001
0.05	25	1000	0.00005	200	0.0100	0.058	0.040	0.0097	r^2, se_y	0.994	0.002
0.025	25	2000	0.00003	200	0.0050	0.039	0.020	0.0047	F, df	613	4
0.01	5	1000	0.00001	200	0.0020	0.028	0.009	0.0019	ss_{reg}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.022	0.004	0.0004			

Regression results are calculated using the function LINEST

Blank	0.019	r^2=	0 994
		m=	0.258
		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
Microsomes	0.037	0.019	0.004	200	220	70400	0.007	0.006	6.075
Microsomes	0.035	0.017	0.004	200	220	70400	0.006		
Microsomes	0.034	0.015	0.003	200	220	70400	0.005		
QC 10	0.026	0.007	0.001	200	200	200	0.000	0.000	0.011
QC 10	0.028	0.009	0.002	200	200	200	0.000		
QC 10	0.033	0.015	0.003	200	200	200	0.000		
QC 100	0.102	0.083	0.021	200	200	200	0.000	0.000	0.107
QC 100	0.101	0.082	0.021	200	200	200	0.000		
QC 100	0.109	0.091	0.023	200	200	200	0.000		

Assay Date	7/28/2005	Test Chemical ID	RC-1 AG	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	2

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.066364
Protein Concentration (dilution added to assay, mg/mL):	0.018957

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	3.30E-05
4	1.00E-05
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-09

Assay Date	7/28/2005	Test Chemical ID	RC-1 AG	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	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	3644.243	7288.486	7205.137	14410.274	1	1628897	0.88	14034	0.0173	1	0.009	15	0.0607
			0.5	2	3560.894	7121.788			1					1		15	
	2	2	0.5	1	3183.795	6367.59	6176.347	12352.694	1	1628897	0.76	11976	0.0147	1	0.009	15	0.0518
			0.5	2	2992.552	5985.104			1					1		15	
	3	2	0.5	1	2625.86	5251.72	5265.09	10530.18	1	1628897	0.65	10154	0.0125	1	0.009	15	0.0439
			0.5	2	2639.23	5278.46			1					1		15	
	4	2	0.5	1	2510.403	5020.806	5074.761	10149.522	1	1628897	0.62	9773	0.0120	1	0.009	15	0.0423
			0.5	2	2564.356	5128.716			1					1		15	
Background control	1	2	0.5	1	96.26371	192.52742	235.31131	470.62262	1	1628897	0.03	94	0.0001	1	0.009	15	0.0004
			0.5	2	139.0476	278.0952			1					1		15	
	2	2	0.5	1	64.64593	129.29186	160.21901	320.43802	1	1628897	0.02	-56	-0.0001	1	0.009	15	-0.0002
			0.5	2	95.57308	191.14616			1					1		15	
	3	2	0.5	1	99.66246	199.3249 2	182.55666	365.11332	1	1628897	0.02	-11	0.0000	1	0.009	15	0.0000
			0.5	2	82.8942	165.7884			1					1		15	
	4	2	0.5	1	98.93823	197.87646	174.91891	349.83382	1	1628897	0.02	-27	0.0000	1	0.009	15	-0.0001
			0.5	2	75.97868	151.95736			1					1		15	
Positive control	1	2	0.5	1	1373.726	2747.452	2641.205	5282.41	1	1628897	0.32	4906	0.0060	1	0.009	15	0.0212
			0.5	2	1267.479	2534.958			1					1		15	
	2	2	0.5	1	1163.193	2326.386	2360.511	4721.022	1	1628897	0.29	4345	0.0053	1	0.009	15	0.0188
			0.5	2	1197.318	2394.636			1					1		15	
	3	2	0.5	1	1198.643	2397.286	2550.82	5101.64	1	1628897	0.31	4725	0.0058	1	0.009	15	0.0204
			0.5	2	1352.177	2704.354			1					1		15	
	4	2	0.5	1	1349.484	2698.968	2638.355	5276.71	1	1628897	0.32	4900	0.0060	1	0.009	15	0.0212
			0.5	2	1288.871	2577.742			1					1		15	
Negative Control	1	2	0.5	1	2410.754	4821.508	4829.589	9659.178	1	1628897	0.59	9283	0.0114	1	0.009	15	0.0401
			0.5	2	2418.835	4837.67			1					1		15	
	2	2	0.5	1	1978.765	3957.53	4117.468	8234.936	1	1628897	0.51	7858	0.0097	1	0.009	15	0.0340
			0.5	2	2138.703	4277.406			1					1		15	
	3	2	0.5	1	2690.05	5380.1	5345.338	10690.676	1	1628897	0.66	10314	0.0127	1	0.009	15	0.0446
			0.5	2	2655.288	5310.576			1					1		15	
	4	2	0.5	1	2805.784	5611.568	5640.231	11280.462	1	1628897	0.69	10904	0.0134	1	0.009	15	0.0471
			0.5	2	2834.447	5668.894			1					1		15	
RC-1 AG	1-1	2	0.5	1	144.9795	289.959	350.878	701.756	1	1628897	0.04	325	0.0004	1	0.009	15	0.0014
			0.5	2	205.8985	411.797			1					1		15	
	1-2	2	0.5	1	246.8971	493.7942	505.4808	1010.9616	1	1628897	0.06	634	0.0008	1	0.009	15	0.0027
			0.5	2	258.5837	517.1674			1					1		15	
	1-3	2	0.5	1	254.0306	508.0612	449.2331	898.4662	1	1628897	0.06	522	0.0006	1	0.009	15	0.0023
			0.5	2	195.2025	390.405			1					1		15	
	2-1	2	0.5	1	1275.356	2550.712	2527.871	5055.742	1	1628897	0.31	4679	0.0058	1	0.009	15	0.0202
			0.5	2	1252.515	2505.03			1					1		15	
	2-2	2	0.5	1	1147.465	2294.93	2346.108	4692.216	1	1628897	0.29	4316	0.0053	1	0.009	15	0.0187
			0.5	2	1198.643	2397.286			1					1		15	
	2-3	2	0.5	1	1161.884	2323.768	2358.554	4717.108	1	1628897	0.29	4341	0.0053	1	0.009	15	0.0188
			0.5	2	1196.67	2393.34			1					1		15	
	3-1	2	0.5	1	1863.685	3727.37	3631.212	7262.424	1	1628897	0.45	6886	0.0085	1	0.009	15	0.0298
			0.5	2	1767.527	3535.054			1					1		15	
	3-2	2	0.5	1	1629.625	3259.25	3280.798	6561.596	1	1628897	0.40	6185	0.0076	1	0.009	15	0.0267
			0.5	2	1651.173	3302.346			1					1		15	
	3-3	2	0.5	1	2176.954	4353.908	4372.233	8744.466	1	1628897	0.54	8368	0.0103	1	0.009	15	0.0362
			0.5	2	2195.279	4390.558			1					1		15	
	4-1	2	0.5	1	2699.971	5399.942	5212.708	10425.416	1	1628897	0.64	10049	0.0124	1	0.009	15	0.0434
			0.5	2	2512.737	5025.474			1					1		15	
	4-2	2	0.5	1	2402.686	4805.372	4828.022	9656.044	1	1628897	0.59	9280	0.0114	1	0.009	15	0.0401
			0.5	2	2425.336	4850.672			1					1		15	
	4-3	2	0.5	1	2771.708	5543.416	5408.292	10816.584	1	1628897	0.66	10440	0.0128	1	0.009	15	0.0451
			0.5	2	2636.584	5273.168			1					1		15	
	5-1	2	0.5	1	3095.522	6191.044	6114.491	12228.982	1	1628897	0.75	11852	0.0146	1	0.009	15	0.0512
			0.5	2	3018.969	6037.938			1					1		15	
	5-2	2	0.5	1	3111.451	6222.902	6276.425	12552.85	1	1628897	0.77	12176	0.0150	1	0.009	15	0.0526
			0.5	2	3164.974	6329.948			1					1		15	
	5-3	2	0.5	1	3163.366	6326.732	6270.578	12541.156	1	1628897	0.77	12165	0.0150	1	0.009	15	0.0526
			0.5	2	3107.212	6214.424			1					1		15	
	6-1	2	0.5	1	3057.23	6114.46	6152.17	12304.34	1	1628897	0.76	11928	0.0147	1	0.009	15	0.0516
			0.5	2	3094.94	6189.88			1					1		15	
	6-2	2	0.5	1	3006.052	6012.104	5880.624	11761.248	1	1628897	0.72	11385	0.0140	1	0.009	15	0.0492
			0.5	2	2874.572	5749.144			1					1		15	
	6-3	2	0.5	1	2986.675	5973.35	5904.031	11808.062	1	1628897	0.72	11432	0.0141	1	0.009	15	0.0494
			0.5	2	2917.356	5834.712			1					1		15	
	7-1	2	0.5	1	2922.55	5845.1	5734.006	11468.012	1	1628897	0.70	11092	0.0136	1	0.009	15	0.0480
			0.5	2	2811.456	5622.912			1					1		15	
	7-2	2	0.5	1	2771.634	5543.268	5508.326	11016.652	1	1628897	0.68	10640	0.0131	1	0.009	15	0.0460
			0.5	2	2736.692	5473.384			1					1		15	
	7-3	2	0.5	1	2820.194	5640.388	5626.341	11252.682	1	1628897	0.69	10876	0.0134	1	0.009	15	0.0470
			0.5	2	2806.147	5612.294			1					1		15	
	8-1	2	0.5	1	3163.366	6326.732	6317.311	12634.622	1	1628897	0.78	12258	0.0151	1	0.009	15	0.0530
			0.5	2	3153.945	6307.89			1					1		15	
	8-2	2	0.5	1	3029.168	6058.336	6032.542	12065.084	1	1628897	0.74	11689	0.0144	1	0.009	15	0.0505
			0.5	2	3003.374	6006.748			1					1		15	
	8-3	2	0.5	1	2981.548	5963.096	5994.787	11989.574	1	1628897	0.74	11613	0.0143	1	0.009	15	0.0502
			0.5	2	3013.239	6026.478			1					1		15	

Assay Date	7/28/2005	Test Chemical ID	RC-1 AG	# Concentrations tested	Microsome 8 type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate # 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0562	0.0063
Full activity	End	0.0431	0.0012
Full activity	Overall	0.0497	0.0084
Background	Beginning	0.0001	0.000459178
Background	End	-0.0001	4.67159E-05
Background	Overall	0.0000	0.000282904
Positive	Beginning	0.0200	0.0017
Positive	End	0.0208	0.0005
Positive	Overall	0.0204	0.0011
Negative	Beginning	0.0371	0.0044
Negative	End	0.0459	0.0018
Negative	Overall	0.0415	0.0058

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC-1 AG	1	1	1.00E-03	-3.00	0.0014
RC-1 AG	1	2	1.00E-03	-3.00	0.0027
RC-1 AG	1	3	1.00E-03	-3.00	0.0023
RC-1 AG	2	1	1.00E-04	-4.00	0.0202
RC-1 AG	2	2	1.00E-04	-4.00	0.0187
RC-1 AG	2	3	1.00E-04	-4.00	0.0188
RC-1 AG	3	1	3.30E-05	-4.48	0.0298
RC-1 AG	3	2	3.30E-05	-4.48	0.0267
RC-1 AG	3	3	3.30E-05	-4.48	0.0362
RC-1 AG	4	1	1.00E-05	-5.00	0.0434
RC-1 AG	4	2	1.00E-05	-5.00	0.0401
RC-1 AG	4	3	1.00E-05	-5.00	0.0451
RC-1 AG	5	1	1.00E-06	-6.00	0.0512
RC-1 AG	5	2	1.00E-06	-6.00	0.0526
RC-1 AG	5	3	1.00E-06	-6.00	0.0526
RC-1 AG	6	1	1.00E-07	-7.00	0.0516
RC-1 AG	6	2	1.00E-07	-7.00	0.0492
RC-1 AG	6	3	1.00E-07	-7.00	0.0494
RC-1 AG	7	1	1.00E-08	-8.00	0.0480
RC-1 AG	7	2	1.00E-08	-8.00	0.0460
RC-1 AG	7	3	1.00E-08	-8.00	0.0470
RC-1 AG	8	1	1.00E-09	-9.00	0.0530
RC-1 AG	8	2	1.00E-09	-9.00	0.0505
RC-1 AG	8	3	1.00E-09	-9.00	0.0502

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.83	5.52	4.55
2	-4.00	40.75	37.58	37.80
3	-4.48	59.96	53.86	72.87
4	-5.00	87.50	80.80	90.91
5	-6.00	103.21	106.03	105.93
6	-7.00	103.86	99.13	99.54
7	-8.00	96.58	92.65	94.71
8	-9.00	106.74	101.78	101.12

Assay Date	7/29/2005	Test Chemical ID	RC-1 AG	# Concentrations tested	8		
Technician ID	TM	Replicate #	3	Microsome type	Placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0202	34226.89	1694400		
2	0.0202	33705.84	1668606		
3	0.0203	34569.55	1702933		
4	0.0202	33478.18	1657336		
5	0.0200	35193.87	1759694		
			Average DPM/g soln	1696594	
			SD	39847	
			CV	2.35	
			µCi/g soln	0.764	

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	17.4	17.4		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.1924 g
Mass of dilution B used in substrate prep	9.1318 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563956 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00865 µg/g soln.
µg/g soln.

a. µCi/g soln	0.764
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.

= 0.563956 + 0.00865
= 0.572607 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.335 µCi/µg ASDN

848583 dpm/nmol

Assay Date	7/29/2005	Test Chemical ID	RC-1 AG	# Concentrations tested	8					
Technician ID	TM	Replicate #	3	Microsome type	Placental	Microsome ID	BAA			

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.496	0.309	0.161	0.112	0.056	0.044	0.027	2	1	
	0.525	0.324	0.169	0.104	0.057	0.045	0.030			
	0.545	0.309	0.171	0.110	0.056	0.045	0.029			

Samples:	Microsomes	QC 10	QC 100
	0.083	0.048	0.246
	0.085	0.047	0.277
	0.089	0.046	0.276

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
0.25	125	1000	0.00025	200	0.0500	0.522	0.493	0.0483
0.125	62.5	1000	0.00013	200	0.0250	0.314	0.285	0.0270
0.05	25	1000	0.00005	200	0.0100	0.167	0.138	0.0119
0.025	25	2000	0.00003	200	0.0050	0.109	0.080	0.0059
0.01	5	1000	0.00001	200	0.0020	0.056	0.028	0.0006
0.005	5	2000	0.00001	200	0.0010	0.045	0.016	-0.0007

Variables	Regression results	
m, b	0.103	-0.002
se_m, se_b	0.005	0.001
r^2, se_y	0.991	0.002
F, df	447	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank 0.029

r^2= 0.991
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
Microsomes	0.083	0.055	0.003	200	220	70400	0.005	0.006	5.698
Microsomes	0.085	0.056	0.003	200	220	70400	0.006		
Microsomes	0.089	0.060	0.004	200	220	70400	0.006		
QC 10	0.048	0.019	0.000	200	200	200	0.000	0.000	-0.002
QC 10	0.047	0.019	0.000	200	200	200	0.000		
QC 10	0.046	0.017	-0.001	200	200	200	0.000		
QC 100	0.246	0.217	0.020	200	200	200	0.000	0.000	0.110
QC 100	0.277	0.248	0.023	200	200	200	0.000		
QC 100	0.276	0.248	0.023	200	200	200	0.000		

Assay Date	7/29/2005	Test Chemical ID	RC-1 AG	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	3

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.689791
Protein Concentration (dilution added to assay, mg/mL):	0.017781

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	3.30E-05
4	1.00E-05
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-09

Assay Date	7/29/2005	Test Chemical ID	RC-1 AG	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	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	3450.458	6900.916	6931.996	13863.992	1	1696594	0.82	13428	0.0158	1	0.009	15	0.0593
			0.5	2	3481.538	6963.076			1					1		15	
	2	2	0.5	1	3784.48	7568.92	7693.836	15387.672	1	1696594	0.91	14952	0.0176	1	0.009	15	0.0661
			0.5	2	3909.376	7818.752			1					1		15	
	3	2	0.5	1	3356.314	6716.628	6599.817	13199.634	1	1696594	0.78	12764	0.0150	1	0.009	15	0.0564
			0.5	2	3241.503	6483.006			1					1		15	
	4	2	0.5	1	3117.875	6235.75	6215.476	12430.952	1	1696594	0.73	11995	0.0141	1	0.009	15	0.0530
			0.5	2	3097.601	6195.202			1					1		15	
Background control	1	2	0.5	1	103.537	207.074	220.2179	440.4358	1	1696594	0.03	5	0.0000	1	0.009	15	0.0000
			0.5	2	116.6809	233.3618			1					1		15	
	2	2	0.5	1	114.1561	228.3122	233.6225	467.245	1	1696594	0.03	32	0.0000	1	0.009	15	0.0001
			0.5	2	119.4664	238.9328			1					1		15	
	3	2	0.5	1	69.52415	139.0483	178.37125	356.7425	1	1696594	0.02	-79	-0.0001	1	0.009	15	-0.0003
			0.5	2	108.8471	217.6942			1					1		15	
	4	2	0.5	1	128.3623	256.7046	238.7891	477.5782	1	1696594	0.03	42	0.0000	1	0.009	15	0.0002
			0.5	2	110.4368	220.8736			1					1		15	
Positive control	1	2	0.5	1	1408.742	2817.484	2890.211	5780.422	1	1696594	0.34	5345	0.0063	1	0.009	15	0.0236
			0.5	2	1481.469	2962.938			1					1		15	
	2	2	0.5	1	1274.308	2548.616	2476.936	4953.872	1	1696594	0.29	4518	0.0053	1	0.009	15	0.0200
			0.5	2	1202.628	2405.256			1					1		15	
	3	2	0.5	1	1630.052	3260.104	3271.892	6543.784	1	1696594	0.39	6108	0.0072	1	0.009	15	0.0270
			0.5	2	1641.84	3283.68			1					1		15	
	4	2	0.5	1	1670.02	3340.04	3387.681	6775.362	1	1696594	0.40	6340	0.0075	1	0.009	15	0.0280
			0.5	2	1717.861	3435.322			1					1		15	
Negative Control	1	2	0.5	1	2369.169	4738.338	4637.163	9274.326	1	1696594	0.55	8839	0.0104	1	0.009	15	0.0391
			0.5	2	2267.994	4535.988			1					1		15	
	2	2	0.5	1	2130.116	4260.232	4169.156	8338.312	1	1696594	0.49	7903	0.0093	1	0.009	15	0.0349
			0.5	2	2039.04	4078.08			1					1		15	
	3	2	0.5	1	3553.98	7107.96	6891.14	13782.28	1	1696594	0.81	13347	0.0157	1	0.009	15	0.0590
			0.5	2	3337.16	6674.32			1					1		15	
	4	2	0.5	1	3206.134	6412.268	6575.382	13150.764	1	1696594	0.78	12715	0.0150	1	0.009	15	0.0562
			0.5	2	3369.248	6738.496			1					1		15	
RC-1 AG	1-1	2	0.5	1	155.0924	310.1848	362.4981	724.9962	1	1696594	0.04	289	0.0003	1	0.009	15	0.0013
			0.5	2	207.4057	414.8114			1					1		15	
	1-2	2	0.5	1	136.3743	272.7486	327.5205	655.041	1	1696594	0.04	220	0.0003	1	0.009	15	0.0010
			0.5	2	191.1462	382.2924			1					1		15	
	1-3	2	0.5	1	299.9933	599.9866	569.3513	1138.7026	1	1696594	0.07	703	0.0008	1	0.009	15	0.0031
			0.5	2	269.358	538.716			1					1		15	
	2-1	2	0.5	1	1375.191	2750.382	2878.483	5756.966	1	1696594	0.34	5321	0.0063	1	0.009	15	0.0235
			0.5	2	1503.292	3006.584			1					1		15	
	2-2	2	0.5	1	1476.051	2952.102	2701.63	5403.26	1	1696594	0.32	4968	0.0059	1	0.009	15	0.0219
			0.5	2	1225.579	2451.158			1					1		15	
	2-3	2	0.5	1	1342.351	2684.702	2815.802	5631.604	1	1696594	0.33	5196	0.0061	1	0.009	15	0.0230
			0.5	2	1473.451	2946.902			1					1		15	
	3-1	2	0.5	1	2253.944	4507.888	4528.816	9057.632	1	1696594	0.53	8622	0.0102	1	0.009	15	0.0381
			0.5	2	2274.872	4549.744			1					1		15	
	3-2	2	0.5	1	2243.503	4487.006	4554.607	9109.214	1	1696594	0.54	8674	0.0102	1	0.009	15	0.0383
			0.5	2	2311.104	4622.208			1					1		15	
	3-3	2	0.5	1	2162.956	4325.912	4298.977	8597.954	1	1696594	0.51	8162	0.0096	1	0.009	15	0.0361
			0.5	2	2136.021	4272.042			1					1		15	
	4-1	2	0.5	1	2852.517	5705.034	5551.499	11102.998	1	1696594	0.65	10667	0.0126	1	0.009	15	0.0471
			0.5	2	2698.982	5397.964			1					1		15	
	4-2	2	0.5	1	2746.219	5492.438	5372.474	10744.948	1	1696594	0.63	10309	0.0121	1	0.009	15	0.0456
			0.5	2	2626.255	5252.51			1					1		15	
	4-3	2	0.5	1	2672.046	5344.092	5391.525	10783.05	1	1696594	0.64	10348	0.0122	1	0.009	15	0.0457
			0.5	2	2719.479	5438.958			1					1		15	
	5-1	2	0.5	1	3607.253	7214.506	7236.395	14472.79	1	1696594	0.85	14037	0.0165	1	0.009	15	0.0620
			0.5	2	3629.142	7258.284			1					1		15	
	5-2	2	0.5	1	3730.256	7460.516	7153.817	14307.634	1	1696594	0.84	13872	0.0163	1	0.009	15	0.0613
			0.5	2	3423.559	6847.118			1					1		15	
	5-3	2	0.5	1	3439.177	6878.354	6968.884	13937.768	1	1696594	0.82	13502	0.0159	1	0.009	15	0.0597
			0.5	2	3529.707	7059.414			1					1		15	
	6-1	2	0.5	1	3773.043	7546.086	7631.695	15263.39	1	1696594	0.90	14828	0.0175	1	0.009	15	0.0655
			0.5	2	3858.652	7717.304			1					1		15	
	6-2	2	0.5	1	3484.183	6968.366	7284.845	14569.69	1	1696594	0.86	14134	0.0167	1	0.009	15	0.0625
			0.5	2	3800.662	7601.324			1					1		15	
	6-3	2	0.5	1	3555.545	7111.09	7026.161	14052.322	1	1696594	0.83	13617	0.0160	1	0.009	15	0.0602
			0.5	2	3470.616	6941.232			1					1		15	
	7-1	2	0.5	1	3520.294	7040.588	7017.913	14035.826	1	1696594	0.83	13600	0.0160	1	0.009	15	0.0601
			0.5	2	3497.619	6995.238			1					1		15	
	7-2	2	0.5	1	3383.155	6766.31	6967.739	13935.478	1	1696594	0.82	13500	0.0159	1	0.009	15	0.0596
			0.5	2	3584.584	7169.168			1					1		15	
	7-3	2	0.5	1	3366.993	6733.986	6661.999	13323.998	1	1696594	0.79	12888	0.0152	1	0.009	15	0.0569
			0.5	2	3295.006	6590.012			1					1		15	
	8-1	2	0.5	1	3385.848	6771.696	6744.778	13489.556	1	1696594	0.80	13054	0.0154	1	0.009	15	0.0577
			0.5	2	3358.93	6717.86			1					1		15	
	8-2	2	0.5	1	3492.299	6984.576	6948.189	13896.378	1	1696594	0.82	13461	0.0159	1	0.009	15	0.0595
			0.5	2	3455.9	6911.8			1					1		15	
	8-3	2	0.5	1	3407.415	6814.83	6792.743	13585.486	1	1696594	0.80	13150	0.0155	1	0.009	15	0.0581
			0.5	2	3385.328	6770.656			1					1		15	

Assay Date	7/29/2005	Test Chemical ID	RC-1 AG	# Concentrations tested		Microsome 8 type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0627	0.0048
Full activity	End	0.0547	0.0024
Full activity	Overall	0.0587	0.0056
Background	Beginning	0.0001	8.37601E-05
Background	End	-0.0001	0.000377527
Background	Overall	0.0000	0.00024208
Positive	Beginning	0.0218	0.0026
Positive	End	0.0275	0.0007
Positive	Overall	0.0246	0.0036
Negative	Beginning	0.0370	0.0029
Negative	End	0.0576	0.0020
Negative	Overall	0.0473	0.0121

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC-1 AG	1	1	1.00E-03	-3.00	0.0013
RC-1 AG	1	2	1.00E-03	-3.00	0.0010
RC-1 AG	1	3	1.00E-03	-3.00	0.0031
RC-1 AG	2	1	1.00E-04	-4.00	0.0235
RC-1 AG	2	2	1.00E-04	-4.00	0.0219
RC-1 AG	2	3	1.00E-04	-4.00	0.0230
RC-1 AG	3	1	3.30E-05	-4.48	0.0381
RC-1 AG	3	2	3.30E-05	-4.48	0.0383
RC-1 AG	3	3	3.30E-05	-4.48	0.0361
RC-1 AG	4	1	1.00E-05	-5.00	0.0471
RC-1 AG	4	2	1.00E-05	-5.00	0.0456
RC-1 AG	4	3	1.00E-05	-5.00	0.0457
RC-1 AG	5	1	1.00E-06	-6.00	0.0620
RC-1 AG	5	2	1.00E-06	-6.00	0.0613
RC-1 AG	5	3	1.00E-06	-6.00	0.0597
RC-1 AG	6	1	1.00E-07	-7.00	0.0655
RC-1 AG	6	2	1.00E-07	-7.00	0.0625
RC-1 AG	6	3	1.00E-07	-7.00	0.0602
RC-1 AG	7	1	1.00E-08	-8.00	0.0601
RC-1 AG	7	2	1.00E-08	-8.00	0.0596
RC-1 AG	7	3	1.00E-08	-8.00	0.0569
RC-1 AG	8	1	1.00E-09	-9.00	0.0577
RC-1 AG	8	2	1.00E-09	-9.00	0.0595
RC-1 AG	8	3	1.00E-09	-9.00	0.0581

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	2.18	1.65	5.29
2	-4.00	40.06	37.39	39.11
3	-4.48	64.90	65.29	61.44
4	-5.00	80.30	77.60	77.89
5	-6.00	105.66	104.42	101.63
6	-7.00	111.61	106.39	102.50
7	-8.00	102.37	101.62	97.01
8	-9.00	98.26	101.32	98.98

Assay Date	7/27/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8		
Technician ID	TM	Replicate #	1	Microsome type	Placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0195	30963.17	1587855		
2	0.0199	31482.74	1582047		
3	0.0200	32769.37	1638469		
4	0.0197	37171.37	1886872		
5	0.0190	37003.84	1947571		
			Average DPM/g soln		1728563
			SD		174935
			CV		10.12
			µCi/g soln		0.779

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	6.1	6.1		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2449 g
Mass of dilution B used in substrate prep	9.1774 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56494 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00881 µg/g soln.
µg/g soln.

a. µCi/g soln	0.779
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.

= 0.564940 + 0.00881
= 0.573755 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.357 µCi/µg ASDN

862843 dpm/nmol

Assay Date	7/27/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8						
Technician ID	TM	Replicate #	1	Microsome type	Placental	Microsome ID	BAA				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.191	0.101	0.055	0.044	0.026	0.022	0.018			
	0.198	0.104	0.055	0.044	0.027	0.022	0.019			
	0.184	0.101	0.057	0.042	0.026	0.023	0.020			

Samples:	Placental	QC 10	QC100
	0.038	0.025	0.096
	0.039	0.026	0.096
	0.042	0.026	0.093

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	
0.25	125	1000	0.00025	200	0.0500	0.191	0.172	0.0502	m, b	0.296	-0.001
0.125	62.5	1000	0.00013	200	0.0250	0.102	0.083	0.0240	se_m, se_b	0.007	0.001
0.05	25	1000	0.00005	200	0.0100	0.056	0.037	0.0103	r^2, se_y	0.998	0.001
0.025	25	2000	0.00003	200	0.0050	0.043	0.024	0.0065	F, df	1825	4
0.01	5	1000	0.00001	200	0.0020	0.026	0.007	0.0016	ss_{reg}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.022	0.003	0.0004			

Regression results are calculated using the function LINEST

Blank	0.019	r^2=	0.998	
		m=	0.296	
		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
Placental	0.038	0.019	0.005	200	220	70400	0.008	0.009	8.803
Placental	0.039	0.020	0.005	200	220	70400	0.008		
Placental	0.042	0.023	0.006	200	220	70400	0.010		
QC 10	0.025	0.006	0.001	200	1	1	0.000	0.000	0.007
QC 10	0.026	0.007	0.002	200	1	1	0.000		
QC 10	0.026	0.007	0.001	200	1	1	0.000		
QC100	0.096	0.077	0.022	200	1	1	0.000	0.000	0.109
QC100	0.096	0.077	0.022	200	1	1	0.000		
QC100	0.093	0.074	0.021	200	1	1	0.000		

Assay Date	7/27/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	1

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.803275
Protein Concentration (dilution added to assay, mg/mL):	0.02751

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	7/27/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	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	3331.875	6663.75	6806.575	13613.15	1	1728563	0.79	12195	0.0141	1	0.014	15	0.0343
			0.5	2	3474.7	6949.4			1					1		15	
	2	2	0.5	1	3590.523	7181.046	7243.196	14486.392	1	1728563	0.84	13068	0.0151	1	0.014	15	0.0367
			0.5	2	3652.673	7305.346			1					1		15	
	3	2	0.5	1	2952.294	5904.588	5925.773	11851.546	1	1728563	0.69	10434	0.0121	1	0.014	15	0.0293
			0.5	2	2973.479	5946.958			1					1		15	
	4	2	0.5	1	3218.811	6437.622	6221.704	12443.408	1	1728563	0.72	11025	0.0128	1	0.014	15	0.0310
			0.5	2	3002.893	6005.786			1					1		15	
Background control	1	2	0.5	1	382.4864	764.9728	767.6663	1535.3326	1	1728563	0.09	117	0.0001	1	0.014	15	0.0003
			0.5	2	385.1799	770.3598			1					1		15	
	2	2	0.5	1	509.0612	1016.1224	986.7083	1973.4166	1	1728563	0.11	556	0.0006	1	0.014	15	0.0016
			0.5	2	478.6471	957.2942			1					1		15	
	3	2	0.5	1	165.7884	331.5768	402.6748	805.3496	1	1728563	0.05	-613	-0.0007	1	0.014	15	-0.0017
			0.5	2	236.8864	473.7728			1					1		15	
	4	2	0.5	1	325.9232	651.8464	678.7822	1357.5644	1	1728563	0.08	-60	-0.0001	1	0.014	15	-0.0002
			0.5	2	352.859	705.718			1					1		15	
Positive control	1	2	0.5	1	2146.396	4292.796	4319.931	8639.862	1	1728563	0.50	7222	0.0084	1	0.014	15	0.0203
			0.5	2	2173.533	4347.066			1					1		15	
	2	2	0.5	1	2167.928	4335.856	4209.475	8418.95	1	1728563	0.49	7001	0.0081	1	0.014	15	0.0197
			0.5	2	2041.547	4083.094			1					1		15	
	3	2	0.5	1	1743.452	3486.904	3356.906	6713.812	1	1728563	0.39	5296	0.0061	1	0.014	15	0.0149
			0.5	2	1613.454	3226.908			1					1		15	
	4	2	0.5	1	1815.65	3631.3	3663.396	7326.792	1	1728563	0.42	5909	0.0068	1	0.014	15	0.0166
			0.5	2	1847.746	3695.492			1					1		15	
Negative Control	1	2	0.5	1	3142.261	6284.522	6188.7	12377.4	1	1728563	0.72	10959	0.0127	1	0.014	15	0.0308
			0.5	2	3045.439	6092.878			1					1		15	
	2	2	0.5	1	2744.766	5489.532	5171.26	10342.52	1	1728563	0.60	8926	0.0103	1	0.014	15	0.0251
			0.5	2	2426.494	4852.988			1					1		15	
	3	2	0.5	1	2906.184	5812.368	5659.023	11318.046	1	1728563	0.65	9900	0.0115	1	0.014	15	0.0278
			0.5	2	2752.839	5505.678			1					1		15	
	4	2	0.5	1	2510.892	5021.784	5061.699	10123.398	1	1728563	0.59	8705	0.0101	1	0.014	15	0.0244
			0.5	2	2550.807	5101.614			1					1		15	
RC#2 KCZ	1-1	2	0.5	1	295.7773	591.5546	624.1172	1248.2344	1	1728563	0.07	-170	-0.0002	1	0.014	15	-0.0005
			0.5	2	328.3399	656.6798			1					1		15	
	1-2	2	0.5	1	344.7819	689.5638	686.5144	1373.0288	1	1728563	0.08	-45	-0.0001	1	0.014	15	-0.0001
			0.5	2	341.7325	683.465			1					1		15	
	1-3	2	0.5	1	458.5504	917.1808	846.47	1692.94	1	1728563	0.10	275	0.0003	1	0.014	15	0.0008
			0.5	2	387.8796	775.7592			1					1		15	
	2-1	2	0.5	1	515.575	1031.15	924.7023	1849.4046	1	1728563	0.11	431	0.0005	1	0.014	15	0.0012
			0.5	2	409.1273	818.2546			1					1		15	
	2-2	2	0.5	1	481.3262	962.6524	972.4792	1944.9584	1	1728563	0.11	527	0.0006	1	0.014	15	0.0015
			0.5	2	491.153	982.306			1					1		15	
	2-3	2	0.5	1	638.3853	1276.7706	1255.2217	2510.4434	1	1728563	0.15	1093	0.0013	1	0.014	15	0.0031
			0.5	2	616.8364	1233.6728			1					1		15	
	3-1	2	0.5	1	1387.824	2775.648	2858.534	5717.068	1	1728563	0.33	4299	0.0050	1	0.014	15	0.0121
			0.5	2	1470.71	2941.42			1					1		15	
	3-2	2	0.5	1	1532.663	3065.326	3254.741	6509.482	1	1728563	0.38	5092	0.0059	1	0.014	15	0.0143
			0.5	2	1722.078	3444.156			1					1		15	
	3-3	2	0.5	1	1249.374	2498.748	2340.222	4680.444	1	1728563	0.27	3263	0.0038	1	0.014	15	0.0092
			0.5	2	1090.848	2181.696			1					1		15	
	4-1	2	0.5	1	2976.2	5952.4	5979.574	11959.148	1	1728563	0.69	10541	0.0122	1	0.014	15	0.0296
			0.5	2	3003.374	6006.748			1					1		15	
	4-2	2	0.5	1	3116.505	6233.01	6219.542	12439.084	1	1728563	0.72	11021	0.0128	1	0.014	15	0.0310
			0.5	2	3103.037	6206.074			1					1		15	
	4-3	2	0.5	1	2647.817	5295.634	5309.812	10619.624	1	1728563	0.61	9202	0.0107	1	0.014	15	0.0258
			0.5	2	2661.995	5323.99			1					1		15	
	5-1	2	0.5	1	3226.943	6453.886	6545.42	13090.84	1	1728563	0.76	11673	0.0135	1	0.014	15	0.0328
			0.5	2	3318.477	6636.954			1					1		15	
	5-2	2	0.5	1	3074.3	6148.6	6166.579	12333.158	1	1728563	0.71	10915	0.0127	1	0.014	15	0.0307
			0.5	2	3092.279	6184.558			1					1		15	
	5-3	2	0.5	1	3636.052	7272.104	6900.473	13800.946	1	1728563	0.80	12383	0.0144	1	0.014	15	0.0348
			0.5	2	3264.421	6528.842			1					1		15	
	6-1	2	0.5	1	3388.02	6776.04	6698.492	13396.984	1	1728563	0.78	11979	0.0139	1	0.014	15	0.0336
			0.5	2	3310.472	6620.944			1					1		15	
	6-2	2	0.5	1	3736.582	7473.164	7163.993	14327.986	1	1728563	0.83	12910	0.0150	1	0.014	15	0.0363
			0.5	2	3427.411	6854.822			1					1		15	
	6-3	2	0.5	1	3628.31	7256.62	7005.633	14011.266	1	1728563	0.81	12593	0.0146	1	0.014	15	0.0354
			0.5	2	3377.323	6754.646			1					1		15	
	7-1	2	0.5	1	3566.357	7132.714	7172.688	14345.376	1	1728563	0.83	12927	0.0150	1	0.014	15	0.0363
			0.5	2	3606.331	7212.662			1					1		15	
	7-2	2	0.5	1	3513.7	7027.4	7269.277	14538.554	1	1728563	0.84	13121	0.0152	1	0.014	15	0.0368
			0.5	2	3755.577	7511.154			1					1		15	
	7-3	2	0.5	1	3560.2	7120.4	6854.518	13709.036	1	1728563	0.79	12291	0.0142	1	0.014	15	0.0345
			0.5	2	3294.318	6588.636			1					1		15	
	8-1	2	0.5	1	3504.403	7008.806	7056.462	14112.924	1	1728563	0.82	12695	0.0147	1	0.014	15	0.0357
			0.5	2	3552.059	7104.118			1					1		15	
	8-2	2	0.5	1	3606.761	7213.522	7104.417	14208.834	1	1728563	0.82	12791	0.0148	1	0.014	15	0.0359
			0.5	2	3497.656	6995.312			1					1		15	
	8-3	2	0.5	1	3663.447	7326.894	7302.531	14605.062	1	1728563	0.84	13187	0.0153	1	0.014	15	0.0370
			0.5	2	3639.084	7278.168			1					1		15	

Assay Date	7/27/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	Microsome 8 type	Placental	Microsome ID BAA	Technician ID TM	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0355	0.0017
Full activity	End	0.0301	0.0012
Full activity	Overall	0.0328	0.0033
Background	Beginning	0.0009	0.000870011
Background	End	-0.0009	0.001096669
Background	Overall	0.0000	0.001357868
Positive	Beginning	0.0200	0.0004
Positive	End	0.0157	0.0012
Positive	Overall	0.0179	0.0026
Negative	Beginning	0.0279	0.0040
Negative	End	0.0261	0.0024
Negative	Overall	0.0270	0.0029

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#2 KCZ	1	1	1.00E-04	-4.00	-0.0005
RC#2 KCZ	1	2	1.00E-04	-4.00	-0.0001
RC#2 KCZ	1	3	1.00E-04	-4.00	0.0008
RC#2 KCZ	2	1	1.00E-05	-5.00	0.0012
RC#2 KCZ	2	2	1.00E-05	-5.00	0.0015
RC#2 KCZ	2	3	1.00E-05	-5.00	0.0031
RC#2 KCZ	3	1	1.00E-06	-6.00	0.0121
RC#2 KCZ	3	2	1.00E-06	-6.00	0.0143
RC#2 KCZ	3	3	1.00E-06	-6.00	0.0092
RC#2 KCZ	4	1	1.00E-07	-7.00	0.0296
RC#2 KCZ	4	2	1.00E-07	-7.00	0.0310
RC#2 KCZ	4	3	1.00E-07	-7.00	0.0258
RC#2 KCZ	5	1	1.00E-08	-8.00	0.0328
RC#2 KCZ	5	2	1.00E-08	-8.00	0.0307
RC#2 KCZ	5	3	1.00E-08	-8.00	0.0348
RC#2 KCZ	6	1	1.00E-09	-9.00	0.0336
RC#2 KCZ	6	2	1.00E-09	-9.00	0.0363
RC#2 KCZ	6	3	1.00E-09	-9.00	0.0354
RC#2 KCZ	7	1	1.00E-10	-10.00	0.0363
RC#2 KCZ	7	2	1.00E-10	-10.00	0.0368
RC#2 KCZ	7	3	1.00E-10	-10.00	0.0345
RC#2 KCZ	8	1	1.00E-11	-11.00	0.0357
RC#2 KCZ	8	2	1.00E-11	-11.00	0.0359
RC#2 KCZ	8	3	1.00E-11	-11.00	0.0370

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	-1.45	-0.38	2.35
2	-5.00	3.69	4.51	9.35
3	-6.00	36.81	43.59	27.93
4	-7.00	90.24	94.35	78.78
5	-8.00	99.93	93.45	106.01
6	-9.00	102.55	110.52	107.81
7	-10.00	110.67	112.33	105.23
8	-11.00	108.68	109.50	112.90

Assay Date	7/28/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	Placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0203	30654.69	1510083
2	0.0199	32692.78	1642853
3	0.0203	33297.46	1640269
4	0.0202	33107.35	1638978
5	0.0204	34931	1712304
		Average DPM/g soln	1628897
		SD	73313
		CV	4.50
		µCi/g soln	0.734

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	17.1	17.1		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2343 g
Mass of dilution B used in substrate prep	9.1771 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.565291 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00831 µg/g soln.
µg/g soln.

a. µCi/g soln	0.734
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.

= 0.565291 + 0.00831
= 0.573597 µg ASDN/g soln.

3) Calculate Solution Specific Activity

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

813317 dpm/nmol

Assay Date	7/28/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8						
Technician ID	TM	Replicate #	2	Microsome type	Placental	Microsome ID	BAA				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	2	1	
	0.206	0.131	0.060	0.040	0.028	0.023	0.019			
	0.219	0.130	0.057	0.039	0.029	0.022	0.018			
	0.201	0.126	0.058	0.038	0.027	0.022	0.018			

Samples:	Microsomes	QC 10	QC 100
	0.037	0.026	0.102
	0.035	0.028	0.101
	0.034	0.033	0.109

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
0.25	125	1000	0.00025	200	0.0500	0.209	0.190	0.0484
0.125	62.5	1000	0.00013	200	0.0250	0.129	0.111	0.0279
0.05	25	1000	0.00005	200	0.0100	0.058	0.040	0.0097
0.025	25	2000	0.00003	200	0.0050	0.039	0.020	0.0047
0.01	5	1000	0.00001	200	0.0020	0.028	0.009	0.0019
0.005	5	2000	0.00001	200	0.0010	0.022	0.004	0.0004

Variables	Regression results			
m, b	0.258	-0.001		
se_m, se_b	0.010	0.001		
r^2, se_y	0.994	0.002		
F, df	613	4		
ss_{reg}, ss_{resid}	0.002	0.000		

Blank 0.019

r^2= 0.994
m= 0.258
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
Microsomes	0.037	0.019	0.004	200	220	70400	0.007	0.006	6.075
Microsomes	0.035	0.017	0.004	200	220	70400	0.006		
Microsomes	0.034	0.015	0.003	200	220	70400	0.005		
QC 10	0.026	0.007	0.001	200	200	200	0.000	0.000	0.011
QC 10	0.028	0.009	0.002	200	200	200	0.000		
QC 10	0.033	0.015	0.003	200	200	200	0.000		
QC 100	0.102	0.083	0.021	200	200	200	0.000	0.000	0.107
QC 100	0.101	0.082	0.021	200	200	200	0.000		
QC 100	0.109	0.091	0.023	200	200	200	0.000		

Assay Date	7/28/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	2

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.066364
Protein Concentration (dilution added to assay, mg/mL):	0.018957

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	7/28/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	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	3644.243	7288.486	7205.137	14410.274	1	1628897	0.88	14034	0.0173	1	0.009	15	0.0607
			0.5	2	3560.894	7121.788			1					1		15	
	2	2	0.5	1	3183.795	6367.59	6176.347	12352.694	1	1628897	0.76	11976	0.0147	1	0.009	15	0.0518
			0.5	2	2992.552	5985.104			1					1		15	
	3	2	0.5	1	2625.86	5251.72	5265.09	10530.18	1	1628897	0.65	10154	0.0125	1	0.009	15	0.0439
			0.5	2	2639.23	5278.46			1					1		15	
	4	2	0.5	1	2510.403	5020.806	5074.761	10149.522	1	1628897	0.62	9773	0.0120	1	0.009	15	0.0423
			0.5	2	2564.358	5128.716			1					1		15	
Background control	1	2	0.5	1	96.26371	192.52742	235.31131	470.62262	1	1628897	0.03	94	0.0001	1	0.009	15	0.0004
			0.5	2	139.0476	278.0952			1					1		15	
	2	2	0.5	1	64.64583	129.29166	160.21901	320.43802	1	1628897	0.02	-56	-0.0001	1	0.009	15	-0.0002
			0.5	2	95.57308	191.14616			1					1		15	
	3	2	0.5	1	99.66246	199.32492	182.55666	365.11332	1	1628897	0.02	-11	0.0000	1	0.009	15	0.0000
			0.5	2	82.8942	165.7884			1					1		15	
	4	2	0.5	1	98.93823	197.87646	174.91691	349.83382	1	1628897	0.02	-27	0.0000	1	0.009	15	-0.0001
			0.5	2	75.97868	151.95736			1					1		15	
Positive control	1	2	0.5	1	1373.726	2747.452	2641.205	5282.41	1	1628897	0.32	4906	0.0060	1	0.009	15	0.0212
			0.5	2	1267.479	2534.958			1					1		15	
	2	2	0.5	1	1163.193	2326.386	2360.511	4721.022	1	1628897	0.29	4345	0.0053	1	0.009	15	0.0188
			0.5	2	1197.318	2394.636			1					1		15	
	3	2	0.5	1	1198.643	2397.286	2550.82	5101.64	1	1628897	0.31	4725	0.0058	1	0.009	15	0.0204
			0.5	2	1352.177	2704.354			1					1		15	
	4	2	0.5	1	1349.484	2698.968	2638.355	5276.71	1	1628897	0.32	4900	0.0060	1	0.009	15	0.0212
			0.5	2	1288.871	2577.742			1					1		15	
Negative Control	1	2	0.5	1	2410.754	4821.508	4829.589	9659.178	1	1628897	0.59	9283	0.0114	1	0.009	15	0.0401
			0.5	2	2418.835	4837.67			1					1		15	
	2	2	0.5	1	1978.765	3957.53	4117.468	8234.936	1	1628897	0.51	7858	0.0097	1	0.009	15	0.0340
			0.5	2	2138.703	4277.405			1					1		15	
	3	2	0.5	1	2690.05	5380.1	5345.338	10690.676	1	1628897	0.66	10314	0.0127	1	0.009	15	0.0446
			0.5	2	2655.288	5310.576			1					1		15	
	4	2	0.5	1	2805.784	5611.568	5640.231	11280.462	1	1628897	0.69	10904	0.0134	1	0.009	15	0.0471
			0.5	2	2834.447	5668.894			1					1		15	
RC#2 KCZ	1-1	2	0.5	1	124.7773	249.5546	242.4348	484.8696	1	1628897	0.03	108	0.0001	1	0.009	15	0.0005
			0.5	2	117.6575	235.315			1					1		15	
	1-2	2	0.5	1	108.8483	217.6966	189.65656	379.31312	1	1628897	0.02	3	0.0000	1	0.009	15	0.0000
			0.5	2	80.80826	161.61652			1					1		15	
	1-3	2	0.5	1	82.89507	165.79014	176.48627	352.97254	1	1628897	0.02	-24	0.0000	1	0.009	15	-0.0001
			0.5	2	93.5912	187.1824			1					1		15	
	2-1	2	0.5	1	210.1015	420.203	433.671	867.342	1	1628897	0.05	491	0.0006	1	0.009	15	0.0021
			0.5	2	223.5695	447.139			1					1		15	
	2-2	2	0.5	1	175.2192	350.4384	347.6101	695.2202	1	1628897	0.04	319	0.0004	1	0.009	15	0.0014
			0.5	2	172.3909	344.7818			1					1		15	
	2-3	2	0.5	1	204.7143	409.4286	416.3714	832.7428	1	1628897	0.05	456	0.0006	1	0.009	15	0.0020
			0.5	2	211.6571	423.3142			1					1		15	
	3-1	2	0.5	1	853.017	1706.034	1685.342	3370.684	1	1628897	0.21	2994	0.0037	1	0.009	15	0.0129
			0.5	2	832.325	1664.65			1					1		15	
	3-2	2	0.5	1	764.7739	1529.5478	1633.8351	3267.6702	1	1628897	0.20	2891	0.0036	1	0.009	15	0.0125
			0.5	2	869.0612	1738.1224			1					1		15	
	3-3	2	0.5	1	864.6484	1729.2968	1739.0576	3478.1152	1	1628897	0.21	3102	0.0038	1	0.009	15	0.0134
			0.5	2	874.4092	1748.8184			1					1		15	
	4-1	2	0.5	1	2243.776	4487.552	4366.959	8733.918	1	1628897	0.54	8357	0.0103	1	0.009	15	0.0361
			0.5	2	2123.183	4246.366			1					1		15	
	4-2	2	0.5	1	2321.062	4642.124	4653.727	9307.454	1	1628897	0.57	8931	0.0110	1	0.009	15	0.0386
			0.5	2	2332.665	4665.33			1					1		15	
	4-3	2	0.5	1	2383.844	4767.688	4611.251	9222.502	1	1628897	0.57	8846	0.0109	1	0.009	15	0.0382
			0.5	2	2227.407	4454.814			1					1		15	
	5-1	2	0.5	1	2663.338	5326.676	5179.168	10358.336	1	1628897	0.64	9982	0.0123	1	0.009	15	0.0432
			0.5	2	2515.83	5031.66			1					1		15	
	5-2	2	0.5	1	2674.753	5349.506	5470.733	10941.466	1	1628897	0.67	10565	0.0130	1	0.009	15	0.0457
			0.5	2	2795.98	5591.96			1					1		15	
	5-3	2	0.5	1	2637.587	5275.174	5371.614	10743.228	1	1628897	0.66	10367	0.0127	1	0.009	15	0.0448
			0.5	2	2734.027	5468.054			1					1		15	
	6-1	2	0.5	1	2845.188	5690.376	5668.105	11336.21	1	1628897	0.70	10960	0.0135	1	0.009	15	0.0474
			0.5	2	2822.917	5645.834			1					1		15	
	6-2	2	0.5	1	2787.9	5575.8	5683.545	11367.09	1	1628897	0.70	10991	0.0135	1	0.009	15	0.0475
			0.5	2	2895.645	5791.29			1					1		15	
	6-3	2	0.5	1	2681.398	5362.796	5644.383	11288.766	1	1628897	0.69	10912	0.0134	1	0.009	15	0.0472
			0.5	2	2962.985	5925.97			1					1		15	
	7-1	2	0.5	1	2817.53	5635.06	5510.396	11020.792	1	1628897	0.68	10644	0.0131	1	0.009	15	0.0460
			0.5	2	2692.866	5385.732			1					1		15	
	7-2	2	0.5	1	2797.055	5594.11	5562.021	11124.042	1	1628897	0.68	10748	0.0132	1	0.009	15	0.0465
			0.5	2	2764.986	5529.932			1					1		15	
	7-3	2	0.5	1	2789.033	5578.066	5685.474	11370.948	1	1628897	0.70	10994	0.0135	1	0.009	15	0.0475
			0.5	2	2896.441	5792.882			1					1		15	
	8-1	2	0.5	1	2868.708	5737.416	5766.798	11533.596	1	1628897	0.71	11157	0.0137	1	0.009	15	0.0482
			0.5	2	2896.09	5796.18			1					1		15	
	8-2	2	0.5	1	2774.431	5548.862	5450.52	10901.04	1	1628897	0.67	10525	0.0129	1	0.009	15	0.0455
			0.5	2	2676.089	5352.178			1					1		15	
	8-3	2	0.5	1	2775.979	5551.958	5538.271	11076.542	1	1628897	0.68	10700	0.0132	1	0.009	15	0.0463
			0.5	2	2762.292	5524.584			1					1		15	

Assay Date	7/28/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	Microsome 8 type	Placental	Microsome ID BAA		Technician ID TM	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0562	0.0063
Full activity	End	0.0431	0.0012
Full activity	Overall	0.0497	0.0084
Background	Beginning	0.0001	0.000459178
Background	End	-0.0001	4.67159E-05
Background	Overall	0.0000	0.000282904
Positive	Beginning	0.0200	0.0017
Positive	End	0.0208	0.0005
Positive	Overall	0.0204	0.0011
Negative	Beginning	0.0371	0.0044
Negative	End	0.0459	0.0018
Negative	Overall	0.0415	0.0058

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#2 KCZ	1	1	1.00E-04	-4.00	0.0005
RC#2 KCZ	1	2	1.00E-04	-4.00	0.0000
RC#2 KCZ	1	3	1.00E-04	-4.00	-0.0001
RC#2 KCZ	2	1	1.00E-05	-5.00	0.0021
RC#2 KCZ	2	2	1.00E-05	-5.00	0.0014
RC#2 KCZ	2	3	1.00E-05	-5.00	0.0020
RC#2 KCZ	3	1	1.00E-06	-6.00	0.0129
RC#2 KCZ	3	2	1.00E-06	-6.00	0.0125
RC#2 KCZ	3	3	1.00E-06	-6.00	0.0134
RC#2 KCZ	4	1	1.00E-07	-7.00	0.0361
RC#2 KCZ	4	2	1.00E-07	-7.00	0.0386
RC#2 KCZ	4	3	1.00E-07	-7.00	0.0382
RC#2 KCZ	5	1	1.00E-08	-8.00	0.0432
RC#2 KCZ	5	2	1.00E-08	-8.00	0.0457
RC#2 KCZ	5	3	1.00E-08	-8.00	0.0448
RC#2 KCZ	6	1	1.00E-09	-9.00	0.0474
RC#2 KCZ	6	2	1.00E-09	-9.00	0.0475
RC#2 KCZ	6	3	1.00E-09	-9.00	0.0472
RC#2 KCZ	7	1	1.00E-10	-10.00	0.0460
RC#2 KCZ	7	2	1.00E-10	-10.00	0.0465
RC#2 KCZ	7	3	1.00E-10	-10.00	0.0475
RC#2 KCZ	8	1	1.00E-11	-11.00	0.0482
RC#2 KCZ	8	2	1.00E-11	-11.00	0.0455
RC#2 KCZ	8	3	1.00E-11	-11.00	0.0463

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.94	0.02	-0.20
2	-5.00	4.27	2.78	3.97
3	-6.00	26.07	25.18	27.01
4	-7.00	72.77	77.77	77.03
5	-8.00	86.92	92.00	90.27
6	-9.00	95.43	95.70	95.02
7	-10.00	92.69	93.59	95.74
8	-11.00	97.15	91.64	93.17

Assay Date	7/29/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8		
Technician ID	TM	Replicate #	3	Microsome type	Placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0202	34226.89	1694400		
2	0.0202	33705.84	1668606		
3	0.0203	34569.55	1702933		
4	0.0202	33478.18	1657336		
5	0.0200	35193.87	1759694		
			Average DPM/g soln		1696594
			SD		39847
			CV		2.35
			µCi/g soln		0.764

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	17.4	17.4		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.1924 g
Mass of dilution B used in substrate prep	9.1318 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563956 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00865 µg/g soln.
µg/g soln.

a. µCi/g soln	0.764
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.

= 0.563956 + 0.00865
= 0.572607 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.335 µCi/µg ASDN

848583 dpm/nmol

Assay Date	7/29/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8
Technician ID	TM	Replicate #	3	Microsome type	Placental

Microsome ID BAA

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.496	0.309	0.161	0.112	0.056	0.044	0.027	2	1	
	0.525	0.324	0.169	0.104	0.057	0.045	0.030			
	0.545	0.309	0.171	0.110	0.056	0.045	0.029			

Samples:	Microsomes	QC 10	QC 100
	0.083	0.048	0.246
	0.085	0.047	0.277
	0.089	0.046	0.276

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	
0.25	125	1000	0.00025	200	0.0500	0.522	0.493	0.0483	m, b	0.103	-0.002
0.125	62.5	1000	0.00013	200	0.0250	0.314	0.285	0.0270	se_m, se_b	0.005	0.001
0.05	25	1000	0.00005	200	0.0100	0.167	0.138	0.0119	r^2, se_y	0.991	0.002
0.025	25	2000	0.00003	200	0.0050	0.109	0.080	0.0059	F, df	447	4
0.01	5	1000	0.00001	200	0.0020	0.056	0.028	0.0006	ss_{reg}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.045	0.016	-0.0007			

Blank 0.029

r^2= 0.991
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
Microsomes	0.083	0.055	0.003	200	220	70400	0.005	0.006	5.698
Microsomes	0.085	0.056	0.003	200	220	70400	0.006		
Microsomes	0.089	0.060	0.004	200	220	70400	0.006		
QC 10	0.048	0.019	0.000	200	200	200	0.000	0.000	-0.002
QC 10	0.047	0.019	0.000	200	200	200	0.000		
QC 10	0.046	0.017	-0.001	200	200	200	0.000		
QC 100	0.246	0.217	0.020	200	200	200	0.000	0.000	0.110
QC 100	0.277	0.248	0.023	200	200	200	0.000		
QC 100	0.276	0.248	0.023	200	200	200	0.000		

Assay Date	7/29/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	3

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.689791
Protein Concentration (dilution added to assay, mg/mL):	0.017781

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	7/29/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	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	3450.458	6900.916	6931.996	13863.992	1	1696594	0.82	13428	0.0158	1	0.009	15	0.0593
			0.5	2	3481.538	6963.076			1					1		15	
	2	2	0.5	1	3784.46	7568.92	7693.836	15387.672	1	1696594	0.91	14952	0.0176	1	0.009	15	0.0661
			0.5	2	3909.376	7818.752			1					1		15	
	3	2	0.5	1	3358.314	6716.628	6599.817	13199.634	1	1696594	0.78	12764	0.0150	1	0.009	15	0.0564
			0.5	2	3241.503	6483.006			1					1		15	
	4	2	0.5	1	3117.875	6235.75	6215.476	12430.952	1	1696594	0.73	11995	0.0141	1	0.009	15	0.0530
			0.5	2	3097.601	6195.202			1					1		15	
Background control	1	2	0.5	1	103.537	207.074	220.2179	440.4358	1	1696594	0.03	5	0.0000	1	0.009	15	0.0000
			0.5	2	116.6809	233.3618			1					1		15	
	2	2	0.5	1	114.1561	228.3122	233.6225	467.245	1	1696594	0.03	32	0.0000	1	0.009	15	0.0001
			0.5	2	119.4664	238.9328			1					1		15	
	3	2	0.5	1	69.52415	139.0483	178.37125	356.7425	1	1696594	0.02	-79	-0.0001	1	0.009	15	-0.0003
			0.5	2	108.8471	217.6942			1					1		15	
	4	2	0.5	1	128.3523	256.7046	238.7891	477.5782	1	1696594	0.03	42	0.0000	1	0.009	15	0.0002
			0.5	2	110.4368	220.8736			1					1		15	
Positive control	1	2	0.5	1	1408.742	2817.484	2890.211	5780.422	1	1696594	0.34	5345	0.0063	1	0.009	15	0.0236
			0.5	2	1481.469	2962.938			1					1		15	
	2	2	0.5	1	1274.308	2548.616	2476.936	4953.872	1	1696594	0.29	4518	0.0053	1	0.009	15	0.0200
			0.5	2	1202.626	2405.256			1					1		15	
	3	2	0.5	1	1630.052	3260.104	3271.892	6543.784	1	1696594	0.39	6108	0.0072	1	0.009	15	0.0270
			0.5	2	1641.84	3283.68			1					1		15	
	4	2	0.5	1	1670.02	3340.04	3387.681	6775.362	1	1696594	0.40	6340	0.0075	1	0.009	15	0.0280
			0.5	2	1717.661	3435.322			1					1		15	
Negative Control	1	2	0.5	1	2369.169	4738.338	4637.163	9274.326	1	1696594	0.55	8639	0.0104	1	0.009	15	0.0391
			0.5	2	2267.994	4535.988			1					1		15	
	2	2	0.5	1	2130.116	4260.232	4169.156	8338.312	1	1696594	0.49	7603	0.0093	1	0.009	15	0.0349
			0.5	2	2039.04	4078.08			1					1		15	
	3	2	0.5	1	3553.98	7107.96	6891.14	13782.28	1	1696594	0.81	13347	0.0157	1	0.009	15	0.0590
			0.5	2	3337.16	6674.32			1					1		15	
	4	2	0.5	1	3206.134	6412.268	6575.382	13150.764	1	1696594	0.78	12715	0.0150	1	0.009	15	0.0562
			0.5	2	3369.248	6738.496			1					1		15	
RC#2 KCZ	1-1	2	0.5	1	127.4321	254.8642	244.9881	489.9762	1	1696594	0.03	54	0.0001	1	0.009	15	0.0002
			0.5	2	117.556	235.112			1					1		15	
	1-2	2	0.5	1	133.9591	267.9182	260.5587	521.1174	1	1696594	0.03	66	0.0001	1	0.009	15	0.0004
			0.5	2	126.5996	253.1992			1					1		15	
	1-3	2	0.5	1	78.11341	156.22682	185.85771	371.71542	1	1696594	0.02	-64	-0.0001	1	0.009	15	-0.0003
			0.5	2	107.7443	215.4886			1					1		15	
	2-1	2	0.5	1	221.9449	443.8898	413.1911	826.3822	1	1696594	0.05	391	0.0005	1	0.009	15	0.0017
			0.5	2	191.2462	382.4924			1					1		15	
	2-2	2	0.5	1	272.0544	544.1088	530.6408	1061.2816	1	1696594	0.06	626	0.0007	1	0.009	15	0.0028
			0.5	2	258.5864	517.1728			1					1		15	
	2-3	2	0.5	1	240.5797	481.1594	470.5466	941.0932	1	1696594	0.06	506	0.0006	1	0.009	15	0.0022
			0.5	2	229.9669	459.9338			1					1		15	
	3-1	2	0.5	1	497.8914	995.7828	1463.2178	2926.4356	1	1696594	0.17	2491	0.0029	1	0.009	15	0.0110
			0.5	2	965.3264	1930.6528			1					1		15	
	3-2	2	0.5	1	915.9184	1831.8368	1830.6169	3661.2338	1	1696594	0.22	3226	0.0038	1	0.009	15	0.0143
			0.5	2	914.6965	1829.397			1					1		15	
	3-3	2	0.5	1	977.7799	1955.5598	2006.7379	4013.4758	1	1696594	0.24	3578	0.0042	1	0.009	15	0.0158
			0.5	2	1028.958	2057.916			1					1		15	
	4-1	2	0.5	1	2626.269	5252.538	5347.962	10695.924	1	1696594	0.63	10260	0.0121	1	0.009	15	0.0453
			0.5	2	2721.693	5443.386			1					1		15	
	4-2	2	0.5	1	2466.343	4932.686	5100.692	10201.384	1	1696594	0.60	9766	0.0115	1	0.009	15	0.0431
			0.5	2	2634.349	5268.698			1					1		15	
	4-3	2	0.5	1	2774.867	5549.734	5440.879	10881.758	1	1696594	0.64	10446	0.0123	1	0.009	15	0.0462
			0.5	2	2665.012	5332.024			1					1		15	
	5-1	2	0.5	1	3283.714	6567.428	6454.091	12908.182	1	1696594	0.76	12473	0.0147	1	0.009	15	0.0551
			0.5	2	3170.377	6340.754			1					1		15	
	5-2	2	0.5	1	3166.057	6332.114	6481.907	12963.814	1	1696594	0.76	12528	0.0148	1	0.009	15	0.0554
			0.5	2	3315.85	6631.7			1					1		15	
	5-3	2	0.5	1	3404.739	6809.478	6623.618	13247.236	1	1696594	0.78	12812	0.0151	1	0.009	15	0.0566
			0.5	2	3218.879	6437.758			1					1		15	
	6-1	2	0.5	1	3272.752	6545.504	6678.279	13356.558	1	1696594	0.79	12921	0.0152	1	0.009	15	0.0571
			0.5	2	3405.527	6811.054			1					1		15	
	6-2	2	0.5	1	3394.672	6789.344	6828.151	13656.302	1	1696594	0.80	13221	0.0156	1	0.009	15	0.0584
			0.5	2	3433.479	6866.958			1					1		15	
	6-3	2	0.5	1	3068.036	6136.072	6202.206	12404.412	1	1696594	0.73	11969	0.0141	1	0.009	15	0.0529
			0.5	2	3134.17	6268.34			1					1		15	
	7-1	2	0.5	1	3497.656	6995.312	7026.302	14052.604	1	1696594	0.83	13617	0.0160	1	0.009	15	0.0602
			0.5	2	3528.646	7057.292			1					1		15	
	7-2	2	0.5	1	3337.213	6674.426	6828.148	13656.296	1	1696594	0.80	13221	0.0156	1	0.009	15	0.0584
			0.5	2	3490.935	6981.87			1					1		15	
	7-3	2	0.5	1	3313.455	6626.91	6597.187	13194.374	1	1696594	0.78	12759	0.0150	1	0.009	15	0.0564
			0.5	2	3283.732	6567.464			1					1		15	
	8-1	2	0.5	1	3261.372	6522.744	6321.328	12642.656	1	1696594	0.75	12207	0.0144	1	0.009	15	0.0539
			0.5	2	3059.956	6119.912			1					1		15	
	8-2	2	0.5	1	3261.977	6523.954	6343.482	12686.964	1	1696594	0.75	12251	0.0144	1	0.009	15	0.0541
			0.5	2	3081.505	6163.01			1					1		15	
	8-3	2	0.5	1	3212.863	6425.726	6302.448	12604.896	1	1696594	0.74	12169	0.0143	1	0.009	15	0.0538
			0.5	2	3089.585	6179.17			1					1		15	

Assay Date	7/29/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0627	0.0048
Full activity	End	0.0547	0.0024
Full activity	Overall	0.0587	0.0056
Background	Beginning	0.0001	8.37601E-05
Background	End	-0.0001	0.000377527
Background	Overall	0.0000	0.00024208
Positive	Beginning	0.0218	0.0026
Positive	End	0.0275	0.0007
Positive	Overall	0.0246	0.0036
Negative	Beginning	0.0370	0.0029
Negative	End	0.0576	0.0020
Negative	Overall	0.0473	0.0121

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#2 KCZ	1	1	1.00E-04	-4.00	0.0002
RC#2 KCZ	1	2	1.00E-04	-4.00	0.0004
RC#2 KCZ	1	3	1.00E-04	-4.00	-0.0003
RC#2 KCZ	2	1	1.00E-05	-5.00	0.0017
RC#2 KCZ	2	2	1.00E-05	-5.00	0.0028
RC#2 KCZ	2	3	1.00E-05	-5.00	0.0022
RC#2 KCZ	3	1	1.00E-06	-6.00	0.0110
RC#2 KCZ	3	2	1.00E-06	-6.00	0.0143
RC#2 KCZ	3	3	1.00E-06	-6.00	0.0158
RC#2 KCZ	4	1	1.00E-07	-7.00	0.0453
RC#2 KCZ	4	2	1.00E-07	-7.00	0.0431
RC#2 KCZ	4	3	1.00E-07	-7.00	0.0462
RC#2 KCZ	5	1	1.00E-08	-8.00	0.0551
RC#2 KCZ	5	2	1.00E-08	-8.00	0.0554
RC#2 KCZ	5	3	1.00E-08	-8.00	0.0566
RC#2 KCZ	6	1	1.00E-09	-9.00	0.0571
RC#2 KCZ	6	2	1.00E-09	-9.00	0.0584
RC#2 KCZ	6	3	1.00E-09	-9.00	0.0529
RC#2 KCZ	7	1	1.00E-10	-10.00	0.0602
RC#2 KCZ	7	2	1.00E-10	-10.00	0.0584
RC#2 KCZ	7	3	1.00E-10	-10.00	0.0564
RC#2 KCZ	8	1	1.00E-11	-11.00	0.0539
RC#2 KCZ	8	2	1.00E-11	-11.00	0.0541
RC#2 KCZ	8	3	1.00E-11	-11.00	0.0538

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.41	0.64	-0.48
2	-5.00	2.94	4.71	3.81
3	-6.00	18.75	24.28	26.93
4	-7.00	77.23	73.51	78.63
5	-8.00	93.89	94.30	96.44
6	-9.00	97.26	99.52	90.09
7	-10.00	102.50	99.52	96.04
8	-11.00	91.89	92.22	91.60

Assay Date	8/5/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	0

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0203	30495.19	1502226	
2	0.0201	33417.8	1662577	
3	0.0201	34409.48	1711914	
4	0.0195	36096.1	1851082	
5	0.0198	36785.36	1857846	
			Average DPM/g soln	1717129
			SD	147420
			CV	8.59
			µCi/g soln	0.773

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	16.7	16.7		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2966 g
Mass of dilution B used in substrate prep	9.1704 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. = 0.00876 µg/g soln.
µg/g soln.

a. µCi/g soln	0.773
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 + 0.00876
= 0.571475 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.353 µCi/µg ASDN

860556 dpm/nmol

Assay Date	8/5/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8				
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	0		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.491	0.332	0.159	0.104	0.053	0.043	0.036			
	0.562	0.343	0.161	0.101	0.057	0.045	0.033			
	0.474	0.329	0.174	0.106	0.063	0.048	0.037			

Samples:	Placental	QC 10	QC 100
	0.100	0.061	0.285
	0.098	0.064	0.276
	0.101	0.065	0.290

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	
0.25	125	1000	0.00025	200	0.0500	0.509	0.473	0.0470	m, b	0.103	-0.002
0.125	62.5	1000	0.00013	200	0.0250	0.334	0.299	0.0291	se_m, se_b	0.007	0.002
0.05	25	1000	0.00005	200	0.0100	0.165	0.130	0.0116	r^2, se_y	0.982	0.003
0.025	25	2000	0.00003	200	0.0050	0.104	0.068	0.0053	F, df	215	4
0.01	5	1000	0.00001	200	0.0020	0.058	0.022	0.0006	ss_{reg}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.046	0.010	-0.0007			

Regression results are calculated using the function LINEST

Blank	0.035	r^2=	0.982	
		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
Placental	0.100	0.065	0.005	200	90	72000	0.020	0.020	19.596
Placental	0.098	0.062	0.005	200	90	72000	0.019		
Placental	0.101	0.066	0.005	200	90	72000	0.020		
QC 10	0.061	0.026	0.001	200	200	200	0.000	0.000	0.006
QC 10	0.064	0.029	0.001	200	200	200	0.000		
QC 10	0.065	0.030	0.001	200	200	200	0.000		
QC 100	0.285	0.250	0.024	200	200	200	0.000	0.000	0.119
QC 100	0.276	0.241	0.023	200	200	200	0.000		
QC 100	0.290	0.255	0.024	200	200	200	0.000		

Assay Date	8/5/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	Replicate #	2

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	19.59606
Protein Concentration (dilution added to assay, mg/mL):	0.024495

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	3.30E-09
7	1.00E-09
8	1.00E-10

Assay Date	8/5/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	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	3592.518	7185.036	7554.166	15108.332	1	1717129	0.88	14726	0.0171	1	0.012	15	0.0466
			0.5	2	3961.648	7923.296			1					1		15	
	2	2	0.5	1	4021.437	8042.874	7912.139	15824.278	1	1717129	0.92	15442	0.0179	1	0.012	15	0.0486
			0.5	2	3890.702	7781.404			1					1		15	
	3	2	0.5	1	3402.81	6805.62	6844.432	13688.864	1	1717129	0.80	13307	0.0155	1	0.012	15	0.0421
			0.5	2	3441.622	6883.244			1					1		15	
	4	2	0.5	1	3320.274	6640.548	6632.045	13264.09	1	1717129	0.77	12882	0.0150	1	0.012	15	0.0407
			0.5	2	3311.771	6623.542			1					1		15	
Background control	1	2	0.5	1	90.98273	181.96546	211.51963	423.03926	1	1717129	0.02	41	0.0000	1	0.012	15	0.0001
			0.5	2	120.5369	241.0738			1					1		15	
	2	2	0.5	1	96.18174	192.36348	181.96545	363.9309	1	1717129	0.02	-18	0.0000	1	0.012	15	-0.0001
			0.5	2	85.76371	171.56742			1					1		15	
	3	2	0.5	1	97.53114	195.06228	170.31732	340.63464	1	1717129	0.02	-41	0.0000	1	0.012	15	-0.0001
			0.5	2	72.78618	145.57236			1					1		15	
	4	2	0.5	1	109.1793	218.3586	200.16251	400.32502	1	1717129	0.02	18	0.0000	1	0.012	15	0.0001
			0.5	2	90.96321	181.96642			1					1		15	
Positive control	1	2	0.5	1	1664.768	3329.536	3396.614	6793.228	1	1717129	0.40	6411	0.0075	1	0.012	15	0.0203
			0.5	2	1731.846	3463.692			1					1		15	
	2	2	0.5	1	1362.149	2724.298	2669.601	5339.202	1	1717129	0.31	4957	0.0058	1	0.012	15	0.0157
			0.5	2	1307.452	2614.904			1					1		15	
	3	2	0.5	1	1679.126	3358.252	3374.068	6748.136	1	1717129	0.39	6366	0.0074	1	0.012	15	0.0201
			0.5	2	1694.942	3389.884			1					1		15	
	4	2	0.5	1	1707.885	3415.77	3427.622	6855.244	1	1717129	0.40	6473	0.0075	1	0.012	15	0.0205
			0.5	2	1719.737	3439.474			1					1		15	
Negative Control	1	2	0.5	1	1141.189	2282.378	2272.05	4544.1	1	1717129	0.26	4162	0.0048	1	0.012	15	0.0132
			0.5	2	1130.861	2261.722			1					1		15	
	2	2	0.5	1	1138.639	2277.278	2240.836	4481.672	1	1717129	0.26	4100	0.0048	1	0.012	15	0.0130
			0.5	2	1102.197	2204.394			1					1		15	
	3	2	0.5	1	2989.448	5978.896	5754.602	11509.204	1	1717129	0.67	11127	0.0129	1	0.012	15	0.0352
			0.5	2	2765.154	5530.308			1					1		15	
	4	2	0.5	1	3251.01	6502.02	6539.403	13078.806	1	1717129	0.76	12897	0.0148	1	0.012	15	0.0402
			0.5	2	3288.393	6576.786			1					1		15	
RC#3 PCZ	1-1	2	0.5	1	135.175	270.35	278.1486	556.2972	1	1717129	0.03	174	0.0002	1	0.012	15	0.0006
			0.5	2	142.9736	285.9472			1					1		15	
	1-2	2	0.5	1	132.5755	265.151	273.9238	547.8476	1	1717129	0.03	166	0.0002	1	0.012	15	0.0005
			0.5	2	141.3483	282.6966			1					1		15	
	1-3	2	0.5	1	125.6429	251.2858	250.4199	500.8398	1	1717129	0.03	119	0.0001	1	0.012	15	0.0004
			0.5	2	124.777	249.554			1					1		15	
	2-1	2	0.5	1	72.78657	145.57314	167.01875	334.0375	1	1717129	0.02	-48	-0.0001	1	0.012	15	-0.0002
			0.5	2	94.23218	188.46436			1					1		15	
	2-2	2	0.5	1	88.38368	176.76736	215.76018	431.52036	1	1717129	0.03	50	0.0001	1	0.012	15	0.0002
			0.5	2	127.3765	254.753			1					1		15	
	2-3	2	0.5	1	86.98769	173.97538	193.56799	387.13598	1	1717129	0.02	5	0.0000	1	0.012	15	0.0000
			0.5	2	106.5803	213.1606			1					1		15	
	3-1	2	0.5	1	114.3789	228.7578	278.1487	556.2974	1	1717129	0.03	174	0.0002	1	0.012	15	0.0006
			0.5	2	163.7698	327.5396			1					1		15	
	3-2	2	0.5	1	138.7307	277.4614	293.1676	586.3352	1	1717129	0.03	204	0.0002	1	0.012	15	0.0006
			0.5	2	154.4369	308.8738			1					1		15	
	3-3	2	0.5	1	147.1677	294.3354	281.4259	562.8518	1	1717129	0.03	181	0.0002	1	0.012	15	0.0006
			0.5	2	134.2582	268.5164			1					1		15	
	4-1	2	0.5	1	463.3106	926.6212	941.8248	1883.2496	1	1717129	0.11	1501	0.0017	1	0.012	15	0.0047
			0.5	2	478.3142	956.6284			1					1		15	
	4-2	2	0.5	1	482.8133	965.6266	913.6732	1827.3464	1	1717129	0.11	1445	0.0017	1	0.012	15	0.0046
			0.5	2	430.8599	861.7198			1					1		15	
	4-3	2	0.5	1	682.7253	1365.4506	1475.5831	2951.1662	1	1717129	0.17	2569	0.0030	1	0.012	15	0.0081
			0.5	2	792.8578	1585.7156			1					1		15	
	5-1	2	0.5	1	2290.226	4580.452	4716.717	9433.434	1	1717129	0.55	9051	0.0105	1	0.012	15	0.0286
			0.5	2	2426.491	4852.982			1					1		15	
	5-2	2	0.5	1	2513.749	5027.498	4916.819	9833.638	1	1717129	0.57	9452	0.0110	1	0.012	15	0.0299
			0.5	2	2403.07	4806.14			1					1		15	
	5-3	2	0.5	1	2354.683	4709.366	4829.439	9658.878	1	1717129	0.56	9277	0.0108	1	0.012	15	0.0293
			0.5	2	2474.756	4949.512			1					1		15	
	6-1	2	0.5	1	2914.078	5828.156	5787.721	11575.442	1	1717129	0.67	11193	0.0130	1	0.012	15	0.0354
			0.5	2	2873.643	5747.286			1					1		15	
	6-2	2	0.5	1	3181.658	6363.316	6396.039	12792.078	1	1717129	0.74	12410	0.0144	1	0.012	15	0.0392
			0.5	2	3214.381	6428.762			1					1		15	
	6-3	2	0.5	1	2960.869	5921.738	5921.738	11843.476	1	1717129	0.69	11461	0.0133	1	0.012	15	0.0362
			0.5	2	2960.869	5921.738			1					1		15	
	7-1	2	0.5	1	3141.089	6282.178	6330.717	12661.434	1	1717129	0.74	12279	0.0143	1	0.012	15	0.0388
			0.5	2	3189.628	6379.256			1					1		15	
	7-2	2	0.5	1	3106.443	6212.886	6262.277	12524.554	1	1717129	0.73	12143	0.0141	1	0.012	15	0.0384
			0.5	2	3155.834	6311.668			1					1		15	
	7-3	2	0.5	1	3039.004	6078.008	6057.232	12114.464	1	1717129	0.71	11732	0.0136	1	0.012	15	0.0371
			0.5	2	3018.228	6036.456			1					1		15	
	8-1	2	0.5	1	3902.803	7805.606	7625.336	15250.672	1	1717129	0.89	14869	0.0173	1	0.012	15	0.0470
			0.5	2	3722.533	7445.066			1					1		15	
	8-2	2	0.5	1	3491.174	6982.348	7091.112	14182.224	1	1717129	0.83	13800	0.0160	1	0.012	15	0.0436
			0.5	2	3599.938	7199.876			1					1		15	
	8-3	2	0.5	1	3774.523	7549.046	7427.896	14855.792	1	1717129	0.87	14474	0.0168	1	0.012	15	0.0458
			0.5	2	3653.373	7306.746			1					1		15	

Assay Date	8/5/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0477	0.0016
Full activity	End	0.0414	0.0009
Full activity	Overall	0.0446	0.0038
Background	Beginning	0.0000	0.000132186
Background	End	0.0000	0.000133487
Background	Overall	0.0000	0.000116312
Positive	Beginning	0.0180	0.0033
Positive	End	0.0203	0.0002
Positive	Overall	0.0191	0.0023
Negative	Beginning	0.0131	0.0001
Negative	End	0.0377	0.0035
Negative	Overall	0.0254	0.0144

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#3 PCZ	1	1	1.00E-04	-4.00	0.0006
RC#3 PCZ	1	2	1.00E-04	-4.00	0.0005
RC#3 PCZ	1	3	1.00E-04	-4.00	0.0004
RC#3 PCZ	2	1	1.00E-05	-5.00	-0.0002
RC#3 PCZ	2	2	1.00E-05	-5.00	0.0002
RC#3 PCZ	2	3	1.00E-05	-5.00	0.0000
RC#3 PCZ	3	1	1.00E-06	-6.00	0.0006
RC#3 PCZ	3	2	1.00E-06	-6.00	0.0006
RC#3 PCZ	3	3	1.00E-06	-6.00	0.0006
RC#3 PCZ	4	1	1.00E-07	-7.00	0.0047
RC#3 PCZ	4	2	1.00E-07	-7.00	0.0046
RC#3 PCZ	4	3	1.00E-07	-7.00	0.0081
RC#3 PCZ	5	1	1.00E-08	-8.00	0.0286
RC#3 PCZ	5	2	1.00E-08	-8.00	0.0299
RC#3 PCZ	5	3	1.00E-08	-8.00	0.0293
RC#3 PCZ	6	1	3.30E-09	-8.48	0.0354
RC#3 PCZ	6	2	3.30E-09	-8.48	0.0392
RC#3 PCZ	6	3	3.30E-09	-8.48	0.0362
RC#3 PCZ	7	1	1.00E-09	-9.00	0.0388
RC#3 PCZ	7	2	1.00E-09	-9.00	0.0384
RC#3 PCZ	7	3	1.00E-09	-9.00	0.0371
RC#3 PCZ	8	1	1.00E-10	-10.00	0.0470
RC#3 PCZ	8	2	1.00E-10	-10.00	0.0436
RC#3 PCZ	8	3	1.00E-10	-10.00	0.0458

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	1.24	1.18	0.84
2	-5.00	-0.34	0.35	0.04
3	-6.00	1.24	1.45	1.28
4	-7.00	10.66	10.26	18.23
5	-8.00	64.24	67.08	65.84
6	-8.48	79.45	88.08	81.35
7	-9.00	87.15	86.18	83.27
8	-10.00	105.53	97.95	102.73

Assay Date	8/8/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8		
Technician ID	TM	Replicate #	3	Microsome type	placental	Microsome ID	0

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0198	29902.82	1510243		
2	0.0200	32737.14	1636857		
3	0.0203	36391.62	1792691		
4	0.0198	36166.82	1826607		
5	0.0205	37264.12	1817762		
			Average DPM/g soln		1716832
			SD		138805
			CV		8.08
			µCi/g soln		0.773

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	18.3	18.3		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2935 g
Mass of dilution B used in substrate prep	9.2097 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.565238 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00875 µg/g soln.
µg/g soln.

a. µCi/g soln	0.773
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.

= 0.565238 + 0.00875
= 0.573992 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.347 µCi/µg ASDN

856633 dpm/nmol

Assay Date	8/8/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8				
Technician ID	TM	Replicate #	3	Microsome type	placental	Microsome ID	0		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.448	0.301	0.163	0.110	0.068	0.061	0.039			
	0.419	0.284	0.172	0.107	0.065	0.053	0.042			
	0.508	0.286	0.179	0.113	0.075	0.054	0.039			

Samples:	Placental	QC 10	QC 100
	0.095	0.067	0.256
	0.101	0.070	0.245
	0.108	0.063	0.239

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
0.25	125	1000	0.00025	200	0.0500	0.458	0.419	0.0477
0.125	62.5	1000	0.00013	200	0.0250	0.291	0.251	0.0274
0.05	25	1000	0.00005	200	0.0100	0.171	0.132	0.0129
0.025	25	2000	0.00003	200	0.0050	0.110	0.070	0.0055
0.01	5	1000	0.00001	200	0.0020	0.069	0.029	0.0005
0.005	5	2000	0.00001	200	0.0010	0.056	0.016	-0.0011

Variables	Regression results			
m, b	0.121	-0.003		
se_m, se_b	0.007	0.002		
r^2, se_y	0.986	0.003		
F, df	272	4		
ss_{reg}, ss_{resid}	0.002	0.000		

Blank 0.040

r^2= 0.986
m= 0.121
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/µL Prep.	average mg/µL	mg/mL
Placental	0.095	0.056	0.004	200	90	72000	0.015	0.018	17.757
Placental	0.101	0.061	0.004	200	90	72000	0.018		
Placental	0.108	0.068	0.005	200	90	72000	0.021		
QC 10	0.067	0.027	0.000	200	200	200	0.000	0.000	0.001
QC 10	0.070	0.030	0.001	200	200	200	0.000		
QC 10	0.063	0.023	0.000	200	200	200	0.000		
QC 100	0.256	0.216	0.023	200	200	200	0.000	0.000	0.110
QC 100	0.245	0.205	0.022	200	200	200	0.000		
QC 100	0.239	0.199	0.021	200	200	200	0.000		

Assay Date	8/8/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	Replicate #	3

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	17.75676
Protein Concentration (dilution added to assay, mg/mL):	0.022196

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	3.30E-09
7	1.00E-09
8	1.00E-10

Assay Date	8/8/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	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	3291.944	6583.888	6376.037	12752.074	1	1716832	0.74	12363	0.0144	1	0.011	15	0.0433
			0.5	2	3084.093	6168.186			1					1		15	
	2	2	0.5	1	3421.133	6842.266	6357.954	12735.908	1	1716832	0.74	12347	0.0144	1	0.011	15	0.0433
			0.5	2	2946.821	5893.642			1					1		15	
	3	2	0.5	1	2936.043	5872.086	6028.878	12057.756	1	1716832	0.70	11669	0.0136	1	0.011	15	0.0409
			0.5	2	3092.835	6185.67			1					1		15	
	4	2	0.5	1	3148.908	6297.816	6364.8	12729.6	1	1716832	0.74	12340	0.0144	1	0.011	15	0.0433
			0.5	2	3215.892	6431.784			1					1		15	
Background control	1	2	0.5	1	134.4348	268.8696	231.96594	463.93188	1	1716832	0.03	75	0.0001	1	0.011	15	0.0003
			0.5	2	97.53114	195.06228			1					1		15	
	2	2	0.5	1	102.3555	204.711	187.3089	374.6178	1	1716832	0.02	-15	0.0000	1	0.011	15	-0.0001
			0.5	2	84.9534	169.9068			1					1		15	
	3	2	0.5	1	92.91779	185.83558	200.99289	401.98578	1	1716832	0.02	13	0.0000	1	0.011	15	0.0000
			0.5	2	108.0751	216.1502			1					1		15	
	4	2	0.5	1	78.52639	157.05278	158.17062	316.34124	1	1716832	0.02	-73	-0.0001	1	0.011	15	-0.0003
			0.5	2	79.64423	159.28846			1					1		15	
Positive control	1	2	0.5	1	1438.615	2877.23	2767.596	5535.192	1	1716832	0.32	5148	0.0060	1	0.011	15	0.0180
			0.5	2	1328.961	2657.962			1					1		15	
	2	2	0.5	1	1250.414	2500.828	2437.113	4874.226	1	1716832	0.28	4485	0.0052	1	0.011	15	0.0157
			0.5	2	1186.699	2373.398			1					1		15	
	3	2	0.5	1	1709.696	3419.392	3422.047	6844.094	1	1716832	0.40	6455	0.0075	1	0.011	15	0.0226
			0.5	2	1712.351	3424.702			1					1		15	
	4	2	0.5	1	1810.579	3621.158	3493.67	6987.34	1	1716832	0.41	6598	0.0077	1	0.011	15	0.0231
			0.5	2	1683.091	3366.182			1					1		15	
Negative Control	1	2	0.5	1	1598.194	3196.388	3217.036	6434.072	1	1716832	0.37	6045	0.0071	1	0.011	15	0.0212
			0.5	2	1618.842	3237.684			1					1		15	
	2	2	0.5	1	1420.799	2841.598	2870.594	5741.188	1	1716832	0.33	5352	0.0062	1	0.011	15	0.0188
			0.5	2	1449.795	2899.59			1					1		15	
	3	2	0.5	1	2933.858	5867.716	5896.623	11793.246	1	1716832	0.69	11404	0.0133	1	0.011	15	0.0400
			0.5	2	2962.765	5925.53			1					1		15	
	4	2	0.5	1	2751.975	5503.95	5615.836	11231.672	1	1716832	0.65	10842	0.0127	1	0.011	15	0.0380
			0.5	2	2863.861	5727.722			1					1		15	
RC#3 PCZ	1-1	2	0.5	1	133.7003	267.4006	271.7503	543.5006	1	1716832	0.03	154	0.0002	1	0.011	15	0.0005
			0.5	2	138.05	276.1			1					1		15	
	1-2	2	0.5	1	159.2885	318.577	294.6637	589.3674	1	1716832	0.03	200	0.0002	1	0.011	15	0.0007
			0.5	2	135.3952	270.7904			1					1		15	
	1-3	2	0.5	1	199.1106	398.2212	436.3498	872.6996	1	1716832	0.05	483	0.0006	1	0.011	15	0.0017
			0.5	2	237.2392	474.4784			1					1		15	
	2-1	2	0.5	1	120.3303	240.6606	242.4515	484.903	1	1716832	0.03	96	0.0001	1	0.011	15	0.0003
			0.5	2	122.1212	244.2424			1					1		15	
	2-2	2	0.5	1	71.67981	143.35962	148.66924	297.33848	1	1716832	0.02	-92	-0.0001	1	0.011	15	-0.0003
			0.5	2	76.98943	153.97886			1					1		15	
	2-3	2	0.5	1	74.87217	149.74434	205.75017	411.50034	1	1716832	0.02	22	0.0000	1	0.011	15	0.0001
			0.5	2	130.878	261.756			1					1		15	
	3-1	2	0.5	1	197.8765	395.753	403.4838	806.9676	1	1716832	0.05	418	0.0005	1	0.011	15	0.0015
			0.5	2	205.6073	411.2146			1					1		15	
	3-2	2	0.5	1	160.7954	321.5908	340.0428	680.0856	1	1716832	0.04	291	0.0003	1	0.011	15	0.0010
			0.5	2	179.2474	358.4948			1					1		15	
	3-3	2	0.5	1	177.995	355.99	333.5193	667.0386	1	1716832	0.04	278	0.0003	1	0.011	15	0.0010
			0.5	2	155.5243	311.0486			1					1		15	
	4-1	2	0.5	1	610.609	1221.218	1164.1312	2328.2624	1	1716832	0.14	1939	0.0023	1	0.011	15	0.0068
			0.5	2	553.5222	1107.0444			1					1		15	
	4-2	2	0.5	1	626.538	1253.076	1256.5432	2513.0864	1	1716832	0.15	2124	0.0025	1	0.011	15	0.0074
			0.5	2	630.0052	1260.0104			1					1		15	
	4-3	2	0.5	1	693.2693	1386.5386	1260.0104	2520.0208	1	1716832	0.15	2131	0.0025	1	0.011	15	0.0075
			0.5	2	566.7411	1133.4822			1					1		15	
	5-1	2	0.5	1	2121.203	4242.406	4271.115	8542.23	1	1716832	0.50	8153	0.0095	1	0.011	15	0.0286
			0.5	2	2149.912	4299.824			1					1		15	
	5-2	2	0.5	1	2090.352	4180.704	4411.402	8822.804	1	1716832	0.51	8434	0.0098	1	0.011	15	0.0296
			0.5	2	2321.05	4642.1			1					1		15	
	5-3	2	0.5	1	2222.602	4445.204	4331.406	8662.812	1	1716832	0.50	8274	0.0097	1	0.011	15	0.0290
			0.5	2	2108.804	4217.608			1					1		15	
	6-1	2	0.5	1	2754.241	5508.482	5443.576	10887.152	1	1716832	0.63	10498	0.0123	1	0.011	15	0.0368
			0.5	2	2689.335	5378.67			1					1		15	
	6-2	2	0.5	1	2447.432	4894.864	5157.245	10314.49	1	1716832	0.60	9925	0.0116	1	0.011	15	0.0348
			0.5	2	2709.813	5419.626			1					1		15	
	6-3	2	0.5	1	2427.76	4855.52	5024.234	10048.468	1	1716832	0.59	9659	0.0113	1	0.011	15	0.0339
			0.5	2	2596.474	5192.948			1					1		15	
	7-1	2	0.5	1	3145.964	6291.928	6214.274	12428.548	1	1716832	0.72	12039	0.0141	1	0.011	15	0.0422
			0.5	2	3068.31	6136.62			1					1		15	
	7-2	2	0.5	1	3192.202	6384.404	6255.867	12511.734	1	1716832	0.73	12123	0.0142	1	0.011	15	0.0425
			0.5	2	3063.685	6127.33			1					1		15	
	7-3	2	0.5	1	3135.854	6271.708	6167.26	12334.52	1	1716832	0.72	11945	0.0139	1	0.011	15	0.0419
			0.5	2	3031.406	6062.812			1					1		15	
	8-1	2	0.5	1	3150.026	6300.052	6424.611	12849.222	1	1716832	0.75	12460	0.0145	1	0.011	15	0.0437
			0.5	2	3274.585	6549.17			1					1		15	
	8-2	2	0.5	1	3095.522	6191.044	6216.552	12433.104	1	1716832	0.72	12044	0.0141	1	0.011	15	0.0422
			0.5	2	3121.03	6242.06			1					1		15	
	8-3	2	0.5	1	3108.796	6217.592	6174.486	12348.972	1	1716832	0.72	11960	0.0140	1	0.011	15	0.0419
			0.5	2	3065.69	6131.38			1					1		15	

Assay Date	8/8/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0433	0.0000
Full activity	End	0.0421	0.0017
Full activity	Overall	0.0427	0.0012
Background	Beginning	0.0001	0.000221434
Background	End	-0.0001	0.000212337
Background	Overall	0.0000	0.000214896
Positive	Beginning	0.0169	0.0016
Positive	End	0.0229	0.0004
Positive	Overall	0.0199	0.0036
Negative	Beginning	0.0200	0.0017
Negative	End	0.0390	0.0014
Negative	Overall	0.0295	0.0111

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#3 PCZ	1	1	1.00E-04	-4.00	0.0005
RC#3 PCZ	1	2	1.00E-04	-4.00	0.0007
RC#3 PCZ	1	3	1.00E-04	-4.00	0.0017
RC#3 PCZ	2	1	1.00E-05	-5.00	0.0003
RC#3 PCZ	2	2	1.00E-05	-5.00	-0.0003
RC#3 PCZ	2	3	1.00E-05	-5.00	0.0001
RC#3 PCZ	3	1	1.00E-06	-6.00	0.0015
RC#3 PCZ	3	2	1.00E-06	-6.00	0.0010
RC#3 PCZ	3	3	1.00E-06	-6.00	0.0010
RC#3 PCZ	4	1	1.00E-07	-7.00	0.0068
RC#3 PCZ	4	2	1.00E-07	-7.00	0.0074
RC#3 PCZ	4	3	1.00E-07	-7.00	0.0075
RC#3 PCZ	5	1	1.00E-08	-8.00	0.0286
RC#3 PCZ	5	2	1.00E-08	-8.00	0.0296
RC#3 PCZ	5	3	1.00E-08	-8.00	0.0290
RC#3 PCZ	6	1	3.30E-09	-8.48	0.0368
RC#3 PCZ	6	2	3.30E-09	-8.48	0.0348
RC#3 PCZ	6	3	3.30E-09	-8.48	0.0339
RC#3 PCZ	7	1	1.00E-09	-9.00	0.0422
RC#3 PCZ	7	2	1.00E-09	-9.00	0.0425
RC#3 PCZ	7	3	1.00E-09	-9.00	0.0419
RC#3 PCZ	8	1	1.00E-10	-10.00	0.0437
RC#3 PCZ	8	2	1.00E-10	-10.00	0.0422
RC#3 PCZ	8	3	1.00E-10	-10.00	0.0419

Percent of control values				
		Replicate		
Level	Log[test substance]	1	2	3
1	-4.00	1.27	1.64	3.97
2	-5.00	0.79	-0.75	0.18
3	-6.00	3.43	2.39	2.28
4	-7.00	15.92	17.44	17.49
5	-8.00	66.94	69.24	67.93
6	-8.48	86.19	81.49	79.31
7	-9.00	98.85	99.53	98.08
8	-10.00	102.30	98.89	98.19

Assay Date	8/15/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8		
Technician ID	TM	Replicate #	4	Microsome type	Placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0198	30520.18	1541423		
2	0.0200	31076.36	1553818		
3	0.0204	31987.64	1568022		
4	0.0202	31947.96	1581582		
5	0.0203	31891.01	1570986		
			Average DPM/g soln	1563166	
			SD	15684	
			CV	1.00	
			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	12.4	12.4		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.1923 g
Mass of dilution B used in substrate prep	9.1029 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562175 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.

= 0.562175 + 0.00797
= 0.570145 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.235 µCi/µg ASDN

785222 dpm/nmol

Assay Date	8/15/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8						
Technician ID	TM	Replicate #	4	Microsome type	Placental	Microsome ID	6-041305				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	2	1	
	0.626	0.398	0.197	0.137	0.091	0.071	0.059			
	0.628	0.412	0.303	0.132	0.087	0.076	0.059			
	0.625	0.408	0.211	0.122	0.089	0.071	0.056			

Samples:	Microsome	QC 10	QC 100
	0.146	0.086	0.306
	0.129	0.082	0.319
	0.129	0.080	0.312

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
0.25	125	1000	0.00025	200	0.0500	0.626	0.568	0.0472
0.125	62.5	1000	0.00013	200	0.0250	0.406	0.347	0.0281
0.05	25	1000	0.00005	200	0.0100	0.237	0.179	0.0135
0.025	25	2000	0.00003	200	0.0050	0.131	0.072	0.0043
0.01	5	1000	0.00001	200	0.0020	0.089	0.031	0.0007
0.005	5	2000	0.00001	200	0.0010	0.073	0.015	-0.0007
			Blank	0.058				

Variables	Regression results	
m, b	0.087	-0.002
se_m, se_b	0.006	0.002
r^2, se_y	0.981	0.003
F, df	205	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

r^2= 0.981
m= 0.087
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
Microsome	0.146	0.088	0.006	200	90	72000	0.022	0.019	18.531
Microsome	0.129	0.071	0.004	200	90	72000	0.017		
Microsome	0.129	0.071	0.004	200	90	72000	0.016		
QC 10	0.086	0.028	0.000	200	200	200	0.000	0.000	0.001
QC 10	0.082	0.024	0.000	200	200	200	0.000		
QC 10	0.080	0.021	0.000	200	200	200	0.000		
QC 100	0.306	0.248	0.019	200	200	200	0.000	0.000	0.100
QC 100	0.319	0.260	0.021	200	200	200	0.000		
QC 100	0.312	0.254	0.020	200	200	200	0.000		

Assay Date	8/15/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	Placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	4

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	18.53052
Protein Concentration (dilution added to assay, mg/mL):	0.023163

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	1.00E-07
5	3.30E-08
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	8/15/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	Placental	Microsome ID	6-041305	Technician ID	TM	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	7348.498	14696.996	14939.842	29879.684	1	1563166	1.91	29480	0.0375	1	0.012	15	0.1081
			0.5	2	7591.344	15182.688			1					1		15	
	2	2	0.5	1	7530.745	15061.49	14901.114	29802.228	1	1563166	1.91	29402	0.0374	1	0.012	15	0.1078
			0.5	2	7370.369	14740.738			1					1		15	
	3	2	0.5	1	7418.883	14837.766	14935.856	29871.712	1	1563166	1.91	29472	0.0375	1	0.012	15	0.1080
			0.5	2	7516.973	15033.946			1					1		15	
	4	2	0.5	1	7619.535	15239.07	15145.731	30291.462	1	1563166	1.94	29892	0.0381	1	0.012	15	0.1096
			0.5	2	7526.196	15052.392			1					1		15	
Background control	1	2	0.5	1	123.0217	246.0434	188.63329	377.26658	1	1563166	0.02	-23	0.0000	1	0.012	15	-0.0001
			0.5	2	65.61159	131.22318			1					1		15	
	2	2	0.5	1	89.54582	179.09164	177.68915	355.3783	1	1563166	0.02	-44	-0.0001	1	0.012	15	-0.0002
			0.5	2	88.14333	176.28666			1					1		15	
	3	2	0.5	1	97.68635	195.3727	245.31245	490.6249	1	1563166	0.03	91	0.0001	1	0.012	15	0.0003
			0.5	2	147.6261	295.2522			1					1		15	
	4	2	0.5	1	90.21593	180.43186	187.9028	375.8056	1	1563166	0.02	-24	0.0000	1	0.012	15	-0.0001
			0.5	2	97.58687	195.37374			1					1		15	
Positive control	1	2	0.5	1	3341.201	6682.402	6465.754	12931.508	1	1563166	0.83	12532	0.0160	1	0.012	15	0.0459
			0.5	2	3124.553	6249.106			1					1		15	
	2	2	0.5	1	3086.495	6172.99	6205.796	12411.592	1	1563166	0.79	12012	0.0153	1	0.012	15	0.0440
			0.5	2	3119.301	6238.602			1					1		15	
	3	2	0.5	1	3078.293	6156.586	6320.082	12640.164	1	1563166	0.81	12240	0.0156	1	0.012	15	0.0449
			0.5	2	3241.789	6483.578			1					1		15	
	4	2	0.5	1	3087.787	6175.574	6226.882	12453.764	1	1563166	0.80	12054	0.0154	1	0.012	15	0.0442
			0.5	2	3139.095	6278.19			1					1		15	
Negative Control	1	2	0.5	1	6384.916	12769.832	12962.514	25925.028	1	1563166	1.66	25525	0.0325	1	0.012	15	0.0936
			0.5	2	6577.598	13155.196			1					1		15	
	2	2	0.5	1	6383.498	12766.996	12856.558	25713.116	1	1563166	1.64	25313	0.0322	1	0.012	15	0.0928
			0.5	2	6473.06	12946.12			1					1		15	
	3	2	0.5	1	6995.873	13991.746	13997.795	27995.59	1	1563166	1.79	27598	0.0351	1	0.012	15	0.1011
			0.5	2	7001.922	14003.844			1					1		15	
	4	2	0.5	1	7061.485	14122.97	14049.635	28099.27	1	1563166	1.80	27700	0.0353	1	0.012	15	0.1015
			0.5	2	6988.15	13976.3			1					1		15	
RC#3 PCZ	1-1	2	0.5	1	115.6887	231.3774	245.1499	490.2998	1	1563166	0.03	91	0.0001	1	0.012	15	0.0003
			0.5	2	129.4612	258.9224			1					1		15	
	1-2	2	0.5	1	90.21642	180.43284	192.13272	384.26544	1	1563166	0.02	-16	0.0000	1	0.012	15	-0.0001
			0.5	2	101.9163	203.8326			1					1		15	
	1-3	2	0.5	1	110.1797	220.3594	173.05781	346.11562	1	1563166	0.02	-54	-0.0001	1	0.012	15	-0.0002
			0.5	2	62.87811	125.75622			1					1		15	
	2-1	2	0.5	1	129.4612	258.9224	255.2174	510.4348	1	1563166	0.03	111	0.0001	1	0.012	15	0.0004
			0.5	2	125.7562	251.5124			1					1		15	
	2-2	2	0.5	1	116.6815	233.363	234.2362	468.4724	1	1563166	0.03	69	0.0001	1	0.012	15	0.0003
			0.5	2	117.5547	235.1094			1					1		15	
	2-3	2	0.5	1	149.2438	298.4876	275.9505	551.901	1	1563166	0.04	152	0.0002	1	0.012	15	0.0006
			0.5	2	126.7067	253.4134			1					1		15	
	3-1	2	0.5	1	278.0966	556.1932	507.4386	1014.8772	1	1563166	0.06	615	0.0008	1	0.012	15	0.0023
			0.5	2	229.342	458.684			1					1		15	
	3-2	2	0.5	1	261.6768	523.3536	534.3731	1068.7462	1	1563166	0.07	669	0.0009	1	0.012	15	0.0025
			0.5	2	272.6963	545.3926			1					1		15	
	3-3	2	0.5	1	253.1979	506.3958	529.3163	1058.6325	1	1563166	0.07	669	0.0008	1	0.012	15	0.0024
			0.5	2	276.1184	552.2368			1					1		15	
	4-1	2	0.5	1	1372.39	2744.78	2840.414	5680.828	1	1563166	0.36	5281	0.0067	1	0.012	15	0.0194
			0.5	2	1468.024	2936.048			1					1		15	
	4-2	2	0.5	1	1150.165	2300.33	2466.82	4933.64	1	1563166	0.32	4534	0.0058	1	0.012	15	0.0166
			0.5	2	1316.655	2633.31			1					1		15	
	4-3	2	0.5	1	1416.467	2832.934	2854.32	5708.64	1	1563166	0.37	5309	0.0068	1	0.012	15	0.0195
			0.5	2	1437.853	2875.706			1					1		15	
	5-1	2	0.5	1	5809.421	11618.842	11716.794	23433.588	1	1563166	1.50	23034	0.0293	1	0.012	15	0.0844
			0.5	2	5907.373	11814.746			1					1		15	
	5-2	2	0.5	1	5918.228	11836.456	11973.429	23946.858	1	1563166	1.53	23547	0.0300	1	0.012	15	0.0863
			0.5	2	6055.201	12110.402			1					1		15	
	5-3	2	0.5	1	5939.376	11878.752	11721.927	23443.854	1	1563166	1.50	23044	0.0293	1	0.012	15	0.0845
			0.5	2	5782.551	11565.102			1					1		15	
	6-1	2	0.5	1	4527.248	9054.496	9027.157	18054.314	1	1563166	1.15	17655	0.0225	1	0.012	15	0.0647
			0.5	2	4499.909	8999.818			1					1		15	
	6-2	2	0.5	1	4781.255	9562.51	9174.544	18349.088	1	1563166	1.17	17949	0.0229	1	0.012	15	0.0658
			0.5	2	4393.289	8786.578			1					1		15	
	6-3	2	0.5	1	4775.828	9551.656	9581.928	19163.856	1	1563166	1.23	18764	0.0239	1	0.012	15	0.0688
			0.5	2	4806.1	9612.2			1					1		15	
	7-1	2	0.5	1	5817.826	11635.652	11768.616	23537.232	1	1563166	1.51	23137	0.0295	1	0.012	15	0.0848
			0.5	2	5950.79	11901.58			1					1		15	
	7-2	2	0.5	1	5896.278	11792.556	12126.711	24253.422	1	1563166	1.55	23854	0.0304	1	0.012	15	0.0874
			0.5	2	6230.433	12460.866			1					1		15	
	7-3	2	0.5	1	5855.816	11711.632	11730.639	23461.278	1	1563166	1.50	23062	0.0294	1	0.012	15	0.0845
			0.5	2	5874.823	11749.646			1					1		15	
	8-1	2	0.5	1	6564.05	13128.1	13667.926	27335.852	1	1563166	1.75	26936	0.0343	1	0.012	15	0.0987
			0.5	2	7103.876	14207.752			1					1		15	
	8-2	2	0.5	1	6732.289	13464.578	13535.13	27070.26	1	1563166	1.73	26670	0.0340	1	0.012	15	0.0978
			0.5	2	6802.841	13605.682			1					1		15	
	8-3	2	0.5	1	5969.855	11979.71	12076.322	24152.644	1	1563166	1.55	23753	0.0302	1	0.012	15	0.0871
			0.5	2	6086.467	12172.934			1					1		15	

Assay Date	8/15/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested		Microsome 8 type	Placental	Microsome ID	6-041305	Technician ID TM	Replicate # 4

Control Type	Portion	Average	SD
Full activity	Beginning	0.1079	0.0002
Full activity	End	0.1088	0.0011
Full activity	Overall	0.1084	0.0008
Background	Beginning	-0.0001	5.67303E-05
Background	End	0.0001	0.00029759
Background	Overall	0.0000	0.000225015
Positive	Beginning	0.0450	0.0013
Positive	End	0.0445	0.0005
Positive	Overall	0.0448	0.0009
Negative	Beginning	0.0932	0.0005
Negative	End	0.1013	0.0003
Negative	Overall	0.0973	0.0047

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#3 PCZ	1	1	1.00E-04	-4.00	0.0003
RC#3 PCZ	1	2	1.00E-04	-4.00	-0.0001
RC#3 PCZ	1	3	1.00E-04	-4.00	-0.0002
RC#3 PCZ	2	1	1.00E-05	-5.00	0.0004
RC#3 PCZ	2	2	1.00E-05	-5.00	0.0003
RC#3 PCZ	2	3	1.00E-05	-5.00	0.0006
RC#3 PCZ	3	1	1.00E-06	-6.00	0.0023
RC#3 PCZ	3	2	1.00E-06	-6.00	0.0025
RC#3 PCZ	3	3	1.00E-06	-6.00	0.0024
RC#3 PCZ	4	1	1.00E-07	-7.00	0.0194
RC#3 PCZ	4	2	1.00E-07	-7.00	0.0166
RC#3 PCZ	4	3	1.00E-07	-7.00	0.0195
RC#3 PCZ	5	1	3.30E-08	-7.48	0.0844
RC#3 PCZ	5	2	3.30E-08	-7.48	0.0863
RC#3 PCZ	5	3	3.30E-08	-7.48	0.0845
RC#3 PCZ	6	1	1.00E-08	-8.00	0.0647
RC#3 PCZ	6	2	1.00E-08	-8.00	0.0658
RC#3 PCZ	6	3	1.00E-08	-8.00	0.0688
RC#3 PCZ	7	1	1.00E-09	-9.00	0.0848
RC#3 PCZ	7	2	1.00E-09	-9.00	0.0874
RC#3 PCZ	7	3	1.00E-09	-9.00	0.0845
RC#3 PCZ	8	1	1.00E-10	-10.00	0.0987
RC#3 PCZ	8	2	1.00E-10	-10.00	0.0978
RC#3 PCZ	8	3	1.00E-10	-10.00	0.0871

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.31	-0.05	-0.18
2	-5.00	0.37	0.23	0.51
3	-6.00	2.08	2.26	2.23
4	-7.00	17.86	15.34	17.96
5	-7.48	77.92	79.65	77.95
6	-8.00	59.72	60.72	63.47
7	-9.00	78.27	80.69	78.01
8	-10.00	91.12	90.22	80.35

Assay Date	9/20/2005	Test Chemical ID	RC#4 NP	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	Placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0203	30593.51	1507069
2	0.0201	32880.06	1635824
3	0.0201	34721.77	1727451
4	0.0204	34491.64	1690767
5	0.0202	35075.27	1736400
		Average DPM/g soln	1659502
		SD	93947
		CV	5.66
		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	16.4	16.4		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.1575 g
Mass of dilution B used in substrate prep	9.0995 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563175 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00846 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/bc

2) Calculate total µg ASDN/g soln.

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

= 0.563175 + 0.00846
= 0.571637 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.308 µCi/µg ASDN

831439 dpm/nmol

Assay Date	9/20/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8					
Technician ID	TM	Replicate #	2	Microsome type	Placental	Microsome ID	BAA			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	2	1	
	0.654	0.438	0.239	0.164	0.103	0.091	0.073			
	0.672	0.432	0.245	0.169	0.098	0.085	0.074			
	0.680	0.440	0.245	0.177	0.101	0.088	0.074			

Samples:	Microsomes	QC 10	QC100
	0.168	0.104	0.347
	0.160	0.107	0.343
	0.156	0.106	0.353

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	
0.25	125	1000	0.00025	200	0.0500	0.669	0.595	0.0475	m, b	0.083	-0.002
0.125	62.5	1000	0.00013	200	0.0250	0.437	0.363	0.0281	se_m, se_b	0.005	0.002
0.05	25	1000	0.00005	200	0.0100	0.243	0.169	0.0121	r^2, se_y	0.985	0.003
0.025	25	2000	0.00003	200	0.0050	0.170	0.096	0.0060	F, df	255	4
0.01	5	1000	0.00001	200	0.0020	0.100	0.027	0.0002	ss_{reg}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.088	0.014	-0.0008			

Regression results are calculated using the function LINEST

Blank	0.074	r^2=	0.985		
		m=	0.083		
		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
Microsomes	0.168	0.095	0.006	200	220	70400	0.009	0.008	8.450
Microsomes	0.160	0.086	0.005	200	220	70400	0.008		
Microsomes	0.156	0.083	0.005	200	220	70400	0.008		
QC 10	0.104	0.030	0.000	200	1	1	0.000	0.000	0.003
QC 10	0.107	0.034	0.001	200	1	1	0.000		
QC 10	0.106	0.032	0.001	200	1	1	0.000		
QC100	0.347	0.273	0.021	200	1	1	0.000	0.000	0.104
QC100	0.343	0.269	0.020	200	1	1	0.000		
QC100	0.353	0.279	0.021	200	1	1	0.000		

Assay Date	9/20/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	2

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.44953
Protein Concentration (dilution added to assay, mg/mL):	0.026405

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
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	9/20/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	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	4176.091	8352.182	8384.954	16769.908	1	1659502	1.01	16241	0.0195	1	0.013	15	0.0493
			0.5	2	4208.863	8417.726			1					1		15	
	2	2	0.5	1	4070.264	8140.528	7939.729	15879.458	1	1659502	0.96	15351	0.0185	1	0.013	15	0.0466
			0.5	2	3869.465	7738.93			1					1		15	
	3	2	0.5	1	3967.623	7935.246	7714.98	15429.96	1	1659502	0.93	14901	0.0179	1	0.013	15	0.0452
			0.5	2	3747.357	7494.714			1					1		15	
	4	2	0.5	1	4015.994	8031.988	7835.47	15670.94	1	1659502	0.94	15142	0.0182	1	0.013	15	0.0460
			0.5	2	3819.476	7638.952			1					1		15	
Background control	1	2	0.5	1	113.9674	227.9348	202.96397	405.92794	1	1659502	0.02	-123	-0.0001	1	0.013	15	-0.0004
			0.5	2	88.99657	177.99314			1					1		15	
	2	2	0.5	1	117.6556	235.3112	268.0155	536.031	1	1659502	0.03	7	0.0000	1	0.013	15	0.0000
			0.5	2	150.3599	300.7198			1					1		15	
	3	2	0.5	1	98.93771	197.87542	248.68121	497.36242	1	1659502	0.03	-32	0.0000	1	0.013	15	-0.0001
			0.5	2	149.7435	299.487			1					1		15	
	4	2	0.5	1	157.7655	315.531	338.2354	676.4708	1	1659502	0.04	148	0.0002	1	0.013	15	0.0004
			0.5	2	180.4699	360.9398			1					1		15	
Positive control	1	2	0.5	1	2340.721	4681.442	4565.45	9130.9	1	1659502	0.55	8602	0.0103	1	0.013	15	0.0261
			0.5	2	2224.729	4449.458			1					1		15	
	2	2	0.5	1	2057.895	4115.79	4164.275	8328.55	1	1659502	0.50	7800	0.0094	1	0.013	15	0.0237
			0.5	2	2106.38	4212.76			1					1		15	
	3	2	0.5	1	1961.903	3923.806	4077.042	8154.084	1	1659502	0.49	7625	0.0092	1	0.013	15	0.0232
			0.5	2	2115.139	4230.278			1					1		15	
	4	2	0.5	1	2018.862	4037.724	4195.275	8390.55	1	1659502	0.51	7862	0.0095	1	0.013	15	0.0239
			0.5	2	2176.413	4352.826			1					1		15	
Negative Control	1	2	0.5	1	3494.926	6989.852	7125.453	14250.906	1	1659502	0.86	13722	0.0165	1	0.013	15	0.0417
			0.5	2	3630.527	7261.054			1					1		15	
	2	2	0.5	1	3618.503	7237.006	7252.143	14504.286	1	1659502	0.87	13975	0.0168	1	0.013	15	0.0424
			0.5	2	3633.64	7267.28			1					1		15	
	3	2	0.5	1	3559.102	7118.204	7071.53	14143.06	1	1659502	0.85	13614	0.0164	1	0.013	15	0.0413
			0.5	2	3512.428	7024.856			1					1		15	
	4	2	0.5	1	3439.702	6879.404	6895.565	13791.13	1	1659502	0.83	13262	0.0160	1	0.013	15	0.0403
			0.5	2	3455.863	6911.726			1					1		15	
RC#4 NYP	1-1	2	0.5	1	868.3276	1736.6552	1673.7081	3347.4162	1	1659502	0.20	2818	0.0034	1	0.013	15	0.0086
			0.5	2	805.3805	1610.761			1					1		15	
	1-2	2	0.5	1	719.9995	1439.979	1438.9712	2877.9424	1	1659502	0.17	2349	0.0028	1	0.013	15	0.0071
			0.5	2	721.9617	1443.9634			1					1		15	
	1-3	2	0.5	1	808.074	1616.148	1612.9499	3225.8998	1	1659502	0.19	2697	0.0032	1	0.013	15	0.0082
			0.5	2	804.8759	1609.7518			1					1		15	
	2-1	2	0.5	1	1659.255	3318.51	3445.099	6890.198	1	1659502	0.42	6361	0.0077	1	0.013	15	0.0193
			0.5	2	1785.844	3571.688			1					1		15	
	2-2	2	0.5	1	1684.624	3369.248	3353.204	6706.408	1	1659502	0.40	6177	0.0074	1	0.013	15	0.0188
			0.5	2	1668.58	3337.16			1					1		15	
	2-3	2	0.5	1	1858.57	3717.14	3785.172	7570.344	1	1659502	0.46	7041	0.0085	1	0.013	15	0.0214
			0.5	2	1926.602	3853.204			1					1		15	
	3-1	2	0.5	1	3387.535	6775.07	7059.275	14118.55	1	1659502	0.85	13590	0.0163	1	0.013	15	0.0413
			0.5	2	3671.74	7343.48			1					1		15	
	3-2	2	0.5	1	3665.963	7331.926	7318.458	14636.916	1	1659502	0.88	14108	0.0170	1	0.013	15	0.0428
			0.5	2	3652.495	7304.99			1					1		15	
	3-3	2	0.5	1	3416.345	6832.69	6837.21	13674.42	1	1659502	0.82	13145	0.0158	1	0.013	15	0.0399
			0.5	2	3420.885	6841.73			1					1		15	
	4-1	2	0.5	1	3790.813	7581.626	7888.256	15776.512	1	1659502	0.95	15248	0.0183	1	0.013	15	0.0463
			0.5	2	4097.443	8194.886			1					1		15	
	4-2	2	0.5	1	3997.294	7994.588	7780.415	15560.83	1	1659502	0.94	15032	0.0181	1	0.013	15	0.0456
			0.5	2	3783.121	7566.242			1					1		15	
	4-3	2	0.5	1	3551.099	7102.198	7455.881	14911.762	1	1659502	0.90	14383	0.0173	1	0.013	15	0.0437
			0.5	2	3904.782	7809.564			1					1		15	
	5-1	2	0.5	1	3795.275	7590.55	7401.998	14803.996	1	1659502	0.89	14275	0.0172	1	0.013	15	0.0433
			0.5	2	3608.723	7213.446			1					1		15	
	5-2	2	0.5	1	3695.612	7391.224	7483.712	14967.424	1	1659502	0.90	14438	0.0174	1	0.013	15	0.0438
			0.5	2	3788.1	7576.2			1					1		15	
	5-3	2	0.5	1	3508.605	7017.21	7000.876	14001.752	1	1659502	0.84	13473	0.0162	1	0.013	15	0.0409
			0.5	2	3492.271	6984.542			1					1		15	
	6-1	2	0.5	1	3820.662	7641.324	7621.324	15242.648	1	1659502	0.92	14714	0.0177	1	0.013	15	0.0447
			0.5	2	3800.662	7601.324			1					1		15	
	6-2	2	0.5	1	3953.625	7907.25	7994.017	15988.034	1	1659502	0.96	15459	0.0186	1	0.013	15	0.0469
			0.5	2	4040.392	8080.784			1					1		15	
	6-3	2	0.5	1	3663.408	7326.816	7540.737	15081.474	1	1659502	0.91	14553	0.0175	1	0.013	15	0.0442
			0.5	2	3877.329	7754.658			1					1		15	
	7-1	2	0.5	1	3724.095	7448.19	7476.919	14953.838	1	1659502	0.90	14425	0.0173	1	0.013	15	0.0438
			0.5	2	3752.824	7505.648			1					1		15	
	7-2	2	0.5	1	3846.453	7692.906	7490.886	14981.772	1	1659502	0.90	14453	0.0174	1	0.013	15	0.0439
			0.5	2	3644.433	7288.866			1					1		15	
	7-3	2	0.5	1	3682.126	7364.252	7348.108	14696.216	1	1659502	0.89	14167	0.0170	1	0.013	15	0.0430
			0.5	2	3665.982	7331.964			1					1		15	
	8-1	2	0.5	1	3377.288	6754.576	6612.853	13225.706	1	1659502	0.80	12697	0.0153	1	0.013	15	0.0386
			0.5	2	3235.565	6471.13			1					1		15	
	8-2	2	0.5	1	3884.794	7769.588	7489.374	14978.748	1	1659502	0.90	14450	0.0174	1	0.013	15	0.0439
			0.5	2	3604.58	7209.16			1					1		15	
	8-3	2	0.5	1	2983.132	5966.264	6148.122	12296.244	1	1659502	0.74	11767	0.0142	1	0.013	15	0.0357
			0.5	2	3164.99	6329.98			1					1		15	

Assay Date	9/20/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0480	0.0019
Full activity	End	0.0456	0.0005
Full activity	Overall	0.0468	0.0018
Background	Beginning	-0.0002	0.000279363
Background	End	0.0002	0.000384589
Background	Overall	0.0000	0.000341516
Positive	Beginning	0.0249	0.0017
Positive	End	0.0235	0.0005
Positive	Overall	0.0242	0.0013
Negative	Beginning	0.0421	0.0005
Negative	End	0.0408	0.0008
Negative	Overall	0.0414	0.0009

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#4 NYP	1	1	1.00E-04	-4.00	0.0086
RC#4 NYP	1	2	1.00E-04	-4.00	0.0071
RC#4 NYP	1	3	1.00E-04	-4.00	0.0082
RC#4 NYP	2	1	3.30E-05	-4.48	0.0193
RC#4 NYP	2	2	3.30E-05	-4.48	0.0188
RC#4 NYP	2	3	3.30E-05	-4.48	0.0214
RC#4 NYP	3	1	1.00E-05	-5.00	0.0413
RC#4 NYP	3	2	1.00E-05	-5.00	0.0428
RC#4 NYP	3	3	1.00E-05	-5.00	0.0399
RC#4 NYP	4	1	1.00E-06	-6.00	0.0463
RC#4 NYP	4	2	1.00E-06	-6.00	0.0456
RC#4 NYP	4	3	1.00E-06	-6.00	0.0437
RC#4 NYP	5	1	1.00E-07	-7.00	0.0433
RC#4 NYP	5	2	1.00E-07	-7.00	0.0438
RC#4 NYP	5	3	1.00E-07	-7.00	0.0409
RC#4 NYP	6	1	1.00E-08	-8.00	0.0447
RC#4 NYP	6	2	1.00E-08	-8.00	0.0469
RC#4 NYP	6	3	1.00E-08	-8.00	0.0442
RC#4 NYP	7	1	1.00E-09	-9.00	0.0438
RC#4 NYP	7	2	1.00E-09	-9.00	0.0439
RC#4 NYP	7	3	1.00E-09	-9.00	0.0430
RC#4 NYP	8	1	1.00E-10	-10.00	0.0386
RC#4 NYP	8	2	1.00E-10	-10.00	0.0439
RC#4 NYP	8	3	1.00E-10	-10.00	0.0357

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	18.29	15.24	17.50
2	-4.48	41.28	40.09	45.70
3	-5.00	88.19	91.56	85.31
4	-6.00	98.95	97.56	93.34
5	-7.00	92.64	93.70	87.44
6	-8.00	95.49	100.33	94.44
7	-9.00	93.62	93.80	91.94
8	-10.00	82.40	93.78	76.37

Assay Date	9/21/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8		
Technician ID	TM	Replicate #	3	Microsome type	Placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0205	28804.71	1405108		
2	0.0201	31256.42	1555046		
3	0.0206	31605.91	1534267		
4	0.0205	32366.37	1578847		
5	0.0204	32936.71	1614545		
			Average DPM/g soln	1537563	
			SD	79830	
			CV	5.19	
			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	13	13		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.1749 g
Mass of dilution B used in substrate prep	9.0994 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562563 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.

= 0.562563 + 0.00784
= 0.570403 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.214 µCi/µg ASDN

772012 dpm/nmol

Assay Date	9/21/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8				
Technician ID	TM	Replicate #	3	Microsome type	Placental	Microsome ID	BAA		

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.602	0.390	0.200	0.120	0.032	0.054	0.035	2	1	
	0.625	0.402	0.185	0.112	0.031	0.042	0.032			
	0.628	0.396	0.199	0.118	0.036	0.042	0.027			

Samples:	Microsomes	QC 10	QC100
	0.113	0.056	0.283
	0.109	0.059	0.280
	0.103	0.059	0.312

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
0.25	125	1000	0.00025	200	0.0500	0.619	0.587	0.0470
0.125	62.5	1000	0.00013	200	0.0250	0.396	0.364	0.0287
0.05	25	1000	0.00005	200	0.0100	0.195	0.163	0.0123
0.025	25	2000	0.00003	200	0.0050	0.117	0.085	0.0059
0.01	5	1000	0.00001	200	0.0020	0.033	0.002	-0.0009
0.005	5	2000	0.00001	200	0.0010	0.046	0.015	0.0001
		Blank	0.031					

Variables	Regression results	
m, b	0.082	-0.001
se_m, se_b	0.006	0.002
r^2, se_y	0.979	0.003
F, df	184	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.979
m= 0.082
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
Microsomes	0.113	0.082	0.006	200	220	70400	0.009	0.008	8.378
Microsomes	0.109	0.078	0.005	200	220	70400	0.008		
Microsomes	0.103	0.072	0.005	200	220	70400	0.008		
QC 10	0.056	0.025	0.001	200	1	1	0.000	0.000	0.006
QC 10	0.059	0.028	0.001	200	1	1	0.000		
QC 10	0.059	0.028	0.001	200	1	1	0.000		
QC100	0.283	0.252	0.020	200	1	1	0.000	0.000	0.101
QC100	0.280	0.249	0.019	200	1	1	0.000		
QC100	0.312	0.281	0.022	200	1	1	0.000		

Assay Date	9/21/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	3

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
3	1.00E-05
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Protein Concentration (stock microsomes, mg/mL):	8.378192
Protein Concentration (dilution added to assay, mg/mL):	0.026182

Assay Date	9/21/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	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	4385.032	8770.064	8992.572	17985.144	1	1537563	1.17	17664	0.0229	1	0.013	15	0.0583
			0.5	2	4607.54	9215.08			1					1		15	
	2	2	0.5	1	4557.514	9115.028	9120.253	18240.506	1	1537563	1.19	17919	0.0232	1	0.013	15	0.0591
			0.5	2	4562.739	9125.478			1					1		15	
	3	2	0.5	1	4341.615	8683.23	8581.321	17162.642	1	1537563	1.12	16841	0.0218	1	0.013	15	0.0555
			0.5	2	4239.706	8479.412			1					1		15	
	4	2	0.5	1	4010.988	8021.976	8105.674	16211.348	1	1537563	1.05	15890	0.0206	1	0.013	15	0.0524
			0.5	2	4094.686	8189.372			1					1		15	
Background control	1	2	0.5	1	78.11341	156.22682	143.23764	286.47528	1	1537563	0.02	-35	0.0000	1	0.013	15	-0.0001
			0.5	2	65.12423	130.24846			1					1		15	
	2	2	0.5	1	64.558	129.116	194.8065	389.613	1	1537563	0.03	68	0.0001	1	0.013	15	0.0002
			0.5	2	130.2485	260.497			1					1		15	
	3	2	0.5	1	94.97284	189.94568	180.54058	361.08116	1	1537563	0.02	40	0.0001	1	0.013	15	0.0001
			0.5	2	85.56774	171.13548			1					1		15	
	4	2	0.5	1	59.25845	118.5169	124.38302	248.76604	1	1537563	0.02	-73	-0.0001	1	0.013	15	-0.0002
			0.5	2	65.12457	130.24914			1					1		15	
Positive control	1	2	0.5	1	2270.688	4541.376	4457.789	8915.578	1	1537563	0.58	8594	0.0111	1	0.013	15	0.0283
			0.5	2	2187.101	4374.202			1					1		15	
	2	2	0.5	1	2208.935	4417.87	4275.942	8551.884	1	1537563	0.56	8230	0.0107	1	0.013	15	0.0271
			0.5	2	2067.007	4134.014			1					1		15	
	3	2	0.5	1	2055.202	4110.404	4048.451	8096.902	1	1537563	0.53	7775	0.0101	1	0.013	15	0.0256
			0.5	2	1993.249	3986.498			1					1		15	
	4	2	0.5	1	2125.235	4250.47	4453.439	8906.878	1	1537563	0.58	8585	0.0111	1	0.013	15	0.0283
			0.5	2	2328.204	4656.408			1					1		15	
Negative Control	1	2	0.5	1	3690.205	7380.41	7372.311	14744.622	1	1537563	0.96	14423	0.0187	1	0.013	15	0.0476
			0.5	2	3682.106	7364.212			1					1		15	
	2	2	0.5	1	3679.43	7358.86	7348.115	14696.23	1	1537563	0.96	14375	0.0186	1	0.013	15	0.0474
			0.5	2	3668.685	7337.37			1					1		15	
	3	2	0.5	1	3712.101	7424.202	7450.79	14901.58	1	1537563	0.97	14580	0.0189	1	0.013	15	0.0481
			0.5	2	3738.689	7477.378			1					1		15	
	4	2	0.5	1	3912.112	7824.224	7820.497	15640.994	1	1537563	1.02	15320	0.0198	1	0.013	15	0.0505
			0.5	2	3908.385	7816.77			1					1		15	
RC#4 NYP	1-1	2	0.5	1	872.72	1745.44	1670.0197	3340.0394	1	1537563	0.22	3019	0.0039	1	0.013	15	0.0100
			0.5	2	797.2997	1594.5994			1					1		15	
	1-2	2	0.5	1	716.3704	1432.7408	1402.5619	2805.1238	1	1537563	0.18	2484	0.0032	1	0.013	15	0.0082
			0.5	2	686.1915	1372.383			1					1		15	
	1-3	2	0.5	1	643.7657	1287.5314	1306.3864	2612.7728	1	1537563	0.17	2291	0.0030	1	0.013	15	0.0076
			0.5	2	662.6207	1325.2414			1					1		15	
	2-1	2	0.5	1	1774.645	3549.29	3684.393	7368.786	1	1537563	0.48	7047	0.0091	1	0.013	15	0.0232
			0.5	2	1909.748	3819.496			1					1		15	
	2-2	2	0.5	1	1744.796	3489.592	3487.542	6975.084	1	1537563	0.45	6654	0.0086	1	0.013	15	0.0219
			0.5	2	1742.746	3485.492			1					1		15	
	2-3	2	0.5	1	1802.28	3604.56	3528.081	7056.162	1	1537563	0.46	6735	0.0087	1	0.013	15	0.0222
			0.5	2	1725.801	3451.602			1					1		15	
	3-1	2	0.5	1	3714.814	7429.628	7135.661	14271.322	1	1537563	0.93	13950	0.0181	1	0.013	15	0.0460
			0.5	2	3420.847	6841.694			1					1		15	
	3-2	2	0.5	1	3592.254	7184.508	7154.454	14308.908	1	1537563	0.93	13967	0.0181	1	0.013	15	0.0461
			0.5	2	3562.2	7124.4			1					1		15	
	3-3	2	0.5	1	3415.478	6830.956	6795.44	13590.88	1	1537563	0.88	13269	0.0172	1	0.013	15	0.0438
			0.5	2	3379.962	6759.924			1					1		15	
	4-1	2	0.5	1	3687.474	7374.948	7450.426	14900.852	1	1537563	0.97	14579	0.0189	1	0.013	15	0.0481
			0.5	2	3762.952	7525.904			1					1		15	
	4-2	2	0.5	1	3813.153	7626.306	7654.219	15308.438	1	1537563	1.00	14987	0.0194	1	0.013	15	0.0494
			0.5	2	3841.066	7682.132			1					1		15	
	4-3	2	0.5	1	4083.875	8167.75	8089.058	16178.116	1	1537563	1.05	15857	0.0205	1	0.013	15	0.0523
			0.5	2	4005.183	8010.366			1					1		15	
	5-1	2	0.5	1	3968.901	7977.802	7935.017	15870.034	1	1537563	1.03	15549	0.0201	1	0.013	15	0.0513
			0.5	2	3946.116	7892.232			1					1		15	
	5-2	2	0.5	1	3839.657	7679.314	7727.743	15455.485	1	1537563	1.01	15134	0.0196	1	0.013	15	0.0499
			0.5	2	3888.086	7776.172			1					1		15	
	5-3	2	0.5	1	4002.3	8004.6	8010.196	16020.392	1	1537563	1.04	15699	0.0203	1	0.013	15	0.0518
			0.5	2	4007.895	8015.792			1					1		15	
	6-1	2	0.5	1	3754.324	7508.648	7655.521	15311.042	1	1537563	1.00	14990	0.0194	1	0.013	15	0.0494
			0.5	2	3901.197	7802.394			1					1		15	
	6-2	2	0.5	1	3730.628	7461.256	7464.457	14928.914	1	1537563	0.97	14607	0.0189	1	0.013	15	0.0482
			0.5	2	3733.829	7467.658			1					1		15	
	6-3	2	0.5	1	3558.238	7116.476	7229.607	14459.214	1	1537563	0.94	14138	0.0183	1	0.013	15	0.0466
			0.5	2	3671.369	7342.738			1					1		15	
	7-1	2	0.5	1	3541.168	7082.336	7278.334	14556.668	1	1537563	0.95	14235	0.0184	1	0.013	15	0.0470
			0.5	2	3737.166	7474.332			1					1		15	
	7-2	2	0.5	1	3902.068	7804.136	7576.131	15152.262	1	1537563	0.99	14831	0.0192	1	0.013	15	0.0489
			0.5	2	3674.063	7348.126			1					1		15	
	7-3	2	0.5	1	3765.645	7531.29	7610.729	15221.458	1	1537563	0.99	14900	0.0193	1	0.013	15	0.0491
			0.5	2	3845.084	7690.168			1					1		15	
	8-1	2	0.5	1	3865.657	7731.314	7783.257	15566.514	1	1537563	1.01	15245	0.0197	1	0.013	15	0.0503
			0.5	2	3917.6	7835.2			1					1		15	
	8-2	2	0.5	1	3720.261	7440.522	7621.657	15243.314	1	1537563	0.99	14922	0.0193	1	0.013	15	0.0492
			0.5	2	3901.396	7802.792			1					1		15	
	8-3	2	0.5	1	3652.514	7305.028	7477.438	14954.876	1	1537563	0.97	14633	0.0190	1	0.013	15	0.0483
			0.5	2	3824.924	7649.848			1					1		15	

Assay Date	9/21/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested		Microsome 8 type	Placental	Microsome ID	BAA	Technician ID TM	Replicate # 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0587	0.0006
Full activity	End	0.0540	0.0022
Full activity	Overall	0.0563	0.0030
Background	Beginning	0.0001	0.00024054
Background	End	-0.0001	0.000261944
Background	Overall	0.0000	0.000214792
Positive	Beginning	0.0277	0.0008
Positive	End	0.0270	0.0019
Positive	Overall	0.0274	0.0013
Negative	Beginning	0.0475	0.0001
Negative	End	0.0493	0.0017
Negative	Overall	0.0484	0.0014

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#4 NYP	1	1	1.00E-04	-4.00	0.0100
RC#4 NYP	1	2	1.00E-04	-4.00	0.0082
RC#4 NYP	1	3	1.00E-04	-4.00	0.0076
RC#4 NYP	2	1	3.30E-05	-4.48	0.0232
RC#4 NYP	2	2	3.30E-05	-4.48	0.0219
RC#4 NYP	2	3	3.30E-05	-4.48	0.0222
RC#4 NYP	3	1	1.00E-05	-5.00	0.0460
RC#4 NYP	3	2	1.00E-05	-5.00	0.0461
RC#4 NYP	3	3	1.00E-05	-5.00	0.0438
RC#4 NYP	4	1	1.00E-06	-6.00	0.0481
RC#4 NYP	4	2	1.00E-06	-6.00	0.0494
RC#4 NYP	4	3	1.00E-06	-6.00	0.0523
RC#4 NYP	5	1	1.00E-07	-7.00	0.0513
RC#4 NYP	5	2	1.00E-07	-7.00	0.0499
RC#4 NYP	5	3	1.00E-07	-7.00	0.0518
RC#4 NYP	6	1	1.00E-08	-8.00	0.0494
RC#4 NYP	6	2	1.00E-08	-8.00	0.0482
RC#4 NYP	6	3	1.00E-08	-8.00	0.0466
RC#4 NYP	7	1	1.00E-09	-9.00	0.0470
RC#4 NYP	7	2	1.00E-09	-9.00	0.0489
RC#4 NYP	7	3	1.00E-09	-9.00	0.0491
RC#4 NYP	8	1	1.00E-10	-10.00	0.0503
RC#4 NYP	8	2	1.00E-10	-10.00	0.0492
RC#4 NYP	8	3	1.00E-10	-10.00	0.0483

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	17.67	14.54	13.42
2	-4.48	41.26	38.96	39.43
3	-5.00	81.68	81.90	77.70
4	-6.00	85.37	87.75	92.85
5	-7.00	91.04	88.61	91.92
6	-8.00	87.77	85.53	82.78
7	-9.00	83.35	86.84	87.24
8	-10.00	89.26	87.37	85.68

Assay Date	10/10/2005	Test Chemical ID	RC#4 NP	# Concentrations tested	8		
Technician ID	TM	Replicate #	6	Microsome type	Placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0204	28731.38	1408401	
2	0.0201	29676.78	1476457	
3	0.0201	31079.11	1546224	
4	0.0200	34948.82	1747441	
5	0.0204	32950.19	1615205	
			Average DPM/g soln	1558746
			SD	130696
			CV	8.38
			µCi/g soln	0.702

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	15.9	15.9		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.1122 g
Mass of dilution B used in substrate prep	4.5537 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56134 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00795 µg/g soln.
µg/g soln.

a. µCi/g soln	0.702
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.561340 + 0.00795
= 0.569288 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.233 µCi/µg ASDN

784181 dpm/nmol

Assay Date	10/10/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8				
Technician ID	TM	Replicate #	6	Microsome type	Placental	Microsome ID	BAA		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	2	1	
	0.580	0.375	0.185	0.112	0.065	0.051	0.029			
	0.604	0.366	0.191	0.119	0.065	0.057	0.030			
	0.620	0.373	0.191	0.116	0.070	0.053	0.035			

Samples:	Microsomes	QC 10	QC100
	0.093	0.061	0.294
	0.087	0.064	0.267
	0.089	0.062	0.287

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
0.25	125	1000	0.00025	200	0.0500	0.601	0.569	0.0480
0.125	62.5	1000	0.00013	200	0.0250	0.371	0.339	0.0277
0.05	25	1000	0.00005	200	0.0100	0.189	0.157	0.0116
0.025	25	2000	0.00003	200	0.0050	0.116	0.084	0.0052
0.01	5	1000	0.00001	200	0.0020	0.067	0.035	0.0008
0.005	5	2000	0.00001	200	0.0010	0.054	0.022	-0.0003
			Blank	0.032				

Variables	Regression results	
m, b	0.088	-0.002
se_m, se_b	0.004	0.001
r^2, se_y	0.991	0.002
F, df	420	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.991
m= 0.088
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
Microsomes	0.093	0.062	0.003	200	140	44800	0.005	0.005	4.602
Microsomes	0.087	0.056	0.003	200	140	44800	0.004		
Microsomes	0.089	0.058	0.003	200	140	44800	0.004		
QC 10	0.061	0.030	0.000	200	1	1	0.000	0.000	0.002
QC 10	0.064	0.033	0.001	200	1	1	0.000		
QC 10	0.062	0.031	0.000	200	1	1	0.000		
QC100	0.294	0.263	0.021	200	1	1	0.000	0.000	0.099
QC100	0.267	0.235	0.018	200	1	1	0.000		
QC100	0.287	0.255	0.020	200	1	1	0.000		

Assay Date	10/10/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	6

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	44.8	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.601825
Protein Concentration (dilution added to assay, mg/mL):	0.014381

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
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	10/10/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	6

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	4441.69	8883.38	8989.335	17978.67	1	1558746	1.15	17582	0.0224	1	0.007	15	0.1039
			0.5	2	4547.645	9095.29			1					1		15	
	2	2	0.5	1	4551.804	9103.608	9034.522	18069.044	1	1558746	1.16	17673	0.0225	1	0.007	15	0.1045
			0.5	2	4482.718	8965.436			1					1		15	
	3	2	0.5	1	4173.378	8346.756	8405.326	16810.652	1	1558746	1.08	16414	0.0209	1	0.007	15	0.0970
			0.5	2	4231.948	8463.896			1					1		15	
	4	2	0.5	1	4049.439	8098.878	8200.439	16400.878	1	1558746	1.05	16004	0.0204	1	0.007	15	0.0946
			0.5	2	4151	8302			1					1		15	
Background control	1	2	0.5	1	123.9515	247.903	261.3234	522.6468	1	1558746	0.03	126	0.0002	1	0.007	15	0.0007
			0.5	2	137.3719	274.7438			1					1		15	
	2	2	0.5	1	92.94975	185.8995	172.23042	344.46084	1	1558746	0.02	-52	-0.0001	1	0.007	15	-0.0003
			0.5	2	79.28067	158.56134			1					1		15	
	3	2	0.5	1	87.48212	174.96424	175.72386	351.44772	1	1558746	0.02	-45	-0.0001	1	0.007	15	-0.0003
			0.5	2	88.24174	176.48348			1					1		15	
	4	2	0.5	1	79.87989	159.75978	183.76549	367.53098	1	1558746	0.02	-29	0.0000	1	0.007	15	-0.0002
			0.5	2	103.8856	207.7712			1					1		15	
Positive control	1	2	0.5	1	2222.804	4445.208	4480.748	8961.496	1	1558746	0.57	8565	0.0109	1	0.007	15	0.0506
			0.5	2	2258.144	4516.288			1					1		15	
	2	2	0.5	1	2296.418	4592.836	4497.086	8994.172	1	1558746	0.58	8598	0.0110	1	0.007	15	0.0508
			0.5	2	2200.668	4401.336			1					1		15	
	3	2	0.5	1	2096.849	4193.698	4194.402	8388.804	1	1558746	0.54	7992	0.0102	1	0.007	15	0.0472
			0.5	2	2097.554	4195.108			1					1		15	
	4	2	0.5	1	2228.072	4456.144	4472.547	8945.094	1	1558746	0.57	8549	0.0109	1	0.007	15	0.0505
			0.5	2	2244.475	4488.95			1					1		15	
Negative Control	1	2	0.5	1	3521.866	7043.332	7201.205	14402.41	1	1558746	0.92	14006	0.0179	1	0.007	15	0.0828
			0.5	2	3679.539	7359.078			1					1		15	
	2	2	0.5	1	3764.485	7528.97	7425.029	14850.058	1	1558746	0.95	14454	0.0184	1	0.007	15	0.0854
			0.5	2	3660.544	7321.088			1					1		15	
	3	2	0.5	1	3593.236	7186.472	7663.236	15326.472	1	1558746	0.98	14930	0.0190	1	0.007	15	0.0883
			0.5	2	4070	8140			1					1		15	
	4	2	0.5	1	3700.979	7401.958	7470.064	14940.128	1	1558746	0.96	14544	0.0185	1	0.007	15	0.0860
			0.5	2	3769.085	7538.17			1					1		15	
RC#4 NYP	1-1	2	0.5	1	825.6169	1651.2338	1672.2384	3344.4728	1	1558746	0.21	2948	0.0038	1	0.007	15	0.0174
			0.5	2	846.6195	1693.239			1					1		15	
	1-2	2	0.5	1	716.3704	1432.7408	1356.7621	2713.5242	1	1558746	0.17	2317	0.0030	1	0.007	15	0.0137
			0.5	2	640.3917	1280.7834			1					1		15	
	1-3	2	0.5	1	606.9105	1213.821	1282.578	2565.156	1	1558746	0.16	2169	0.0028	1	0.007	15	0.0128
			0.5	2	675.6675	1351.335			1					1		15	
	2-1	2	0.5	1	1955.69	3911.38	3898.573	7797.146	1	1558746	0.50	7401	0.0094	1	0.007	15	0.0438
			0.5	2	1942.883	3885.766			1					1		15	
	2-2	2	0.5	1	1828.742	3653.484	3488.681	6977.362	1	1558746	0.45	6581	0.0084	1	0.007	15	0.0389
			0.5	2	1661.939	3323.878			1					1		15	
	2-3	2	0.5	1	1724.734	3449.468	3435.157	6870.314	1	1558746	0.44	6474	0.0083	1	0.007	15	0.0383
			0.5	2	1710.423	3420.846			1					1		15	
	3-1	2	0.5	1	3595.929	7191.858	7321.597	14643.194	1	1558746	0.94	14247	0.0182	1	0.007	15	0.0842
			0.5	2	3725.668	7451.336			1					1		15	
	3-2	2	0.5	1	3493.573	6987.146	7140.522	14281.044	1	1558746	0.92	13885	0.0177	1	0.007	15	0.0821
			0.5	2	3646.949	7293.898			1					1		15	
	3-3	2	0.5	1	3459.662	6919.324	7038.819	14077.638	1	1558746	0.90	13681	0.0174	1	0.007	15	0.0809
			0.5	2	3579.157	7158.314			1					1		15	
	4-1	2	0.5	1	3663.496	7326.992	7353.72	14707.44	1	1558746	0.94	14311	0.0182	1	0.007	15	0.0846
			0.5	2	3690.224	7380.448			1					1		15	
	4-2	2	0.5	1	3926.49	7852.98	7729.845	15459.69	1	1558746	0.99	15063	0.0192	1	0.007	15	0.0890
			0.5	2	3803.355	7606.71			1					1		15	
	4-3	2	0.5	1	3853.263	7706.526	7730.592	15461.184	1	1558746	0.99	15065	0.0192	1	0.007	15	0.0891
			0.5	2	3877.329	7754.658			1					1		15	
	5-1	2	0.5	1	4018.75	8037.5	7988.573	15977.146	1	1558746	1.03	15581	0.0199	1	0.007	15	0.0921
			0.5	2	3969.823	7939.646			1					1		15	
	5-2	2	0.5	1	3858.652	7717.304	7880.138	15760.276	1	1558746	1.01	15364	0.0196	1	0.007	15	0.0908
			0.5	2	4021.486	8042.972			1					1		15	
	5-3	2	0.5	1	3857.456	7714.912	7585.658	15171.716	1	1558746	0.97	14775	0.0188	1	0.007	15	0.0873
			0.5	2	3728.402	7456.804			1					1		15	
	6-1	2	0.5	1	3805.512	7611.024	7818.049	15636.098	1	1558746	1.00	15240	0.0194	1	0.007	15	0.0901
			0.5	2	4012.537	8025.074			1					1		15	
	6-2	2	0.5	1	3896.641	7793.282	7657.605	15315.21	1	1558746	0.98	14919	0.0190	1	0.007	15	0.0882
			0.5	2	3760.984	7521.928			1					1		15	
	6-3	2	0.5	1	3733.322	7466.644	7495.093	14990.186	1	1558746	0.96	14594	0.0186	1	0.007	15	0.0863
			0.5	2	3761.771	7523.542			1					1		15	
	7-1	2	0.5	1	3680.023	7360.046	7419.923	14839.846	1	1558746	0.95	14443	0.0184	1	0.007	15	0.0854
			0.5	2	3739.9	7479.8			1					1		15	
	7-2	2	0.5	1	3738.709	7477.418	7481.343	14962.686	1	1558746	0.96	14566	0.0186	1	0.007	15	0.0861
			0.5	2	3742.634	7485.268			1					1		15	
	7-3	2	0.5	1	3816.448	7632.896	7420.478	14840.956	1	1558746	0.95	14444	0.0184	1	0.007	15	0.0854
			0.5	2	3604.03	7208.06			1					1		15	
	8-1	2	0.5	1	3590.562	7181.124	7267.319	14534.638	1	1558746	0.93	14138	0.0180	1	0.007	15	0.0836
			0.5	2	3676.757	7353.514			1					1		15	
	8-2	2	0.5	1	3988.901	7977.802	7909.235	15818.47	1	1558746	1.01	15422	0.0197	1	0.007	15	0.0912
			0.5	2	3920.334	7840.668			1					1		15	
	8-3	2	0.5	1	3746.79	7493.58	7428.934	14857.868	1	1558746	0.95	14461	0.0184	1	0.007	15	0.0855
			0.5	2	3682.144	7364.288			1					1		15	

Assay Date	10/10/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Placental	Microsome ID	BAA	Technician ID	TM	Replicate #	6

Control Type	Portion	Average	SD
Full activity	Beginning	0.1042	0.0004
Full activity	End	0.0958	0.0017
Full activity	Overall	0.1000	0.0049
Background	Beginning	0.0002	0.000744853
Background	End	-0.0002	6.72313E-05
Background	Overall	0.0000	0.000500345
Positive	Beginning	0.0507	0.0001
Positive	End	0.0489	0.0023
Positive	Overall	0.0498	0.0017
Negative	Beginning	0.0841	0.0019
Negative	End	0.0871	0.0016
Negative	Overall	0.0856	0.0022

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#4 NYP	1	1	1.00E-04	-4.00	0.0174
RC#4 NYP	1	2	1.00E-04	-4.00	0.0137
RC#4 NYP	1	3	1.00E-04	-4.00	0.0128
RC#4 NYP	2	1	3.30E-05	-4.48	0.0438
RC#4 NYP	2	2	3.30E-05	-4.48	0.0389
RC#4 NYP	2	3	3.30E-05	-4.48	0.0383
RC#4 NYP	3	1	1.00E-05	-5.00	0.0842
RC#4 NYP	3	2	1.00E-05	-5.00	0.0821
RC#4 NYP	3	3	1.00E-05	-5.00	0.0809
RC#4 NYP	4	1	1.00E-06	-6.00	0.0846
RC#4 NYP	4	2	1.00E-06	-6.00	0.0890
RC#4 NYP	4	3	1.00E-06	-6.00	0.0891
RC#4 NYP	5	1	1.00E-07	-7.00	0.0921
RC#4 NYP	5	2	1.00E-07	-7.00	0.0908
RC#4 NYP	5	3	1.00E-07	-7.00	0.0873
RC#4 NYP	6	1	1.00E-08	-8.00	0.0901
RC#4 NYP	6	2	1.00E-08	-8.00	0.0882
RC#4 NYP	6	3	1.00E-08	-8.00	0.0863
RC#4 NYP	7	1	1.00E-09	-9.00	0.0854
RC#4 NYP	7	2	1.00E-09	-9.00	0.0861
RC#4 NYP	7	3	1.00E-09	-9.00	0.0854
RC#4 NYP	8	1	1.00E-10	-10.00	0.0836
RC#4 NYP	8	2	1.00E-10	-10.00	0.0912
RC#4 NYP	8	3	1.00E-10	-10.00	0.0855

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	17.42	13.70	12.82
2	-4.48	43.74	38.90	38.27
3	-5.00	84.21	82.07	80.87
4	-6.00	84.59	89.03	89.04
5	-7.00	92.09	90.81	87.33
6	-8.00	90.08	88.18	86.26
7	-9.00	85.37	86.10	85.38
8	-10.00	83.57	91.16	85.48

Assay Date	9/19/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8		
Technician ID	TM	Replicate #	1	Microsome type	Placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0206	29595.2	1436660		
2	0.0206	33722.76	1637027		
3	0.0201	35735.11	1777866		
4	0.0202	34956.41	1730515		
5	0.0201	35468.46	1764600		
			Average DPM/g soln		1669334
			SD		141226
			CV		8.46
			µCi/g soln		0.752

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	11.4	11.4		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.332 g
Mass of dilution B used in substrate prep	9.1614 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.560948 µg/g

Calculation of Substrate Solution Specific Activity

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

a. µCi/g soln	0.752
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.

= 0.560948 + 0.00851
= 0.569460 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.320 µCi/µg ASDN

839562 dpm/nmol

Assay Date	9/19/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8					
Technician ID	TM	Replicate #	1	Microsome type	Placental	Microsome ID	6-041305			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	2	1	
	0.597	0.368	0.182	0.103	0.055	0.041	0.030			
	0.612	0.371	0.180	0.114	0.054	0.046	0.032			
	0.584	0.379	0.186	0.110	0.051	0.041	0.032			

Samples:	Microsomes	QC 10	QC100
	0.118	0.063	0.304
	0.117	0.052	0.312
	0.101	0.055	0.316

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	
0.25	125	1000	0.00025	200	0.0500	0.598	0.567	0.0477	m, b	0.087	-0.001
0.125	62.5	1000	0.00013	200	0.0250	0.373	0.342	0.0282	se_m, se_b	0.005	0.001
0.05	25	1000	0.00005	200	0.0100	0.183	0.151	0.0117	r^2, se_y	0.987	0.002
0.025	25	2000	0.00003	200	0.0050	0.109	0.078	0.0053	F, df	311	4
0.01	5	1000	0.00001	200	0.0020	0.053	0.022	0.0005	ss_{reg}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.042	0.011	-0.0004			

Regression results are calculated using the function LINEST

Blank	0.031	r^2=	0.987	
		m=	0.087	
		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
Microsomes	0.118	0.087	0.006	200	90	72000	0.025	0.022	22.408
Microsomes	0.117	0.086	0.006	200	90	72000	0.024		
Microsomes	0.101	0.069	0.005	200	90	72000	0.018		
QC 10	0.063	0.032	0.001	200	1	1	0.000	0.000	0.004
QC 10	0.052	0.021	0.000	200	1	1	0.000		
QC 10	0.055	0.024	0.001	200	1	1	0.000		
QC100	0.304	0.273	0.022	200	1	1	0.000	0.000	0.114
QC100	0.312	0.280	0.023	200	1	1	0.000		
QC100	0.316	0.285	0.023	200	1	1	0.000		

Assay Date	9/19/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	1

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	22.40835
Protein Concentration (dilution added to assay, mg/mL):	0.02801

Test Chemical Concentrations	
Level	Final Concentration (M)
1	3.30E-05
2	1.00E-05
3	3.30E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/19/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	6	Microsome type	Placental	Microsome ID	6-041305	Technician ID	TM	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	2091.139	4182.278	4272.882	8545.764	1	1669334	0.51	8167	0.0097	1	0.014	15	0.0232
			0.5	2	2181.743	4363.486			1					1		15	
	2	2	0.5	1	2195.429	4390.858	4505.589	9011.178	1	1669334	0.54	8632	0.0103	1	0.014	15	0.0245
			0.5	2	2310.16	4620.32			1					1		15	
	3	2	0.5	1	1583.875	3167.75	3263.6	6527.2	1	1669334	0.39	6148	0.0073	1	0.014	15	0.0174
			0.5	2	1679.725	3359.45			1					1		15	
	4	2	0.5	1	1672.734	3745.468	3593.163	7186.326	1	1669334	0.43	6808	0.0081	1	0.014	15	0.0193
			0.5	2	1720.429	3440.858			1					1		15	
Background control	1	2	0.5	1	98.42102	196.84204	212.39262	424.78524	1	1669334	0.03	46	0.0001	1	0.014	15	0.0001
			0.5	2	113.9716	227.9432			1					1		15	
	2	2	0.5	1	81.4083	162.8166	193.7201	387.4402	1	1669334	0.02	9	0.0000	1	0.014	15	0.0000
			0.5	2	112.3118	224.6236			1					1		15	
	3	2	0.5	1	93.59319	187.18638	169.01583	338.03166	1	1669334	0.02	-41	0.0000	1	0.014	15	-0.0001
			0.5	2	75.42264	150.84528			1					1		15	
	4	2	0.5	1	108.5444	217.0888	182.36017	364.72034	1	1669334	0.02	-14	0.0000	1	0.014	15	0.0000
			0.5	2	73.81577	147.63154			1					1		15	
Positive control	1	2	0.5	1	971.4724	1942.9448	1935.9448	3871.8896	1	1669334	0.23	3493	0.0042	1	0.014	15	0.0099
			0.5	2	964.4724	1928.9448			1					1		15	
	2	2	0.5	1	1080.16	2160.32	2047.1859	4094.3718	1	1669334	0.25	3716	0.0044	1	0.014	15	0.0105
			0.5	2	967.0259	1934.0518			1					1		15	
	3	2	0.5	1	732.6747	1465.3494	1575.792	3151.584	1	1669334	0.19	2773	0.0033	1	0.014	15	0.0079
			0.5	2	843.1173	1686.2346			1					1		15	
	4	2	0.5	1	853.892	1707.784	1696.2307	3392.4614	1	1669334	0.20	3014	0.0036	1	0.014	15	0.0085
			0.5	2	842.3387	1684.6774			1					1		15	
Negative Control	1	2	0.5	1	1727.845	3455.69	3468.749	6937.498	1	1669334	0.42	6559	0.0078	1	0.014	15	0.0186
			0.5	2	1740.904	3481.808			1					1		15	
	2	2	0.5	1	1620.034	3240.068	3357.457	6714.914	1	1669334	0.40	6336	0.0075	1	0.014	15	0.0180
			0.5	2	1737.423	3474.846			1					1		15	
	3	2	0.5	1	1556.947	3113.894	3193.263	6386.526	1	1669334	0.38	6008	0.0072	1	0.014	15	0.0170
			0.5	2	1636.316	3272.632			1					1		15	
	4	2	0.5	1	1470.749	2941.498	3033.083	6066.166	1	1669334	0.36	5687	0.0068	1	0.014	15	0.0161
			0.5	2	1562.334	3124.668			1					1		15	
RC#5 DBA	1-1	2	0.5	1	1468.056	2936.112	3035.082	6070.164	1	1669334	0.36	5691	0.0068	1	0.014	15	0.0161
			0.5	2	1567.026	3134.052			1					1		15	
	1-2	2	0.5	1	1866.525	3733.05	3736.212	7472.424	1	1669334	0.45	7094	0.0084	1	0.014	15	0.0201
			0.5	2	1869.687	3739.374			1					1		15	
	1-3	2	0.5	1	1782.851	3565.702	3579.535	7159.07	1	1669334	0.43	6780	0.0081	1	0.014	15	0.0192
			0.5	2	1796.684	3593.368			1					1		15	
	2-1	2	0.5	1	1718.568	3437.136	3377.875	6755.75	1	1669334	0.40	6377	0.0076	1	0.014	15	0.0181
			0.5	2	1659.307	3318.614			1					1		15	
	2-2	2	0.5	1	1706.079	3412.158	3416.566	6833.132	1	1669334	0.41	6454	0.0077	1	0.014	15	0.0183
			0.5	2	1710.487	3420.974			1					1		15	
	2-3	2	0.5	1	1656.613	3313.226	3124.684	6249.368	1	1669334	0.37	5871	0.0070	1	0.014	15	0.0166
			0.5	2	1468.071	2936.142			1					1		15	
	3-1	2	0.5	1	1692.304	3384.608	3433.819	6867.638	1	1669334	0.41	6469	0.0077	1	0.014	15	0.0184
			0.5	2	1741.515	3483.03			1					1		15	
	3-2	2	0.5	1	1693.302	3386.604	3261.777	6523.554	1	1669334	0.39	6145	0.0073	1	0.014	15	0.0174
			0.5	2	1568.475	3136.95			1					1		15	
	3-3	2	0.5	1	1574.745	3149.49	3099.368	6198.736	1	1669334	0.37	5820	0.0069	1	0.014	15	0.0165
			0.5	2	1524.623	3049.246			1					1		15	
	4-1	2	0.5	1	1413.798	2827.596	2733.701	5467.402	1	1669334	0.33	5089	0.0061	1	0.014	15	0.0144
			0.5	2	1319.903	2639.806			1					1		15	
	4-2	2	0.5	1	1244.48	2488.96	2606.72	5213.44	1	1669334	0.31	4835	0.0058	1	0.014	15	0.0137
			0.5	2	1362.24	2724.48			1					1		15	
	4-3	2	0.5	1	1700.506	3401.012	3605.47	7210.94	1	1669334	0.43	6832	0.0081	1	0.014	15	0.0194
			0.5	2	1904.964	3809.928			1					1		15	
	5-1	2	0.5	1	1626.698	3253.396	3369.788	6739.576	1	1669334	0.40	6361	0.0076	1	0.014	15	0.0180
			0.5	2	1743.09	3486.18			1					1		15	
	5-2	2	0.5	1	1739.443	3478.886	3463.407	6926.814	1	1669334	0.41	6548	0.0078	1	0.014	15	0.0186
			0.5	2	1723.964	3447.928			1					1		15	
	5-3	2	0.5	1	1818.074	3636.148	3628.236	7256.472	1	1669334	0.43	6878	0.0082	1	0.014	15	0.0195
			0.5	2	1810.162	3620.324			1					1		15	
	6-1	2	0.5	1	1799.143	3598.286	3378.481	6756.962	1	1669334	0.40	6378	0.0076	1	0.014	15	0.0181
			0.5	2	1579.338	3158.676			1					1		15	
	6-2	2	0.5	1	1731.302	3462.604	3351.344	6702.688	1	1669334	0.40	6324	0.0075	1	0.014	15	0.0179
			0.5	2	1620.042	3240.084			1					1		15	
	6-3	2	0.5	1	1555.616	3111.232	3096.409	6192.816	1	1669334	0.37	5814	0.0069	1	0.014	15	0.0165
			0.5	2	1540.793	3081.586			1					1		15	
	7-1	2	0.5	1	1511.497	3022.994	3131.539	6263.078	1	1669334	0.38	5884	0.0070	1	0.014	15	0.0167
			0.5	2	1620.042	3240.084			1					1		15	
	7-2	2	0.5	1	1520.074	3040.148	2988.153	5976.306	1	1669334	0.36	5598	0.0067	1	0.014	15	0.0159
			0.5	2	1468.079	2936.158			1					1		15	
	7-3	2	0.5	1	1663.784	3327.568	3229.554	6459.108	1	1669334	0.39	6080	0.0072	1	0.014	15	0.0172
			0.5	2	1565.77	3131.54			1					1		15	
	8-1	2	0.5	1	1473.506	2947.012	3069.126	6138.252	1	1669334	0.37	5760	0.0069	1	0.014	15	0.0163
			0.5	2	1595.62	3191.24			1					1		15	
	8-2	2	0.5	1	1505.775	3011.55	3169.236	6338.472	1	1669334	0.39	5960	0.0071	1	0.014	15	0.0169
			0.5	2	1663.461	3326.922			1					1		15	
	8-3	2	0.5	1	1557.629	3115.258	3192.701	6385.402	1	1669334	0.38	6007	0.0072	1	0.014	15	0.0170
			0.5	2	1635.072	3270.144			1					1		15	

Assay Date	9/19/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0238	0.0009
Full activity	End	0.0184	0.0013
Full activity	Overall	0.0211	0.0033
Background	Beginning	0.0001	7.48607E-05
Background	End	-0.0001	5.34993E-05
Background	Overall	0.0000	0.000104155
Positive	Beginning	0.0102	0.0004
Positive	End	0.0082	0.0005
Positive	Overall	0.0092	0.0012
Negative	Beginning	0.0183	0.0004
Negative	End	0.0166	0.0006
Negative	Overall	0.0174	0.0011

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#5 DBA	1	1	3.30E-05	-4.48	0.0161
RC#5 DBA	1	2	3.30E-05	-4.48	0.0201
RC#5 DBA	1	3	3.30E-05	-4.48	0.0192
RC#5 DBA	2	1	1.00E-05	-5.00	0.0181
RC#5 DBA	2	2	1.00E-05	-5.00	0.0183
RC#5 DBA	2	3	1.00E-05	-5.00	0.0166
RC#5 DBA	3	1	3.30E-06	-5.48	0.0184
RC#5 DBA	3	2	3.30E-06	-5.48	0.0174
RC#5 DBA	3	3	3.30E-06	-5.48	0.0165
RC#5 DBA	4	1	1.00E-06	-6.00	0.0144
RC#5 DBA	4	2	1.00E-06	-6.00	0.0137
RC#5 DBA	4	3	1.00E-06	-6.00	0.0194
RC#5 DBA	5	1	1.00E-07	-7.00	0.0180
RC#5 DBA	5	2	1.00E-07	-7.00	0.0186
RC#5 DBA	5	3	1.00E-07	-7.00	0.0195
RC#5 DBA	6	1	1.00E-08	-8.00	0.0181
RC#5 DBA	6	2	1.00E-08	-8.00	0.0179
RC#5 DBA	6	3	1.00E-08	-8.00	0.0165
RC#5 DBA	7	1	1.00E-09	-9.00	0.0167
RC#5 DBA	7	2	1.00E-09	-9.00	0.0159
RC#5 DBA	7	3	1.00E-09	-9.00	0.0172
RC#5 DBA	8	1	1.00E-10	-10.00	0.0163
RC#5 DBA	8	2	1.00E-10	-10.00	0.0169
RC#5 DBA	8	3	1.00E-10	-10.00	0.0170

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	76.51	95.36	91.15
2	-5.00	85.73	86.77	78.92
3	-5.48	87.23	82.60	78.24
4	-6.00	68.41	64.99	91.84
5	-7.00	85.51	88.03	92.46
6	-8.00	85.74	85.01	78.16
7	-9.00	79.10	75.25	81.74
8	-10.00	77.42	80.12	80.75

Assay Date	9/20/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	Placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0203	30593.51	1507069		
2	0.0201	32880.06	1635824		
3	0.0201	34721.77	1727451		
4	0.0204	34491.64	1690767		
5	0.0202	35075.27	1736400		
			Average DPM/g soln		1659502
			SD		93947
			CV		5.66
			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	16.4	16.4		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.1575 g
Mass of dilution B used in substrate prep	9.0995 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563175 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00846 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.

= 0.563175 + 0.00846
= 0.571637 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.308 µCi/µg ASDN

831439 dpm/nmol

Assay Date	9/20/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8				
Technician ID	TM	Replicate #	2	Microsome type	Placental	Microsome ID	6-041305		

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks
	0.654	0.438	0.239	0.164	0.103	0.091	0.073
	0.672	0.432	0.245	0.169	0.098	0.085	0.074
	0.680	0.440	0.245	0.177	0.101	0.088	0.074

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
2	1	

Samples:	Microsomes	QC 10	QC100
	0.157	0.104	0.347
	0.157	0.107	0.343
	0.157	0.106	0.353

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
0.25	125	1000	0.00025	200	0.0500	0.669	0.595	0.0475
0.125	62.5	1000	0.00013	200	0.0250	0.437	0.363	0.0281
0.05	25	1000	0.00005	200	0.0100	0.243	0.169	0.0121
0.025	25	2000	0.00003	200	0.0050	0.170	0.096	0.0060
0.01	5	1000	0.00001	200	0.0020	0.100	0.027	0.0002
0.005	5	2000	0.00001	200	0.0010	0.088	0.014	-0.0008
			Blank	0.074				

Variables	Regression results	
m, b	0.083	-0.002
se_m, se_b	0.005	0.002
r^2, se_y	0.985	0.003
F, df	255	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.985
m= 0.083
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
Microsomes	0.157	0.083	0.005	200	90	72000	0.020	0.020	19.594
Microsomes	0.157	0.084	0.005	200	90	72000	0.020		
Microsomes	0.157	0.083	0.005	200	90	72000	0.020		
QC 10	0.104	0.030	0.000	200	1	1	0.000	0.000	0.003
QC 10	0.107	0.034	0.001	200	1	1	0.000		
QC 10	0.106	0.032	0.001	200	1	1	0.000		
QC100	0.347	0.273	0.021	200	1	1	0.000	0.000	0.104
QC100	0.343	0.269	0.020	200	1	1	0.000		
QC100	0.353	0.279	0.021	200	1	1	0.000		

Assay Date	9/20/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	2

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	19.59415
Protein Concentration (dilution added to assay, mg/mL):	0.024493

Test Chemical Concentrations	
Level	Final Concentration (M)
1	3.30E-05
2	1.00E-05
3	3.30E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/20/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Placental	Microsome ID	6-041305	Technician ID	TM	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	4121.221	8242.442	8434.438	16868.876	1	1659502	1.02	16344	0.0197	1	0.012	15	0.0535
			0.5	2	4313.217	8626.434			1					1		15	
	2	2	0.5	1	4786.542	9573.084	9565.108	19130.216	1	1659502	1.15	18605	0.0224	1	0.012	15	0.0609
			0.5	2	4778.566	9557.132			1					1		15	
	3	2	0.5	1	4099.672	8199.344	8311.276	16622.552	1	1659502	1.00	16097	0.0194	1	0.012	15	0.0527
			0.5	2	4211.604	8423.208			1					1		15	
	4	2	0.5	1	4521.804	9043.608	8668.113	17336.226	1	1659502	1.04	16811	0.0202	1	0.012	15	0.0550
			0.5	2	4146.309	8292.618			1					1		15	
Background control	1	2	0.5	1	137.374	274.748	254.0568	508.1136	1	1659502	0.03	-17	0.0000	1	0.012	15	-0.0001
			0.5	2	116.6828	233.3656			1					1		15	
	2	2	0.5	1	125.6796	251.3592	234.2217	468.4434	1	1659502	0.03	-57	-0.0001	1	0.012	15	-0.0002
			0.5	2	108.5421	217.0842			1					1		15	
	3	2	0.5	1	119.3963	238.7926	263.2146	526.4292	1	1659502	0.03	1	0.0000	1	0.012	15	0.0000
			0.5	2	143.8183	287.6366			1					1		15	
	4	2	0.5	1	148.1485	296.297	298.9906	597.9812	1	1659502	0.04	73	0.0001	1	0.012	15	0.0002
			0.5	2	150.8421	301.6842			1					1		15	
Positive control	1	2	0.5	1	2413.998	4827.996	4890.154	9780.308	1	1659502	0.59	9255	0.0111	1	0.012	15	0.0303
			0.5	2	2476.156	4952.312			1					1		15	
	2	2	0.5	1	2303.035	4606.07	4821.559	9643.118	1	1659502	0.56	9118	0.0110	1	0.012	15	0.0298
			0.5	2	2518.524	5037.048			1					1		15	
	3	2	0.5	1	2246.822	4493.644	4423.258	8846.516	1	1659502	0.53	8321	0.0100	1	0.012	15	0.0272
			0.5	2	2176.436	4352.872			1					1		15	
	4	2	0.5	1	2117.176	4234.352	4331.322	8662.644	1	1659502	0.52	8137	0.0098	1	0.012	15	0.0266
			0.5	2	2214.146	4428.292			1					1		15	
Negative Control	1	2	0.5	1	3359.378	6718.756	6996.065	13992.13	1	1659502	0.84	13467	0.0162	1	0.012	15	0.0441
			0.5	2	3636.687	7273.374			1					1		15	
	2	2	0.5	1	3568.322	7136.644	6895.138	13790.276	1	1659502	0.83	13265	0.0160	1	0.012	15	0.0434
			0.5	2	3326.816	6653.632			1					1		15	
	3	2	0.5	1	3571.805	7143.61	7219.549	14439.098	1	1659502	0.87	13914	0.0167	1	0.012	15	0.0456
			0.5	2	3647.744	7295.488			1					1		15	
	4	2	0.5	1	3676.948	7353.896	7281.015	14562.03	1	1659502	0.88	14037	0.0169	1	0.012	15	0.0460
			0.5	2	3604.067	7208.134			1					1		15	
RC#5 DBA	1-1	2	0.5	1	3155.879	6311.758	6094.622	12189.244	1	1659502	0.73	11684	0.0140	1	0.012	15	0.0382
			0.5	2	2938.743	5877.486			1					1		15	
	1-2	2	0.5	1	2427.68	4855.36	4636.451	9272.902	1	1659502	0.55	8748	0.0105	1	0.012	15	0.0286
			0.5	2	2208.771	4417.542			1					1		15	
	1-3	2	0.5	1	4276.582	8553.164	8335.872	16671.744	1	1659502	1.00	16147	0.0194	1	0.012	15	0.0529
			0.5	2	4059.29	8118.58			1					1		15	
	2-1	2	0.5	1	4262.434	8524.868	8637.176	17274.356	1	1659502	1.04	16749	0.0201	1	0.012	15	0.0548
			0.5	2	4374.744	8749.488			1					1		15	
	2-2	2	0.5	1	4256.699	8513.398	8526.999	17053.998	1	1659502	1.03	16529	0.0199	1	0.012	15	0.0541
			0.5	2	4260.3	8520.6			1					1		15	
	2-3	2	0.5	1	4158.146	8316.292	8516.135	17032.27	1	1659502	1.03	16507	0.0199	1	0.012	15	0.0540
			0.5	2	4357.989	8715.978			1					1		15	
	3-1	2	0.5	1	4083.159	8166.318	8441.148	16882.296	1	1659502	1.02	16357	0.0197	1	0.012	15	0.0535
			0.5	2	4357.989	8715.978			1					1		15	
	3-2	2	0.5	1	4071.206	8142.412	7935.206	15870.412	1	1659502	0.96	15345	0.0185	1	0.012	15	0.0502
			0.5	2	3864	7728			1					1		15	
	3-3	2	0.5	1	3950.448	7900.896	7796.941	15593.882	1	1659502	0.94	15069	0.0181	1	0.012	15	0.0493
			0.5	2	3846.493	7692.986			1					1		15	
	4-1	2	0.5	1	3734.472	7468.944	7272.238	14544.476	1	1659502	0.88	14019	0.0169	1	0.012	15	0.0459
			0.5	2	3537.766	7075.532			1					1		15	
	4-2	2	0.5	1	3450.531	6901.062	6993.645	13987.29	1	1659502	0.84	13462	0.0162	1	0.012	15	0.0441
			0.5	2	3543.114	7086.228			1					1		15	
	4-3	2	0.5	1	3088.526	6177.052	6059.592	12119.184	1	1659502	0.73	11594	0.0139	1	0.012	15	0.0380
			0.5	2	2971.066	5942.132			1					1		15	
	5-1	2	0.5	1	4026.934	8053.868	8222.109	16444.218	1	1659502	0.99	15919	0.0191	1	0.012	15	0.0521
			0.5	2	4195.175	8390.35			1					1		15	
	5-2	2	0.5	1	4109.013	8218.026	8249.111	16498.222	1	1659502	0.99	15973	0.0192	1	0.012	15	0.0523
			0.5	2	4140.096	8280.196			1					1		15	
	5-3	2	0.5	1	4054.069	8108.138	8150.712	16301.424	1	1659502	0.98	15776	0.0190	1	0.012	15	0.0516
			0.5	2	4096.643	8193.286			1					1		15	
	6-1	2	0.5	1	3804.421	7608.842	7760.802	15521.604	1	1659502	0.94	14996	0.0180	1	0.012	15	0.0491
			0.5	2	3956.381	7912.762			1					1		15	
	6-2	2	0.5	1	4097.351	8194.7016	8181.7808	16363.562	1	1659502	0.99	15838	0.0190	1	0.012	15	0.0519
			0.5	2	4084.43	8168.86			1					1		15	
	6-3	2	0.5	1	3928.198	7856.396	7835.755	15671.51	1	1659502	0.94	15146	0.0182	1	0.012	15	0.0496
			0.5	2	3907.557	7815.114			1					1		15	
	7-1	2	0.5	1	3294.288	6588.576	7025.462	14050.924	1	1659502	0.85	13526	0.0163	1	0.012	15	0.0443
			0.5	2	3731.174	7462.348			1					1		15	
	7-2	2	0.5	1	3653.093	7306.186	7433.112	14866.224	1	1659502	0.90	14341	0.0172	1	0.012	15	0.0469
			0.5	2	3780.019	7560.038			1					1		15	
	7-3	2	0.5	1	3627.87	7255.74	7011.706	14023.412	1	1659502	0.85	13498	0.0162	1	0.012	15	0.0442
			0.5	2	3363.836	6767.672			1					1		15	
	8-1	2	0.5	1	3891.276	7782.552	7829.374	15658.748	1	1659502	0.94	15134	0.0182	1	0.012	15	0.0495
			0.5	2	3838.098	7676.196			1					1		15	
	8-2	2	0.5	1	3873.45	7746.9	7676.866	15353.732	1	1659502	0.93	14828	0.0178	1	0.012	15	0.0485
			0.5	2	3803.416	7606.832			1					1		15	
	8-3	2	0.5	1	3555.789	7113.578	7070.877	14141.754	1	1659502	0.85	13617	0.0164	1	0.012	15	0.0446
			0.5	2	3514.088	7028.176			1					1		15	

Assay Date	9/20/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Placental	Microsome ID	6-041305	Technician ID TM	Replicate # 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0572	0.0052
Full activity	End	0.0539	0.0017
Full activity	Overall	0.0555	0.0037
Background	Beginning	-0.0001	9.18314E-05
Background	End	0.0001	0.000165634
Background	Overall	0.0000	0.000177425
Positive	Beginning	0.0301	0.0003
Positive	End	0.0269	0.0004
Positive	Overall	0.0285	0.0018
Negative	Beginning	0.0438	0.0005
Negative	End	0.0458	0.0003
Negative	Overall	0.0448	0.0012

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#5 DBA	1	1	3.30E-05	-4.48	0.0382
RC#5 DBA	1	2	3.30E-05	-4.48	0.0286
RC#5 DBA	1	3	3.30E-05	-4.48	0.0529
RC#5 DBA	2	1	1.00E-05	-5.00	0.0548
RC#5 DBA	2	2	1.00E-05	-5.00	0.0541
RC#5 DBA	2	3	1.00E-05	-5.00	0.0540
RC#5 DBA	3	1	3.30E-06	-5.48	0.0535
RC#5 DBA	3	2	3.30E-06	-5.48	0.0502
RC#5 DBA	3	3	3.30E-06	-5.48	0.0493
RC#5 DBA	4	1	1.00E-06	-6.00	0.0459
RC#5 DBA	4	2	1.00E-06	-6.00	0.0441
RC#5 DBA	4	3	1.00E-06	-6.00	0.0380
RC#5 DBA	5	1	1.00E-07	-7.00	0.0521
RC#5 DBA	5	2	1.00E-07	-7.00	0.0523
RC#5 DBA	5	3	1.00E-07	-7.00	0.0516
RC#5 DBA	6	1	1.00E-08	-8.00	0.0491
RC#5 DBA	6	2	1.00E-08	-8.00	0.0519
RC#5 DBA	6	3	1.00E-08	-8.00	0.0496
RC#5 DBA	7	1	1.00E-09	-9.00	0.0443
RC#5 DBA	7	2	1.00E-09	-9.00	0.0469
RC#5 DBA	7	3	1.00E-09	-9.00	0.0442
RC#5 DBA	8	1	1.00E-10	-10.00	0.0495
RC#5 DBA	8	2	1.00E-10	-10.00	0.0485
RC#5 DBA	8	3	1.00E-10	-10.00	0.0446

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	68.76	51.57	95.18
2	-5.00	98.73	97.43	97.30
3	-5.48	96.42	90.46	88.83
4	-6.00	82.64	79.36	68.34
5	-7.00	93.84	94.16	93.00
6	-8.00	88.40	93.36	89.28
7	-9.00	79.73	84.54	79.57
8	-10.00	89.21	87.41	80.27

Assay Date	9/21/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8		
Technician ID	TM	Replicate #	3	Microsome type	Placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0205	28804.71	1405108		
2	0.0201	31256.42	1555046		
3	0.0206	31605.91	1534267		
4	0.0205	32366.37	1578847		
5	0.0204	32936.71	1614545		
			Average DPM/g soln	1537563	
			SD	79830	
			CV	5.19	
			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	13	13		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.1749 g
Mass of dilution B used in substrate prep	9.0994 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562563 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/bc

2) Calculate total µg ASDN/g soln.

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

= 0.562563 + 0.00784
= 0.570403 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.214 µCi/µg ASDN

772012 dpm/nmol

Assay Date	9/21/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8				
Technician ID	TM	Replicate #	3	Microsome type	Placental	Microsome ID	6-041305		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	2	1	
	0.602	0.390	0.200	0.120	0.032	0.054	0.035			
	0.625	0.402	0.186	0.112	0.031	0.042	0.032			
	0.628	0.396	0.199	0.118	0.036	0.042	0.027			

Samples:	Microsomes	QC 10	QC100
	0.108	0.056	0.283
	0.103	0.059	0.280
	0.106	0.059	0.312

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	
0.25	125	1000	0.00025	200	0.0500	0.619	0.587	0.0390	m, b	0.066	0.000
0.125	62.5	1000	0.00013	200	0.0250	0.396	0.364	0.0243	se_m, se_b	0.004	0.001
0.05	25	1000	0.00005	200	0.0100	0.195	0.163	0.0111	r^2, se_y	0.987	0.001
0.025	25	2000	0.00003	200	0.0050	0.117	0.085	0.0059	F, df	225	3
0.01	5	1000	0.00001	200	0.0020	0.033	0.002	0.0004	ss_{reg}, ss_{resid}	0.000	0.000
0.005	5	2000	0.00001	200	0.0010	0.046	0.015	0.0013			

Regression results are calculated using the function LINEST

Blank	0.031	r^2=	0.987		
		m=	0.066		
		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
Microsomes	0.108	0.076	0.005	200	90	72000	0.021	0.021	20.822
Microsomes	0.103	0.072	0.005	200	90	72000	0.020		
Microsomes	0.106	0.075	0.005	200	90	72000	0.021		
QC 10	0.056	0.025	0.002	200	1	1	0.000	0.000	0.010
QC 10	0.059	0.028	0.002	200	1	1	0.000		
QC 10	0.059	0.028	0.002	200	1	1	0.000		
QC100	0.283	0.252	0.017	200	1	1	0.000	0.000	0.087
QC100	0.280	0.249	0.017	200	1	1	0.000		
QC100	0.312	0.281	0.019	200	1	1	0.000		

Assay Date	9/21/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	3

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	20.82183
Protein Concentration (dilution added to assay, mg/mL):	0.026027

Test Chemical Concentrations	
Level	Final Concentration (M)
1	3.30E-05
2	1.00E-05
3	3.30E-06
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	RC#5 DBA	# Concentrations tested	6	Microsome type	Placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	3

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 (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4430.986	8861.972	8760.248	17520.496	1	1537563	1.14	17088	0.0221	1	0.013	15	0.0567
			0.5	2	4329.262	8658.524			1					1		15	
	2	2	0.5	1	4200.908	8401.816	8586.009	17172.018	1	1537563	1.12	16740	0.0217	1	0.013	15	0.0555
			0.5	2	4385.101	8770.202			1					1		15	
	3	2	0.5	1	4414.95	8829.9	8653.519	17307.038	1	1537563	1.13	16875	0.0219	1	0.013	15	0.0560
			0.5	2	4238.569	8477.138			1					1		15	
	4	2	0.5	1	3975.766	7951.532	7925.315	15850.63	1	1537563	1.03	15418	0.0200	1	0.013	15	0.0512
			0.5	2	3949.549	7899.098			1					1		15	
Background control	1	2	0.5	1	97.6879	195.3758	223.4454	446.8908	1	1537563	0.03	15	0.0000	1	0.013	15	0.0000
			0.5	2	125.7575	251.515			1					1		15	
	2	2	0.5	1	97.6879	195.3758	208.1259	416.2518	1	1537563	0.03	-16	0.0000	1	0.013	15	-0.0001
			0.5	2	110.438	220.876			1					1		15	
	3	2	0.5	1	110.438	220.876	202.73514	405.47028	1	1537563	0.03	-27	0.0000	1	0.013	15	-0.0001
			0.5	2	92.29714	184.59428			1					1		15	
	4	2	0.5	1	128.4914	256.9828	230.1047	460.2094	1	1537563	0.03	28	0.0000	1	0.013	15	0.0001
			0.5	2	101.6133	203.2266			1					1		15	
Positive control	1	2	0.5	1	2168.129	4336.258	4339.178	8678.356	1	1537563	0.56	8246	0.0107	1	0.013	15	0.0274
			0.5	2	2171.049	4342.098			1					1		15	
	2	2	0.5	1	2363.504	4727.008	4591.119	9182.238	1	1537563	0.60	8750	0.0113	1	0.013	15	0.0290
			0.5	2	2227.615	4455.23			1					1		15	
	3	2	0.5	1	2249.956	4499.932	4464.112	8928.224	1	1537563	0.58	8496	0.0110	1	0.013	15	0.0282
			0.5	2	2214.146	4428.292			1					1		15	
	4	2	0.5	1	2287.525	4575.05	4588.618	9177.236	1	1537563	0.60	8745	0.0113	1	0.013	15	0.0290
			0.5	2	2301.093	4602.186			1					1		15	
Negative Control	1	2	0.5	1	3507.078	7014.156	7240.926	14481.852	1	1537563	0.94	14050	0.0182	1	0.013	15	0.0466
			0.5	2	3733.848	7467.696			1					1		15	
	2	2	0.5	1	3652.442	7304.884	7391.718	14783.436	1	1537563	0.96	14351	0.0186	1	0.013	15	0.0476
			0.5	2	3739.276	7478.552			1					1		15	
	3	2	0.5	1	3901.416	7802.832	7755.97	15511.94	1	1537563	1.01	15080	0.0195	1	0.013	15	0.0500
			0.5	2	3854.554	7709.108			1					1		15	
	4	2	0.5	1	3955.895	7911.79	7877.81	15755.62	1	1537563	1.02	15323	0.0198	1	0.013	15	0.0508
			0.5	2	3921.915	7843.83			1					1		15	
RC#5 DBA	1-1	2	0.5	1	3622.612	7245.224	7267.084	14534.168	1	1537563	0.95	14102	0.0183	1	0.013	15	0.0468
			0.5	2	3644.472	7288.944			1					1		15	
	1-2	2	0.5	1	3870.736	7741.472	7892.243	15784.486	1	1537563	1.03	15352	0.0199	1	0.013	15	0.0509
			0.5	2	4021.507	8043.014			1					1		15	
	1-3	2	0.5	1	3970.4	7940.8	8019.042	16038.084	1	1537563	1.04	15606	0.0202	1	0.013	15	0.0518
			0.5	2	4048.642	8097.284			1					1		15	
	2-1	2	0.5	1	4107.775	8215.55	8191.307	16382.614	1	1537563	1.07	15950	0.0207	1	0.013	15	0.0529
			0.5	2	4083.532	8167.064			1					1		15	
	2-2	2	0.5	1	4101.991	8203.982	8213.045	16426.09	1	1537563	1.07	15994	0.0207	1	0.013	15	0.0531
			0.5	2	4111.054	8222.108			1					1		15	
	2-3	2	0.5	1	3948.24	7896.48	7991.455	15982.91	1	1537563	1.04	15551	0.0201	1	0.013	15	0.0516
			0.5	2	4043.215	8086.43			1					1		15	
	3-1	2	0.5	1	3733.361	7466.722	7654.465	15308.93	1	1537563	1.00	14877	0.0193	1	0.013	15	0.0494
			0.5	2	3921.104	7842.208			1					1		15	
	3-2	2	0.5	1	3934.045	7868.09	7955.574	15911.148	1	1537563	1.03	15479	0.0201	1	0.013	15	0.0514
			0.5	2	4021.529	8043.058			1					1		15	
	3-3	2	0.5	1	3709.446	7418.892	7413.465	14826.93	1	1537563	0.96	14395	0.0186	1	0.013	15	0.0478
			0.5	2	3704.019	7408.038			1					1		15	
	4-1	2	0.5	1	3660.772	7321.544	7490.93	14981.86	1	1537563	0.97	14550	0.0188	1	0.013	15	0.0483
			0.5	2	3830.158	7660.316			1					1		15	
	4-2	2	0.5	1	3904.823	7809.646	8073.981	16147.962	1	1537563	1.05	15716	0.0204	1	0.013	15	0.0521
			0.5	2	4169.158	8338.316			1					1		15	
	4-3	2	0.5	1	4141.819	8283.638	8117.195	16234.39	1	1537563	1.06	15802	0.0205	1	0.013	15	0.0524
			0.5	2	3975.376	7950.752			1					1		15	
	5-1	2	0.5	1	4000.03	8000.06	8092.089	16184.178	1	1537563	1.05	15752	0.0204	1	0.013	15	0.0523
			0.5	2	4092.059	8184.118			1					1		15	
	5-2	2	0.5	1	4137.405	8274.81	8372.176	16744.352	1	1537563	1.09	16312	0.0211	1	0.013	15	0.0541
			0.5	2	4234.771	8469.542			1					1		15	
	5-3	2	0.5	1	3964.521	7929.042	8188.356	16376.712	1	1537563	1.07	15945	0.0207	1	0.013	15	0.0529
			0.5	2	4223.835	8447.67			1					1		15	
	6-1	2	0.5	1	4239.233	8478.466	8306.483	16612.966	1	1537563	1.08	16181	0.0210	1	0.013	15	0.0537
			0.5	2	4067.25	8134.5			1					1		15	
	6-2	2	0.5	1	4032.375	8064.75	7895.051	15790.102	1	1537563	1.03	15358	0.0199	1	0.013	15	0.0510
			0.5	2	3862.676	7725.352			1					1		15	
	6-3	2	0.5	1	4121.93	8243.86	7923.658	15847.316	1	1537563	1.03	15415	0.0200	1	0.013	15	0.0511
			0.5	2	3801.728	7603.456			1					1		15	
	7-1	2	0.5	1	3725.747	7451.494	7682.149	15364.296	1	1537563	1.00	14932	0.0193	1	0.013	15	0.0495
			0.5	2	3956.402	7912.804			1					1		15	
	7-2	2	0.5	1	4070.372	8140.744	7959.984	15919.968	1	1537563	1.04	15488	0.0201	1	0.013	15	0.0514
			0.5	2	3889.612	7779.224			1					1		15	
	7-3	2	0.5	1	4143.916	8287.832	7951.781	15903.562	1	1537563	1.03	15471	0.0200	1	0.013	15	0.0513
			0.5	2	3807.865	7615.73			1					1		15	
	8-1	2	0.5	1	4029.681	8059.362	8422.97	16845.94	1	1537563	1.10	16414	0.0213	1	0.013	15	0.0545
			0.5	2	4393.289	8786.578			1					1		15	
	8-2	2	0.5	1	3952.265	7904.53	8000.929	16001.858	1	1537563	1.04	15570	0.0202	1	0.013	15	0.0517
			0.5	2	4048.664	8097.328			1					1		15	
	8-3	2	0.5	1	4067.659	8135.318	8089.187	16178.374	1	1537563	1.05	15746	0.0204	1	0.013	15	0.0522
			0.5	2	4021.528	8043.056			1					1		15	

Assay Date	9/21/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Placental	Microsome ID	6-041305	Technician ID TM	Replicate # 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0561	0.0008
Full activity	End	0.0536	0.0034
Full activity	Overall	0.0548	0.0025
Background	Beginning	0.0000	7.18813E-05
Background	End	0.0000	0.000128422
Background	Overall	0.0000	8.50035E-05
Positive	Beginning	0.0282	0.0012
Positive	End	0.0286	0.0006
Positive	Overall	0.0284	0.0008
Negative	Beginning	0.0471	0.0007
Negative	End	0.0504	0.0006
Negative	Overall	0.0488	0.0020

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#5 DBA	1	1	3.30E-05	-4.48	0.0468
RC#5 DBA	1	2	3.30E-05	-4.48	0.0509
RC#5 DBA	1	3	3.30E-05	-4.48	0.0518
RC#5 DBA	2	1	1.00E-05	-5.00	0.0529
RC#5 DBA	2	2	1.00E-05	-5.00	0.0531
RC#5 DBA	2	3	1.00E-05	-5.00	0.0516
RC#5 DBA	3	1	3.30E-06	-5.48	0.0494
RC#5 DBA	3	2	3.30E-06	-5.48	0.0514
RC#5 DBA	3	3	3.30E-06	-5.48	0.0478
RC#5 DBA	4	1	1.00E-06	-6.00	0.0483
RC#5 DBA	4	2	1.00E-06	-6.00	0.0521
RC#5 DBA	4	3	1.00E-06	-6.00	0.0524
RC#5 DBA	5	1	1.00E-07	-7.00	0.0523
RC#5 DBA	5	2	1.00E-07	-7.00	0.0541
RC#5 DBA	5	3	1.00E-07	-7.00	0.0529
RC#5 DBA	6	1	1.00E-08	-8.00	0.0537
RC#5 DBA	6	2	1.00E-08	-8.00	0.0510
RC#5 DBA	6	3	1.00E-08	-8.00	0.0511
RC#5 DBA	7	1	1.00E-09	-9.00	0.0495
RC#5 DBA	7	2	1.00E-09	-9.00	0.0514
RC#5 DBA	7	3	1.00E-09	-9.00	0.0513
RC#5 DBA	8	1	1.00E-10	-10.00	0.0545
RC#5 DBA	8	2	1.00E-10	-10.00	0.0517
RC#5 DBA	8	3	1.00E-10	-10.00	0.0522

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	85.31	92.87	94.41
2	-5.00	96.49	96.75	94.07
3	-5.48	90.00	93.64	87.08
4	-6.00	88.02	95.07	95.60
5	-7.00	95.29	98.68	96.46
6	-8.00	97.89	92.91	93.25
7	-9.00	90.33	93.69	93.59
8	-10.00	99.29	94.19	95.26

Assay Date	8/3/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8		
Technician ID	TM	Replicate #	1	Microsome type	placental	Microsome ID	0

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0198	26764.19	1351727		
2	0.0205	29446.78	1436428		
3	0.0203	31405.98	1547093		
4	0.0203	31530.1	1553207		
5	0.0201	30775.5	1531119		
			Average DPM/g soln	1483915	
			SD	87678	
			CV	5.91	
			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	14	14		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.3306 g
Mass of dilution B used in substrate prep	9.2077 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563831 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00757 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/bc

2) Calculate total µg ASDN/g soln.

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

= 0.563831 + 0.00757
= 0.571398 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.170 µCi/µg ASDN

743778 dpm/nmol

Assay Date	8/3/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8				
Technician ID	TM	Replicate #	1	Microsome type	placental	Microsome ID	0		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.505	0.294	0.155	0.094	0.047	0.047	0.029			
	0.510	0.343	0.168	0.100	0.049	0.045	0.030			
	0.504	0.282	0.159	0.108	0.050	0.044	0.030			

Samples:	Placental	QC 10	QC 100
	0.086	0.059	0.288
	0.086	0.060	0.288
	0.089	0.060	0.282

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
0.25	125	1000	0.00025	200	0.0500	0.506	0.477	0.0482
0.125	62.5	1000	0.00013	200	0.0250	0.306	0.277	0.0272
0.05	25	1000	0.00005	200	0.0100	0.161	0.131	0.0119
0.025	25	2000	0.00003	200	0.0050	0.100	0.071	0.0056
0.01	5	1000	0.00001	200	0.0020	0.049	0.019	0.0002
0.005	5	2000	0.00001	200	0.0010	0.045	0.015	-0.0002

Variables	Regression results			
m, b	0.105	-0.002		
se_m, se_b	0.005	0.001		
r^2, se_y	0.991	0.002		
F, df	424	4		
ss_{reg}, ss_{resid}	0.002	0.000		

Blank 0.030

r^2= 0.991
m= 0.105
b= -0.002

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
Placental	0.086	0.056	0.004	200	90	72000	0.016	0.017	16.798
Placental	0.086	0.056	0.004	200	90	72000	0.016		
Placental	0.089	0.059	0.004	200	90	72000	0.018		
QC 10	0.059	0.029	0.001	200	200	200	0.000	0.000	0.007
QC 10	0.060	0.031	0.001	200	200	200	0.000		
QC 10	0.060	0.031	0.001	200	200	200	0.000		
QC 100	0.288	0.258	0.025	200	200	200	0.000	0.000	0.126
QC 100	0.288	0.258	0.025	200	200	200	0.000		
QC 100	0.282	0.253	0.025	200	200	200	0.000		

Assay Date	8/3/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	Replicate #	1

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	16.79839
Protein Concentration (dilution added to assay, mg/mL):	0.020998

Test Chemical Concentrations	
Level	Final Concentration (M)
1	3.33E-05
2	1.00E-05
3	3.30E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	8/3/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	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	3235.287	6470.574	6562.655	13125.31	1	1483915	0.88	12645	0.0170	1	0.010	15	0.0540
			0.5	2	3327.368	6654.736			1					1		15	
	2	2	0.5	1	2999.708	5999.416	5994.339	11988.678	1	1483915	0.81	11509	0.0155	1	0.010	15	0.0491
			0.5	2	2994.631	5989.262			1					1		15	
	3	2	0.5	1	2701.876	5403.752	5397.564	10795.128	1	1483915	0.73	10315	0.0139	1	0.010	15	0.0440
			0.5	2	2695.688	5391.376			1					1		15	
	4	2	0.5	1	2947.357	5894.714	5983.58	11967.16	1	1483915	0.81	11487	0.0154	1	0.010	15	0.0490
			0.5	2	3036.223	6072.446			1					1		15	
Background control	1	2	0.5	1	134.4348	268.8696	311.0453	622.0906	1	1483915	0.04	142	0.0002	1	0.010	15	0.0006
			0.5	2	176.6105	353.221			1					1		15	
	2	2	0.5	1	100.1677	200.3354	250.4192	500.8384	1	1483915	0.03	21	0.0000	1	0.010	15	0.0001
			0.5	2	150.2515	300.503			1					1		15	
	3	2	0.5	1	104.7024	209.4048	206.7873	413.5746	1	1483915	0.03	-66	-0.0001	1	0.010	15	-0.0003
			0.5	2	102.0849	204.1698			1					1		15	
	4	2	0.5	1	76.99943	153.97886	191.36833	382.73666	1	1483915	0.03	-97	-0.0001	1	0.010	15	-0.0004
			0.5	2	114.3789	228.7578			1					1		15	
Positive control	1	2	0.5	1	62.82145	125.6429	162.84195	325.6839	1	1483915	0.02	-154	-0.0002	1	0.010	15	-0.0007
			0.5	2	100.0205	200.041			1					1		15	
	2	2	0.5	1	107.32	214.64	264.3736	528.7472	1	1483915	0.04	49	0.0001	1	0.010	15	0.0002
			0.5	2	157.0536	314.1072			1					1		15	
	3	2	0.5	1	983.2245	1966.449	2000.7165	4001.433	1	1483915	0.27	3522	0.0047	1	0.010	15	0.0150
			0.5	2	1017.492	2034.984			1					1		15	
	4	2	0.5	1	948.9567	1897.9134	1845.1936	3690.3872	1	1483915	0.25	3211	0.0043	1	0.010	15	0.0137
			0.5	2	896.2369	1792.4738			1					1		15	
Negative Control	1	2	0.5	1	1308.78	2617.56	2795.554	5591.108	1	1483915	0.38	5111	0.0069	1	0.010	15	0.0218
			0.5	2	1486.774	2973.548			1					1		15	
	2	2	0.5	1	1289	2578	2576.84	5153.68	1	1483915	0.35	4674	0.0063	1	0.010	15	0.0200
			0.5	2	1287.84	2575.68			1					1		15	
	3	2	0.5	1	110.7116	221.4232	181.38573	362.77146	1	1483915	0.02	-117	-0.0002	1	0.010	15	-0.0005
			0.5	2	70.67413	141.34826			1					1		15	
	4	2	0.5	1	1718.283	3436.566	3272.796	6545.592	1	1483915	0.44	6066	0.0082	1	0.010	15	0.0259
			0.5	2	1554.513	3109.026			1					1		15	
RC#6 FRM	1-1	2	0.5	1	251.2684	502.5768	464.4513	928.9026	1	1483915	0.06	449	0.0006	1	0.010	15	0.0019
			0.5	2	213.1629	426.3258			1					1		15	
	1-2	2	0.5	1	410.9813	821.9226	903.0678	1806.1356	1	1483915	0.12	1326	0.0018	1	0.010	15	0.0057
			0.5	2	492.1055	984.213			1					1		15	
	1-3	2	0.5	1	1283.741	2567.482	2549.03	5098.06	1	1483915	0.34	4618	0.0062	1	0.010	15	0.0197
			0.5	2	1265.289	2530.578			1					1		15	
	2-1	2	0.5	1	944.9763	1889.9526	1919.8066	3839.6132	1	1483915	0.26	3360	0.0045	1	0.010	15	0.0143
			0.5	2	974.8303	1949.6606			1					1		15	
	2-2	2	0.5	1	877.7942	1755.5884	1862.0073	3724.0146	1	1483915	0.25	3244	0.0044	1	0.010	15	0.0138
			0.5	2	984.2131	1968.4262			1					1		15	
	2-3	2	0.5	1	1063.215	2126.43	2237.981	4475.962	1	1483915	0.30	3996	0.0054	1	0.010	15	0.0171
			0.5	2	1174.766	2349.532			1					1		15	
	3-1	2	0.5	1	1536.229	3072.458	3211.485	6422.97	1	1483915	0.43	5943	0.0080	1	0.010	15	0.0254
			0.5	2	1675.256	3350.512			1					1		15	
	3-2	2	0.5	1	1639.604	3279.208	3147.342	6294.684	1	1483915	0.42	5815	0.0078	1	0.010	15	0.0248
			0.5	2	1507.738	3015.476			1					1		15	
	3-3	2	0.5	1	1629.916	3259.832	3195.234	6390.468	1	1483915	0.43	5911	0.0079	1	0.010	15	0.0252
			0.5	2	1565.318	3130.636			1					1		15	
	4-1	2	0.5	1	2536.443	5072.886	5130.791	10261.582	1	1483915	0.69	9782	0.0132	1	0.010	15	0.0418
			0.5	2	2594.348	5188.696			1					1		15	
	4-2	2	0.5	1	2395.783	4793.566	4985.932	9971.864	1	1483915	0.67	9492	0.0128	1	0.010	15	0.0405
			0.5	2	2589.149	5178.298			1					1		15	
	4-3	2	0.5	1	2344.367	4688.734	4728.152	9456.304	1	1483915	0.64	8976	0.0121	1	0.010	15	0.0383
			0.5	2	2383.785	4767.57			1					1		15	
	5-1	2	0.5	1	2828.308	5656.616	5453.851	10907.702	1	1483915	0.74	10428	0.0140	1	0.010	15	0.0445
			0.5	2	2625.543	5251.086			1					1		15	
	5-2	2	0.5	1	2800.819	5601.638	5501.503	11003.006	1	1483915	0.74	10523	0.0141	1	0.010	15	0.0449
			0.5	2	2700.664	5401.368			1					1		15	
	5-3	2	0.5	1	2646.395	5292.79	5350.964	10701.928	1	1483915	0.72	10222	0.0137	1	0.010	15	0.0436
			0.5	2	2704.569	5409.138			1					1		15	
	6-1	2	0.5	1	2868.892	5737.784	5889.786	11779.572	1	1483915	0.79	11300	0.0152	1	0.010	15	0.0482
			0.5	2	3020.894	6041.788			1					1		15	
	6-2	2	0.5	1	2997.154	5994.308	5882.667	11765.334	1	1483915	0.79	11286	0.0152	1	0.010	15	0.0482
			0.5	2	2885.513	5771.026			1					1		15	
	6-3	2	0.5	1	2861.039	5722.078	5593.178	11186.356	1	1483915	0.75	10707	0.0144	1	0.010	15	0.0457
			0.5	2	2732.139	5464.278			1					1		15	
	7-1	2	0.5	1	3111.675	6223.35	6053.859	12107.718	1	1483915	0.82	11628	0.0156	1	0.010	15	0.0496
			0.5	2	2942.184	5884.368			1					1		15	
	7-2	2	0.5	1	2957.503	5915.006	5842.1	11684.2	1	1483915	0.79	11204	0.0151	1	0.010	15	0.0478
			0.5	2	2884.597	5769.194			1					1		15	
	7-3	2	0.5	1	2929.706	5859.412	5828.802	11657.604	1	1483915	0.79	11178	0.0150	1	0.010	15	0.0477
			0.5	2	2899.098	5798.192			1					1		15	
	8-1	2	0.5	1	3002.389	6004.778	5846.668	11693.336	1	1483915	0.79	11214	0.0151	1	0.010	15	0.0479
			0.5	2	2844.279	5688.558			1					1		15	
	8-2	2	0.5	1	2802.327	5604.654	5650.181	11300.362	1	1483915	0.76	10921	0.0145	1	0.010	15	0.0462
			0.5	2	2847.854	5695.708			1					1		15	
	8-3	2	0.5	1	2897.685	5795.37	5863.428	11726.856	1	1483915	0.79	11247	0.0151	1	0.010	15	0.0480
			0.5	2	2965.743	5931.486			1					1		15	

Assay Date	8/3/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID TM	Replicate # 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0516	0.0034
Full activity	End	0.0465	0.0035
Full activity	Overall	0.0490	0.0041
Background	Beginning	0.0003	0.000365984
Background	End	-0.0003	9.30804E-05
Background	Overall	0.0000	0.000457734
Positive	Beginning	-0.0002	0.0006
Positive	End	0.0144	0.0009
Positive	Overall	0.0071	0.0085
Negative	Beginning	0.0209	0.0013
Negative	End	0.0127	0.0187
Negative	Overall	0.0168	0.0118

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#6 FRM	1	1	3.33E-05	-4.48	0.0019
RC#6 FRM	1	2	3.33E-05	-4.48	0.0057
RC#6 FRM	1	3	3.33E-05	-4.48	0.0197
RC#6 FRM	2	1	1.00E-05	-5.00	0.0143
RC#6 FRM	2	2	1.00E-05	-5.00	0.0138
RC#6 FRM	2	3	1.00E-05	-5.00	0.0171
RC#6 FRM	3	1	3.30E-06	-5.48	0.0254
RC#6 FRM	3	2	3.30E-06	-5.48	0.0248
RC#6 FRM	3	3	3.30E-06	-5.48	0.0252
RC#6 FRM	4	1	1.00E-06	-6.00	0.0418
RC#6 FRM	4	2	1.00E-06	-6.00	0.0405
RC#6 FRM	4	3	1.00E-06	-6.00	0.0383
RC#6 FRM	5	1	1.00E-07	-7.00	0.0445
RC#6 FRM	5	2	1.00E-07	-7.00	0.0449
RC#6 FRM	5	3	1.00E-07	-7.00	0.0436
RC#6 FRM	6	1	1.00E-08	-8.00	0.0482
RC#6 FRM	6	2	1.00E-08	-8.00	0.0482
RC#6 FRM	6	3	1.00E-08	-8.00	0.0457
RC#6 FRM	7	1	1.00E-09	-9.00	0.0496
RC#6 FRM	7	2	1.00E-09	-9.00	0.0478
RC#6 FRM	7	3	1.00E-09	-9.00	0.0477
RC#6 FRM	8	1	1.00E-10	-10.00	0.0479
RC#6 FRM	8	2	1.00E-10	-10.00	0.0462
RC#6 FRM	8	3	1.00E-10	-10.00	0.0480

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	3.91	11.54	40.20
2	-5.00	29.24	28.24	34.78
3	-5.48	51.73	50.61	51.45
4	-6.00	85.14	82.62	78.13
5	-7.00	90.76	91.59	88.97
6	-8.00	98.35	98.23	93.19
7	-9.00	101.21	97.52	97.29
8	-10.00	97.60	94.18	97.89

Assay Date	8/5/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	0

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0203	30495.19	1502226		
2	0.0201	33417.8	1662577		
3	0.0201	34409.48	1711914		
4	0.0195	36096.1	1851082		
5	0.0198	36785.36	1857846		
			Average DPM/g soln		1717129
			SD		147420
			CV		8.59
			µCi/g soln		0.773

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	16.7	16.7		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2966 g
Mass of dilution B used in substrate prep	9.1704 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. = 0.00876 µg/g soln.
µg/g soln.

a. µCi/g soln 0.773
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.562719 + 0.00876
= 0.571475 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.353 µCi/µg ASDN

860556 dpm/nmol

Assay Date	8/5/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8				
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	0		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.491	0.332	0.159	0.104	0.053	0.043	0.036			
	0.562	0.343	0.161	0.101	0.057	0.045	0.033			
	0.474	0.329	0.174	0.106	0.063	0.048	0.037			

Samples:	Placental	QC 10	QC 100
	0.100	0.061	0.285
	0.098	0.064	0.276
	0.101	0.065	0.290

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	
0.25	125	1000	0.00025	200	0.0500	0.509	0.473	0.0470	m, b	0.103	-0.002
0.125	62.5	1000	0.00013	200	0.0250	0.334	0.299	0.0291	se_m, se_b	0.007	0.002
0.05	25	1000	0.00005	200	0.0100	0.165	0.130	0.0116	r^2, se_y	0.982	0.003
0.025	25	2000	0.00003	200	0.0050	0.104	0.068	0.0053	F, df	215	4
0.01	5	1000	0.00001	200	0.0020	0.058	0.022	0.0006	ss_{regr}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.046	0.010	-0.0007			

Regression results are calculated using the function LINEST

Blank	0.035	r^2=	0.982	
		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
Placental	0.100	0.065	0.005	200	90	72000	0.020	0.020	19.596
Placental	0.098	0.062	0.005	200	90	72000	0.019		
Placental	0.101	0.066	0.005	200	90	72000	0.020		
QC 10	0.061	0.026	0.001	200	200	200	0.000	0.000	0.006
QC 10	0.064	0.029	0.001	200	200	200	0.000		
QC 10	0.065	0.030	0.001	200	200	200	0.000		
QC 100	0.285	0.250	0.024	200	200	200	0.000	0.000	0.119
QC 100	0.276	0.241	0.023	200	200	200	0.000		
QC 100	0.290	0.255	0.024	200	200	200	0.000		

Assay Date	8/5/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	Replicate # 2

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	19.59606
Protein Concentration (dilution added to assay, mg/mL):	0.024495

Test Chemical Concentrations	
Level	Final Concentration (M)
1	3.30E-05
2	1.00E-05
3	3.30E-06
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	RC#6 FRM	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	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	3592.518	7185.036	7554.166	15108.332	1	1717129	0.88	14726	0.0171	1	0.012	15	0.0466
			0.5	2	3961.648	7923.296			1					1		15	
	2	2	0.5	1	4021.437	8042.874	7912.139	15824.278	1	1717129	0.92	15442	0.0179	1	0.012	15	0.0488
			0.5	2	3890.702	7781.404			1					1		15	
	3	2	0.5	1	3402.81	6805.62	6844.432	13688.864	1	1717129	0.80	13307	0.0155	1	0.012	15	0.0421
			0.5	2	3441.622	6883.244			1					1		15	
	4	2	0.5	1	3320.274	6640.548	6632.045	13264.09	1	1717129	0.77	12882	0.0150	1	0.012	15	0.0407
			0.5	2	3311.771	6623.542			1					1		15	
Background control	1	2	0.5	1	90.98273	181.96546	211.51963	423.03926	1	1717129	0.02	41	0.0000	1	0.012	15	0.0001
			0.5	2	120.5359	241.0718			1					1		15	
	2	2	0.5	1	96.18174	192.36348	181.96545	363.9309	1	1717129	0.02	-18	0.0000	1	0.012	15	-0.0001
			0.5	2	85.78371	171.56742			1					1		15	
	3	2	0.5	1	97.5314	195.06228	170.31732	340.63464	1	1717129	0.02	-41	0.0000	1	0.012	15	-0.0001
			0.5	2	72.78618	145.57236			1					1		15	
	4	2	0.5	1	109.1793	218.3586	200.16251	400.32502	1	1717129	0.02	18	0.0000	1	0.012	15	0.0001
			0.5	2	90.98321	181.96642			1					1		15	
Positive control	1	2	0.5	1	1664.769	3329.536	3396.614	6793.228	1	1717129	0.40	6411	0.0075	1	0.012	15	0.0203
			0.5	2	1731.846	3463.692			1					1		15	
	2	2	0.5	1	1362.149	2724.298	2669.601	5339.202	1	1717129	0.31	4957	0.0058	1	0.012	15	0.0157
			0.5	2	1307.452	2614.904			1					1		15	
	3	2	0.5	1	1679.126	3358.252	3374.068	6748.136	1	1717129	0.39	6366	0.0074	1	0.012	15	0.0201
			0.5	2	1694.942	3389.884			1					1		15	
	4	2	0.5	1	1707.885	3415.77	3427.622	6855.244	1	1717129	0.40	6473	0.0075	1	0.012	15	0.0205
			0.5	2	1719.737	3439.474			1					1		15	
Negative Control	1	2	0.5	1	1141.189	2282.378	2272.05	4544.1	1	1717129	0.26	4162	0.0048	1	0.012	15	0.0132
			0.5	2	1130.861	2261.722			1					1		15	
	2	2	0.5	1	1138.639	2277.278	2240.836	4481.672	1	1717129	0.26	4100	0.0048	1	0.012	15	0.0130
			0.5	2	1102.197	2204.394			1					1		15	
	3	2	0.5	1	2989.448	5978.896	5754.602	11509.204	1	1717129	0.67	11127	0.0129	1	0.012	15	0.0352
			0.5	2	2765.154	5530.308			1					1		15	
	4	2	0.5	1	3251.01	6502.02	6539.403	13078.806	1	1717129	0.76	12697	0.0148	1	0.012	15	0.0402
			0.5	2	3288.393	6576.786			1					1		15	
RC#6 FRM	1-1	2	0.5	1	565.399	1130.798	1143.8859	2287.7718	1	1717129	0.13	1906	0.0022	1	0.012	15	0.0060
			0.5	2	578.4669	1156.9338			1					1		15	
	1-2	2	0.5	1	539.2231	1078.4462	1063.7908	2127.5816	1	1717129	0.12	1746	0.0020	1	0.012	15	0.0055
			0.5	2	524.5677	1049.1354			1					1		15	
	1-3	2	0.5	1	504.3122	1008.6244	1084.0114	2168.0228	1	1717129	0.13	1786	0.0021	1	0.012	15	0.0056
			0.5	2	579.6992	1159.3984			1					1		15	
	2-1	2	0.5	1	1138.651	2277.302	2351.219	4702.438	1	1717129	0.27	4320	0.0050	1	0.012	15	0.0137
			0.5	2	1212.568	2425.136			1					1		15	
	2-2	2	0.5	1	1276.378	2552.756	2399.383	4798.766	1	1717129	0.28	4417	0.0051	1	0.012	15	0.0140
			0.5	2	1123.005	2246.01			1					1		15	
	2-3	2	0.5	1	1162.484	2324.968	2389.47	4778.94	1	1717129	0.28	4397	0.0051	1	0.012	15	0.0139
			0.5	2	1226.986	2453.972			1					1		15	
	3-1	2	0.5	1	1845.213	3690.426	3719.527	7439.054	1	1717129	0.43	7057	0.0082	1	0.012	15	0.0223
			0.5	2	1874.314	3748.628			1					1		15	
	3-2	2	0.5	1	2086.701	4173.402	4233.123	8466.246	1	1717129	0.49	8084	0.0094	1	0.012	15	0.0256
			0.5	2	2146.422	4292.844			1					1		15	
	3-3	2	0.5	1	1944.868	3889.736	3953.514	7907.028	1	1717129	0.46	7525	0.0087	1	0.012	15	0.0238
			0.5	2	2008.646	4017.292			1					1		15	
	4-1	2	0.5	1	2696.115	5392.23	5500.839	11001.678	1	1717129	0.64	10620	0.0123	1	0.012	15	0.0336
			0.5	2	2804.724	5609.448			1					1		15	
	4-2	2	0.5	1	2565.236	5130.472	5237.505	10475.01	1	1717129	0.61	10093	0.0117	1	0.012	15	0.0319
			0.5	2	2672.269	5344.538			1					1		15	
	4-3	2	0.5	1	2802.088	5604.176	5669.389	11338.778	1	1717129	0.66	10957	0.0127	1	0.012	15	0.0347
			0.5	2	2867.301	5734.602			1					1		15	
	5-1	2	0.5	1	3392.394	6784.788	6665.225	13330.45	1	1717129	0.78	12948	0.0150	1	0.012	15	0.0410
			0.5	2	3272.831	6545.662			1					1		15	
	5-2	2	0.5	1	3313.866	6627.732	6550.897	13101.794	1	1717129	0.76	12720	0.0148	1	0.012	15	0.0402
			0.5	2	3237.031	6474.062			1					1		15	
	5-3	2	0.5	1	3041.966	6083.932	6117.632	12235.264	1	1717129	0.71	11853	0.0138	1	0.012	15	0.0375
			0.5	2	3075.666	6151.332			1					1		15	
	6-1	2	0.5	1	3039.02	6078.04	6093.745	12187.49	1	1717129	0.71	11806	0.0137	1	0.012	15	0.0373
			0.5	2	3054.725	6109.45			1					1		15	
	6-2	2	0.5	1	3347.761	6695.522	6789.907	13579.814	1	1717129	0.79	13198	0.0153	1	0.012	15	0.0417
			0.5	2	3442.146	6884.292			1					1		15	
	6-3	2	0.5	1	3254.651	6509.302	6699.07	13398.14	1	1717129	0.78	13016	0.0151	1	0.012	15	0.0412
			0.5	2	3444.419	6888.838			1					1		15	
	7-1	2	0.5	1	3402.882	6805.764	6849.901	13699.802	1	1717129	0.80	13318	0.0155	1	0.012	15	0.0421
			0.5	2	3447.019	6894.038			1					1		15	
	7-2	2	0.5	1	3315.18	6630.36	6708.078	13416.156	1	1717129	0.78	13034	0.0151	1	0.012	15	0.0412
			0.5	2	3392.898	6785.796			1					1		15	
	7-3	2	0.5	1	3248.051	6496.102	6580.689	13161.378	1	1717129	0.77	12779	0.0149	1	0.012	15	0.0404
			0.5	2	3332.638	6665.276			1					1		15	
	8-1	2	0.5	1	3359.717	6719.434	6746.894	13493.788	1	1717129	0.79	13112	0.0152	1	0.012	15	0.0415
			0.5	2	3387.177	6774.354			1					1		15	
	8-2	2	0.5	1	3371.471	6742.942	6667.715	13335.43	1	1717129	0.78	12953	0.0151	1	0.012	15	0.0410
			0.5	2	3296.244	6592.488			1					1		15	
	8-3	2	0.5	1	3214.415	6428.83	6486.417	12972.834	1	1717129	0.76	12591	0.0146	1	0.012	15	0.0398
			0.5	2	3272.002	6544.004			1					1		15	

Assay Date	8/5/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID TM	Replicate # 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0477	0.0016
Full activity	End	0.0414	0.0009
Full activity	Overall	0.0446	0.0038
Background	Beginning	0.0000	0.000132186
Background	End	0.0000	0.000133487
Background	Overall	0.0000	0.000116312
Positive	Beginning	0.0180	0.0033
Positive	End	0.0203	0.0002
Positive	Overall	0.0191	0.0023
Negative	Beginning	0.0131	0.0001
Negative	End	0.0377	0.0035
Negative	Overall	0.0254	0.0144

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#6 FRM	1	1	3.30E-05	-4.48	0.0060
RC#6 FRM	1	2	3.30E-05	-4.48	0.0055
RC#6 FRM	1	3	3.30E-05	-4.48	0.0056
RC#6 FRM	2	1	1.00E-05	-5.00	0.0137
RC#6 FRM	2	2	1.00E-05	-5.00	0.0140
RC#6 FRM	2	3	1.00E-05	-5.00	0.0139
RC#6 FRM	3	1	3.30E-06	-5.48	0.0223
RC#6 FRM	3	2	3.30E-06	-5.48	0.0256
RC#6 FRM	3	3	3.30E-06	-5.48	0.0238
RC#6 FRM	4	1	1.00E-06	-6.00	0.0336
RC#6 FRM	4	2	1.00E-06	-6.00	0.0319
RC#6 FRM	4	3	1.00E-06	-6.00	0.0347
RC#6 FRM	5	1	1.00E-07	-7.00	0.0410
RC#6 FRM	5	2	1.00E-07	-7.00	0.0402
RC#6 FRM	5	3	1.00E-07	-7.00	0.0375
RC#6 FRM	6	1	1.00E-08	-8.00	0.0373
RC#6 FRM	6	2	1.00E-08	-8.00	0.0417
RC#6 FRM	6	3	1.00E-08	-8.00	0.0412
RC#6 FRM	7	1	1.00E-09	-9.00	0.0421
RC#6 FRM	7	2	1.00E-09	-9.00	0.0412
RC#6 FRM	7	3	1.00E-09	-9.00	0.0404
RC#6 FRM	8	1	1.00E-10	-10.00	0.0415
RC#6 FRM	8	2	1.00E-10	-10.00	0.0410
RC#6 FRM	8	3	1.00E-10	-10.00	0.0398

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	13.53	12.39	12.68
2	-5.00	30.66	31.35	31.21
3	-5.48	50.09	57.38	53.41
4	-6.00	75.37	71.64	77.77
5	-7.00	91.90	90.28	84.13
6	-8.00	83.79	93.67	92.38
7	-9.00	94.52	92.51	90.70
8	-10.00	93.06	91.94	89.36

Assay Date	8/8/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8		
Technician ID	TM	Replicate #	3	Microsome type	placental	Microsome ID	0

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0198	29902.82	1510243
2	0.0200	32737.14	1636857
3	0.0203	36391.62	1792691
4	0.0198	36166.82	1826607
5	0.0205	37264.12	1817762
		Average DPM/g soln	1716832
		SD	138805
		CV	8.08
		µCi/g soln	0.773

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	18.3	18.3		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2935 g
Mass of dilution B used in substrate prep	9.2097 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.565238 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00875 µg/g soln.
µg/g soln.

a. µCi/g soln	0.773
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.565238 + 0.00875
= 0.573992 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.347 µCi/µg ASDN

856633 dpm/nmol

Assay Date	8/8/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8				
Technician ID	TM	Replicate #	3	Microsome type	placental	Microsome ID	0		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.448	0.301	0.163	0.110	0.068	0.061	0.039			
	0.419	0.284	0.172	0.107	0.065	0.053	0.042			
	0.508	0.286	0.179	0.113	0.075	0.054	0.039			

Samples:	Placental	QC 10	QC 100
	0.095	0.067	0.256
	0.101	0.070	0.245
	0.108	0.063	0.239

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	
0.25	125	1000	0.00025	200	0.0500	0.458	0.419	0.0477	m, b	0.121	-0.003
0.125	62.5	1000	0.00013	200	0.0250	0.291	0.251	0.0274	se_m, se_b	0.007	0.002
0.05	25	1000	0.00005	200	0.0100	0.171	0.132	0.0129	r^2, se_y	0.986	0.003
0.025	25	2000	0.00003	200	0.0050	0.110	0.070	0.0055	F, df	272	4
0.01	5	1000	0.00001	200	0.0020	0.069	0.029	0.0005	ss_{reg}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.056	0.016	-0.0011			
			Blank	0.040							

r^2= 0.986
m= 0.121
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/µL Prep.	average mg/µL	mg/mL
Placental	0.095	0.056	0.004	200	90	72000	0.015	0.018	17.757
Placental	0.101	0.061	0.004	200	90	72000	0.018		
Placental	0.108	0.068	0.005	200	90	72000	0.021		
QC 10	0.067	0.027	0.000	200	200	200	0.000	0.000	0.001
QC 10	0.070	0.030	0.001	200	200	200	0.000		
QC 10	0.063	0.023	0.000	200	200	200	0.000		
QC 100	0.256	0.216	0.023	200	200	200	0.000	0.000	0.110
QC 100	0.245	0.205	0.022	200	200	200	0.000		
QC 100	0.239	0.199	0.021	200	200	200	0.000		

Assay Date	8/8/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	Replicate #	3

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	17.75676
Protein Concentration (dilution added to assay, mg/mL):	0.022196

Test Chemical Concentrations	
Level	Final Concentration (M)
1	3.30E-05
2	1.00E-05
3	3.30E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	8/8/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	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	3291.944	6583.888	6376.037	12752.074	1	1716832	0.74	12363	0.0144	1	0.011	15	0.0433
			0.5	2	3084.093	6168.186			1					1		15	
	2	2	0.5	1	3421.133	6842.266	6367.954	12735.908	1	1716832	0.74	12347	0.0144	1	0.011	15	0.0433
			0.5	2	2946.821	5893.642			1					1		15	
	3	2	0.5	1	2936.043	5872.086	6028.878	12057.756	1	1716832	0.70	11669	0.0136	1	0.011	15	0.0409
			0.5	2	3092.835	6185.67			1					1		15	
	4	2	0.5	1	3148.908	6297.816	6364.8	12729.6	1	1716832	0.74	12340	0.0144	1	0.011	15	0.0433
			0.5	2	3215.892	6431.784			1					1		15	
Background control	1	2	0.5	1	134.4348	268.8696	231.96594	463.93188	1	1716832	0.03	75	0.0001	1	0.011	15	0.0003
			0.5	2	97.53114	195.06228			1					1		15	
	2	2	0.5	1	102.3555	204.711	187.3089	374.6178	1	1716832	0.02	-15	0.0000	1	0.011	15	-0.0001
			0.5	2	84.9534	169.9068			1					1		15	
	3	2	0.5	1	92.91779	185.83558	200.99289	401.98578	1	1716832	0.02	13	0.0000	1	0.011	15	0.0000
			0.5	2	108.0751	216.1502			1					1		15	
	4	2	0.5	1	78.52639	157.05278	158.17062	316.34124	1	1716832	0.02	-73	-0.0001	1	0.011	15	-0.0003
			0.5	2	79.64423	159.28846			1					1		15	
Positive control	1	2	0.5	1	1438.615	2877.23	2767.596	5535.192	1	1716832	0.32	5146	0.0060	1	0.011	15	0.0180
			0.5	2	1328.981	2657.962			1					1		15	
	2	2	0.5	1	1250.414	2500.828	2437.113	4874.226	1	1716832	0.28	4485	0.0052	1	0.011	15	0.0157
			0.5	2	1186.699	2373.398			1					1		15	
	3	2	0.5	1	1709.696	3419.392	3422.047	6844.094	1	1716832	0.40	6455	0.0075	1	0.011	15	0.0226
			0.5	2	1712.351	3424.702			1					1		15	
	4	2	0.5	1	1810.579	3621.158	3493.67	6987.34	1	1716832	0.41	6598	0.0077	1	0.011	15	0.0231
			0.5	2	1683.091	3366.182			1					1		15	
Negative Control	1	2	0.5	1	1598.194	3196.388	3217.036	6434.072	1	1716832	0.37	6045	0.0071	1	0.011	15	0.0212
			0.5	2	1618.842	3237.684			1					1		15	
	2	2	0.5	1	1420.799	2841.598	2870.594	5741.188	1	1716832	0.33	5352	0.0062	1	0.011	15	0.0188
			0.5	2	1449.795	2899.59			1					1		15	
	3	2	0.5	1	2933.858	5867.716	5896.623	11793.246	1	1716832	0.69	11404	0.0133	1	0.011	15	0.0400
			0.5	2	2962.765	5925.53			1					1		15	
	4	2	0.5	1	2751.975	5503.95	5615.836	11231.672	1	1716832	0.65	10842	0.0127	1	0.011	15	0.0380
			0.5	2	2863.861	5727.722			1					1		15	
RC#6 FRM	1-1	2	0.5	1	445.4872	890.9744	955.9168	1911.8336	1	1716832	0.11	1523	0.0018	1	0.011	15	0.0053
			0.5	2	510.4296	1020.8592			1					1		15	
	1-2	2	0.5	1	515.8648	1031.3296	1089.1093	2178.2186	1	1716832	0.13	1789	0.0021	1	0.011	15	0.0063
			0.5	2	573.4445	1146.889			1					1		15	
	1-3	2	0.5	1	496.4543	992.9086	941.4442	1882.8884	1	1716832	0.11	1494	0.0017	1	0.011	15	0.0052
			0.5	2	444.9899	889.9798			1					1		15	
	2-1	2	0.5	1	1014.867	2029.734	2058.73	4117.46	1	1716832	0.24	3728	0.0044	1	0.011	15	0.0131
			0.5	2	1043.863	2087.726			1					1		15	
	2-2	2	0.5	1	1019.023	2038.046	2155.056	4310.112	1	1716832	0.25	3921	0.0046	1	0.011	15	0.0137
			0.5	2	1136.033	2272.066			1					1		15	
	2-3	2	0.5	1	1109.857	2219.714	2346.15	4692.3	1	1716832	0.27	4303	0.0050	1	0.011	15	0.0151
			0.5	2	1236.293	2472.586			1					1		15	
	3-1	2	0.5	1	1981.514	3963.028	4033.702	8067.404	1	1716832	0.47	7678	0.0090	1	0.011	15	0.0269
			0.5	2	2052.188	4104.376			1					1		15	
	3-2	2	0.5	1	2061.367	4122.734	4024.108	8048.216	1	1716832	0.47	7659	0.0089	1	0.011	15	0.0269
			0.5	2	1962.741	3925.482			1					1		15	
	3-3	2	0.5	1	1876.845	3753.69	3637.001	7274.002	1	1716832	0.42	6885	0.0080	1	0.011	15	0.0241
			0.5	2	1760.158	3520.312			1					1		15	
	4-1	2	0.5	1	2831.802	5663.604	5537.931	11075.862	1	1716832	0.65	10687	0.0125	1	0.011	15	0.0375
			0.5	2	2706.129	5412.258			1					1		15	
	4-2	2	0.5	1	2625.633	5251.266	5291.088	10582.176	1	1716832	0.62	10193	0.0119	1	0.011	15	0.0357
			0.5	2	2665.455	5330.91			1					1		15	
	4-3	2	0.5	1	2538.023	5076.046	5189.658	10379.716	1	1716832	0.60	9990	0.0117	1	0.011	15	0.0350
			0.5	2	2651.835	5303.67			1					1		15	
	5-1	2	0.5	1	3439.51	6879.02	6499.928	12999.856	1	1716832	0.76	12611	0.0147	1	0.011	15	0.0442
			0.5	2	3060.418	6120.836			1					1		15	
	5-2	2	0.5	1	2865.352	5730.704	5883.593	11767.186	1	1716832	0.69	11378	0.0133	1	0.011	15	0.0399
			0.5	2	3018.241	6036.482			1					1		15	
	5-3	2	0.5	1	2955.257	5910.514	6018.311	12036.622	1	1716832	0.70	11647	0.0136	1	0.011	15	0.0408
			0.5	2	3063.054	6126.108			1					1		15	
	6-1	2	0.5	1	3156.6	6313.2	6414.719	12829.438	1	1716832	0.75	12440	0.0145	1	0.011	15	0.0436
			0.5	2	3258.119	6516.238			1					1		15	
	6-2	2	0.5	1	3131.607	6263.214	6397.072	12794.144	1	1716832	0.75	12405	0.0145	1	0.011	15	0.0435
			0.5	2	3265.465	6530.93			1					1		15	
	6-3	2	0.5	1	3111.484	6222.968	6240.455	12480.91	1	1716832	0.73	12092	0.0141	1	0.011	15	0.0424
			0.5	2	3128.971	6257.942			1					1		15	
	7-1	2	0.5	1	3094.702	6189.404	6316.97	12633.94	1	1716832	0.74	12245	0.0143	1	0.011	15	0.0429
			0.5	2	3222.268	6444.536			1					1		15	
	7-2	2	0.5	1	3046.888	6093.776	6023.101	12046.202	1	1716832	0.70	11657	0.0136	1	0.011	15	0.0409
			0.5	2	2976.213	5952.426			1					1		15	
	7-3	2	0.5	1	3084.158	6168.316	6405.559	12811.118	1	1716832	0.75	12422	0.0145	1	0.011	15	0.0436
			0.5	2	3321.401	6642.802			1					1		15	
	8-1	2	0.5	1	3118.427	6236.854	6203.363	12406.726	1	1716832	0.72	12018	0.0140	1	0.011	15	0.0421
			0.5	2	3084.936	6169.872			1					1		15	
	8-2	2	0.5	1	3163.239	6326.478	6170.952	12341.904	1	1716832	0.72	11953	0.0140	1	0.011	15	0.0419
			0.5	2	3007.713	6015.426			1					1		15	
	8-3	2	0.5	1	3021.219	6042.438	6258.954	12517.908	1	1716832	0.73	12129	0.0142	1	0.011	15	0.0426
			0.5	2	3237.735	6475.47			1					1		15	

Assay Date	8/8/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	placental	Microsome ID	0	Technician ID	TM	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0433	0.0000
Full activity	End	0.0421	0.0017
Full activity	Overall	0.0427	0.0012
Background	Beginning	0.0001	0.000221434
Background	End	-0.0001	0.000212337
Background	Overall	0.0000	0.000214896
Positive	Beginning	0.0169	0.0016
Positive	End	0.0229	0.0004
Positive	Overall	0.0199	0.0036
Negative	Beginning	0.0200	0.0017
Negative	End	0.0390	0.0014
Negative	Overall	0.0295	0.0111

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#6 FRM	1	1	3.30E-05	-4.48	0.0053
RC#6 FRM	1	2	3.30E-05	-4.48	0.0063
RC#6 FRM	1	3	3.30E-05	-4.48	0.0052
RC#6 FRM	2	1	1.00E-05	-5.00	0.0131
RC#6 FRM	2	2	1.00E-05	-5.00	0.0137
RC#6 FRM	2	3	1.00E-05	-5.00	0.0151
RC#6 FRM	3	1	3.30E-06	-5.48	0.0269
RC#6 FRM	3	2	3.30E-06	-5.48	0.0269
RC#6 FRM	3	3	3.30E-06	-5.48	0.0241
RC#6 FRM	4	1	1.00E-06	-6.00	0.0375
RC#6 FRM	4	2	1.00E-06	-6.00	0.0357
RC#6 FRM	4	3	1.00E-06	-6.00	0.0350
RC#6 FRM	5	1	1.00E-07	-7.00	0.0442
RC#6 FRM	5	2	1.00E-07	-7.00	0.0399
RC#6 FRM	5	3	1.00E-07	-7.00	0.0408
RC#6 FRM	6	1	1.00E-08	-8.00	0.0436
RC#6 FRM	6	2	1.00E-08	-8.00	0.0435
RC#6 FRM	6	3	1.00E-08	-8.00	0.0424
RC#6 FRM	7	1	1.00E-09	-9.00	0.0429
RC#6 FRM	7	2	1.00E-09	-9.00	0.0409
RC#6 FRM	7	3	1.00E-09	-9.00	0.0436
RC#6 FRM	8	1	1.00E-10	-10.00	0.0421
RC#6 FRM	8	2	1.00E-10	-10.00	0.0419
RC#6 FRM	8	3	1.00E-10	-10.00	0.0425

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	12.50	14.69	12.26
2	-5.00	30.61	32.19	35.33
3	-5.48	63.04	62.88	56.53
4	-6.00	87.74	83.69	82.03
5	-7.00	103.54	93.42	95.63
6	-8.00	102.14	101.85	99.28
7	-9.00	100.53	95.71	101.99
8	-10.00	98.67	98.14	99.58

Assay Date	8/29/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8		
Technician ID	TM	Replicate #	1	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0198	29440.4	1486889		
2	0.0198	32671.58	1650080		
3	0.0203	33623.9	1656350		
4	0.0198	34041.22	1719254		
5	0.0200	33986.36	1699318		
			Average DPM/g soln		1642378
			SD		91629
			CV		5.58
			µCi/g soln		0.740

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	17.5	17.5		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.4349 g
Mass of dilution B used in substrate prep	9.1897 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.559158 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00837 µg/g soln.
µg/g soln.

a. µCi/g soln	0.740
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.559158 + 0.00837
= 0.567532 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.304 µCi/µg ASDN

828811 dpm/nmol

Assay Date 8/29/2005 | Test Chemical ID RC#7 ECZ | # Concentrations tested 8

Technician ID TM | Replicate # 1 | Microsome type placental | Microsome ID 6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.581	0.365	0.181	0.110	0.068	0.054	0.040			
	0.580	0.368	0.183	0.113	0.071	0.055	0.039			
	0.589	0.368	0.183	0.110	0.069	0.054	0.039			

Samples:	Placental	QC 10	QC 100
	0.112	0.071	0.306
	0.105	0.070	0.302
	0.103	0.068	0.295

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
0.25	125	1000	0.00025	200	0.0500	0.583	0.544	0.0478
0.125	62.5	1000	0.00013	200	0.0250	0.367	0.328	0.0282
0.05	25	1000	0.00005	200	0.0100	0.182	0.143	0.0113
0.025	25	2000	0.00003	200	0.0050	0.111	0.071	0.0049
0.01	5	1000	0.00001	200	0.0020	0.069	0.030	0.0011
0.005	5	2000	0.00001	200	0.0010	0.054	0.015	-0.0003

Variables	Regression results			
m, b	0.091	-0.002		
se_m, se_b	0.005	0.001		
r^2, se_y	0.990	0.002		
F, df	377	4		
ss_{reg}, ss_{resid}	0.002	0.000		

Blank 0.040

r^2= 0.990
m= 0.091
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
Placental	0.112	0.072	0.005	200	90	72000	0.020	0.018	17.857
Placental	0.105	0.065	0.004	200	90	72000	0.017		
Placental	0.103	0.064	0.004	200	90	72000	0.017		
QC 10	0.071	0.031	0.001	200	200	200	0.000	0.000	0.005
QC 10	0.070	0.030	0.001	200	200	200	0.000		
QC 10	0.068	0.029	0.001	200	200	200	0.000		
QC 100	0.306	0.266	0.023	200	200	200	0.000	0.000	0.111
QC 100	0.302	0.262	0.022	200	200	200	0.000		
QC 100	0.295	0.255	0.022	200	200	200	0.000		

Assay Date	8/29/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate # 1

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	17.85727
Protein Concentration (dilution added to assay, mg/mL):	0.022322

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
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	RC#7 ECZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	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	4037.702	8075.404	8088.825	16177.65	1	1642378	0.99	15790	0.0191	1	0.011	15	0.0569
			0.5	2	4051.123	8102.246			1					1		15	
	2	2	0.5	1	3902.027	7804.054	7660.238	15320.476	1	1642378	0.93	14933	0.0180	1	0.011	15	0.0538
			0.5	2	3758.211	7516.422			1					1		15	
	3	2	0.5	1	3261.639	6523.278	6713.001	13426.002	1	1642378	0.82	13038	0.0157	1	0.011	15	0.0470
			0.5	2	3451.362	6902.724			1					1		15	
	4	2	0.5	1	3288.848	6577.696	6422.952	12845.904	1	1642378	0.78	12458	0.0150	1	0.011	15	0.0449
			0.5	2	3134.104	6268.208			1					1		15	
Background control	1	2	0.5	1	86.83231	173.66462	199.14001	398.28002	1	1642378	0.02	11	0.0000	1	0.011	15	0.0000
			0.5	2	112.3077	224.6154			1					1		15	
	2	2	0.5	1	70.55125	141.1025	153.18562	306.37124	1	1642378	0.02	-81	-0.0001	1	0.011	15	-0.0003
			0.5	2	82.63437	165.26874			1					1		15	
	3	2	0.5	1	95.68357	191.36714	226.70917	453.41834	1	1642378	0.03	66	0.0001	1	0.011	15	0.0002
			0.5	2	131.0256	262.0512			1					1		15	
	4	2	0.5	1	113.9674	227.9348	195.98232	391.96464	1	1642378	0.02	4	0.0000	1	0.011	15	0.0000
			0.5	2	82.01492	164.02984			1					1		15	
Positive control	1	2	0.5	1	1229.226	2458.452	2523.577	5047.154	1	1642378	0.31	4660	0.0056	1	0.011	15	0.0188
			0.5	2	1294.351	2588.702			1					1		15	
	2	2	0.5	1	1395.275	2790.55	2685.643	5371.286	1	1642378	0.33	4984	0.0060	1	0.011	15	0.0180
			0.5	2	1290.368	2580.736			1					1		15	
	3	2	0.5	1	1595.552	3191.104	3237.234	6474.468	1	1642378	0.39	6087	0.0073	1	0.011	15	0.0219
			0.5	2	1641.682	3283.364			1					1		15	
	4	2	0.5	1	1766.055	3532.11	3383.315	6766.63	1	1642378	0.41	6379	0.0077	1	0.011	15	0.0230
			0.5	2	1617.26	3234.52			1					1		15	
Negative Control	1	2	0.5	1	2400.303	4800.606	4714.088	9428.176	1	1642378	0.57	9041	0.0109	1	0.011	15	0.0326
			0.5	2	2313.785	4627.57			1					1		15	
	2	2	0.5	1	2398.755	4797.51	4754.094	9508.188	1	1642378	0.58	9121	0.0110	1	0.011	15	0.0329
			0.5	2	2355.339	4710.678			1					1		15	
	3	2	0.5	1	3198.582	6397.164	6372.56	12745.12	1	1642378	0.78	12358	0.0149	1	0.011	15	0.0445
			0.5	2	3173.978	6347.956			1					1		15	
	4	2	0.5	1	3221.975	6443.95	6309.762	12619.524	1	1642378	0.77	12232	0.0148	1	0.011	15	0.0441
			0.5	2	3087.787	6175.574			1					1		15	
RC#7 ECZ	1-1	2	0.5	1	71.0796	142.1592	163.33942	326.67884	1	1642378	0.02	-61	-0.0001	1	0.011	15	-0.0002
			0.5	2	92.25982	184.51964			1					1		15	
	1-2	2	0.5	1	92.95025	185.9005	177.06949	354.13898	1	1642378	0.02	-33	0.0000	1	0.011	15	-0.0001
			0.5	2	84.11924	168.23848			1					1		15	
	1-3	2	0.5	1	128.3523	256.7046	249.5634	499.1268	1	1642378	0.03	112	0.0001	1	0.011	15	0.0004
			0.5	2	121.2111	242.4222			1					1		15	
	2-1	2	0.5	1	80.8074	161.6148	199.3249	398.6498	1	1642378	0.02	11	0.0000	1	0.011	15	0.0000
			0.5	2	118.5175	237.035			1					1		15	
	2-2	2	0.5	1	65.61194	131.22388	133.45004	266.90008	1	1642378	0.02	-121	-0.0001	1	0.011	15	-0.0004
			0.5	2	67.8351	135.6702			1					1		15	
	2-3	2	0.5	1	92.95025	185.9005	174.96517	349.93034	1	1642378	0.02	-38	0.0000	1	0.011	15	-0.0001
			0.5	2	82.01492	164.02984			1					1		15	
	3-1	2	0.5	1	91.58173	183.16346	158.19383	316.38766	1	1642378	0.02	-71	-0.0001	1	0.011	15	-0.0003
			0.5	2	66.6121	133.2242			1					1		15	
	3-2	2	0.5	1	94.97334	189.94668	155.57219	311.14438	1	1642378	0.02	-76	-0.0001	1	0.011	15	-0.0003
			0.5	2	60.59885	121.1977			1					1		15	
	3-3	2	0.5	1	80.22061	160.44122	162.23597	324.47194	1	1642378	0.02	-63	-0.0001	1	0.011	15	-0.0002
			0.5	2	82.01536	164.03072			1					1		15	
	4-1	2	0.5	1	73.81383	147.62766	161.29688	322.59376	1	1642378	0.02	-65	-0.0001	1	0.011	15	-0.0002
			0.5	2	87.46305	174.9661			1					1		15	
	4-2	2	0.5	1	84.11969	168.23938	165.52584	331.05168	1	1642378	0.02	-56	-0.0001	1	0.011	15	-0.0002
			0.5	2	81.40615	162.8123			1					1		15	
	4-3	2	0.5	1	111.2551	222.5102	194.75653	389.51306	1	1642378	0.02	2	0.0000	1	0.011	15	0.0000
			0.5	2	83.50143	167.00286			1					1		15	
	5-1	2	0.5	1	134.6797	269.3594	233.09813	466.19626	1	1642378	0.03	79	0.0001	1	0.011	15	0.0003
			0.5	2	98.41843	196.83686			1					1		15	
	5-2	2	0.5	1	173.6665	347.333	299.4234	598.8468	1	1642378	0.04	211	0.0003	1	0.011	15	0.0008
			0.5	2	125.7569	251.5138			1					1		15	
	5-3	2	0.5	1	145.9889	291.9778	277.2135	554.427	1	1642378	0.03	167	0.0002	1	0.011	15	0.0006
			0.5	2	131.2246	262.4492			1					1		15	
	6-1	2	0.5	1	549.5029	1099.0058	1025.192	2050.384	1	1642378	0.12	1663	0.0020	1	0.011	15	0.0060
			0.5	2	475.6891	951.3782			1					1		15	
	6-2	2	0.5	1	423.312	846.624	874.3965	1748.793	1	1642378	0.11	1361	0.0016	1	0.011	15	0.0049
			0.5	2	451.0845	902.169			1					1		15	
	6-3	2	0.5	1	455.8745	911.749	980.7728	1961.5456	1	1642378	0.12	1574	0.0019	1	0.011	15	0.0057
			0.5	2	524.8983	1049.7966			1					1		15	
	7-1	2	0.5	1	1624.237	3248.474	3344.62	6689.24	1	1642378	0.41	6302	0.0076	1	0.011	15	0.0227
			0.5	2	1720.383	3440.766			1					1		15	
	7-2	2	0.5	1	1738.726	3477.452	3433.71	6867.42	1	1642378	0.42	6480	0.0078	1	0.011	15	0.0234
			0.5	2	1694.994	3389.988			1					1		15	
	7-3	2	0.5	1	1316.655	2633.31	2525.684	5051.768	1	1642378	0.31	4664	0.0056	1	0.011	15	0.0168
			0.5	2	1209.229	2418.458			1					1		15	
	8-1	2	0.5	1	3239.607	6479.214	6334.32	12668.64	1	1642378	0.77	12281	0.0148	1	0.011	15	0.0443
			0.5	2	3094.713	6189.426			1					1		15	
	8-2	2	0.5	1	2938.762	5877.524	5804.259	11608.518	1	1642378	0.71	11221	0.0135	1	0.011	15	0.0404
			0.5	2	2865.497	5730.994			1					1		15	
	8-3	2	0.5	1	3050.971	6101.942	5913.307	11826.614	1	1642378	0.72	11439	0.0138	1	0.011	15	0.0412
			0.5	2	2862.336	5724.672			1					1		15	

Assay Date	8/29/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0554	0.0022
Full activity	End	0.0459	0.0015
Full activity	Overall	0.0506	0.0056
Background	Beginning	-0.0001	0.000234191
Background	End	0.0001	0.000156589
Background	Overall	0.0000	0.000218831
Positive	Beginning	0.0174	0.0008
Positive	End	0.0225	0.0007
Positive	Overall	0.0199	0.0030
Negative	Beginning	0.0327	0.0002
Negative	End	0.0443	0.0003
Negative	Overall	0.0385	0.0067

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#7 ECZ	1	1	1.00E-04	-4.00	-0.0002
RC#7 ECZ	1	2	1.00E-04	-4.00	-0.0001
RC#7 ECZ	1	3	1.00E-04	-4.00	0.0004
RC#7 ECZ	2	1	3.30E-05	-4.48	0.0000
RC#7 ECZ	2	2	3.30E-05	-4.48	-0.0004
RC#7 ECZ	2	3	3.30E-05	-4.48	-0.0001
RC#7 ECZ	3	1	1.00E-05	-5.00	-0.0003
RC#7 ECZ	3	2	1.00E-05	-5.00	-0.0003
RC#7 ECZ	3	3	1.00E-05	-5.00	-0.0002
RC#7 ECZ	4	1	1.00E-06	-6.00	-0.0002
RC#7 ECZ	4	2	1.00E-06	-6.00	-0.0002
RC#7 ECZ	4	3	1.00E-06	-6.00	0.0000
RC#7 ECZ	5	1	1.00E-07	-7.00	0.0003
RC#7 ECZ	5	2	1.00E-07	-7.00	0.0008
RC#7 ECZ	5	3	1.00E-07	-7.00	0.0006
RC#7 ECZ	6	1	1.00E-08	-8.00	0.0060
RC#7 ECZ	6	2	1.00E-08	-8.00	0.0049
RC#7 ECZ	6	3	1.00E-08	-8.00	0.0057
RC#7 ECZ	7	1	1.00E-09	-9.00	0.0227
RC#7 ECZ	7	2	1.00E-09	-9.00	0.0234
RC#7 ECZ	7	3	1.00E-09	-9.00	0.0168
RC#7 ECZ	8	1	1.00E-10	-10.00	0.0443
RC#7 ECZ	8	2	1.00E-10	-10.00	0.0404
RC#7 ECZ	8	3	1.00E-10	-10.00	0.0412

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	-0.43	-0.24	0.79
2	-4.48	0.08	-0.86	-0.27
3	-5.00	-0.51	-0.54	-0.45
4	-6.00	-0.46	-0.40	0.01
5	-7.00	0.56	1.50	1.19
6	-8.00	11.83	9.69	11.20
7	-9.00	44.84	46.10	33.19
8	-10.00	87.38	79.84	81.39

Assay Date	9/5/2005	Test Chemical ID	RC-7	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0200	31549.44	1577472		
2	0.0203	32266.18	1589467		
3	0.0203	33770.23	1663558		
4	0.0202	34875.67	1726518		
5	0.0205	33650.29	1641478		
			Average DPM/g soln	1639699	
			SD	60219	
			CV	3.67	
			µCi/g soln	0.739	

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	14.2	14.2		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.4094 g
Mass of dilution B used in substrate prep	9.2532 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563896 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00836 µg/g soln.
µg/g soln.

a. µCi/g soln	0.739
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.563896 + 0.00836
= 0.572257 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.291 µCi/µg ASDN

820627 dpm/nmol

Assay Date	9/5/2005	Test Chemical ID	RC-7	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	6-041305

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank
	0.302	0.178	0.091	0.060	0.039	0.034	0.029
	0.304	0.180	0.088	0.061	0.039	0.033	0.028
	0.298	0.183	0.090	0.060	0.039	0.033	0.029

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
2	1	

Samples:	Placental	QC 10	QC 100
	0.044	0.038	0.132
	0.057	0.039	0.131
	0.056	0.036	0.132

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
0.25	125	1000	0.00025	200	0.0500	0.301	0.272	0.0488
0.125	62.5	1000	0.00013	200	0.0250	0.180	0.152	0.0269
0.05	25	1000	0.00005	200	0.0100	0.090	0.061	0.0105
0.025	25	2000	0.00003	200	0.0050	0.060	0.032	0.0052
0.01	5	1000	0.00001	200	0.0020	0.039	0.011	0.0013
0.005	5	2000	0.00001	200	0.0010	0.034	0.005	0.0003

Variables	Regression results	
m, b	0.182	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.997	0.001
F, df	1160	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	Blank	0.029

r^2= 0.997
m= 0.182
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
Placental	0.044	0.015	0.002	200	90	72000	0.008	0.015	14.782
Placental	0.057	0.029	0.005	200	90	72000	0.019		
Placental	0.056	0.027	0.004	200	90	72000	0.017		
QC 10	0.038	0.009	0.001	200	200	200	0.000	0.000	0.005
QC 10	0.039	0.010	0.001	200	200	200	0.000		
QC 10	0.036	0.007	0.001	200	200	200	0.000		
QC 100	0.132	0.103	0.018	200	200	200	0.000	0.000	0.090
QC 100	0.131	0.102	0.018	200	200	200	0.000		
QC 100	0.132	0.103	0.018	200	200	200	0.000		

Assay Date	9/5/2005	Test Chemical ID	RC-7	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	2

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	14.78169
Protein Concentration (dilution added to assay, mg/mL):	0.018477

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	1.00E-08
5	3.30E-09
6	1.00E-09
7	3.30E-10
8	1.00E-10

Assay Date	9/5/2005	Test Chemical ID	RC-7	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	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	3638.816	7277.632	7242.807	14485.614	1	1639699	0.88	14104	0.0172	1	0.009	15	0.0620
			0.5	2	3603.991	7207.982			1					1		15	
	2	2	0.5	1	3731.076	7462.152	7481.146	14962.292	1	1639699	0.91	14581	0.0178	1	0.009	15	0.0641
			0.5	2	3750.07	7500.14			1					1		15	
	3	2	0.5	1	3910.167	7820.334	7636.359	15272.718	1	1639699	0.93	14891	0.0181	1	0.009	15	0.0655
			0.5	2	3726.192	7452.384			1					1		15	
	4	2	0.5	1	3801.181	7602.362	7491.554	14983.108	1	1639699	0.91	14602	0.0178	1	0.009	15	0.0642
			0.5	2	3690.373	7380.746			1					1		15	
Background control	1	2	0.5	1	100.3999	200.7999	183.90044	367.80088	1	1639699	0.02	-14	0.0000	1	0.009	15	-0.0001
			0.5	2	83.50054	167.00108			1					1		15	
	2	2	0.5	1	120.2879	240.5758	242.3958	484.7916	1	1639699	0.03	103	0.0001	1	0.009	15	0.0005
			0.5	2	122.1079	244.2158			1					1		15	
	3	2	0.5	1	89.54582	179.09164	189.20775	378.4155	1	1639699	0.02	-3	0.0000	1	0.009	15	0.0000
			0.5	2	99.66193	199.32386			1					1		15	
	4	2	0.5	1	79.28067	158.56134	147.11877	294.23754	1	1639699	0.02	-87	-0.0001	1	0.009	15	-0.0004
			0.5	2	67.8381	135.6762			1					1		15	
Positive control	1	2	0.5	1	1285.058	2570.116	2591.829	5183.658	1	1639699	0.32	4802	0.0059	1	0.009	15	0.0211
			0.5	2	1306.771	2613.542			1					1		15	
	2	2	0.5	1	1082.696	2165.392	2340.258	4680.516	1	1639699	0.29	4299	0.0052	1	0.009	15	0.0189
			0.5	2	1257.562	2515.124			1					1		15	
	3	2	0.5	1	1683.488	3366.976	3259.232	6518.464	1	1639699	0.40	6137	0.0075	1	0.009	15	0.0270
			0.5	2	1575.744	3151.488			1					1		15	
	4	2	0.5	1	1702.277	3404.554	3482.349	6964.698	1	1639699	0.42	6583	0.0080	1	0.009	15	0.0289
			0.5	2	1780.072	3560.144			1					1		15	
Negative Control	1	2	0.5	1	2327.547	4655.094	4674.745	9349.49	1	1639699	0.57	8968	0.0109	1	0.009	15	0.0394
			0.5	2	2347.198	4694.396			1					1		15	
	2	2	0.5	1	1980.873	3961.746	3948.178	7896.356	1	1639699	0.48	7515	0.0092	1	0.009	15	0.0330
			0.5	2	1967.305	3934.61			1					1		15	
	3	2	0.5	1	3414.554	6829.108	6885.151	13770.302	1	1639699	0.84	13389	0.0163	1	0.009	15	0.0589
			0.5	2	3470.597	6941.194			1					1		15	
	4	2	0.5	1	3532.109	7064.218	6943.009	13886.018	1	1639699	0.85	13505	0.0165	1	0.009	15	0.0594
			0.5	2	3410.9	6821.8			1					1		15	
RC-7	1-1	2	0.5	1	100.4004	200.8008	196.80767	393.61534	1	1639699	0.02	12	0.0000	1	0.009	15	0.0001
			0.5	2	96.40727	192.81454			1					1		15	
	1-2	2	0.5	1	84.74876	169.49752	141.73276	283.46552	1	1639699	0.02	-98	-0.0001	1	0.009	15	-0.0004
			0.5	2	56.984	113.968			1					1		15	
	1-3	2	0.5	1	83.26512	166.53024	170.09788	340.19576	1	1639699	0.02	-41	-0.0001	1	0.009	15	-0.0002
			0.5	2	86.83278	173.66552			1					1		15	
	2-1	2	0.5	1	111.2545	222.509	208.94137	417.88274	1	1639699	0.03	37	0.0000	1	0.009	15	0.0002
			0.5	2	97.68687	195.37374			1					1		15	
	2-2	2	0.5	1	100.4004	200.8008	170.95203	341.90406	1	1639699	0.02	-39	0.0000	1	0.009	15	-0.0002
			0.5	2	70.55163	141.10326			1					1		15	
	2-3	2	0.5	1	94.97334	189.94668	157.38439	314.76878	1	1639699	0.02	-67	-0.0001	1	0.009	15	-0.0003
			0.5	2	62.41105	124.8221			1					1		15	
	3-1	2	0.5	1	154.6709	309.3418	305.5114	611.0228	1	1639699	0.04	230	0.0003	1	0.009	15	0.0010
			0.5	2	150.8405	301.681			1					1		15	
	3-2	2	0.5	1	162.516	325.032	303.62	607.24	1	1639699	0.04	226	0.0003	1	0.009	15	0.0010
			0.5	2	141.104	282.208			1					1		15	
	3-3	2	0.5	1	141.104	282.208	265.9268	531.8536	1	1639699	0.03	151	0.0002	1	0.009	15	0.0007
			0.5	2	124.8228	249.6456			1					1		15	
	4-1	2	0.5	1	484.847	969.694	978.711	1957.422	1	1639699	0.12	1576	0.0019	1	0.009	15	0.0069
			0.5	2	493.864	987.728			1					1		15	
	4-2	2	0.5	1	457.2484	914.4968	880.1427	1760.2854	1	1639699	0.11	1379	0.0017	1	0.009	15	0.0061
			0.5	2	422.8943	845.7886			1					1		15	
	4-3	2	0.5	1	496.315	996.63	992.179	1984.358	1	1639699	0.12	1603	0.0020	1	0.009	15	0.0070
			0.5	2	493.864	987.728			1					1		15	
	5-1	2	0.5	1	1071.667	2143.334	2232.192	4464.384	1	1639699	0.27	4083	0.0050	1	0.009	15	0.0180
			0.5	2	1160.525	2321.05			1					1		15	
	5-2	2	0.5	1	1231.946	2463.892	2314.648	4629.296	1	1639699	0.28	4248	0.0052	1	0.009	15	0.0187
			0.5	2	1082.702	2165.404			1					1		15	
	5-3	2	0.5	1	1232.964	2465.928	2413.985	4827.97	1	1639699	0.29	4447	0.0054	1	0.009	15	0.0196
			0.5	2	1181.021	2362.042			1					1		15	
	6-1	2	0.5	1	2167.939	4335.878	4158.505	8317.01	1	1639699	0.51	7936	0.0097	1	0.009	15	0.0349
			0.5	2	1990.566	3981.132			1					1		15	
	6-2	2	0.5	1	2102.992	4205.984	4235.391	8470.782	1	1639699	0.52	8089	0.0099	1	0.009	15	0.0356
			0.5	2	2132.399	4264.798			1					1		15	
	6-3	2	0.5	1	2116.73	4237.46	4212.156	8424.312	1	1639699	0.51	8043	0.0098	1	0.009	15	0.0354
			0.5	2	2093.426	4186.852			1					1		15	
	7-1	2	0.5	1	2583.484	5166.968	5218.33	10436.66	1	1639699	0.64	10055	0.0123	1	0.009	15	0.0442
			0.5	2	2634.846	5269.692			1					1		15	
	7-2	2	0.5	1	2681.902	5363.804	5159.363	10318.726	1	1639699	0.63	9937	0.0121	1	0.009	15	0.0437
			0.5	2	2477.461	4954.922			1					1		15	
	7-3	2	0.5	1	3005.168	6010.336	5938.719	11877.438	1	1639699	0.72	11496	0.0140	1	0.009	15	0.0505
			0.5	2	2933.551	5867.102			1					1		15	
	8-1	2	0.5	1	3763.678	7527.356	7266.856	14533.712	1	1639699	0.89	14152	0.0172	1	0.009	15	0.0622
			0.5	2	3503.178	7006.356			1					1		15	
	8-2	2	0.5	1	3539.454	7078.908	7054.179	14108.358	1	1639699	0.86	13727	0.0167	1	0.009	15	0.0604
			0.5	2	3515.725	7031.45			1					1		15	
	8-3	2	0.5	1	3560.181	7120.362	7041.669	14083.338	1	1639699	0.86	13702	0.0167	1	0.009	15	0.0602
			0.5	2	3481.488	6962.976			1					1		15	

Assay Date	9/5/2005	Test Chemical ID	RC-7	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0631	0.0015
Full activity	End	0.0648	0.0009
Full activity	Overall	0.0639	0.0014
Background	Beginning	0.0002	0.000363718
Background	End	-0.0002	0.000261705
Background	Overall	0.0000	0.000345088
Positive	Beginning	0.0200	0.0016
Positive	End	0.0280	0.0014
Positive	Overall	0.0240	0.0047
Negative	Beginning	0.0362	0.0045
Negative	End	0.0591	0.0004
Negative	Overall	0.0477	0.0135

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC-7	1	1	1.00E-05	-5.00	0.0001
RC-7	1	2	1.00E-05	-5.00	-0.0004
RC-7	1	3	1.00E-05	-5.00	-0.0002
RC-7	2	1	1.00E-06	-6.00	0.0002
RC-7	2	2	1.00E-06	-6.00	-0.0002
RC-7	2	3	1.00E-06	-6.00	-0.0003
RC-7	3	1	1.00E-07	-7.00	0.0010
RC-7	3	2	1.00E-07	-7.00	0.0010
RC-7	3	3	1.00E-07	-7.00	0.0007
RC-7	4	1	1.00E-08	-8.00	0.0069
RC-7	4	2	1.00E-08	-8.00	0.0061
RC-7	4	3	1.00E-08	-8.00	0.0070
RC-7	5	1	3.30E-09	-8.48	0.0180
RC-7	5	2	3.30E-09	-8.48	0.0187
RC-7	5	3	3.30E-09	-8.48	0.0196
RC-7	6	1	1.00E-09	-9.00	0.0349
RC-7	6	2	1.00E-09	-9.00	0.0356
RC-7	6	3	1.00E-09	-9.00	0.0354
RC-7	7	1	3.30E-10	-9.48	0.0442
RC-7	7	2	3.30E-10	-9.48	0.0437
RC-7	7	3	3.30E-10	-9.48	0.0505
RC-7	8	1	1.00E-10	-10.00	0.0622
RC-7	8	2	1.00E-10	-10.00	0.0604
RC-7	8	3	1.00E-10	-10.00	0.0602

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	0.08	-0.67	-0.28
2	-6.00	0.25	-0.27	-0.46
3	-7.00	1.58	1.55	1.04
4	-8.00	10.84	9.48	11.02
5	-8.48	28.07	29.21	30.57
6	-9.00	54.56	55.62	55.30
7	-9.48	69.13	68.32	79.04
8	-10.00	97.30	94.38	94.21

Assay Date	9/6/2005	Test Chemical ID	RC-7	# Concentrations tested	8		
Technician ID	TM	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	31944.67	1589287	
2	0.0201	32385.38	1611213	
3	0.0202	33291.23	1648081	
4	0.0205	34850.95	1700046	
5	0.0198	36743.6	1855737	
			Average DPM/g soln	1680873
			SD	106373
			CV	6.33
			µCi/g soln	0.757

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	15.8	15.8		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.318 g
Mass of dilution B used in substrate prep	9.1948 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563476 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00857 µg/g soln.
µg/g soln.

a. µCi/g soln 0.757
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.563476 + 0.00857
= 0.572047 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.324 µCi/µg ASDN

841543 dpm/nmol

Assay Date	9/6/2005	Test Chemical ID	RC-7	# Concentrations tested	8
Technician ID	TM	Replicate #	3	Microsome type	placental

Microsome ID 6-041305

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.267	0.154	0.082	0.052	0.035	0.031	0.031			
	0.263	0.162	0.083	0.054	0.035	0.030	0.026			
	0.266	0.157	0.083	0.054	0.035	0.031	0.027			

Samples:	Placental	QC 10	QC 100
	0.054	0.034	0.115
	0.050	0.040	0.121
	0.048	0.033	0.120

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
0.25	125	1000	0.00025	200	0.0500	0.265	0.237	0.0489
0.125	62.5	1000	0.00013	200	0.0250	0.158	0.130	0.0266
0.05	25	1000	0.00005	200	0.0100	0.082	0.054	0.0110
0.025	25	2000	0.00003	200	0.0050	0.053	0.026	0.0050
0.01	5	1000	0.00001	200	0.0020	0.035	0.007	0.0012
0.005	5	2000	0.00001	200	0.0010	0.031	0.003	0.0003

Variables	Regression results	
m, b	0.207	0.000
se_m, se_b	0.006	0.001
r^2, se_y	0.997	0.001
F, df	1199	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank	0.028		r^2=	0.997								
			m=	0.207								
			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
Placental	0.054	0.026	0.005	200	90	72000	0.020	0.018	17.669
Placental	0.050	0.022	0.004	200	90	72000	0.017		
Placental	0.048	0.020	0.004	200	90	72000	0.015		
QC 10	0.034	0.006	0.001	200	200	200	0.000	0.000	0.007
QC 10	0.040	0.012	0.002	200	200	200	0.000		
QC 10	0.033	0.005	0.001	200	200	200	0.000		
QC 100	0.115	0.087	0.018	200	200	200	0.000	0.000	0.092
QC 100	0.121	0.093	0.019	200	200	200	0.000		
QC 100	0.120	0.092	0.019	200	200	200	0.000		

Assay Date	9/6/2005	Test Chemical ID	RC-7	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	3

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	17.66908
Protein Concentration (dilution added to assay, mg/mL):	0.022086

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	1.00E-08
5	3.30E-09
6	1.00E-09
7	3.30E-10
8	1.00E-10

Assay Date	9/6/2005	Test Chemical ID	RC-7	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	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	6797.342	13594.684	12994.904	25989.808	1	1680873	1.55	25628	0.0305	1	0.011	15	0.0919
			0.5	2	6197.562	12395.124			1					1		15	
	2	2	0.5	1	6657.575	13315.15	13036.948	26073.896	1	1680873	1.55	25712	0.0306	1	0.011	15	0.0922
			0.5	2	6379.373	12758.746			1					1		15	
	3	2	0.5	1	6404.163	12808.326	12869.639	25739.278	1	1680873	1.53	25378	0.0302	1	0.011	15	0.0910
			0.5	2	6465.476	12930.952			1					1		15	
	4	2	0.5	1	6282.966	12565.932	12772.518	25545.036	1	1680873	1.52	25184	0.0299	1	0.011	15	0.0903
			0.5	2	6489.552	12979.104			1					1		15	
Background control	1	2	0.5	1	99.91762	199.83524	163.27063	326.54126	1	1680873	0.02	-35	0.0000	1	0.011	15	-0.0001
			0.5	2	63.35301	126.70602			1					1		15	
	2	2	0.5	1	79.28067	158.56134	162.54535	325.0907	1	1680873	0.02	-36	0.0000	1	0.011	15	-0.0001
			0.5	2	83.26458	166.52936			1					1		15	
	3	2	0.5	1	122.1079	244.2158	255.0699	510.1398	1	1680873	0.03	149	0.0002	1	0.011	15	0.0005
			0.5	2	132.962	265.924			1					1		15	
	4	2	0.5	1	62.87777	125.75554	142.15886	284.31772	1	1680873	0.02	-77	-0.0001	1	0.011	15	-0.0003
			0.5	2	79.28109	158.56218			1					1		15	
Positive control	1	2	0.5	1	2200.668	4401.336	4298.222	8596.444	1	1680873	0.51	8235	0.0098	1	0.011	15	0.0295
			0.5	2	2097.554	4195.108			1					1		15	
	2	2	0.5	1	2005.294	4010.588	3848.614	7697.228	1	1680873	0.46	7336	0.0087	1	0.011	15	0.0263
			0.5	2	1843.32	3686.64			1					1		15	
	3	2	0.5	1	2523.116	5046.232	4850.663	9701.326	1	1680873	0.58	9340	0.0111	1	0.011	15	0.0335
			0.5	2	2327.547	4655.094			1					1		15	
	4	2	0.5	1	2434.972	4869.944	4701.318	9402.636	1	1680873	0.56	9041	0.0107	1	0.011	15	0.0324
			0.5	2	2266.346	4532.692			1					1		15	
Negative Control	1	2	0.5	1	4390.536	8781.072	8866.823	17733.646	1	1680873	1.06	17372	0.0206	1	0.011	15	0.0623
			0.5	2	4476.287	8952.574			1					1		15	
	2	2	0.5	1	3810.96	7621.92	7818.935	15637.67	1	1680873	0.93	15276	0.0182	1	0.011	15	0.0548
			0.5	2	4007.875	8015.75			1					1		15	
	3	2	0.5	1	6313.299	12626.598	12450.311	24900.622	1	1680873	1.48	24539	0.0292	1	0.011	15	0.0880
			0.5	2	6137.012	12274.024			1					1		15	
	4	2	0.5	1	6144.966	12289.932	12381.139	24762.278	1	1680873	1.47	24401	0.0290	1	0.011	15	0.0875
			0.5	2	6236.173	12472.346			1					1		15	
RC-7	1-1	2	0.5	1	160.0979	320.1958	263.9835	527.967	1	1680873	0.03	166	0.0002	1	0.011	15	0.0006
			0.5	2	103.8656	207.7712			1					1		15	
	1-2	2	0.5	1	107.4262	214.8504	176.28754	352.57508	1	1680873	0.02	-9	0.0000	1	0.011	15	0.0000
			0.5	2	68.86234	137.72468			1					1		15	
	1-3	2	0.5	1	90.21642	180.43284	158.56219	317.12438	1	1680873	0.02	-44	-0.0001	1	0.011	15	-0.0002
			0.5	2	68.34577	136.69154			1					1		15	
	2-1	2	0.5	1	95.68408	191.36816	167.30091	334.60182	1	1680873	0.02	-27	0.0000	1	0.011	15	-0.0001
			0.5	2	71.61683	143.23366			1					1		15	
	2-2	2	0.5	1	83.50098	167.00196	175.79715	351.5943	1	1680873	0.02	-10	0.0000	1	0.011	15	0.0000
			0.5	2	92.29617	184.59234			1					1		15	
	2-3	2	0.5	1	112.0871	224.1742	198.28166	396.56332	1	1680873	0.02	35	0.0000	1	0.011	15	0.0001
			0.5	2	86.19456	172.38912			1					1		15	
	3-1	2	0.5	1	142.1592	284.3184	300.7214	601.4428	1	1680873	0.04	240	0.0003	1	0.011	15	0.0009
			0.5	2	158.5622	317.1244			1					1		15	
	3-2	2	0.5	1	225.8685	451.737	498.5634	997.1268	1	1680873	0.06	635	0.0008	1	0.011	15	0.0023
			0.5	2	272.6949	545.3898			1					1		15	
	3-3	2	0.5	1	276.7809	553.5618	494.387	988.774	1	1680873	0.06	627	0.0007	1	0.011	15	0.0022
			0.5	2	217.6061	435.2122			1					1		15	
	4-1	2	0.5	1	721.7351	1443.4702	1451.6718	2903.3436	1	1680873	0.17	2542	0.0030	1	0.011	15	0.0091
			0.5	2	729.9387	1459.8734			1					1		15	
	4-2	2	0.5	1	675.671	1351.342	1468.0243	2936.0486	1	1680873	0.17	2575	0.0031	1	0.011	15	0.0092
			0.5	2	792.3533	1584.7066			1					1		15	
	4-3	2	0.5	1	868.3323	1736.6646	1815.3573	3630.7146	1	1680873	0.22	3269	0.0039	1	0.011	15	0.0117
			0.5	2	947.025	1894.05			1					1		15	
	5-1	2	0.5	1	1989.024	3978.048	4098.108	8196.216	1	1680873	0.49	7835	0.0093	1	0.011	15	0.0281
			0.5	2	2109.084	4218.168			1					1		15	
	5-2	2	0.5	1	1607.501	3215.002	3386.913	6773.826	1	1680873	0.40	6412	0.0076	1	0.011	15	0.0230
			0.5	2	1779.412	3558.824			1					1		15	
	5-3	2	0.5	1	1985.179	3970.358	4142.183	8284.366	1	1680873	0.49	7923	0.0094	1	0.011	15	0.0284
			0.5	2	2157.004	4314.008			1					1		15	
	6-1	2	0.5	1	3731.699	7463.398	7418.985	14839.97	1	1680873	0.88	14478	0.0172	1	0.011	15	0.0519
			0.5	2	3688.286	7376.572			1					1		15	
	6-2	2	0.5	1	3560.163	7120.326	7239.919	14479.838	1	1680873	0.86	14118	0.0168	1	0.011	15	0.0506
			0.5	2	3679.756	7359.512			1					1		15	
	6-3	2	0.5	1	3353.222	6706.444	6761.426	13522.852	1	1680873	0.80	13161	0.0156	1	0.011	15	0.0472
			0.5	2	3408.204	6816.408			1					1		15	
	7-1	2	0.5	1	4550.604	9101.208	9266.733	18533.466	1	1680873	1.10	18172	0.0216	1	0.011	15	0.0652
			0.5	2	4716.129	9432.258			1					1		15	
	7-2	2	0.5	1	4193.926	8387.852	8616.808	17233.616	1	1680873	1.03	16872	0.0200	1	0.011	15	0.0605
			0.5	2	4422.882	8845.764			1					1		15	
	7-3	2	0.5	1	5212.707	10425.414	10403.706	20807.412	1	1680873	1.24	20446	0.0243	1	0.011	15	0.0733
			0.5	2	5190.999	10381.998			1					1		15	
	8-1	2	0.5	1	5952.844	11905.688	11957.905	23915.81	1	1680873	1.42	23554	0.0280	1	0.011	15	0.0845
			0.5	2	6005.061	12010.122			1					1		15	
	8-2	2	0.5	1	6082.806	12165.612	12304.95	24609.9	1	1680873	1.46	24248	0.0288	1	0.011	15	0.0870
			0.5	2	6222.144	12444.288			1					1		15	
	8-3	2	0.5	1	6178.49	12356.98	12080.436	24160.872	1	1680873	1.44	23799	0.0283	1	0.011	15	0.0854
			0.5	2	5901.946	11803.892			1					1		15	

Assay Date	9/6/2005	Test Chemical ID	RC-7	# Concentrations tested		Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0921	0.0002
Full activity	End	0.0907	0.0005
Full activity	Overall	0.0914	0.0009
Background	Beginning	-0.0001	3.679E-06
Background	End	0.0001	0.000572743
Background	Overall	0.0000	0.000362242
Positive	Beginning	0.0279	0.0023
Positive	End	0.0330	0.0008
Positive	Overall	0.0304	0.0032
Negative	Beginning	0.0586	0.0053
Negative	End	0.0878	0.0004
Negative	Overall	0.0732	0.0171

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC-7	1	1	1.00E-05	-5.00	0.0006
RC-7	1	2	1.00E-05	-5.00	0.0000
RC-7	1	3	1.00E-05	-5.00	-0.0002
RC-7	2	1	1.00E-06	-6.00	-0.0001
RC-7	2	2	1.00E-06	-6.00	0.0000
RC-7	2	3	1.00E-06	-6.00	0.0001
RC-7	3	1	1.00E-07	-7.00	0.0009
RC-7	3	2	1.00E-07	-7.00	0.0023
RC-7	3	3	1.00E-07	-7.00	0.0022
RC-7	4	1	1.00E-08	-8.00	0.0091
RC-7	4	2	1.00E-08	-8.00	0.0092
RC-7	4	3	1.00E-08	-8.00	0.0117
RC-7	5	1	3.30E-09	-8.48	0.0281
RC-7	5	2	3.30E-09	-8.48	0.0230
RC-7	5	3	3.30E-09	-8.48	0.0284
RC-7	6	1	1.00E-09	-9.00	0.0519
RC-7	6	2	1.00E-09	-9.00	0.0506
RC-7	6	3	1.00E-09	-9.00	0.0472
RC-7	7	1	3.30E-10	-9.48	0.0652
RC-7	7	2	3.30E-10	-9.48	0.0605
RC-7	7	3	3.30E-10	-9.48	0.0733
RC-7	8	1	1.00E-10	-10.00	0.0845
RC-7	8	2	1.00E-10	-10.00	0.0870
RC-7	8	3	1.00E-10	-10.00	0.0854

Percent of control values				
		Replicate		
Level	Log[test substance]	1	2	3
1	-5.00	0.65	-0.04	-0.17
2	-6.00	-0.11	-0.04	0.14
3	-7.00	0.94	2.49	2.46
4	-8.00	9.98	10.11	12.83
5	-8.48	30.75	25.17	31.10
6	-9.00	56.83	55.42	51.66
7	-9.48	71.33	66.23	80.26
8	-10.00	92.46	95.18	93.42

Assay Date	8/29/2005	Test Chemical ID	RC-8	# Concentrations tested	8		
Technician ID	TM	Replicate #	1	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0198	29440.4	1486889		
2	0.0198	32671.58	1650080		
3	0.0203	33623.9	1656350		
4	0.0198	34041.22	1719254		
5	0.0200	33986.36	1699318		
			Average DPM/g soln		1642378
			SD		91629
			CV		5.58
			µCi/g soln		0.740

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	17.5	17.5		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.4349 g
Mass of dilution B used in substrate prep	9.1897 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.559158 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00837 µg/g soln.
µg/g soln.

a. µCi/g soln	0.740
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.559158 + 0.00837
= 0.567532 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.304 µCi/µg ASDN

828811 dpm/nmol

Assay Date	8/29/2005	Test Chemical ID	RC-8	# Concentrations tested	8
Technician ID	TM	Replicate #	1	Microsome type	placental
Microsome ID	6-041305				

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank
	0.581	0.365	0.181	0.110	0.068	0.054	0.040
	0.580	0.368	0.183	0.113	0.071	0.055	0.039
	0.589	0.368	0.183	0.110	0.069	0.054	0.039

Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
2	1	

Samples:	Placental	QC 10	QC 100
	0.112	0.071	0.306
	0.105	0.070	0.302
	0.103	0.068	0.295

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
0.25	125	1000	0.00025	200	0.0500	0.583	0.544	0.0478
0.125	62.5	1000	0.00013	200	0.0250	0.367	0.328	0.0282
0.05	25	1000	0.00005	200	0.0100	0.182	0.143	0.0113
0.025	25	2000	0.00003	200	0.0050	0.111	0.071	0.0049
0.01	5	1000	0.00001	200	0.0020	0.069	0.030	0.0011
0.005	5	2000	0.00001	200	0.0010	0.054	0.015	-0.0003

Variables	Regression results	
m, b	0.091	-0.002
se_m, se_b	0.005	0.001
r^2, se_y	0.990	0.002
F, df	377	4
ss_{reg}, ss_{resid}	0.002	0.000

Blank 0.040

r^2= 0.990
m= 0.091
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
Placental	0.112	0.072	0.005	200	90	72000	0.020	0.018	17.857
Placental	0.105	0.065	0.004	200	90	72000	0.017		
Placental	0.103	0.064	0.004	200	90	72000	0.017		
QC 10	0.071	0.031	0.001	200	200	200	0.000	0.000	0.005
QC 10	0.070	0.030	0.001	200	200	200	0.000		
QC 10	0.068	0.029	0.001	200	200	200	0.000		
QC 100	0.306	0.266	0.023	200	200	200	0.000	0.000	0.111
QC 100	0.302	0.262	0.022	200	200	200	0.000		
QC 100	0.295	0.255	0.022	200	200	200	0.000		

Assay Date	8/29/2005	Test Chemical ID	RC-8	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	1

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	17.85727
Protein Concentration (dilution added to assay, mg/mL):	0.022322

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
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	RC-8	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	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	4037.702	8075.404	8088.825	16177.65	1	1642378	0.99	15790	0.0191	1	0.011	15	0.0569
			0.5	2	4051.123	8102.246			1					1		15	
	2	2	0.5	1	3902.027	7804.054	7660.236	15320.476	1	1642376	0.93	14933	0.0180	1	0.011	15	0.0538
			0.5	2	3758.211	7516.422			1					1		15	
	3	2	0.5	1	3261.639	6523.278	6713.001	13426.002	1	1642378	0.82	13036	0.0157	1	0.011	15	0.0470
			0.5	2	3451.362	6902.724			1					1		15	
	4	2	0.5	1	3288.848	6577.696	6422.952	12845.904	1	1642378	0.78	12458	0.0150	1	0.011	15	0.0449
			0.5	2	3134.104	6268.208			1					1		15	
Background control	1	2	0.5	1	86.83231	173.66462	199.14001	398.28002	1	1642378	0.02	11	0.0000	1	0.011	15	0.0000
			0.5	2	112.3077	224.6154			1					1		15	
	2	2	0.5	1	70.55125	141.1025	153.18562	306.37124	1	1642378	0.02	-81	-0.0001	1	0.011	15	-0.0003
			0.5	2	82.63437	165.26874			1					1		15	
	3	2	0.5	1	95.68357	191.36714	226.70917	453.41834	1	1642378	0.03	68	0.0001	1	0.011	15	0.0002
			0.5	2	131.0256	262.0512			1					1		15	
	4	2	0.5	1	113.9674	227.9348	195.98232	391.96464	1	1642378	0.02	4	0.0000	1	0.011	15	0.0000
			0.5	2	82.01492	164.02984			1					1		15	
Positive control	1	2	0.5	1	1229.226	2458.452	2523.577	5047.154	1	1642378	0.31	4660	0.0056	1	0.011	15	0.0168
			0.5	2	1294.351	2588.702			1					1		15	
	2	2	0.5	1	1395.275	2790.55	2665.643	5371.286	1	1642378	0.33	4984	0.0060	1	0.011	15	0.0180
			0.5	2	1290.368	2580.736			1					1		15	
	3	2	0.5	1	1595.552	3191.104	3237.234	6474.468	1	1642376	0.39	6087	0.0073	1	0.011	15	0.0219
			0.5	2	1641.682	3283.364			1					1		15	
	4	2	0.5	1	1766.055	3532.11	3383.315	6766.63	1	1642378	0.41	6379	0.0077	1	0.011	15	0.0230
			0.5	2	1617.26	3234.52			1					1		15	
Negative Control	1	2	0.5	1	2400.303	4800.606	4714.088	9428.176	1	1642378	0.57	9041	0.0109	1	0.011	15	0.0326
			0.5	2	2313.785	4627.57			1					1		15	
	2	2	0.5	1	2398.755	4797.51	4754.094	9508.188	1	1642378	0.58	9121	0.0110	1	0.011	15	0.0329
			0.5	2	2355.339	4710.678			1					1		15	
	3	2	0.5	1	3198.582	6397.164	6372.56	12745.12	1	1642378	0.78	12358	0.0149	1	0.011	15	0.0445
			0.5	2	3173.978	6347.956			1					1		15	
	4	2	0.5	1	3221.975	6443.95	6309.762	12619.524	1	1642378	0.77	12232	0.0148	1	0.011	15	0.0441
			0.5	2	3087.787	6175.574			1					1		15	
RC-8	1-1	2	0.5	1	793.3025	1586.605	1514.9412	3029.8824	1	1642378	0.18	2642	0.0032	1	0.011	15	0.0095
			0.5	2	721.6387	1443.2774			1					1		15	
	1-2	2	0.5	1	804.3206	1608.6412	1523.3257	3046.6514	1	1642378	0.19	2659	0.0032	1	0.011	15	0.0096
			0.5	2	719.0051	1438.0102			1					1		15	
	1-3	2	0.5	1	678.3882	1356.7764	1316.7735	2633.547	1	1642378	0.16	2246	0.0027	1	0.011	15	0.0081
			0.5	2	638.3659	1276.7706			1					1		15	
	2-1	2	0.5	1	202.3056	404.6112	373.2594	746.5188	1	1642378	0.05	359	0.0004	1	0.011	15	0.0013
			0.5	2	170.9538	341.9076			1					1		15	
	2-2	2	0.5	1	347.202	694.404	667.4012	1334.8024	1	1642378	0.08	947	0.0011	1	0.011	15	0.0034
			0.5	2	320.1992	640.3984			1					1		15	
	2-3	2	0.5	1	338.9986	677.9972	658.9602	1317.7204	1	1642378	0.08	930	0.0011	1	0.011	15	0.0034
			0.5	2	319.8616	639.7232			1					1		15	
	3-1	2	0.5	1	779.15	1558.3	1522.6634	3045.3268	1	1642378	0.19	2658	0.0032	1	0.011	15	0.0096
			0.5	2	743.5134	1487.0268			1					1		15	
	3-2	2	0.5	1	778.7696	1557.5792	1664.5601	3329.1202	1	1642378	0.20	2942	0.0035	1	0.011	15	0.0106
			0.5	2	885.7705	1771.541			1					1		15	
	3-3	2	0.5	1	803.8661	1607.7322	1578.2276	3156.4552	1	1642378	0.19	2769	0.0033	1	0.011	15	0.0100
			0.5	2	774.3615	1548.723			1					1		15	
	4-1	2	0.5	1	2522.92	5045.84	4848.78	9697.56	1	1642378	0.59	9310	0.0112	1	0.011	15	0.0335
			0.5	2	2325.86	4651.72			1					1		15	
	4-2	2	0.5	1	2550.717	5101.434	5093.065	10186.13	1	1642378	0.62	9799	0.0118	1	0.011	15	0.0353
			0.5	2	2542.348	5084.696			1					1		15	
	4-3	2	0.5	1	2231.126	4462.256	4335.55	8671.1	1	1642378	0.53	8284	0.0100	1	0.011	15	0.0299
			0.5	2	2104.422	4208.844			1					1		15	
	5-1	2	0.5	1	2979.86	5959.72	5938.532	11877.064	1	1642378	0.72	11490	0.0139	1	0.011	15	0.0414
			0.5	2	2958.672	5917.344			1					1		15	
	5-2	2	0.5	1	2838.331	5676.662	5763.588	11527.176	1	1642378	0.70	11140	0.0134	1	0.011	15	0.0401
			0.5	2	2925.257	5850.514			1					1		15	
	5-3	2	0.5	1	2716.223	5432.446	5393.628	10787.256	1	1642378	0.66	10400	0.0125	1	0.011	15	0.0375
			0.5	2	2677.405	5354.81			1					1		15	
	6-1	2	0.5	1	3042.738	6085.476	6320.584	12641.168	1	1642378	0.77	12254	0.0148	1	0.011	15	0.0442
			0.5	2	3277.846	6555.692			1					1		15	
	6-2	2	0.5	1	3031.006	6062.012	6112.033	12224.066	1	1642378	0.74	11837	0.0143	1	0.011	15	0.0427
			0.5	2	3081.027	6162.054			1					1		15	
	6-3	2	0.5	1	2313.774	4627.548	4611.891	9223.782	1	1642378	0.56	8836	0.0107	1	0.011	15	0.0318
			0.5	2	2298.117	4596.234			1					1		15	
	7-1	2	0.5	1	2652.577	5305.154	5276.555	10553.11	1	1642378	0.64	10166	0.0123	1	0.011	15	0.0366
			0.5	2	2623.978	5247.956			1					1		15	
	7-2	2	0.5	1	2628.402	5256.804	5240.152	10480.304	1	1642378	0.64	10093	0.0122	1	0.011	15	0.0364
			0.5	2	2611.75	5223.5			1					1		15	
	7-3	2	0.5	1	2542.572	5085.144	5074.099	10148.198	1	1642378	0.62	9761	0.0118	1	0.011	15	0.0352
			0.5	2	2531.527	5063.054			1					1		15	
	8-1	2	0.5	1	3209.517	6419.034	6183.925	12367.85	1	1642378	0.75	11980	0.0145	1	0.011	15	0.0432
			0.5	2	2974.408	5948.816			1					1		15	
	8-2	2	0.5	1	3169.396	6338.792	6430.856	12861.712	1	1642378	0.78	12474	0.0151	1	0.011	15	0.0450
			0.5	2	3261.46	6522.92			1					1		15	
	8-3	2	0.5	1	2886.925	5773.85	5626.224	11252.448	1	1642378	0.69	10865	0.0131	1	0.011	15	0.0392
			0.5	2	2739.299	5478.598			1					1		15	

Assay Date	8/29/2005	Test Chemical ID	RC-8	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0554	0.0022
Full activity	End	0.0459	0.0015
Full activity	Overall	0.0506	0.0056
Background	Beginning	-0.0001	0.000234191
Background	End	0.0001	0.000156589
Background	Overall	0.0000	0.000218831
Positive	Beginning	0.0174	0.0008
Positive	End	0.0225	0.0007
Positive	Overall	0.0199	0.0030
Negative	Beginning	0.0327	0.0002
Negative	End	0.0443	0.0003
Negative	Overall	0.0385	0.0067

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC-8	1	1	1.00E-04	-4.00	0.0095
RC-8	1	2	1.00E-04	-4.00	0.0096
RC-8	1	3	1.00E-04	-4.00	0.0081
RC-8	2	1	3.30E-05	-4.48	0.0013
RC-8	2	2	3.30E-05	-4.48	0.0034
RC-8	2	3	3.30E-05	-4.48	0.0034
RC-8	3	1	1.00E-05	-5.00	0.0096
RC-8	3	2	1.00E-05	-5.00	0.0106
RC-8	3	3	1.00E-05	-5.00	0.0100
RC-8	4	1	1.00E-06	-6.00	0.0335
RC-8	4	2	1.00E-06	-6.00	0.0353
RC-8	4	3	1.00E-06	-6.00	0.0299
RC-8	5	1	1.00E-07	-7.00	0.0414
RC-8	5	2	1.00E-07	-7.00	0.0401
RC-8	5	3	1.00E-07	-7.00	0.0375
RC-8	6	1	1.00E-08	-8.00	0.0442
RC-8	6	2	1.00E-08	-8.00	0.0427
RC-8	6	3	1.00E-08	-8.00	0.0318
RC-8	7	1	1.00E-09	-9.00	0.0366
RC-8	7	2	1.00E-09	-9.00	0.0364
RC-8	7	3	1.00E-09	-9.00	0.0352
RC-8	8	1	1.00E-10	-10.00	0.0432
RC-8	8	2	1.00E-10	-10.00	0.0450
RC-8	8	3	1.00E-10	-10.00	0.0392

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	18.80	18.92	15.98
2	-4.48	2.55	6.74	6.62
3	-5.00	18.91	20.93	19.70
4	-6.00	66.24	69.72	58.94
5	-7.00	81.75	79.26	73.99
6	-8.00	87.18	84.22	62.87
7	-9.00	72.33	71.81	69.45
8	-10.00	85.24	88.75	77.30

Assay Date	9/5/2005	Test Chemical ID	RC-8	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0200	31549.44	1577472		
2	0.0203	32266.18	1589467		
3	0.0203	33770.23	1663558		
4	0.0202	34875.67	1726518		
5	0.0205	33650.29	1641478		
			Average DPM/g soln		1639699
			SD		60219
			CV		3.67
			µCi/g soln		0.739

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	14.2	14.2		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.4094 g
Mass of dilution B used in substrate prep	9.2532 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563896 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00836 µg/g soln.
µg/g soln.

a. µCi/g soln	0.739
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.563896 + 0.00836
= 0.572257 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.291 µCi/µg ASDN

820627 dpm/nmol

Assay Date	9/5/2005	Test Chemical ID	RC-8	# Concentrations tested	8					
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	6-041305			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.302	0.178	0.091	0.060	0.039	0.034	0.029			
	0.304	0.180	0.088	0.061	0.039	0.033	0.028			
	0.298	0.183	0.090	0.060	0.039	0.033	0.029			

Samples:	Placental	QC 10	QC 100
	0.044	0.038	0.132
	0.057	0.039	0.131
	0.056	0.036	0.132

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	
0.25	125	1000	0.00025	200	0.0500	0.301	0.272	0.0488	m, b	0.182	-0.001
0.125	62.5	1000	0.00013	200	0.0250	0.180	0.152	0.0269	se_m, se_b	0.005	0.001
0.05	25	1000	0.00005	200	0.0100	0.090	0.061	0.0105	r^2, se_y	0.997	0.001
0.025	25	2000	0.00003	200	0.0050	0.060	0.032	0.0052	F, df	1160	4
0.01	5	1000	0.00001	200	0.0020	0.039	0.011	0.0013	ss_{regr}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.034	0.005	0.0003			

Regression results are calculated using the function LINEST

Blank	0.029	r^2=	0.997
		m=	0.182
		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
Placental	0.044	0.015	0.002	200	90	72000	0.008	0.015	14.782
Placental	0.057	0.029	0.005	200	90	72000	0.019		
Placental	0.056	0.027	0.004	200	90	72000	0.017		
QC 10	0.038	0.009	0.001	200	200	200	0.000	0.000	0.005
QC 10	0.039	0.010	0.001	200	200	200	0.000		
QC 10	0.036	0.007	0.001	200	200	200	0.000		
QC 100	0.132	0.103	0.018	200	200	200	0.000	0.000	0.090
QC 100	0.131	0.102	0.018	200	200	200	0.000		
QC 100	0.132	0.103	0.018	200	200	200	0.000		

Assay Date	9/5/2005	Test Chemical ID	RC-8	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	2

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	14.78169
Protein Concentration (dilution added to assay, mg/mL):	0.018477

Test Chemical Concentrations	
Level	Final Concentration (M)
1	3.30E-05
2	1.00E-05
3	3.30E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/5/2005	Test Chemical ID	RC-8	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	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	3638.816	7277.632	7242.807	14485.614	1	1639699	0.88	14104	0.0172	1	0.009	15	0.0620
	2	2	0.5	1	3731.076	7462.152	7481.146	14962.292	1	1639699	0.91	14581	0.0178	1	0.009	15	0.0641
			0.5	2	3750.07	7500.14			1					1		15	
	3	2	0.5	1	3910.167	7820.334	7636.359	15272.718	1	1639699	0.93	14891	0.0181	1	0.009	15	0.0655
			0.5	2	3726.192	7452.384			1					1		15	
	4	2	0.5	1	3801.181	7602.362	7491.554	14983.108	1	1639699	0.91	14602	0.0178	1	0.009	15	0.0642
			0.5	2	3690.373	7380.746			1					1		15	
Background control	1	2	0.5	1	100.3999	200.7998	183.90044	367.80088	1	1639699	0.02	-14	0.0000	1	0.009	15	-0.0001
			0.5	2	83.50054	167.00108			1					1		15	
	2	2	0.5	1	120.2879	240.5758	242.3958	484.7916	1	1639699	0.03	103	0.0001	1	0.009	15	0.0005
			0.5	2	122.1079	244.2158			1					1		15	
	3	2	0.5	1	89.54582	179.09164	189.20775	378.4155	1	1639699	0.02	-3	0.0000	1	0.009	15	0.0000
			0.5	2	99.86193	199.32386			1					1		15	
	4	2	0.5	1	79.28067	158.56134	147.11877	294.23754	1	1639699	0.02	-87	0.0001	1	0.009	15	-0.0004
			0.5	2	67.8381	135.6762			1					1		15	
Positive control	1	2	0.5	1	1295.058	2570.116	2591.829	5183.658	1	1639699	0.32	4802	0.0059	1	0.009	15	0.0211
			0.5	2	1306.771	2613.542			1					1		15	
	2	2	0.5	1	1082.696	2165.392	2340.258	4680.516	1	1639699	0.29	4299	0.0052	1	0.009	15	0.0189
			0.5	2	1257.562	2515.124			1					1		15	
	3	2	0.5	1	1583.488	3366.976	3259.232	6518.464	1	1639699	0.40	6137	0.0075	1	0.009	15	0.0270
			0.5	2	1575.744	3151.488			1					1		15	
	4	2	0.5	1	1702.277	3404.554	3482.349	6964.698	1	1639699	0.42	6583	0.0080	1	0.009	15	0.0289
			0.5	2	1760.072	3560.144			1					1		15	
Negative Control	1	2	0.5	1	2327.547	4655.094	4674.745	9349.49	1	1639699	0.57	8968	0.0109	1	0.009	15	0.0394
			0.5	2	2347.198	4694.396			1					1		15	
	2	2	0.5	1	1980.873	3961.746	3948.178	7896.356	1	1639699	0.48	7515	0.0092	1	0.009	15	0.0330
			0.5	2	1967.305	3934.61			1					1		15	
	3	2	0.5	1	3414.554	6829.108	6885.151	13770.302	1	1639699	0.84	13389	0.0163	1	0.009	15	0.0589
			0.5	2	3470.597	6941.194			1					1		15	
	4	2	0.5	1	3532.109	7064.218	6943.009	13886.018	1	1639699	0.85	13505	0.0165	1	0.009	15	0.0594
			0.5	2	3410.9	6821.8			1					1		15	
RC-8	1-1	2	0.5	1	466.731	933.462	957.036	1914.072	1	1639699	0.12	1533	0.0019	1	0.009	15	0.0067
			0.5	2	490.305	980.61			1					1		15	
	1-2	2	0.5	1	355.3334	710.6668	727.0901	1454.1802	1	1639699	0.09	1073	0.0013	1	0.009	15	0.0047
			0.5	2	371.7567	743.5134			1					1		15	
	1-3	2	0.5	1	399.6768	799.3536	782.4172	1564.8344	1	1639699	0.10	1184	0.0014	1	0.009	15	0.0052
			0.5	2	382.7404	765.4808			1					1		15	
	2-1	2	0.5	1	852.9642	1705.9284	1698.7573	3397.5146	1	1639699	0.21	3016	0.0037	1	0.009	15	0.0133
			0.5	2	845.7931	1691.5862			1					1		15	
	2-2	2	0.5	1	773.3625	1546.725	1626.3267	3252.6534	1	1639699	0.20	2871	0.0035	1	0.009	15	0.0126
			0.5	2	852.9642	1705.9284			1					1		15	
	2-3	2	0.5	1	792.8193	1585.6386	1479.3481	2958.6962	1	1639699	0.18	2577	0.0031	1	0.009	15	0.0113
			0.5	2	686.5288	1373.0576			1					1		15	
	3-1	2	0.5	1	1595.569	3191.138	3205.812	6411.624	1	1639699	0.39	6030	0.0073	1	0.009	15	0.0265
			0.5	2	1610.243	3220.486			1					1		15	
	3-2	2	0.5	1	1594.616	3189.232	3308.746	6617.492	1	1639699	0.40	6238	0.0076	1	0.009	15	0.0274
			0.5	2	1714.13	3428.26			1					1		15	
	3-3	2	0.5	1	1643.024	3286.048	3233.141	6466.282	1	1639699	0.39	6085	0.0074	1	0.009	15	0.0268
			0.5	2	1590.117	3180.234			1					1		15	
	4-1	2	0.5	1	2808.482	5616.964	5540.925	11081.85	1	1639699	0.68	10701	0.0130	1	0.009	15	0.0470
			0.5	2	2732.443	5464.886			1					1		15	
	4-2	2	0.5	1	2716.223	5432.446	5332.046	10664.092	1	1639699	0.65	10283	0.0125	1	0.009	15	0.0452
			0.5	2	2615.823	5231.646			1					1		15	
	4-3	2	0.5	1	2808.795	5617.59	5414.98	10829.96	1	1639699	0.66	10449	0.0127	1	0.009	15	0.0459
			0.5	2	2606.185	5212.37			1					1		15	
	5-1	2	0.5	1	3264.177	6528.354	6649.451	13298.902	1	1639699	0.81	12918	0.0157	1	0.009	15	0.0568
			0.5	2	3385.274	6770.548			1					1		15	
	5-2	2	0.5	1	3302.45	6604.9	6731.776	13463.552	1	1639699	0.82	13082	0.0159	1	0.009	15	0.0575
			0.5	2	3429.326	6858.652			1					1		15	
	5-3	2	0.5	1	3316.393	6632.786	6473.951	12947.902	1	1639699	0.79	12567	0.0153	1	0.009	15	0.0553
			0.5	2	3157.558	6315.116			1					1		15	
	6-1	2	0.5	1	3432.081	6864.162	6791.424	13582.848	1	1639699	0.83	13202	0.0161	1	0.009	15	0.0580
			0.5	2	3359.343	6718.686			1					1		15	
	6-2	2	0.5	1	3414.554	6829.108	6978.868	13957.736	1	1639699	0.85	13576	0.0165	1	0.009	15	0.0597
			0.5	2	3564.314	7128.628			1					1		15	
	6-3	2	0.5	1	3504.771	7009.542	6945.133	13890.266	1	1639699	0.85	13509	0.0165	1	0.009	15	0.0594
			0.5	2	3440.362	6880.724			1					1		15	
	7-1	2	0.5	1	3466.604	6933.208	6968.265	13936.53	1	1639699	0.85	13555	0.0165	1	0.009	15	0.0596
			0.5	2	3501.661	7003.322			1					1		15	
	7-2	2	0.5	1	3499.911	6999.822	6944.538	13889.076	1	1639699	0.85	13508	0.0165	1	0.009	15	0.0594
			0.5	2	3444.627	6889.254			1					1		15	
	7-3	2	0.5	1	3572.578	7145.156	6958.693	13917.386	1	1639699	0.85	13536	0.0165	1	0.009	15	0.0595
			0.5	2	3386.115	6772.23			1					1		15	
	8-1	2	0.5	1	3585.951	7171.902	7199.658	14399.316	1	1639699	0.88	14018	0.0171	1	0.009	15	0.0616
			0.5	2	3613.707	7227.414			1					1		15	
	8-2	2	0.5	1	3570.383	7140.766	7150.783	14301.566	1	1639699	0.87	13920	0.0170	1	0.009	15	0.0612
			0.5	2	3580.4	7160.8			1					1		15	
	8-3	2	0.5	1	3439.159	6878.318	7020.477	14040.954	1	1639699	0.86	13660	0.0166	1	0.009	15	0.0601
			0.5	2	3581.318	7162.636			1					1		15	

Assay Date	9/5/2005	Test Chemical ID	RC-8	# Concentrations tested	8	Microsome type	placental	Microsome ID 6-041305	Technician ID TM	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0631	0.0015
Full activity	End	0.0648	0.0009
Full activity	Overall	0.0639	0.0014
Background	Beginning	0.0002	0.000363718
Background	End	-0.0002	0.000261705
Background	Overall	0.0000	0.000345088
Positive	Beginning	0.0200	0.0016
Positive	End	0.0280	0.0014
Positive	Overall	0.0240	0.0047
Negative	Beginning	0.0362	0.0045
Negative	End	0.0591	0.0004
Negative	Overall	0.0477	0.0135

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC-8	1	1	3.30E-05	-4.48	0.0067
RC-8	1	2	3.30E-05	-4.48	0.0047
RC-8	1	3	3.30E-05	-4.48	0.0052
RC-8	2	1	1.00E-05	-5.00	0.0133
RC-8	2	2	1.00E-05	-5.00	0.0126
RC-8	2	3	1.00E-05	-5.00	0.0113
RC-8	3	1	3.30E-06	-5.48	0.0265
RC-8	3	2	3.30E-06	-5.48	0.0274
RC-8	3	3	3.30E-06	-5.48	0.0268
RC-8	4	1	1.00E-06	-6.00	0.0470
RC-8	4	2	1.00E-06	-6.00	0.0452
RC-8	4	3	1.00E-06	-6.00	0.0459
RC-8	5	1	1.00E-07	-7.00	0.0568
RC-8	5	2	1.00E-07	-7.00	0.0575
RC-8	5	3	1.00E-07	-7.00	0.0553
RC-8	6	1	1.00E-08	-8.00	0.0580
RC-8	6	2	1.00E-08	-8.00	0.0597
RC-8	6	3	1.00E-08	-8.00	0.0594
RC-8	7	1	1.00E-09	-9.00	0.0596
RC-8	7	2	1.00E-09	-9.00	0.0594
RC-8	7	3	1.00E-09	-9.00	0.0595
RC-8	8	1	1.00E-10	-10.00	0.0616
RC-8	8	2	1.00E-10	-10.00	0.0612
RC-8	8	3	1.00E-10	-10.00	0.0601

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	10.54	7.38	8.14
2	-5.00	20.74	19.74	17.72
3	-5.48	41.46	42.88	41.84
4	-6.00	73.57	70.70	71.84
5	-7.00	88.81	89.95	86.40
6	-8.00	90.77	93.34	92.88
7	-9.00	93.20	92.87	93.07
8	-10.00	96.38	95.71	93.92

Assay Date	9/6/2005	Test Chemical ID	RC-8	# Concentrations tested	8		
Technician ID	TM	Replicate #	3	Microsome type	placental	Microsome ID	6-041305

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0201	31944.67	1589287		
2	0.0201	32385.38	1611213		
3	0.0202	33291.23	1648081		
4	0.0205	34850.95	1700046		
5	0.0198	36743.6	1855737		
			Average DPM/g soln		1680873
			SD		106373
			CV		6.33
			µCi/g soln		0.757

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	15.8	15.8		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.318 g
Mass of dilution B used in substrate prep	9.1948 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563476 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00857 µg/g soln.
µg/g soln.

a. µCi/g soln	0.757
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.

= 0.563476 + 0.00857
= 0.572047 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.324 µCi/µg ASDN

841543 dpm/nmol

Assay Date	9/6/2005	Test Chemical ID	RC-8	# Concentrations tested	8
Technician ID	TM	Replicate #	3	Microsome type	placental
Microsome ID	6-041305				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.267	0.154	0.082	0.052	0.035	0.031	0.031			
	0.263	0.162	0.083	0.054	0.035	0.030	0.026			
	0.266	0.157	0.083	0.054	0.035	0.031	0.027			

Samples:	Placental	Q 10	Q 100
	0.054	0.034	0.115
	0.050	0.040	0.121
	0.048	0.033	0.120

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	
0.25	125	1000	0.00025	200	0.0500	0.265	0.237	0.0489	m,b	0.207	0.000
0.125	62.5	1000	0.00013	200	0.0250	0.158	0.130	0.0266	se_m,se_b	0.006	0.001
0.05	25	1000	0.00005	200	0.0100	0.082	0.054	0.0110	r^2,se_y	0.997	0.001
0.025	25	2000	0.00003	200	0.0050	0.053	0.026	0.0050	F,df	1199	4
0.01	5	1000	0.00001	200	0.0020	0.035	0.007	0.0012	ss_{reg},ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.031	0.003	0.0003			

Blank 0.028

r^2= 0.997
m= 0.207
b= 0.000

Regression results are calculated using the function LINST

	A_{raw}	A_{adj}	mg protein measured	µL diluted usome	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
Placental	0.054	0.026	0.005	200	90	72000	0.020	0.018	17.669
Placental	0.050	0.022	0.004	200	90	72000	0.017		
Placental	0.048	0.020	0.004	200	90	72000	0.015		
Q 10	0.034	0.006	0.001	200	200	200	0.000	0.000	0.007
Q 10	0.040	0.012	0.002	200	200	200	0.000		
Q 10	0.033	0.005	0.001	200	200	200	0.000		
Q 100	0.115	0.087	0.018	200	200	200	0.000	0.000	0.092
Q 100	0.121	0.093	0.019	200	200	200	0.000		
Q 100	0.120	0.092	0.019	200	200	200	0.000		

Assay Date	9/6/2005	Test Chemical ID	RC-8	# Concentrations tested	8	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	3

Microsome Dilution Details		
Dilution A	0.09	mL microsome Stock used
	72	mL total volume
	800	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	800	total dilution factor

Protein Concentration (stock microsomes,mg/mL):	17.66908
Protein Concentration (dilution added to assay,mg/mL):	0.022086

Test Chemical Concentrations	
Level	Final Concentration (M)
1	3.30E-05
2	1.00E-05
3	3.30E-06
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	RC-8	# Concentrations tested		8 Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate %turnover			Calculate nmol ^3H_2Oformed					
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_2Oformed	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	6797.342	13594.684	12994.904	25989.808	1	1680873	1.55	25628	0.0305	1	0.011	15	0.0919
			0.5	2	6197.562	12395.124			1					1		15	
	2	2	0.5	1	6657.575	13315.15	13036.948	26073.896	1	1680873	1.55	25712	0.0306	1	0.011	15	0.0922
			0.5	2	6379.373	12758.746			1					1		15	
	3	2	0.5	1	6404.163	12808.326	12869.639	25739.278	1	1680873	1.53	25378	0.0302	1	0.011	15	0.0910
			0.5	2	6465.476	12930.952			1					1		15	
	4	2	0.5	1	6282.966	12565.932	12772.518	25545.036	1	1680873	1.52	25184	0.0299	1	0.011	15	0.0903
			0.5	2	6489.552	12979.104			1					1		15	
Background control	1	2	0.5	1	99.91762	199.83524	163.27063	326.54126	1	1680873	0.02	-35	0.0000	1	0.011	15	-0.0001
			0.5	2	63.35301	126.70602			1					1		15	
	2	2	0.5	1	79.28067	158.56134	162.54535	325.0907	1	1680873	0.02	-36	0.0000	1	0.011	15	-0.0001
			0.5	2	83.26468	166.52936			1					1		15	
	3	2	0.5	1	122.1079	244.2158	255.0699	510.1398	1	1680873	0.03	149	0.0002	1	0.011	15	0.0005
			0.5	2	132.962	265.924			1					1		15	
	4	2	0.5	1	62.87777	125.75554	142.15886	284.31772	1	1680873	0.02	-77	-0.0001	1	0.011	15	-0.0003
			0.5	2	79.28109	158.56218			1					1		15	
Positive control	1	2	0.5	1	2200.668	4401.336	4298.222	8596.444	1	1680873	0.51	8235	0.0098	1	0.011	15	0.0295
			0.5	2	2097.554	4195.108			1					1		15	
	2	2	0.5	1	2005.294	4010.588	3848.614	7697.228	1	1680873	0.46	7336	0.0087	1	0.011	15	0.0263
			0.5	2	1843.32	3686.64			1					1		15	
	3	2	0.5	1	2523.116	5046.232	4850.663	9701.326	1	1680873	0.58	9340	0.0111	1	0.011	15	0.0335
			0.5	2	2327.547	4655.094			1					1		15	
	4	2	0.5	1	2434.972	4869.944	4701.318	9402.636	1	1680873	0.56	9041	0.0107	1	0.011	15	0.0324
			0.5	2	2266.346	4532.692			1					1		15	
Negative Control	1	2	0.5	1	4390.536	8781.072	8866.823	17733.646	1	1680873	1.06	17372	0.0206	1	0.011	15	0.0623
			0.5	2	4476.287	8952.574			1					1		15	
	2	2	0.5	1	3810.96	7621.92	7818.835	15637.67	1	1680873	0.93	15276	0.0182	1	0.011	15	0.0548
			0.5	2	4007.875	8015.75			1					1		15	
	3	2	0.5	1	6313.299	12626.598	12450.311	24900.622	1	1680873	1.48	24539	0.0292	1	0.011	15	0.0880
			0.5	2	6137.012	12274.024			1					1		15	
	4	2	0.5	1	6144.966	12289.932	12381.139	24762.278	1	1680873	1.47	24401	0.0290	1	0.011	15	0.0875
			0.5	2	6236.173	12472.346			1					1		15	
RC-8	1-1	2	0.5	1	795.071	1590.142	1619.991	3239.982	1	1680873	0.19	2876	0.0034	1	0.011	15	0.0103
			0.5	2	824.92	1649.84			1					1		15	
	1-2	2	0.5	1	801.0209	1602.0418	1473.5504	2947.1008	1	1680873	0.18	2586	0.0031	1	0.011	15	0.0093
			0.5	2	672.5295	1345.059			1					1		15	
	1-3	2	0.5	1	713.3121	1426.6242	1491.179	2982.358	1	1680873	0.18	2621	0.0031	1	0.011	15	0.0094
			0.5	2	777.8669	1555.7338			1					1		15	
	2-1	2	0.5	1	1829.003	3658.006	3365.432	6730.864	1	1680873	0.40	6369	0.0076	1	0.011	15	0.0228
			0.5	2	1536.429	3072.858			1					1		15	
	2-2	2	0.5	1	1752.955	3505.91	3464.351	6928.702	1	1680873	0.41	6567	0.0076	1	0.011	15	0.0236
			0.5	2	1711.396	3422.792			1					1		15	
	2-3	2	0.5	1	1837.075	3674.15	3871.541	7743.082	1	1680873	0.46	7382	0.0088	1	0.011	15	0.0265
			0.5	2	2034.466	4068.932			1					1		15	
	3-1	2	0.5	1	3522.191	7044.382	6890.972	13781.944	1	1680873	0.82	13420	0.0159	1	0.011	15	0.0481
			0.5	2	3368.781	6737.562			1					1		15	
	3-2	2	0.5	1	3502.074	7004.148	6848.819	13697.639	1	1680873	0.81	13336	0.0158	1	0.011	15	0.0478
			0.5	2	3346.745	6693.49			1					1		15	
	3-3	2	0.5	1	3329.841	6659.682	6815.512	13631.024	1	1680873	0.81	13270	0.0158	1	0.011	15	0.0476
			0.5	2	3485.671	6971.342			1					1		15	
	4-1	2	0.5	1	5071.63	10143.26	10186.778	20373.556	1	1680873	1.21	20012	0.0238	1	0.011	15	0.0718
			0.5	2	5115.148	10230.296			1					1		15	
	4-2	2	0.5	1	5183.397	10366.794	10295.73	20591.46	1	1680873	1.23	20230	0.0240	1	0.011	15	0.0726
			0.5	2	5112.333	10224.666			1					1		15	
	4-3	2	0.5	1	5137.512	10275.024	10231.547	20463.094	1	1680873	1.22	20102	0.0239	1	0.011	15	0.0721
			0.5	2	5094.035	10188.07			1					1		15	
	5-1	2	0.5	1	6296.079	12592.158	12596.54	25193.08	1	1680873	1.50	24832	0.0295	1	0.011	15	0.0891
			0.5	2	6300.461	12600.922			1					1		15	
	5-2	2	0.5	1	5596.211	11192.422	11309.091	22618.182	1	1680873	1.35	22257	0.0264	1	0.011	15	0.0798
			0.5	2	5712.88	11425.76			1					1		15	
	5-3	2	0.5	1	5820.387	11640.774	11638.244	23276.488	1	1680873	1.38	22915	0.0272	1	0.011	15	0.0822
			0.5	2	5817.857	11635.714			1					1		15	
	6-1	2	0.5	1	6604.787	13209.574	13201.869	26403.738	1	1680873	1.57	26042	0.0309	1	0.011	15	0.0934
			0.5	2	6597.082	13194.164			1					1		15	
	6-2	2	0.5	1	6682.507	13365.014	13388.7	26777.4	1	1680873	1.59	26416	0.0314	1	0.011	15	0.0947
			0.5	2	6706.193	13412.386			1					1		15	
	6-3	2	0.5	1	6509.354	13018.708	13202.879	26405.758	1	1680873	1.57	26044	0.0309	1	0.011	15	0.0934
			0.5	2	6693.525	13387.05			1					1		15	
	7-1	2	0.5	1	6583.214	13166.428	13364.884	26729.768	1	1680873	1.59	26368	0.0313	1	0.011	15	0.0946
			0.5	2	6781.67	13563.34			1					1		15	
	7-2	2	0.5	1	6416.402	12832.804	12910.727	25821.454	1	1680873	1.54	25460	0.0303	1	0.011	15	0.0913
			0.5	2	6494.325	12988.65			1					1		15	
	7-3	2	0.5	1	6401.305	12802.61	12802.849	25605.698	1	1680873	1.52	25244	0.0300	1	0.011	15	0.0905
			0.5	2	6401.544	12803.088			1					1		15	
	8-1	2	0.5	1	6743.107	13486.214	14473.008	28946.016	1	1680873	1.72	28584	0.0340	1	0.011	15	0.1025
			0.5	2	7729.901	15459.802			1					1		15	
	8-2	2	0.5	1	6449.209	12898.418	12999.571	25999.142	1	1680873	1.55	25638	0.0305	1	0.011	15	0.0920
			0.5	2	6550.362	13100.724			1					1		15	
	8-3	2	0.5	1	6039.128	12078.256	12280.563	24561.126	1	1680873	1.46	24200	0.0288	1	0.011	15	0.0868
			0.5	2	6241.435	12482.87			1					1		15	

Assay Date	9/6/2005	Test Chemical ID	RC-8	# Concentrations tested	Microsome type	placental	Microsome ID	6-041305	Technician ID	TM	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0921	0.0002
Full activity	End	0.0907	0.0005
Full activity	Overall	0.0914	0.0009
Background	Beginning	-0.0001	3.679E-06
Background	End	0.0001	0.000572743
Background	Overall	0.0000	0.000362242
Positive	Beginning	0.0279	0.0023
Positive	End	0.0330	0.0008
Positive	Overall	0.0304	0.0032
Negative	Beginning	0.0586	0.0053
Negative	End	0.0878	0.0004
Negative	Overall	0.0732	0.0171

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC-8	1	1	3.30E-05	-4.48	0.0103
RC-8	1	2	3.30E-05	-4.48	0.0093
RC-8	1	3	3.30E-05	-4.48	0.0094
RC-8	2	1	1.00E-05	-5.00	0.0228
RC-8	2	2	1.00E-05	-5.00	0.0236
RC-8	2	3	1.00E-05	-5.00	0.0265
RC-8	3	1	3.30E-06	-5.48	0.0481
RC-8	3	2	3.30E-06	-5.48	0.0478
RC-8	3	3	3.30E-06	-5.48	0.0476
RC-8	4	1	1.00E-06	-6.00	0.0718
RC-8	4	2	1.00E-06	-6.00	0.0726
RC-8	4	3	1.00E-06	-6.00	0.0721
RC-8	5	1	1.00E-07	-7.00	0.0891
RC-8	5	2	1.00E-07	-7.00	0.0798
RC-8	5	3	1.00E-07	-7.00	0.0822
RC-8	6	1	1.00E-08	-8.00	0.0934
RC-8	6	2	1.00E-08	-8.00	0.0947
RC-8	6	3	1.00E-08	-8.00	0.0934
RC-8	7	1	1.00E-09	-9.00	0.0946
RC-8	7	2	1.00E-09	-9.00	0.0913
RC-8	7	3	1.00E-09	-9.00	0.0905
RC-8	8	1	1.00E-10	-10.00	0.1025
RC-8	8	2	1.00E-10	-10.00	0.0920
RC-8	8	3	1.00E-10	-10.00	0.0868

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	11.30	10.15	10.29
2	-5.00	25.00	25.78	28.98
3	-5.48	52.68	52.35	52.09
4	-6.00	78.55	79.41	78.91
5	-7.00	97.47	87.37	89.95
6	-8.00	102.22	103.69	102.23
7	-9.00	103.50	99.94	99.09
8	-10.00	112.20	100.64	94.99

Assay Date	9/8/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8		
Technician ID	TM	Replicate #	1	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	30258.43	1520524	
2	0.0201	31448.17	1564586	
3	0.0203	32934.57	1622393	
4	0.0201	33755.44	1679375	
5	0.0201	34761.64	1729435	
			Average DPM/g soln	1623262
			SD	84291
			CV	5.19
			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	11.5	11.5		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.3288 g
Mass of dilution B used in substrate prep	9.1883 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562705 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00828 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/bc

2) Calculate total µg ASDN/g soln.

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

= 0.562705 + 0.00828
= 0.570982 µg ASDN/g soln.

3) Calculate Solution Specific Activity

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

814215 dpm/nmol

Assay Date	9/8/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8					
Technician ID	TM	Replicate #	1	Microsome type	placental	Microsome ID	BAA			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.628	0.390	0.192	0.124	0.077	0.061	0.044			
	0.635	0.400	0.195	0.125	0.073	0.061	0.039			
	0.629	0.405	0.190	0.119	0.076	0.061	0.040			

Samples:	Placental	QC 10	QC 100
	0.102	0.074	0.307
	0.098	0.072	0.305
	0.103	0.067	0.310

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
0.25	125	1000	0.00025	200	0.0500	0.631	0.589	0.0479
0.125	62.5	1000	0.00013	200	0.0250	0.398	0.357	0.0283
0.05	25	1000	0.00005	200	0.0100	0.193	0.151	0.0109
0.025	25	2000	0.00003	200	0.0050	0.123	0.082	0.0051
0.01	5	1000	0.00001	200	0.0020	0.075	0.034	0.0010
0.005	5	2000	0.00001	200	0.0010	0.061	0.020	-0.0002
			Blank	0.041				

Variables	Regression results	
m, b	0.084	-0.002
se_m, se_b	0.004	0.001
r^2, se_y	0.990	0.002
F, df	390	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.990
m= 0.084
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
Placental	0.102	0.061	0.003	200	220	70400	0.005	0.005	5.186
Placental	0.098	0.057	0.003	200	220	70400	0.005		
Placental	0.103	0.062	0.003	200	220	70400	0.005		
QC 10	0.074	0.033	0.001	200	200	200	0.000	0.000	0.003
QC 10	0.072	0.031	0.001	200	200	200	0.000		
QC 10	0.067	0.026	0.000	200	200	200	0.000		
QC 100	0.307	0.266	0.021	200	200	200	0.000	0.000	0.103
QC 100	0.305	0.264	0.020	200	200	200	0.000		
QC 100	0.310	0.269	0.021	200	200	200	0.000		

Assay Date	9/8/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	1

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.186269
Protein Concentration (dilution added to assay, mg/mL):	0.016207

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
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	RC#9 DCF	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	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	5489.487	10978.974	10817.361	21634.722	1	1623262	1.33	21216	0.0261	1	0.008	15	0.1072
			0.5	2	5327.874	10655.748			1					1		15	
	2	2	0.5	1	5361.354	10722.708	10766.665	21533.33	1	1623262	1.33	21115	0.0259	1	0.008	15	0.1067
			0.5	2	5405.311	10810.622			1					1		15	
	3	2	0.5	1	4246.643	8493.286	8416.283	16832.566	1	1623262	1.04	16414	0.0202	1	0.008	15	0.0829
			0.5	2	4169.64	8339.28			1					1		15	
	4	2	0.5	1	4401.391	8802.782	8697.629	17395.258	1	1623262	1.07	16977	0.0209	1	0.008	15	0.0858
			0.5	2	4296.238	8592.476			1					1		15	
Background control	1	2	0.5	1	86.19411	172.38822	207.40461	414.80922	1	1623262	0.03	-3	0.0000	1	0.008	15	0.0000
			0.5	2	121.2105	242.421			1					1		15	
	2	2	0.5	1	91.58124	183.16248	167.00109	334.00218	1	1623262	0.02	-84	-0.0001	1	0.008	15	-0.0004
			0.5	2	75.41995	150.8399			1					1		15	
	3	2	0.5	1	124.7753	249.5506	262.1472	524.2944	1	1623262	0.03	106	0.0001	1	0.008	15	0.0005
			0.5	2	137.3719	274.7438			1					1		15	
	4	2	0.5	1	104.2857	208.5714	199.96976	399.93956	1	1623262	0.02	-18	0.0000	1	0.008	15	-0.0001
			0.5	2	95.68408	191.36816			1					1		15	
Positive control	1	2	0.5	1	2340.159	4680.318	4616.234	9232.468	1	1623262	0.57	8814	0.0108	1	0.008	15	0.0445
			0.5	2	2276.075	4552.15			1					1		15	
	2	2	0.5	1	2168.106	4336.212	4379.628	8759.256	1	1623262	0.54	8341	0.0102	1	0.008	15	0.0421
			0.5	2	2211.522	4423.044			1					1		15	
	3	2	0.5	1	2162.945	4325.89	4194.181	8388.362	1	1623262	0.52	7970	0.0098	1	0.008	15	0.0403
			0.5	2	2031.236	4062.472			1					1		15	
	4	2	0.5	1	2127.403	4254.806	4170.687	8341.374	1	1623262	0.51	7923	0.0097	1	0.008	15	0.0400
			0.5	2	2043.284	4086.568			1					1		15	
Negative Control	1	2	0.5	1	3677.002	7354.004	7065.526	14131.052	1	1623262	0.87	13713	0.0168	1	0.008	15	0.0693
			0.5	2	3388.524	6777.048			1					1		15	
	2	2	0.5	1	3622.555	7245.11	7069.916	14139.832	1	1623262	0.87	13722	0.0169	1	0.008	15	0.0693
			0.5	2	3447.361	6894.722			1					1		15	
	3	2	0.5	1	3926.515	7857.03	7979.66	15959.32	1	1623262	0.98	15541	0.0191	1	0.008	15	0.0785
			0.5	2	4051.145	8102.29			1					1		15	
	4	2	0.5	1	3970.337	7940.674	7706.333	15412.666	1	1623262	0.95	14994	0.0184	1	0.008	15	0.0758
			0.5	2	3735.996	7471.992			1					1		15	
RC#9 DCF	1-1	2	0.5	1	2106.38	4212.76	4080.206	8160.412	1	1623262	0.50	7742	0.0095	1	0.008	15	0.0391
			0.5	2	1973.826	3947.652			1					1		15	
	1-2	2	0.5	1	2444.885	4889.77	4901.43	9802.86	1	1623262	0.60	9385	0.0115	1	0.008	15	0.0474
			0.5	2	2456.545	4913.09			1					1		15	
	1-3	2	0.5	1	2782.468	5564.936	5477.866	10955.732	1	1623262	0.67	10537	0.0129	1	0.008	15	0.0532
			0.5	2	2695.398	5390.796			1					1		15	
	2-1	2	0.5	1	3423.54	6847.08	6868.629	13737.258	1	1623262	0.85	13319	0.0164	1	0.008	15	0.0673
			0.5	2	3445.089	6890.178			1					1		15	
	2-2	2	0.5	1	3405.473	6810.946	6523.312	13046.624	1	1623262	0.80	12628	0.0155	1	0.008	15	0.0638
			0.5	2	3117.839	6235.678			1					1		15	
	2-3	2	0.5	1	3071.709	6143.418	6234.751	12469.502	1	1623262	0.77	12051	0.0148	1	0.008	15	0.0609
			0.5	2	3163.042	6326.084			1					1		15	
	3-1	2	0.5	1	3454.135	6908.27	6700.379	13400.758	1	1623262	0.83	12982	0.0159	1	0.008	15	0.0656
			0.5	2	3246.244	6492.488			1					1		15	
	3-2	2	0.5	1	3655.132	7310.264	7157.188	14314.376	1	1623262	0.88	13895	0.0171	1	0.008	15	0.0702
			0.5	2	3502.056	7004.112			1					1		15	
	3-3	2	0.5	1	3313.121	6626.242	6599.791	13199.582	1	1623262	0.81	12781	0.0157	1	0.008	15	0.0646
			0.5	2	3286.67	6573.34			1					1		15	
	4-1	2	0.5	1	4238.322	8476.644	8534.605	17069.21	1	1623262	1.05	16651	0.0205	1	0.008	15	0.0841
			0.5	2	4296.283	8592.566			1					1		15	
	4-2	2	0.5	1	4211.412	8422.824	8713.172	17426.344	1	1623262	1.07	17008	0.0209	1	0.008	15	0.0859
			0.5	2	4501.76	9003.52			1					1		15	
	4-3	2	0.5	1	4140.055	8280.11	8436.338	16872.676	1	1623262	1.04	16454	0.0202	1	0.008	15	0.0831
			0.5	2	4296.283	8592.566			1					1		15	
	5-1	2	0.5	1	4512.614	9025.228	8897.766	17795.572	1	1623262	1.10	17377	0.0213	1	0.008	15	0.0878
			0.5	2	4385.172	8770.344			1					1		15	
	5-2	2	0.5	1	4431.208	8862.416	8767.086	17534.172	1	1623262	1.08	17116	0.0210	1	0.008	15	0.0865
			0.5	2	4335.876	8671.756			1					1		15	
	5-3	2	0.5	1	4256.573	8517.146	8356.016	16712.032	1	1623262	1.03	16294	0.0200	1	0.008	15	0.0823
			0.5	2	4097.443	8194.886			1					1		15	
	6-1	2	0.5	1	4272.041	8544.082	8657.119	17314.238	1	1623262	1.07	16896	0.0208	1	0.008	15	0.0854
			0.5	2	4385.078	8770.156			1					1		15	
	6-2	2	0.5	1	3885.787	7771.574	8033.03	16066.06	1	1623262	0.99	15648	0.0192	1	0.008	15	0.0791
			0.5	2	4147.243	8294.486			1					1		15	
	6-3	2	0.5	1	4133.574	8267.148	7875.544	15751.088	1	1623262	0.97	15333	0.0188	1	0.008	15	0.0775
			0.5	2	3741.97	7483.94			1					1		15	
	7-1	2	0.5	1	4330.807	8661.614	8890.861	17781.722	1	1623262	1.10	17363	0.0213	1	0.008	15	0.0877
			0.5	2	4560.054	9120.108			1					1		15	
	7-2	2	0.5	1	4471.911	8943.822	8975.601	17951.202	1	1623262	1.11	17533	0.0215	1	0.008	15	0.0885
			0.5	2	4503.69	9007.38			1					1		15	
	7-3	2	0.5	1	4480.772	8961.544	9199.95	18399.9	1	1623262	1.13	17982	0.0221	1	0.008	15	0.0908
			0.5	2	4719.178	9438.356			1					1		15	
	8-1	2	0.5	1	4271.109	8542.218	8773.752	17547.504	1	1623262	1.08	17129	0.0210	1	0.008	15	0.0865
			0.5	2	4502.643	9005.286			1					1		15	
	8-2	2	0.5	1	4468.585	8937.17	8794.498	17588.996	1	1623262	1.08	17171	0.0211	1	0.008	15	0.0867
			0.5	2	4325.913	8651.826			1					1		15	
	8-3	2	0.5	1	4360.679	8721.358	8673.858	17347.716	1	1623262	1.07	16929	0.0208	1	0.008	15	0.0855
			0.5	2	4313.179	8626.358			1					1		15	

Assay Date	9/8/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.1069	0.0004
Full activity	End	0.0843	0.0020
Full activity	Overall	0.0956	0.0131
Background	Beginning	-0.0002	0.000288668
Background	End	0.0002	0.000444235
Background	Overall	0.0000	0.00039876
Positive	Beginning	0.0433	0.0017
Positive	End	0.0401	0.0002
Positive	Overall	0.0417	0.0021
Negative	Beginning	0.0693	0.0000
Negative	End	0.0771	0.0020
Negative	Overall	0.0732	0.0047

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#9 DCF	1	1	1.00E-04	-4.00	0.0391
RC#9 DCF	1	2	1.00E-04	-4.00	0.0474
RC#9 DCF	1	3	1.00E-04	-4.00	0.0532
RC#9 DCF	2	1	3.30E-05	-4.48	0.0673
RC#9 DCF	2	2	3.30E-05	-4.48	0.0638
RC#9 DCF	2	3	3.30E-05	-4.48	0.0609
RC#9 DCF	3	1	1.00E-05	-5.00	0.0656
RC#9 DCF	3	2	1.00E-05	-5.00	0.0702
RC#9 DCF	3	3	1.00E-05	-5.00	0.0646
RC#9 DCF	4	1	1.00E-06	-6.00	0.0841
RC#9 DCF	4	2	1.00E-06	-6.00	0.0859
RC#9 DCF	4	3	1.00E-06	-6.00	0.0831
RC#9 DCF	5	1	1.00E-07	-7.00	0.0878
RC#9 DCF	5	2	1.00E-07	-7.00	0.0865
RC#9 DCF	5	3	1.00E-07	-7.00	0.0823
RC#9 DCF	6	1	1.00E-08	-8.00	0.0854
RC#9 DCF	6	2	1.00E-08	-8.00	0.0791
RC#9 DCF	6	3	1.00E-08	-8.00	0.0775
RC#9 DCF	7	1	1.00E-09	-9.00	0.0877
RC#9 DCF	7	2	1.00E-09	-9.00	0.0886
RC#9 DCF	7	3	1.00E-09	-9.00	0.0908
RC#9 DCF	8	1	1.00E-10	-10.00	0.0865
RC#9 DCF	8	2	1.00E-10	-10.00	0.0867
RC#9 DCF	8	3	1.00E-10	-10.00	0.0855

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	40.90	49.57	55.66
2	-4.48	70.36	66.71	63.66
3	-5.00	68.58	73.41	67.52
4	-6.00	87.96	89.84	86.92
5	-7.00	91.79	90.41	86.07
6	-8.00	89.25	82.66	80.99
7	-9.00	91.72	92.62	94.99
8	-10.00	90.48	90.70	89.43

Assay Date	9/12/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	28991.12	1456840		
2	0.0205	31876.95	1554973		
3	0.0199	32398.9	1628085		
4	0.0200	32617.59	1630880		
5	0.0201	33249.76	1654217		
			Average DPM/g soln	1584999	
			SD	80745	
			CV	5.09	
			µCi/g soln	0.714	

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	13.8	13.8		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2713 g
Mass of dilution B used in substrate prep	9.145 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562033 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00808 µg/g soln.
µg/g soln.

a. µCi/g soln	0.714
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.562033 + 0.00808
= 0.570115 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.252 µCi/µg ASDN

796232 dpm/nmol

Assay Date	9/12/2005	Test Chemical ID	RC#9 DCF		# Concentrations tested	8					
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	BAA				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.625	0.405	0.200	0.162	0.081	0.061	0.043			
	0.632	0.405	0.177	0.162	0.089	0.058	0.044			
	0.629	0.405	0.199	0.122	0.101	0.068	0.045			

Samples:	Placental	QC 10	QC 100
	0.109	0.073	0.332
	0.100	0.063	0.315
	0.101	0.071	0.317

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	
0.25	125	1000	0.00025	200	0.0500	0.628	0.585	0.0477	m, b	0.086	-0.003
0.125	62.5	1000	0.00013	200	0.0250	0.405	0.361	0.0285	se_m, se_b	0.005	0.001
0.05	25	1000	0.00005	200	0.0100	0.192	0.148	0.0101	r^2, se_y	0.987	0.002
0.025	25	2000	0.00003	200	0.0050	0.148	0.104	0.0064	F, df	302	4
0.01	5	1000	0.00001	200	0.0020	0.090	0.047	0.0014	ss_{reg}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.062	0.019	-0.0010			

Blank 0.044

r^2= 0.987
m= 0.086
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/µL Prep.	average mg/µL	mg/mL
Placental	0.109	0.065	0.003	200	220	70400	0.005	0.004	4.004
Placental	0.100	0.056	0.002	200	220	70400	0.004		
Placental	0.101	0.057	0.002	200	220	70400	0.004		
QC 10	0.073	0.029	0.000	200	200	200	0.000	0.000	-0.002
QC 10	0.063	0.019	-0.001	200	200	200	0.000		
QC 10	0.071	0.028	0.000	200	200	200	0.000		
QC 100	0.332	0.288	0.022	200	200	200	0.000	0.000	0.106
QC 100	0.315	0.271	0.021	200	200	200	0.000		
QC 100	0.317	0.273	0.021	200	200	200	0.000		

Assay Date	9/12/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	2

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.004164
Protein Concentration (dilution added to assay, mg/mL):	0.012513

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
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	9/12/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	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	5085.452	10170.904	10062.029	20124.058	1	1584999	1.27	19765	0.0248	1	0.006	15	0.1323
			0.5	2	4976.577	9953.154			1					1		15	
	2	2	0.5	1	4659.096	9318.192	9272.062	18544.124	1	1584999	1.17	18185	0.0228	1	0.006	15	0.1217
			0.5	2	4612.966	9225.932			1					1		15	
	3	2	0.5	1	4573.675	9147.35	9156.008	18312.016	1	1584999	1.16	17953	0.0225	1	0.006	15	0.1201
			0.5	2	4582.333	9164.666			1					1		15	
	4	2	0.5	1	4577.691	9155.382	9079.403	18158.806	1	1584999	1.15	17799	0.0224	1	0.006	15	0.1191
			0.5	2	4501.712	9003.424			1					1		15	
Background control	1	2	0.5	1	110.1792	220.3584	193.07295	386.1459	1	1584999	0.02	27	0.0000	1	0.006	15	0.0002
			0.5	2	82.89375	165.7875			1					1		15	
	2	2	0.5	1	60.14396	120.28792	156.55072	313.10144	1	1584999	0.02	-46	-0.0001	1	0.006	15	-0.0003
			0.5	2	96.40676	192.81352			1					1		15	
	3	2	0.5	1	84.74831	169.49662	183.16569	366.33138	1	1584999	0.02	7	0.0000	1	0.006	15	0.0000
			0.5	2	98.41738	196.83476			1					1		15	
	4	2	0.5	1	92.94975	185.8995	185.9	371.8	1	1584999	0.02	12	0.0000	1	0.006	15	0.0001
			0.5	2	92.95025	185.9005			1					1		15	
Positive control	1	2	0.5	1	2032.816	4065.632	3949.943	7899.886	1	1584999	0.50	7541	0.0095	1	0.006	15	0.0505
			0.5	2	1917.127	3834.254			1					1		15	
	2	2	0.5	1	1826.199	3652.398	3586.786	7173.572	1	1584999	0.45	6814	0.0086	1	0.006	15	0.0456
			0.5	2	1760.587	3521.174			1					1		15	
	3	2	0.5	1	2112.697	4225.394	4291.501	8583.002	1	1584999	0.54	8224	0.0103	1	0.006	15	0.0550
			0.5	2	2178.804	4357.608			1					1		15	
	4	2	0.5	1	2195.266	4390.532	4363.052	8726.104	1	1584999	0.55	8367	0.0105	1	0.006	15	0.0560
			0.5	2	2167.786	4335.572			1					1		15	
Negative Control	1	2	0.5	1	3153.115	6306.23	6227.13	12454.26	1	1584999	0.79	12095	0.0152	1	0.006	15	0.0809
			0.5	2	3074.015	6148.03			1					1		15	
	2	2	0.5	1	2988.625	5977.25	5792.699	11585.398	1	1584999	0.73	11226	0.0141	1	0.006	15	0.0751
			0.5	2	2804.074	5608.148			1					1		15	
	3	2	0.5	1	4409.944	8819.888	8990.667	17981.334	1	1584999	1.13	17622	0.0221	1	0.006	15	0.1179
			0.5	2	4580.723	9161.446			1					1		15	
	4	2	0.5	1	4299.765	8599.53	8709.242	17418.484	1	1584999	1.10	17059	0.0214	1	0.006	15	0.1141
			0.5	2	4409.477	8818.954			1					1		15	
RC#9 DCF	1-1	2	0.5	1	1548.025	3096.05	3021.469	6042.938	1	1584999	0.38	5684	0.0071	1	0.006	15	0.0380
			0.5	2	1473.444	2946.888			1					1		15	
	1-2	2	0.5	1	3372.237	6744.474	6397.816	12795.632	1	1584999	0.81	12436	0.0156	1	0.006	15	0.0832
			0.5	2	3025.579	6051.158			1					1		15	
	1-3	2	0.5	1	2832.919	5665.838	5543.341	11086.682	1	1584999	0.70	10727	0.0135	1	0.006	15	0.0718
			0.5	2	2710.422	5420.844			1					1		15	
	2-1	2	0.5	1	3216.135	6432.27	6693.842	13387.684	1	1584999	0.84	13028	0.0164	1	0.006	15	0.0872
			0.5	2	3477.707	6955.414			1					1		15	
	2-2	2	0.5	1	3393.536	6787.072	6820.872	13641.744	1	1584999	0.86	13282	0.0167	1	0.006	15	0.0889
			0.5	2	3427.336	6854.672			1					1		15	
	2-3	2	0.5	1	3087.787	6175.574	6127.807	12255.614	1	1584999	0.77	11896	0.0149	1	0.006	15	0.0796
			0.5	2	3040.02	6080.04			1					1		15	
	3-1	2	0.5	1	3229.094	6458.188	6460.115	12920.23	1	1584999	0.82	12561	0.0158	1	0.006	15	0.0840
			0.5	2	3231.021	6462.042			1					1		15	
	3-2	2	0.5	1	2925.199	5850.398	6047.25	12094.5	1	1584999	0.76	11735	0.0147	1	0.006	15	0.0785
			0.5	2	3122.051	6244.102			1					1		15	
	3-3	2	0.5	1	2971.69	5943.38	5815.478	11630.956	1	1584999	0.73	11272	0.0142	1	0.006	15	0.0754
			0.5	2	2843.788	5687.576			1					1		15	
	4-1	2	0.5	1	3391.684	6783.368	6653.022	13306.044	1	1584999	0.84	12947	0.0163	1	0.006	15	0.0866
			0.5	2	3261.338	6522.676			1					1		15	
	4-2	2	0.5	1	4647.537	9295.074	9139.245	18278.49	1	1584999	1.15	17919	0.0225	1	0.006	15	0.1199
			0.5	2	4491.708	8983.416			1					1		15	
	4-3	2	0.5	1	4529.981	9059.962	9051.301	18102.602	1	1584999	1.14	17743	0.0223	1	0.006	15	0.1187
			0.5	2	4521.32	9042.64			1					1		15	
	5-1	2	0.5	1	4651.005	9302.01	9774.39	19548.78	1	1584999	1.23	19189	0.0241	1	0.006	15	0.1284
			0.5	2	5123.385	10246.77			1					1		15	
	5-2	2	0.5	1	4819.77	9639.54	9643.018	19286.036	1	1584999	1.22	18927	0.0238	1	0.006	15	0.1266
			0.5	2	4823.248	9646.496			1					1		15	
	5-3	2	0.5	1	4743.222	9486.444	9330.615	18661.23	1	1584999	1.18	18302	0.0230	1	0.006	15	0.1225
			0.5	2	4587.393	9174.786			1					1		15	
	6-1	2	0.5	1	4611.046	9222.092	9184.769	18369.538	1	1584999	1.16	18010	0.0226	1	0.006	15	0.1205
			0.5	2	4573.723	9147.446			1					1		15	
	6-2	2	0.5	1	4729.552	9459.104	9122.841	18245.682	1	1584999	1.15	17886	0.0225	1	0.006	15	0.1197
			0.5	2	4393.269	8786.578			1					1		15	
	6-3	2	0.5	1	4269.488	8538.976	8569.826	17139.652	1	1584999	1.08	16780	0.0211	1	0.006	15	0.1123
			0.5	2	4300.338	8600.676			1					1		15	
	7-1	2	0.5	1	4503.621	9007.242	8817.181	17634.362	1	1584999	1.11	17275	0.0217	1	0.006	15	0.1156
			0.5	2	4313.56	8627.12			1					1		15	
	7-2	2	0.5	1	4308.051	8616.102	8470.113	16940.226	1	1584999	1.07	16581	0.0208	1	0.006	15	0.1109
			0.5	2	4162.062	8324.124			1					1		15	
	7-3	2	0.5	1	4626.583	9253.166	9220.604	18441.208	1	1584999	1.16	18082	0.0227	1	0.006	15	0.1210
			0.5	2	4594.021	9188.042			1					1		15	
	8-1	2	0.5	1	4792.109	9584.218	9626.271	19252.542	1	1584999	1.21	18893	0.0237	1	0.006	15	0.1264
			0.5	2	4834.162	9668.324			1					1		15	
	8-2	2	0.5	1	4919.645	9839.29	9679.27	19358.54	1	1584999	1.22	18999	0.0239	1	0.006	15	0.1271
			0.5	2	4759.625	9519.25			1					1		15	
	8-3	2	0.5	1	4801.133	9602.266	9273.068	18546.136	1	1584999	1.17	18187	0.0228	1	0.006	15	0.1217
			0.5	2	4471.935	8943.87			1					1		15	

Assay Date	9/12/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.1270	0.0075
Full activity	End	0.1196	0.0007
Full activity	Overall	0.1233	0.0061
Background	Beginning	-0.0001	0.000345605
Background	End	0.0001	2.58744E-05
Background	Overall	0.0000	0.000213725
Positive	Beginning	0.0480	0.0034
Positive	End	0.0555	0.0007
Positive	Overall	0.0518	0.0048
Negative	Beginning	0.0780	0.0041
Negative	End	0.1160	0.0027
Negative	Overall	0.0970	0.0221

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#9 DCF	1	1	1.00E-04	-4.00	0.0380
RC#9 DCF	1	2	1.00E-04	-4.00	0.0832
RC#9 DCF	1	3	1.00E-04	-4.00	0.0718
RC#9 DCF	2	1	3.30E-05	-4.48	0.0872
RC#9 DCF	2	2	3.30E-05	-4.48	0.0889
RC#9 DCF	2	3	3.30E-05	-4.48	0.0796
RC#9 DCF	3	1	1.00E-05	-5.00	0.0840
RC#9 DCF	3	2	1.00E-05	-5.00	0.0785
RC#9 DCF	3	3	1.00E-05	-5.00	0.0754
RC#9 DCF	4	1	1.00E-06	-6.00	0.0866
RC#9 DCF	4	2	1.00E-06	-6.00	0.1199
RC#9 DCF	4	3	1.00E-06	-6.00	0.1187
RC#9 DCF	5	1	1.00E-07	-7.00	0.1284
RC#9 DCF	5	2	1.00E-07	-7.00	0.1266
RC#9 DCF	5	3	1.00E-07	-7.00	0.1225
RC#9 DCF	6	1	1.00E-08	-8.00	0.1205
RC#9 DCF	6	2	1.00E-08	-8.00	0.1197
RC#9 DCF	6	3	1.00E-08	-8.00	0.1123
RC#9 DCF	7	1	1.00E-09	-9.00	0.1156
RC#9 DCF	7	2	1.00E-09	-9.00	0.1109
RC#9 DCF	7	3	1.00E-09	-9.00	0.1210
RC#9 DCF	8	1	1.00E-10	-10.00	0.1264
RC#9 DCF	8	2	1.00E-10	-10.00	0.1271
RC#9 DCF	8	3	1.00E-10	-10.00	0.1217

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	30.85	67.50	58.22
2	-4.48	70.71	72.09	64.56
3	-5.00	68.17	63.69	61.17
4	-6.00	70.27	97.25	96.30
5	-7.00	104.15	102.72	99.33
6	-8.00	97.75	97.07	91.07
7	-9.00	93.76	89.99	98.14
8	-10.00	102.54	103.11	98.70

Assay Date	9/13/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8		
Technician ID	TM	Replicate #	3	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	29618.38	1488361		
2	0.0199	31708.7	1593402		
3	0.0205	34115.73	1664182		
4	0.0203	34217.05	1685569		
5	0.0201	34583.58	1720576		
			Average DPM/g soln	1630418	
			SD	92006	
			CV	5.64	
			µCi/g soln	0.734	

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	11.3	11.3		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2263 g
Mass of dilution B used in substrate prep	9.1031 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.561009 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00831 µg/g soln.
µg/g soln.

a. µCi/g soln	0.734
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.561009 + 0.00831
= 0.569323 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.290 µCi/µg ASDN

820188 dpm/nmol

Assay Date	9/13/2005	Test Chemical ID	RC#9 DCF		# Concentrations tested	8					
Technician ID	TM	Replicate #	3	Microsome type	placental	Microsome ID	BAA				

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.512	0.401	0.187	0.120	0.066	0.048	0.041			
	0.640	0.401	0.200	0.122	0.080	0.048	0.038			
	0.631	0.401	0.179	0.124	0.073	0.051	0.037			

Samples:	Placental	QC 10	QC 100
	0.102	0.081	0.315
	0.099	0.075	0.321
	0.096	0.084	0.324

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
0.25	125	1000	0.00025	200	0.0500	0.594	0.556	0.0466
0.125	62.5	1000	0.00013	200	0.0250	0.401	0.363	0.0297
0.05	25	1000	0.00005	200	0.0100	0.189	0.150	0.0112
0.025	25	2000	0.00003	200	0.0050	0.122	0.083	0.0054
0.01	5	1000	0.00001	200	0.0020	0.073	0.035	0.0011
0.005	5	2000	0.00001	200	0.0010	0.049	0.011	-0.0010

Blank	0.038	r^2=	0.978		
		m=	0.087		
		b=	-0.002		

Variables	Regression results	
m, b	0.087	-0.002
se_m, se_b	0.007	0.002
r^2, se_y	0.978	0.003
F, df	177	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
Placental	0.102	0.064	0.004	200	220	70400	0.006	0.005	5.421
Placental	0.099	0.061	0.003	200	220	70400	0.005		
Placental	0.096	0.058	0.003	200	220	70400	0.005		
QC 10	0.081	0.042	0.002	200	200	200	0.000	0.000	0.009
QC 10	0.075	0.037	0.001	200	200	200	0.000		
QC 10	0.084	0.046	0.002	200	200	200	0.000		
QC 100	0.315	0.277	0.022	200	200	200	0.000	0.000	0.113
QC 100	0.321	0.282	0.023	200	200	200	0.000		
QC 100	0.324	0.285	0.023	200	200	200	0.000		

Assay Date	9/13/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	3

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.420536
Protein Concentration (dilution added to assay, mg/mL):	0.016939

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
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	9/13/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	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	5754.431	11508.862	11375.903	22751.806	1	1630418	1.40	22360	0.0273	1	0.008	15	0.1073
			0.5	2	5621.472	11242.944			1					1		15	
	2	2	0.5	1	6019.863	12039.726	12331.103	24662.206	1	1630418	1.51	24271	0.0296	1	0.008	15	0.1165
			0.5	2	6311.24	12622.48			1					1		15	
	3	2	0.5	1	5624.166	11248.332	11406.187	22812.374	1	1630418	1.40	22421	0.0273	1	0.008	15	0.1076
			0.5	2	5782.021	11564.042			1					1		15	
	4	2	0.5	1	5618.057	11236.114	11083.065	22166.13	1	1630418	1.36	21775	0.0265	1	0.008	15	0.1045
			0.5	2	5465.008	10930.016			1					1		15	
Background control	1	2	0.5	1	106.9597	213.9194	225.4766	450.9532	1	1630418	0.03	60	0.0001	1	0.008	15	0.0003
			0.5	2	118.5169	237.0338			1					1		15	
	2	2	0.5	1	101.1512	202.3024	192.73244	385.46488	1	1630418	0.02	-6	0.0000	1	0.008	15	0.0000
			0.5	2	91.55124	183.16248			1					1		15	
	3	2	0.5	1	94.2748	188.5496	177.77534	355.55068	1	1630418	0.02	-36	0.0000	1	0.008	15	-0.0002
			0.5	2	83.50054	167.00108			1					1		15	
	4	2	0.5	1	116.6809	233.3618	186.71399	373.42798	1	1630418	0.02	-18	0.0000	1	0.008	15	-0.0001
			0.5	2	70.03309	140.06618			1					1		15	
Positive control	1	2	0.5	1	2410.754	4821.508	4779.66	9559.32	1	1630418	0.59	9168	0.0112	1	0.008	15	0.0440
			0.5	2	2368.906	4737.812			1					1		15	
	2	2	0.5	1	2349.912	4699.824	4755.683	9511.366	1	1630418	0.58	9120	0.0111	1	0.008	15	0.0438
			0.5	2	2405.771	4811.542			1					1		15	
	3	2	0.5	1	2192.528	4385.056	4453.406	8906.812	1	1630418	0.55	8515	0.0104	1	0.008	15	0.0409
			0.5	2	2260.878	4521.756			1					1		15	
	4	2	0.5	1	2374.334	4748.668	4805.652	9611.304	1	1630418	0.59	9220	0.0112	1	0.008	15	0.0442
			0.5	2	2431.318	4862.636			1					1		15	
Negative Control	1	2	0.5	1	5546.942	11093.884	11124.792	22249.584	1	1630418	1.36	21858	0.0267	1	0.008	15	0.1049
			0.5	2	5577.85	11155.7			1					1		15	
	2	2	0.5	1	5372.777	10745.554	10755.057	21510.114	1	1630418	1.32	21119	0.0257	1	0.008	15	0.1013
			0.5	2	5382.28	10764.56			1					1		15	
	3	2	0.5	1	5494.886	10989.772	10601.914	21203.828	1	1630418	1.30	20812	0.0254	1	0.008	15	0.0999
			0.5	2	5107.028	10214.056			1					1		15	
	4	2	0.5	1	4897.911	9795.822	9583.305	19166.61	1	1630418	1.18	18775	0.0229	1	0.008	15	0.0901
			0.5	2	4685.394	9370.788			1					1		15	
RC#9 DCF	1-1	2	0.5	1	1603.115	3206.23	3103.439	6206.878	1	1630418	0.38	5816	0.0071	1	0.008	15	0.0279
			0.5	2	1500.324	3000.648			1					1		15	
	1-2	2	0.5	1	2036.704	4073.408	3997.903	7995.806	1	1630418	0.49	7604	0.0093	1	0.008	15	0.0365
			0.5	2	1961.199	3922.398			1					1		15	
	1-3	2	0.5	1	2230.264	4460.568	4696.2	9392.4	1	1630418	0.58	9001	0.0110	1	0.008	15	0.0432
			0.5	2	2465.916	4931.832			1					1		15	
	2-1	2	0.5	1	3037.286	6074.572	5858.599	11717.198	1	1630418	0.72	11326	0.0138	1	0.008	15	0.0543
			0.5	2	2821.313	5642.626			1					1		15	
	2-2	2	0.5	1	2936.033	5872.066	6123.679	12247.358	1	1630418	0.75	11856	0.0145	1	0.008	15	0.0569
			0.5	2	3187.646	6375.292			1					1		15	
	2-3	2	0.5	1	2637.015	5274.03	5130.744	10261.488	1	1630418	0.63	9870	0.0120	1	0.008	15	0.0474
			0.5	2	2493.729	4987.456			1					1		15	
	3-1	2	0.5	1	4127.27	8254.54	8308.811	16617.622	1	1630418	1.02	16226	0.0198	1	0.008	15	0.0779
			0.5	2	4181.541	8363.082			1					1		15	
	3-2	2	0.5	1	4393.196	8786.392	8577.472	17154.944	1	1630418	1.05	16764	0.0204	1	0.008	15	0.0804
			0.5	2	4184.276	8368.552			1					1		15	
	3-3	2	0.5	1	4134.667	8269.334	8306.515	16613.03	1	1630418	1.02	16222	0.0198	1	0.008	15	0.0778
			0.5	2	4171.848	8343.696			1					1		15	
	4-1	2	0.5	1	5318.955	10637.91	10390.558	20781.116	1	1630418	1.27	20390	0.0249	1	0.008	15	0.0978
			0.5	2	5071.603	10143.206			1					1		15	
	4-2	2	0.5	1	5309.075	10618.15	10388.506	20777.012	1	1630418	1.27	20386	0.0249	1	0.008	15	0.0978
			0.5	2	5079.431	10158.862			1					1		15	
	4-3	2	0.5	1	5525.042	11050.084	11169.029	22338.058	1	1630418	1.37	21947	0.0268	1	0.008	15	0.1053
			0.5	2	5643.987	11287.974			1					1		15	
	5-1	2	0.5	1	5399.941	10799.882	11073.95	22147.9	1	1630418	1.36	21757	0.0265	1	0.008	15	0.1044
			0.5	2	5674.009	11348.018			1					1		15	
	5-2	2	0.5	1	5516.481	11032.962	10883.24	21766.48	1	1630418	1.34	21375	0.0261	1	0.008	15	0.1026
			0.5	2	5366.759	10733.518			1					1		15	
	5-3	2	0.5	1	5658.227	11316.454	11161.361	22322.722	1	1630418	1.37	21931	0.0267	1	0.008	15	0.1052
			0.5	2	5503.134	11006.268			1					1		15	
	6-1	2	0.5	1	5456.926	10913.852	11129.636	22259.272	1	1630418	1.37	21868	0.0267	1	0.008	15	0.1049
			0.5	2	5672.71	11345.42			1					1		15	
	6-2	2	0.5	1	5465.303	10930.606	10739.361	21478.722	1	1630418	1.32	21087	0.0257	1	0.008	15	0.1012
			0.5	2	5274.058	10548.116			1					1		15	
	6-3	2	0.5	1	5634.303	11268.606	11083.445	22166.89	1	1630418	1.36	21776	0.0265	1	0.008	15	0.1045
			0.5	2	5449.142	10898.284			1					1		15	
	7-1	2	0.5	1	5744.561	11489.122	11337.164	22674.328	1	1630418	1.39	22283	0.0272	1	0.008	15	0.1069
			0.5	2	5592.603	11185.206			1					1		15	
	7-2	2	0.5	1	5163.533	10327.066	10770.65	21541.3	1	1630418	1.32	21150	0.0258	1	0.008	15	0.1015
			0.5	2	5607.117	11214.234			1					1		15	
	7-3	2	0.5	1	5610.757	11221.514	11314.615	22629.23	1	1630418	1.39	22238	0.0271	1	0.008	15	0.1067
			0.5	2	5703.858	11407.716			1					1		15	
	8-1	2	0.5	1	5743.909	11487.818	11539.927	23079.654	1	1630418	1.42	22689	0.0277	1	0.008	15	0.1089
			0.5	2	5796.116	11592.236			1					1		15	
	8-2	2	0.5	1	5381.802	10763.604	10922.526	21845.052	1	1630418	1.34	21454	0.0262	1	0.008	15	0.1029
			0.5	2	5540.724	11081.448			1					1		15	
	8-3	2	0.5	1	5612.769	11225.538	11294.949	22589.898	1	1630418	1.39	22199	0.0271	1	0.008	15	0.1065
			0.5	2	5682.18	11364.36			1					1		15	

Assay Date	9/13/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.1119	0.0065
Full activity	End	0.1060	0.0022
Full activity	Overall	0.1090	0.0052
Background	Beginning	0.0001	0.000222204
Background	End	-0.0001	6.06583E-05
Background	Overall	0.0000	0.000199584
Positive	Beginning	0.0439	0.0002
Positive	End	0.0426	0.0024
Positive	Overall	0.0432	0.0016
Negative	Beginning	0.1031	0.0025
Negative	End	0.0950	0.0069
Negative	Overall	0.0990	0.0063

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#9 DCF	1	1	1.00E-04	-4.00	0.0279
RC#9 DCF	1	2	1.00E-04	-4.00	0.0365
RC#9 DCF	1	3	1.00E-04	-4.00	0.0432
RC#9 DCF	2	1	3.30E-05	-4.48	0.0543
RC#9 DCF	2	2	3.30E-05	-4.48	0.0569
RC#9 DCF	2	3	3.30E-05	-4.48	0.0474
RC#9 DCF	3	1	1.00E-05	-5.00	0.0779
RC#9 DCF	3	2	1.00E-05	-5.00	0.0804
RC#9 DCF	3	3	1.00E-05	-5.00	0.0778
RC#9 DCF	4	1	1.00E-06	-6.00	0.0978
RC#9 DCF	4	2	1.00E-06	-6.00	0.0978
RC#9 DCF	4	3	1.00E-06	-6.00	0.1053
RC#9 DCF	5	1	1.00E-07	-7.00	0.1044
RC#9 DCF	5	2	1.00E-07	-7.00	0.1026
RC#9 DCF	5	3	1.00E-07	-7.00	0.1052
RC#9 DCF	6	1	1.00E-08	-8.00	0.1049
RC#9 DCF	6	2	1.00E-08	-8.00	0.1012
RC#9 DCF	6	3	1.00E-08	-8.00	0.1045
RC#9 DCF	7	1	1.00E-09	-9.00	0.1069
RC#9 DCF	7	2	1.00E-09	-9.00	0.1015
RC#9 DCF	7	3	1.00E-09	-9.00	0.1067
RC#9 DCF	8	1	1.00E-10	-10.00	0.1089
RC#9 DCF	8	2	1.00E-10	-10.00	0.1029
RC#9 DCF	8	3	1.00E-10	-10.00	0.1065

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	25.61	33.49	39.64
2	-4.48	49.88	52.21	43.47
3	-5.00	71.46	73.83	71.44
4	-6.00	89.80	89.78	96.65
5	-7.00	95.82	94.14	96.59
6	-8.00	96.31	92.87	95.90
7	-9.00	98.13	93.14	97.93
8	-10.00	99.92	94.48	97.76

Assay Date	9/8/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8		
Technician ID	TM	Replicate #	1	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	30258.43	1520524		
2	0.0201	31448.17	1564586		
3	0.0203	32934.57	1622393		
4	0.0201	33755.44	1679375		
5	0.0201	34761.64	1729435		
			Average DPM/g soln	1623262	
			SD	84291	
			CV	5.19	
			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	11.5	11.5		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.3288 g
Mass of dilution B used in substrate prep	9.1883 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562705 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00828 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/bc

2) Calculate total µg ASDN/g soln.

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

= 0.562705 + 0.00828
= 0.570982 µg ASDN/g soln.

3) Calculate Solution Specific Activity

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

814215 dpm/nmol

Assay Date	9/8/2005	Test Chemical ID	RC#10 ATZ		# Concentrations tested		8				
Technician ID	TM	Replicate #		1	Microsome type	placental	Microsome ID	BAA			

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.628	0.390	0.192	0.124	0.077	0.061	0.044			
	0.635	0.400	0.195	0.125	0.073	0.061	0.039			
	0.629	0.405	0.190	0.119	0.076	0.061	0.040			

Samples:	Placental	QC 10	QC 100
	0.102	0.074	0.307
	0.098	0.072	0.305
	0.103	0.067	0.310

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
0.25	125	1000	0.00025	200	0.0500	0.631	0.589	0.0479
0.125	62.5	1000	0.00013	200	0.0250	0.398	0.357	0.0283
0.05	25	1000	0.00005	200	0.0100	0.193	0.151	0.0109
0.025	25	2000	0.00003	200	0.0050	0.123	0.082	0.0051
0.01	5	1000	0.00001	200	0.0020	0.075	0.034	0.0010
0.005	5	2000	0.00001	200	0.0010	0.061	0.020	-0.0002
			Blank	0.041				

Variables	Regression results	
m, b	0.084	-0.002
se_m, se_b	0.004	0.001
r^2, se_y	0.990	0.002
F, df	390	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.990
m= 0.084
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
Placental	0.102	0.061	0.003	200	220	70400	0.005	0.005	5.186
Placental	0.098	0.057	0.003	200	220	70400	0.005		
Placental	0.103	0.062	0.003	200	220	70400	0.005		
QC 10	0.074	0.033	0.001	200	200	200	0.000	0.000	0.003
QC 10	0.072	0.031	0.001	200	200	200	0.000		
QC 10	0.067	0.026	0.000	200	200	200	0.000		
QC 100	0.307	0.266	0.021	200	200	200	0.000	0.000	0.103
QC 100	0.305	0.264	0.020	200	200	200	0.000		
QC 100	0.310	0.269	0.021	200	200	200	0.000		

Assay Date	9/8/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	1

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.186269
Protein Concentration (dilution added to assay, mg/mL):	0.016207

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
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	RC#10 ATZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	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	5489.487	10978.974	10817.361	21634.722	1	1623262	1.33	21216	0.0261	1	0.008	15	0.1072
			0.5	2	5327.874	10655.748			1					1		15	
	2	2	0.5	1	5361.354	10722.708	10766.665	21533.33	1	1623262	1.33	21115	0.0259	1	0.008	15	0.1067
			0.5	2	5405.311	10810.622			1					1		15	
	3	2	0.5	1	4246.643	8493.286	8416.283	16832.566	1	1623262	1.04	16414	0.0202	1	0.008	15	0.0829
			0.5	2	4169.64	8339.28			1					1		15	
	4	2	0.5	1	4401.391	8802.782	8697.629	17395.256	1	1623262	1.07	16977	0.0209	1	0.008	15	0.0858
			0.5	2	4296.238	8592.476			1					1		15	
Background control	1	2	0.5	1	86.19411	172.38822	207.40461	414.80922	1	1623262	0.03	-3	0.0000	1	0.008	15	0.0000
			0.5	2	121.2105	242.421			1					1		15	
	2	2	0.5	1	91.58124	183.16248	167.00109	334.00218	1	1623262	0.02	-64	-0.0001	1	0.008	15	-0.0004
			0.5	2	75.41985	150.8397			1					1		15	
	3	2	0.5	1	124.7753	249.5506	262.1472	524.2944	1	1623262	0.03	106	0.0001	1	0.008	15	0.0005
			0.5	2	137.3719	274.7438			1					1		15	
	4	2	0.5	1	104.2657	208.5714	199.96978	399.93956	1	1623262	0.02	-18	0.0000	1	0.008	15	-0.0001
			0.5	2	95.68408	191.36816			1					1		15	
Positive control	1	2	0.5	1	2340.159	4680.318	4616.234	9232.468	1	1623262	0.57	8814	0.0108	1	0.008	15	0.0445
			0.5	2	2276.075	4552.15			1					1		15	
	2	2	0.5	1	2168.106	4336.212	4379.628	8759.256	1	1623262	0.54	8341	0.0102	1	0.008	15	0.0421
			0.5	2	2211.522	4423.044			1					1		15	
	3	2	0.5	1	2162.945	4325.89	4194.181	8388.362	1	1623262	0.52	7970	0.0098	1	0.008	15	0.0403
			0.5	2	2031.236	4062.472			1					1		15	
	4	2	0.5	1	2127.403	4254.806	4170.687	8341.374	1	1623262	0.51	7923	0.0097	1	0.008	15	0.0400
			0.5	2	2043.284	4086.568			1					1		15	
Negative Control	1	2	0.5	1	3677.002	7354.004	7065.526	14131.052	1	1623262	0.87	13713	0.0168	1	0.008	15	0.0693
			0.5	2	3388.524	6777.048			1					1		15	
	2	2	0.5	1	3622.555	7245.11	7059.916	14139.832	1	1623262	0.87	13722	0.0169	1	0.008	15	0.0693
			0.5	2	3447.361	6894.722			1					1		15	
	3	2	0.5	1	3928.515	7857.03	7979.66	15959.32	1	1623262	0.98	15541	0.0191	1	0.008	15	0.0785
			0.5	2	4051.145	8102.29			1					1		15	
	4	2	0.5	1	3970.337	7940.674	7706.333	15412.666	1	1623262	0.95	14994	0.0184	1	0.008	15	0.0758
			0.5	2	3735.996	7471.992			1					1		15	
RC#10 ATZ	1-1	2	0.5	1	4108.318	8216.636	8181.361	16362.722	1	1623262	1.01	15944	0.0196	1	0.008	15	0.0806
			0.5	2	4073.043	8146.086			1					1		15	
	1-2	2	0.5	1	4027.096	8054.192	8290.087	16580.174	1	1623262	1.02	16162	0.0198	1	0.008	15	0.0817
			0.5	2	4262.991	8525.982			1					1		15	
	1-3	2	0.5	1	3894.958	7789.916	7619.888	15239.776	1	1623262	0.94	14822	0.0182	1	0.008	15	0.0749
			0.5	2	3724.93	7449.86			1					1		15	
	2-1	2	0.5	1	3781.827	7563.654	7663.908	15327.816	1	1623262	0.94	14910	0.0183	1	0.008	15	0.0753
			0.5	2	3882.081	7764.162			1					1		15	
	2-2	2	0.5	1	4262.087	8524.174	8515.972	17031.944	1	1623262	1.05	16614	0.0204	1	0.008	15	0.0839
			0.5	2	4253.885	8507.77			1					1		15	
	2-3	2	0.5	1	4474.648	8949.296	8824.473	17648.946	1	1623262	1.09	17231	0.0212	1	0.008	15	0.0870
			0.5	2	4349.825	8699.65			1					1		15	
	3-1	2	0.5	1	4667.311	9334.622	9022.876	18045.752	1	1623262	1.11	17627	0.0216	1	0.008	15	0.0891
			0.5	2	4355.565	8711.13			1					1		15	
	3-2	2	0.5	1	4179.516	8359.032	8455.272	16910.544	1	1623262	1.04	16492	0.0203	1	0.008	15	0.0833
			0.5	2	4275.756	8551.512			1					1		15	
	3-3	2	0.5	1	4608.023	9216.046	8965.542	17931.084	1	1623262	1.10	17513	0.0215	1	0.008	15	0.0885
			0.5	2	4357.519	8715.038			1					1		15	
	4-1	2	0.5	1	4460.211	8920.422	8759.444	17518.888	1	1623262	1.08	17101	0.0210	1	0.008	15	0.0864
			0.5	2	4299.233	8598.466			1					1		15	
	4-2	2	0.5	1	4097.991	8195.982	8173.682	16347.364	1	1623262	1.01	15929	0.0196	1	0.008	15	0.0805
			0.5	2	4075.691	8151.382			1					1		15	
	4-3	2	0.5	1	4151.67	8303.34	8394.553	16789.106	1	1623262	1.03	16371	0.0201	1	0.008	15	0.0827
			0.5	2	4242.883	8485.766			1					1		15	
	5-1	2	0.5	1	3983.17	7966.34	8144.729	16289.458	1	1623262	1.00	15871	0.0195	1	0.008	15	0.0802
			0.5	2	4161.559	8323.118			1					1		15	
	5-2	2	0.5	1	4046.329	8092.658	8149.787	16299.574	1	1623262	1.00	15881	0.0195	1	0.008	15	0.0802
			0.5	2	4103.458	8206.916			1					1		15	
	5-3	2	0.5	1	4007.854	8015.708	7917.211	15834.422	1	1623262	0.98	15416	0.0189	1	0.008	15	0.0779
			0.5	2	3909.357	7818.714			1					1		15	
	6-1	2	0.5	1	4374.178	8748.356	8615.152	17230.304	1	1623262	1.06	16812	0.0206	1	0.008	15	0.0849
			0.5	2	4240.974	8481.948			1					1		15	
	6-2	2	0.5	1	4010.589	8021.178	7869.22	15738.44	1	1623262	0.97	15320	0.0188	1	0.008	15	0.0774
			0.5	2	3858.631	7717.262			1					1		15	
	6-3	2	0.5	1	4026.902	8053.804	8067.339	16134.678	1	1623262	0.99	15716	0.0193	1	0.008	15	0.0794
			0.5	2	4040.437	8080.874			1					1		15	
	7-1	2	0.5	1	3896.621	7793.242	7988.615	15977.23	1	1623262	0.98	15559	0.0191	1	0.008	15	0.0786
			0.5	2	4081.994	8163.988			1					1		15	
	7-2	2	0.5	1	4018.731	8037.462	8154.142	16308.284	1	1623262	1.00	15890	0.0195	1	0.008	15	0.0803
			0.5	2	4135.411	8270.822			1					1		15	
	7-3	2	0.5	1	4034.963	8069.966	8032.256	16064.512	1	1623262	0.99	15646	0.0192	1	0.008	15	0.0790
			0.5	2	3997.273	7994.546			1					1		15	
	8-1	2	0.5	1	4032.297	8064.594	7964.067	15928.134	1	1623262	0.98	15510	0.0190	1	0.008	15	0.0784
			0.5	2	3931.77	7863.54			1					1		15	
	8-2	2	0.5	1	4118.484	8236.968	8013.401	16026.802	1	1623262	0.99	15609	0.0192	1	0.008	15	0.0789
			0.5	2	3894.917	7789.834			1					1		15	
	8-3	2	0.5	1	3972.599	7945.198	8045.599	16091.198	1	1623262	0.99	15673	0.0192	1	0.008	15	0.0792
			0.5	2	4073	8146			1					1		15	

Assay Date	9/8/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.1069	0.0004
Full activity	End	0.0843	0.0020
Full activity	Overall	0.0956	0.0131
Background	Beginning	-0.0002	0.000288668
Background	End	0.0002	0.000444235
Background	Overall	0.0000	0.00039876
Positive	Beginning	0.0433	0.0017
Positive	End	0.0401	0.0002
Positive	Overall	0.0417	0.0021
Negative	Beginning	0.0693	0.0000
Negative	End	0.0771	0.0020
Negative	Overall	0.0732	0.0047

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#10 ATZ	1	1	1.00E-04	-4.00	0.0806
RC#10 ATZ	1	2	1.00E-04	-4.00	0.0817
RC#10 ATZ	1	3	1.00E-04	-4.00	0.0749
RC#10 ATZ	2	1	3.30E-05	-4.48	0.0753
RC#10 ATZ	2	2	3.30E-05	-4.48	0.0839
RC#10 ATZ	2	3	3.30E-05	-4.48	0.0870
RC#10 ATZ	3	1	1.00E-05	-5.00	0.0891
RC#10 ATZ	3	2	1.00E-05	-5.00	0.0833
RC#10 ATZ	3	3	1.00E-05	-5.00	0.0885
RC#10 ATZ	4	1	1.00E-06	-6.00	0.0864
RC#10 ATZ	4	2	1.00E-06	-6.00	0.0805
RC#10 ATZ	4	3	1.00E-06	-6.00	0.0827
RC#10 ATZ	5	1	1.00E-07	-7.00	0.0802
RC#10 ATZ	5	2	1.00E-07	-7.00	0.0802
RC#10 ATZ	5	3	1.00E-07	-7.00	0.0779
RC#10 ATZ	6	1	1.00E-08	-8.00	0.0849
RC#10 ATZ	6	2	1.00E-08	-8.00	0.0774
RC#10 ATZ	6	3	1.00E-08	-8.00	0.0794
RC#10 ATZ	7	1	1.00E-09	-9.00	0.0786
RC#10 ATZ	7	2	1.00E-09	-9.00	0.0803
RC#10 ATZ	7	3	1.00E-09	-9.00	0.0790
RC#10 ATZ	8	1	1.00E-10	-10.00	0.0784
RC#10 ATZ	8	2	1.00E-10	-10.00	0.0789
RC#10 ATZ	8	3	1.00E-10	-10.00	0.0792

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	84.23	85.37	78.29
2	-4.48	78.76	87.76	91.02
3	-5.00	93.12	87.12	92.51
4	-6.00	90.33	84.14	86.48
5	-7.00	83.84	83.89	81.43
6	-8.00	88.81	80.93	83.02
7	-9.00	82.19	83.94	82.65
8	-10.00	81.93	82.45	82.79

Assay Date	9/12/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8		
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	28991.12	1456840		
2	0.0205	31876.95	1554973		
3	0.0199	32398.9	1628085		
4	0.0200	32617.59	1630880		
5	0.0201	33249.76	1654217		
			Average DPM/g soln		1584999
			SD		80745
			CV		5.09
			µCi/g soln		0.714

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	13.8	13.8		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2713 g
Mass of dilution B used in substrate prep	9.145 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562033 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00808 µg/g soln.
µg/g soln.

a. µCi/g soln 0.714
b. Specific activity of [^{3}H]ASDN (µCi/mmol) 25300000
c. Molecular wt of ASDN (mg/mmol) 286.4

Formula=a/bc

2) Calculate total µg ASDN/g soln.

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

= 0.562033 + 0.00808
= 0.570115 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.252 µCi/µg ASDN

796232 dpm/nmol

Assay Date	9/12/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8				
Technician ID	TM	Replicate #	2	Microsome type	placental	Microsome ID	BAA		

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.625	0.405	0.200	0.162	0.081	0.061	0.043			
	0.632	0.405	0.177	0.162	0.089	0.058	0.044			
	0.629	0.405	0.199	0.122	0.101	0.068	0.045			

Samples:	Placental	QC 10	QC 100
	0.109	0.073	0.332
	0.100	0.063	0.315
	0.101	0.071	0.317

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
0.25	125	1000	0.00025	200	0.0500	0.628	0.585	0.0477
0.125	62.5	1000	0.00013	200	0.0250	0.405	0.361	0.0285
0.05	25	1000	0.00005	200	0.0100	0.192	0.148	0.0101
0.025	25	2000	0.00003	200	0.0050	0.148	0.104	0.0064
0.01	5	1000	0.00001	200	0.0020	0.090	0.047	0.0014
0.005	5	2000	0.00001	200	0.0010	0.062	0.019	-0.0010

			Variables	Regression results	
			m, b	0.086	-0.003
			se_m, se_b	0.005	0.001
			r^2, se_y	0.987	0.002
			F, df	302	4
			ss_{reg}, ss_{resid}	0.002	0.000

Blank	0.044	r^2=	0.987
		m=	0.086
		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/µL Prep.	average mg/µL	mg/mL
Placental	0.109	0.065	0.003	200	220	70400	0.005	0.004	4.004
Placental	0.100	0.056	0.002	200	220	70400	0.004		
Placental	0.101	0.057	0.002	200	220	70400	0.004		
QC 10	0.073	0.029	0.000	200	200	200	0.000	0.000	-0.002
QC 10	0.063	0.019	-0.001	200	200	200	0.000		
QC 10	0.071	0.028	0.000	200	200	200	0.000		
QC 100	0.332	0.288	0.022	200	200	200	0.000	0.000	0.106
QC 100	0.315	0.271	0.021	200	200	200	0.000		
QC 100	0.317	0.273	0.021	200	200	200	0.000		

Assay Date	9/12/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	2

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.004164
Protein Concentration (dilution added to assay, mg/mL):	0.012513

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
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	9/12/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	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	5085.452	10170.904	10062.029	20124.058	1	1584999	1.27	19765	0.0248	1	0.006	15	0.1323
			0.5	2	4976.577	9953.154			1					1		15	
	2	2	0.5	1	4659.096	9318.192	9272.062	18544.124	1	1584999	1.17	18185	0.0228	1	0.006	15	0.1217
			0.5	2	4612.966	9225.932			1					1		15	
	3	2	0.5	1	4573.675	9147.35	9156.008	18312.016	1	1584999	1.16	17953	0.0225	1	0.006	15	0.1201
			0.5	2	4582.333	9164.666			1					1		15	
	4	2	0.5	1	4577.691	9155.382	9079.403	18158.806	1	1584999	1.15	17799	0.0224	1	0.006	15	0.1191
			0.5	2	4501.712	9003.424			1					1		15	
Background control	1	2	0.5	1	110.1792	220.3584	193.07295	386.1459	1	1584999	0.02	27	0.0000	1	0.006	15	0.0002
			0.5	2	82.89375	165.7875			1					1		16	
	2	2	0.5	1	60.14396	120.28792	156.55072	313.10144	1	1584999	0.02	-46	-0.0001	1	0.006	15	-0.0003
			0.5	2	96.40676	192.81352			1					1		15	
	3	2	0.5	1	84.74831	169.49662	183.16569	366.33138	1	1584999	0.02	7	0.0000	1	0.006	15	0.0000
			0.5	2	98.41738	196.83476			1					1		15	
	4	2	0.5	1	92.94975	185.8995	185.9	371.8	1	1584999	0.02	12	0.0000	1	0.006	15	0.0001
			0.5	2	92.95025	185.9005			1					1		15	
Positive control	1	2	0.5	1	2032.818	4065.632	3949.943	7899.886	1	1584999	0.50	7541	0.0095	1	0.006	15	0.0505
			0.5	2	1917.127	3834.254			1					1		15	
	2	2	0.5	1	1826.199	3652.398	3586.786	7173.572	1	1584999	0.45	6814	0.0086	1	0.006	15	0.0456
			0.5	2	1760.587	3521.174			1					1		15	
	3	2	0.5	1	2112.697	4225.394	4291.501	8583.002	1	1584999	0.54	8224	0.0103	1	0.006	15	0.0550
			0.5	2	2178.804	4357.608			1					1		15	
	4	2	0.5	1	2195.266	4390.532	4363.052	8726.104	1	1584999	0.55	8367	0.0105	1	0.006	15	0.0560
			0.5	2	2167.786	4335.572			1					1		15	
Negative Control	1	2	0.5	1	3153.115	6306.23	6227.13	12454.26	1	1584999	0.79	12095	0.0152	1	0.006	15	0.0809
			0.5	2	3074.015	6148.03			1					1		16	
	2	2	0.5	1	2988.625	5977.25	5792.699	11585.398	1	1584999	0.73	11226	0.0141	1	0.006	15	0.0751
			0.5	2	2804.074	5608.148			1					1		15	
	3	2	0.5	1	4409.944	8819.888	8990.667	17981.334	1	1584999	1.13	17622	0.0221	1	0.006	15	0.1179
			0.5	2	4580.723	9161.446			1					1		15	
	4	2	0.5	1	4299.765	8599.53	8709.242	17418.484	1	1584999	1.10	17059	0.0214	1	0.006	15	0.1141
			0.5	2	4409.477	8818.954			1					1		15	
RC#10 ATZ	1-1	2	0.5	1	4491.982	8983.964	8920.183	17840.366	1	1584999	1.13	17481	0.0220	1	0.006	15	0.1170
			0.5	2	4428.201	8856.402			1					1		15	
	1-2	2	0.5	1	4339.403	8678.806	8534.555	17069.11	1	1584999	1.08	16710	0.0210	1	0.006	15	0.1118
			0.5	2	4195.152	8390.304			1					1		15	
	1-3	2	0.5	1	4528.435	9056.87	8886.694	17773.388	1	1584999	1.12	17414	0.0219	1	0.006	15	0.1165
			0.5	2	4358.259	8716.518			1					1		15	
	2-1	2	0.5	1	4474.648	8949.296	8649.742	17299.484	1	1584999	1.09	16940	0.0213	1	0.006	15	0.1134
			0.5	2	4175.094	8350.188			1					1		15	
	2-2	2	0.5	1	4430.986	8861.972	8587.224	17174.448	1	1584999	1.08	16815	0.0211	1	0.006	15	0.1125
			0.5	2	4156.238	8312.476			1					1		15	
	2-3	2	0.5	1	4187.012	8374.024	8585.675	17171.35	1	1584999	1.08	16812	0.0211	1	0.006	15	0.1125
			0.5	2	4398.663	8797.326			1					1		15	
	3-1	2	0.5	1	4790.115	9580.23	9251.196	18502.392	1	1584999	1.17	18143	0.0228	1	0.006	15	0.1214
			0.5	2	4461.081	8922.162			1					1		15	
	3-2	2	0.5	1	4654.556	9309.112	9254.028	18508.056	1	1584999	1.17	18149	0.0228	1	0.006	15	0.1214
			0.5	2	4599.472	9198.944			1					1		15	
	3-3	2	0.5	1	4613.04	9226.08	9118.788	18237.576	1	1584999	1.15	17878	0.0225	1	0.006	15	0.1196
			0.5	2	4505.748	9011.496			1					1		15	
	4-1	2	0.5	1	4525.263	9050.526	8965.051	17930.102	1	1584999	1.13	17571	0.0221	1	0.006	15	0.1176
			0.5	2	4439.788	8879.576			1					1		15	
	4-2	2	0.5	1	4577.764	9155.528	9342.758	18685.516	1	1584999	1.18	18326	0.0230	1	0.006	15	0.1226
			0.5	2	4764.994	9529.988			1					1		15	
	4-3	2	0.5	1	4463.31	8926.62	8992.229	17984.458	1	1584999	1.13	17625	0.0221	1	0.006	15	0.1179
			0.5	2	4528.919	9057.838			1					1		15	
	5-1	2	0.5	1	4642.094	9284.188	9092.321	18184.642	1	1584999	1.15	17825	0.0224	1	0.006	15	0.1193
			0.5	2	4450.227	8900.454			1					1		15	
	5-2	2	0.5	1	4100.178	8200.356	8291.433	16582.866	1	1584999	1.05	16224	0.0204	1	0.006	15	0.1086
			0.5	2	4191.255	8382.51			1					1		15	
	5-3	2	0.5	1	3760.278	7520.556	7715.772	15431.544	1	1584999	0.97	15072	0.0189	1	0.006	15	0.1009
			0.5	2	3955.494	7910.988			1					1		15	
	6-1	2	0.5	1	4379.697	8759.394	8700.959	17401.918	1	1584999	1.10	17043	0.0214	1	0.006	15	0.1140
			0.5	2	4321.262	8642.524			1					1		15	
	6-2	2	0.5	1	4638.418	9276.836	9220.391	18440.782	1	1584999	1.16	18081	0.0227	1	0.006	15	0.1210
			0.5	2	4581.973	9163.946			1					1		15	
	6-3	2	0.5	1	3534.033	7068.066	6934.133	13868.266	1	1584999	0.87	13509	0.0170	1	0.006	15	0.0904
			0.5	2	3400.1	6800.2			1					1		15	
	7-1	2	0.5	1	4476.357	8952.714	9118.475	18236.95	1	1584999	1.15	17878	0.0225	1	0.006	15	0.1196
			0.5	2	4642.118	9284.236			1					1		15	
	7-2	2	0.5	1	4406.833	8813.666	8982.119	17964.238	1	1584999	1.13	17605	0.0221	1	0.006	15	0.1178
			0.5	2	4575.286	9150.572			1					1		15	
	7-3	2	0.5	1	4501.808	9003.616	9070.112	18140.224	1	1584999	1.14	17781	0.0223	1	0.006	15	0.1190
			0.5	2	4568.304	9136.608			1					1		15	
	8-1	2	0.5	1	4298.29	8596.58	8844.723	17689.446	1	1584999	1.12	17330	0.0218	1	0.006	15	0.1160
			0.5	2	4546.433	9092.866			1					1		15	
	8-2	2	0.5	1	4412.473	8824.946	8679.172	17358.344	1	1584999	1.10	16999	0.0213	1	0.006	15	0.1137
			0.5	2	4266.699	8533.398			1					1		15	
	8-3	2	0.5	1	4360.976	8721.952	8782.008	17564.016	1	1584999	1.11	17205	0.0216	1	0.006	15	0.1151
			0.5	2	4421.032	8842.064			1					1		15	

Assay Date	9/12/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.1270	0.0075
Full activity	End	0.1196	0.0007
Full activity	Overall	0.1233	0.0061
Background	Beginning	-0.0001	0.000345605
Background	End	0.0001	2.58744E-05
Background	Overall	0.0000	0.000213725
Positive	Beginning	0.0480	0.0034
Positive	End	0.0555	0.0007
Positive	Overall	0.0518	0.0048
Negative	Beginning	0.0780	0.0041
Negative	End	0.1160	0.0027
Negative	Overall	0.0970	0.0221

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#10 ATZ	1	1	1.00E-04	-4.00	0.1170
RC#10 ATZ	1	2	1.00E-04	-4.00	0.1118
RC#10 ATZ	1	3	1.00E-04	-4.00	0.1165
RC#10 ATZ	2	1	3.30E-05	-4.48	0.1134
RC#10 ATZ	2	2	3.30E-05	-4.48	0.1125
RC#10 ATZ	2	3	3.30E-05	-4.48	0.1125
RC#10 ATZ	3	1	1.00E-05	-5.00	0.1214
RC#10 ATZ	3	2	1.00E-05	-5.00	0.1214
RC#10 ATZ	3	3	1.00E-05	-5.00	0.1196
RC#10 ATZ	4	1	1.00E-06	-6.00	0.1176
RC#10 ATZ	4	2	1.00E-06	-6.00	0.1226
RC#10 ATZ	4	3	1.00E-06	-6.00	0.1179
RC#10 ATZ	5	1	1.00E-07	-7.00	0.1193
RC#10 ATZ	5	2	1.00E-07	-7.00	0.1086
RC#10 ATZ	5	3	1.00E-07	-7.00	0.1009
RC#10 ATZ	6	1	1.00E-08	-8.00	0.1140
RC#10 ATZ	6	2	1.00E-08	-8.00	0.1210
RC#10 ATZ	6	3	1.00E-08	-8.00	0.0904
RC#10 ATZ	7	1	1.00E-09	-9.00	0.1196
RC#10 ATZ	7	2	1.00E-09	-9.00	0.1178
RC#10 ATZ	7	3	1.00E-09	-9.00	0.1190
RC#10 ATZ	8	1	1.00E-10	-10.00	0.1160
RC#10 ATZ	8	2	1.00E-10	-10.00	0.1137
RC#10 ATZ	8	3	1.00E-10	-10.00	0.1151

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	94.87	90.69	94.51
2	-4.48	91.94	91.26	91.24
3	-5.00	98.47	98.50	97.03
4	-6.00	95.36	99.46	95.66
5	-7.00	96.74	88.05	81.80
6	-8.00	92.49	98.13	73.32
7	-9.00	97.03	95.55	96.50
8	-10.00	94.06	92.26	93.37

Assay Date	9/13/2005	Test Chemical ID	RC#0 ATZ	# Concentrations tested	8		
Technician ID	TM	Replicate #	3	Microsome type	placental	Microsome ID	BAA

Aliquot #	Weight of aliquot (g)	DPM/aliq.	DPM/g soln.		
1	0.0199	29618.38	1488361		
2	0.0199	31708.7	1593402		
3	0.0205	34115.73	1664182		
4	0.0203	34217.05	1685569		
5	0.0201	34583.58	1720576		
			Average DPM/g soln		1630418
			SD		92006
			CV		5.64
			µCi/g soln		0.734

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	11.3	11.3		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	16.2263 g
Mass of dilution B used in substrate prep	9.1031 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.561009 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00831 µg/g soln.
µg/g soln.

a. µCi/g soln 0.734
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.

= 0.561009 + 0.00831
= 0.569323 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)(µg ASDN/g soln.)
= 1.290 µCi/µg ASDN

820188 dpm/nmol

Assay Date 9/13/2005 | Test Chemical ID RC#10 ATZ | # Concentrations tested 8

Technician ID TM | Replicate # 3 | Microsome type placental | Microsome ID BAA

								Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	2	1	
	0.512	0.401	0.187	0.120	0.066	0.048	0.041			
	0.640	0.401	0.200	0.122	0.080	0.048	0.038			
	0.631	0.401	0.179	0.124	0.073	0.051	0.037			

Samples:	Placental	QC 10	QC 100
	0.102	0.081	0.315
	0.099	0.075	0.321
	0.096	0.084	0.324

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
0.25	125	1000	0.00025	200	0.0500	0.594	0.556	0.0466
0.125	62.5	1000	0.00013	200	0.0250	0.401	0.363	0.0297
0.05	25	1000	0.00005	200	0.0100	0.189	0.150	0.0112
0.025	25	2000	0.00003	200	0.0050	0.122	0.083	0.0054
0.01	5	1000	0.00001	200	0.0020	0.073	0.035	0.0011
0.005	5	2000	0.00001	200	0.0010	0.049	0.011	-0.0010

			Variables	Regression results	
Blank	0.038		m, b	0.087	-0.002
r^2=	0.978		se_m, se_b	0.007	0.002
m=	0.087		r^2, se_y	0.978	0.003
b=	-0.002		F, df	177	4
			ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
Placental	0.102	0.064	0.004	200	220	70400	0.006	0.005	5.421
Placental	0.099	0.061	0.003	200	220	70400	0.005		
Placental	0.096	0.058	0.003	200	220	70400	0.005		
QC 10	0.081	0.042	0.002	200	200	200	0.000	0.000	0.009
QC 10	0.075	0.037	0.001	200	200	200	0.000		
QC 10	0.084	0.046	0.002	200	200	200	0.000		
QC 100	0.315	0.277	0.022	200	200	200	0.000	0.000	0.113
QC 100	0.321	0.282	0.023	200	200	200	0.000		
QC 100	0.324	0.285	0.023	200	200	200	0.000		

Assay Date	9/13/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	Replicate #	3

Microsome Dilution Details		
Dilution A	0.22	mL microsome Stock used
	70.4	mL total volume
	320	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	320	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.420536
Protein Concentration (dilution added to assay, mg/mL):	0.016939

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	3.30E-05
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	9/13/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	placental	Microsome ID	BAA	Technician ID	TM	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	5754.431	11508.862	11375.903	22751.806	1	1630418	1.40	22360	0.0273	1	0.008	15	0.1073
			0.5	2	5621.472	11242.944			1					1		15	
	2	2	0.5	1	6019.863	12039.726	12331.103	24662.206	1	1630418	1.51	24271	0.0296	1	0.008	15	0.1165
			0.5	2	6311.24	12622.48			1					1		15	
	3	2	0.5	1	5624.166	11248.332	11406.187	22812.374	1	1630418	1.40	22421	0.0273	1	0.008	15	0.1076
			0.5	2	5782.021	11564.042			1					1		15	
	4	2	0.5	1	5618.057	11236.114	11083.065	22166.13	1	1630418	1.36	21775	0.0265	1	0.008	15	0.1045
			0.5	2	5465.008	10930.016			1					1		15	
Background control	1	2	0.5	1	106.9597	213.9194	225.4766	450.9532	1	1630418	0.03	60	0.0001	1	0.008	15	0.0003
			0.5	2	118.8169	237.0338			1					1		15	
	2	2	0.5	1	101.1512	202.3024	192.73244	385.46488	1	1630418	0.02	-6	0.0000	1	0.008	15	0.0000
			0.5	2	91.58124	183.16248			1					1		15	
	3	2	0.5	1	94.2748	188.5496	177.77534	355.55068	1	1630418	0.02	-36	0.0000	1	0.008	15	-0.0002
			0.5	2	83.50054	167.00108			1					1		15	
	4	2	0.5	1	116.6809	233.3618	186.71399	373.42798	1	1630418	0.02	-18	0.0000	1	0.008	15	-0.0001
			0.5	2	70.03309	140.06618			1					1		15	
Positive control	1	2	0.5	1	2410.754	4821.508	4779.66	9559.32	1	1630418	0.59	9168	0.0112	1	0.008	15	0.0440
			0.5	2	2368.906	4737.812			1					1		15	
	2	2	0.5	1	2349.912	4699.824	4755.683	9511.366	1	1630418	0.58	9120	0.0111	1	0.008	15	0.0438
			0.5	2	2405.771	4811.542			1					1		15	
	3	2	0.5	1	2192.528	4385.056	4453.406	8906.812	1	1630418	0.55	8515	0.0104	1	0.008	15	0.0409
			0.5	2	2260.878	4521.756			1					1		15	
	4	2	0.5	1	2374.334	4748.668	4805.652	9611.304	1	1630418	0.59	9220	0.0112	1	0.008	15	0.0442
			0.5	2	2431.318	4862.636			1					1		15	
Negative Control	1	2	0.5	1	5546.942	11093.884	11124.792	22249.584	1	1630418	1.36	21856	0.0267	1	0.008	15	0.1049
			0.5	2	5577.85	11155.7			1					1		15	
	2	2	0.5	1	5372.777	10745.554	10755.057	21510.114	1	1630418	1.32	21119	0.0257	1	0.008	15	0.1013
			0.5	2	5382.28	10764.56			1					1		15	
	3	2	0.5	1	5494.886	10989.772	10601.914	21203.828	1	1630418	1.30	20812	0.0254	1	0.008	15	0.0999
			0.5	2	5107.028	10214.056			1					1		15	
	4	2	0.5	1	4897.911	9795.822	9583.305	19166.61	1	1630418	1.18	18775	0.0229	1	0.008	15	0.0901
			0.5	2	4585.394	9370.788			1					1		15	
RC#10 ATZ	1-1	2	0.5	1	5186.131	10372.262	10512.807	21025.614	1	1630418	1.29	20634	0.0252	1	0.008	15	0.0990
			0.5	2	5326.676	10653.352			1					1		15	
	1-2	2	0.5	1	5486.856	10973.712	11104.937	22209.874	1	1630418	1.36	21819	0.0266	1	0.008	15	0.1047
			0.5	2	5618.081	11236.162			1					1		15	
	1-3	2	0.5	1	5012.806	10025.612	10289.585	20579.17	1	1630418	1.26	20188	0.0246	1	0.008	15	0.0969
			0.5	2	5276.779	10553.558			1					1		15	
	2-1	2	0.5	1	5511.226	11022.452	11027.878	22055.756	1	1630418	1.35	21664	0.0264	1	0.008	15	0.1040
			0.5	2	5516.652	11033.304			1					1		15	
	2-2	2	0.5	1	5260.724	10501.448	10530.197	21060.394	1	1630418	1.29	20669	0.0252	1	0.008	15	0.0992
			0.5	2	5279.473	10558.946			1					1		15	
	2-3	2	0.5	1	5244.456	10488.912	10424.265	20848.53	1	1630418	1.28	20457	0.0249	1	0.008	15	0.0982
			0.5	2	5179.809	10359.618			1					1		15	
	3-1	2	0.5	1	5691.585	11383.17	11286.21	22572.42	1	1630418	1.38	22181	0.0270	1	0.008	15	0.1064
			0.5	2	5594.625	11189.25			1					1		15	
	3-2	2	0.5	1	5655.044	11310.088	11214.652	22429.304	1	1630418	1.38	22038	0.0269	1	0.008	15	0.1057
			0.5	2	5559.608	11119.216			1					1		15	
	3-3	2	0.5	1	5455.03	10910.06	10858.408	21716.816	1	1630418	1.33	21325	0.0260	1	0.008	15	0.1023
			0.5	2	5403.378	10806.756			1					1		15	
	4-1	2	0.5	1	5420.268	10840.536	10827.165	21654.33	1	1630418	1.33	21263	0.0259	1	0.008	15	0.1020
			0.5	2	5406.897	10813.794			1					1		15	
	4-2	2	0.5	1	5519.204	11038.408	10938.745	21877.49	1	1630418	1.34	21486	0.0262	1	0.008	15	0.1031
			0.5	2	5419.541	10839.082			1					1		15	
	4-3	2	0.5	1	5278.543	10557.086	11042.865	22085.73	1	1630418	1.35	21694	0.0265	1	0.008	15	0.1041
			0.5	2	5764.322	11528.644			1					1		15	
	5-1	2	0.5	1	5375.548	10751.096	10919.336	21838.672	1	1630418	1.34	21447	0.0261	1	0.008	15	0.1029
			0.5	2	5543.788	11087.576			1					1		15	
	5-2	2	0.5	1	5408.796	10817.532	11046.489	22092.978	1	1630418	1.36	21702	0.0265	1	0.008	15	0.1041
			0.5	2	5637.723	11275.446			1					1		15	
	5-3	2	0.5	1	5694.629	11389.258	11216.688	22433.376	1	1630418	1.38	22042	0.0269	1	0.008	15	0.1058
			0.5	2	5522.059	11044.118			1					1		15	
	6-1	2	0.5	1	5621.591	11243.182	10752.943	21505.886	1	1630418	1.32	21115	0.0257	1	0.008	15	0.1013
			0.5	2	5131.352	10262.704			1					1		15	
	6-2	2	0.5	1	5551.958	11103.916	11328.634	22657.268	1	1630418	1.39	22266	0.0271	1	0.008	15	0.1068
			0.5	2	5776.676	11553.352			1					1		15	
	6-3	2	0.5	1	5600.042	11200.084	11030.386	22060.772	1	1630418	1.35	21669	0.0264	1	0.008	15	0.1040
			0.5	2	5430.344	10860.688			1					1		15	
	7-1	2	0.5	1	5209.047	10418.094	10676.795	21353.59	1	1630418	1.31	20962	0.0256	1	0.008	15	0.1006
			0.5	2	5467.748	10935.496			1					1		15	
	7-2	2	0.5	1	5462.411	10924.822	10725.75	21451.5	1	1630418	1.32	21060	0.0257	1	0.008	15	0.1011
			0.5	2	5263.339	10526.678			1					1		15	
	7-3	2	0.5	1	5559.638	11119.276	10835.536	21671.072	1	1630418	1.33	21280	0.0259	1	0.008	15	0.1021
			0.5	2	5275.898	10551.796			1					1		15	
	8-1	2	0.5	1	5163.918	10327.836	10143.384	20286.768	1	1630418	1.24	19895	0.0243	1	0.008	15	0.0955
			0.5	2	4979.466	9958.932			1					1		15	
	8-2	2	0.5	1	5568.239	11136.478	11039.79	22077.58	1	1630418	1.35	21686	0.0264	1	0.008	15	0.1041
			0.5	2	5470.551	10941.102			1					1		15	
	8-3	2	0.5	1	5617.083	11234.166	11530.453	23060.906	1	1630418	1.41	22670	0.0276	1	0.008	15	0.1088
			0.5	2	5913.37	11826.74			1					1		15	

Assay Date	9/13/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	placental	Microsome ID BAA		Technician ID TM	Replicate # 3

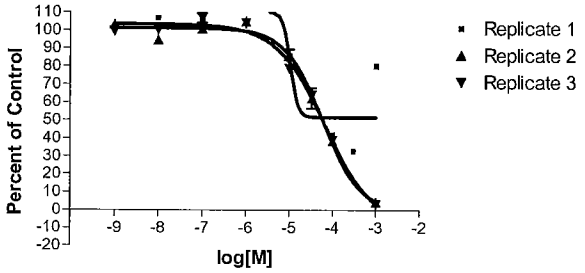
Control Type	Portion	Average	SD
Full activity	Beginning	0.1119	0.0065
Full activity	End	0.1060	0.0022
Full activity	Overall	0.1090	0.0052
Background	Beginning	0.0001	0.000222204
Background	End	-0.0001	6.06583E-05
Background	Overall	0.0000	0.000199584
Positive	Beginning	0.0439	0.0002
Positive	End	0.0426	0.0024
Positive	Overall	0.0432	0.0016
Negative	Beginning	0.1031	0.0025
Negative	End	0.0950	0.0069
Negative	Overall	0.0990	0.0063

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#10 ATZ	1	1	1.00E-04	-4.00	0.0990
RC#10 ATZ	1	2	1.00E-04	-4.00	0.1047
RC#10 ATZ	1	3	1.00E-04	-4.00	0.0969
RC#10 ATZ	2	1	3.30E-05	-4.48	0.1040
RC#10 ATZ	2	2	3.30E-05	-4.48	0.0992
RC#10 ATZ	2	3	3.30E-05	-4.48	0.0982
RC#10 ATZ	3	1	1.00E-05	-5.00	0.1064
RC#10 ATZ	3	2	1.00E-05	-5.00	0.1057
RC#10 ATZ	3	3	1.00E-05	-5.00	0.1023
RC#10 ATZ	4	1	1.00E-06	-6.00	0.1020
RC#10 ATZ	4	2	1.00E-06	-6.00	0.1031
RC#10 ATZ	4	3	1.00E-06	-6.00	0.1041
RC#10 ATZ	5	1	1.00E-07	-7.00	0.1029
RC#10 ATZ	5	2	1.00E-07	-7.00	0.1041
RC#10 ATZ	5	3	1.00E-07	-7.00	0.1058
RC#10 ATZ	6	1	1.00E-08	-8.00	0.1013
RC#10 ATZ	6	2	1.00E-08	-8.00	0.1068
RC#10 ATZ	6	3	1.00E-08	-8.00	0.1040
RC#10 ATZ	7	1	1.00E-09	-9.00	0.1006
RC#10 ATZ	7	2	1.00E-09	-9.00	0.1011
RC#10 ATZ	7	3	1.00E-09	-9.00	0.1021
RC#10 ATZ	8	1	1.00E-10	-10.00	0.0955
RC#10 ATZ	8	2	1.00E-10	-10.00	0.1041
RC#10 ATZ	8	3	1.00E-10	-10.00	0.1088

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	90.87	96.09	88.91
2	-4.48	95.41	91.03	90.09
3	-5.00	97.68	97.05	93.92
4	-6.00	93.64	94.62	95.54
5	-7.00	94.45	95.57	97.07
6	-8.00	92.99	98.06	95.43
7	-9.00	92.32	92.75	93.72
8	-10.00	87.62	95.51	99.84

Appendix 5: Prism Output for Task 7

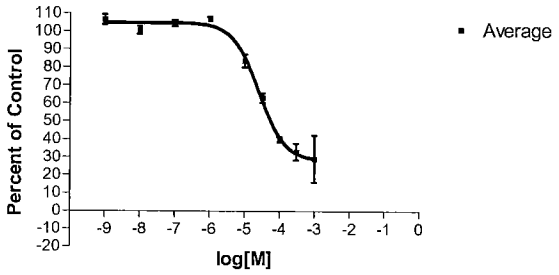
IVT 4-16 Task 7 Aminoglutethimide Replicate 1, 2 and 3



log[Aminoglutethimide]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.00	87.10	93.61	59.51	2.83	5.52	4.55	2.18	1.65	5.29
-3.52	27.71	42.16	29.16						
-4.00	42.05	49.30	34.63	40.75	37.58	37.80	40.06	37.39	39.11
-4.48				59.96	53.86	72.87	64.90	65.29	61.44
-5.00	63.90	99.62	94.08	87.50	80.80	90.91	80.30	77.60	77.89
-6.00	111.55	109.74	112.52	103.21	106.03	105.93	105.66	104.42	101.63
-7.00	113.39	100.88	103.42	103.86	99.13	99.54	111.61	106.39	102.50
-8.00	99.67	113.77	106.62	96.58	92.65	94.71	102.37	101.62	97.01
-9.00	115.45	110.57	123.88	106.74	101.78	101.12	98.26	101.32	98.98

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	51.69	-1.148	-9.120
TOP	110.1	101.3	103.3
LOGEC50	-4.971	-4.228	-4.170
HILLSLOPE	-5.155	-0.9889	-0.7764
EC50	1.069e-005	5.910e-005	6.763e-005
Std. Error			
BOTTOM	6.749	5.055	6.095
TOP	5.128	1.618	1.371
LOGEC50	38.00	0.06845	0.08325
HILLSLOPE	6527	0.1367	0.09117
95% Confidence Intervals			
BOTTOM	37.61 to 65.77	-11.69 to 9.397	-21.84 to 3.595
TOP	99.42 to 120.8	97.89 to 104.6	100.4 to 106.2
LOGEC50	-84.24 to 74.30	-4.371 to -4.086	-4.343 to -3.996
HILLSLOPE	-13620 to 13611	-1.274 to -0.7037	-0.9666 to -0.5862
EC50		4.254e-005 to 8.211e-005	4.534e-005 to 0.0001009
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.7493	0.9805	0.9884
Absolute Sum of Squares	5876	554.6	338.5
Sy.x	17.14	5.266	4.114
Data			
Number of X values	9	9	9
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	3	3	3

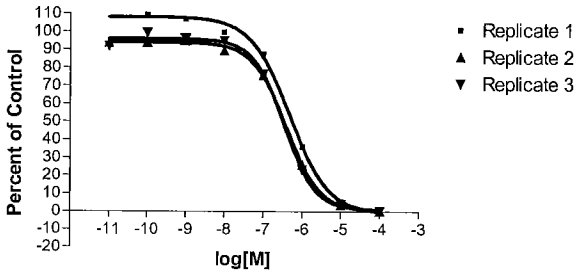
IVT 4-16 Task 7 Aminoglutethimide Replicate 1, 2 and 3



log[Aminoglutethimide]	Average								
	Y	Y	Y	Y	Y	Y	Y	Y	Y
-3.00	87.10	93.61	59.51	2.83	5.52	4.55	2.18	1.65	5.29
-3.52	27.71	42.16	29.16						
-4.00	42.05	49.30	34.63	40.75	37.58	37.80	40.06	37.39	39.11
-4.48				59.96	53.86	72.87	64.90	65.29	61.44
-5.00	63.90	99.62	94.08	87.50	80.80	90.91	80.30	77.60	77.89
-6.00	111.55	109.74	112.52	103.21	106.03	105.93	105.66	104.42	101.63
-7.00	113.39	100.88	103.42	103.86	99.13	99.54	111.61	106.39	102.50
-8.00	99.67	113.77	106.62	96.58	92.65	94.71	102.37	101.62	97.01

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	28.03
TOP	104.8
LOGEC50	-4.595
HILLSLOPE	-1.166
EC50	2.543e-005
Std. Error	
BOTTOM	5.457
TOP	2.656
LOGEC50	0.1117
HILLSLOPE	0.3026
95% Confidence Intervals	
BOTTOM	17.13 to 38.93
TOP	99.50 to 110.1
LOGEC50	-4.818 to -4.372
HILLSLOPE	-1.770 to -0.5615
EC50	1.522e-005 to 4.250e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.8146
Absolute Sum of Squares	15370
Sy.x	15.03
Data	
Number of X values	9
Number of Y replicates	9
Total number of values	72
Number of missing values	9

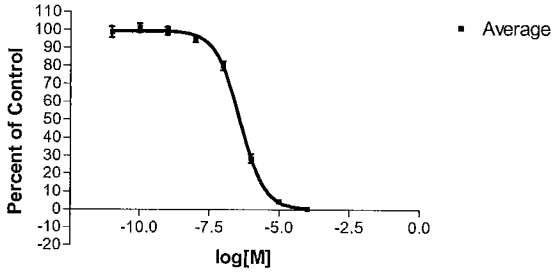
IVT 4-16 Task 7 Ketoconazole Replicate 1, 2 and 3



log[Ketoconazole]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	-1.45	-0.38	2.35	0.94	0.02	-0.20	0.41	0.64	-0.48
-5.00	3.69	4.51	9.35	4.27	2.78	3.97	2.94	4.71	3.81
-6.00	36.81	43.59	27.93	26.07	25.18	27.01	18.75	24.28	26.93
-7.00	90.24	94.35	78.78	72.77	77.77	77.03	77.23	73.51	78.63
-8.00	99.93	93.45	106.01	86.92	92.00	90.27	93.89	94.30	96.44
-9.00	102.55	110.52	107.81	95.43	95.70	95.02	97.26	99.52	90.09
-10.00	110.67	112.33	105.23	92.69	93.59	95.74	102.50	99.52	96.04
-11.00	108.68	109.50	112.90	97.15	91.64	93.17	91.89	92.22	91.60

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-1.234	0.01524	0.7018
TOP	108.0	94.00	95.93
LOGEC50	-6.315	-6.404	-6.460
HILLSLOPE	-0.8716	-1.014	-1.097
EC50	4.847e-007	3.946e-007	3.471e-007
Std. Error			
BOTTOM	2.880	0.9471	1.524
TOP	1.647	0.5999	1.002
LOGEC50	0.06309	0.02468	0.03977
HILLSLOPE	0.09383	0.04492	0.07879
95% Confidence Intervals			
BOTTOM	-7.241 to 4.774	-1.960 to 1.991	-2.478 to 3.882
TOP	104.6 to 111.5	92.75 to 95.25	93.84 to 98.02
LOGEC50	-6.446 to -6.183	-6.455 to -6.352	-6.542 to -6.377
HILLSLOPE	-1.067 to -0.6759	-1.108 to -0.9202	-1.262 to -0.9330
EC50	3.580e-007 to 6.562e-007	3.505e-007 to 4.443e-007	2.868e-007 to 4.202e-007
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9890	0.9980	0.9947
Absolute Sum of Squares	529.0	74.73	214.1
Sy.x	5.143	1.933	3.272
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

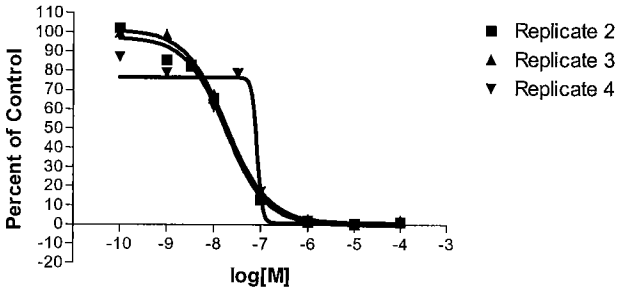
IVT 4-16 Task 7 Ketoconazole Replicate 1, 2 and 3



log[Ketoconazole]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	-1.45	-0.38	2.35	0.94	0.02	-0.20	0.41	0.64	-0.48
-5.00	3.69	4.51	9.35	4.27	2.78	3.97	2.94	4.71	3.81
-6.00	36.81	43.59	27.93	26.07	25.18	27.01	18.75	24.28	26.93
-7.00	90.24	94.35	78.78	72.77	77.77	77.03	77.23	73.51	78.63
-8.00	99.93	93.45	106.01	86.92	92.00	90.27	93.89	94.30	96.44
-9.00	102.55	110.52	107.81	95.43	95.70	95.02	97.26	99.52	90.09
-10.00	110.67	112.33	105.23	92.69	93.59	95.74	102.50	99.52	96.04
-11.00	108.68	109.50	112.90	97.15	91.64	93.17	91.89	92.22	91.60

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.01102
TOP	99.27
LOGEC50	-6.392
HILLSLOPE	-0.9880
EC50	4.055e-007
Std. Error	
BOTTOM	1.789
TOP	1.119
LOGEC50	0.04404
HILLSLOPE	0.07760
95% Confidence Intervals	
BOTTOM	-3.563 to 3.585
TOP	97.03 to 101.5
LOGEC50	-6.480 to -6.304
HILLSLOPE	-1.143 to -0.8330
EC50	3.312e-007 to 4.966e-007
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9794
Absolute Sum of Squares	2624
Sy.x	6.212
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

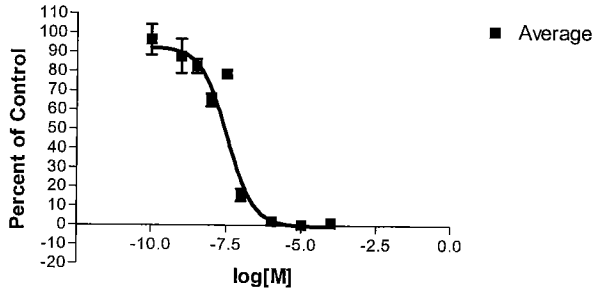
IVT 4-16 Task 7 Prochloraz Replicates 2, 3 and 4



log[Prochloraz]	Replicate 2			Replicate 3			Replicate 4		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	1.24	1.18	0.84	1.27	1.64	3.97	0.31	-0.05	-0.18
-5.00	-0.34	0.35	0.04	0.79	-0.75	0.18	0.37	0.23	0.51
-6.00	1.24	1.45	1.28	3.43	2.39	2.28	2.08	2.26	2.23
-7.00	10.66	10.26	18.23	15.92	17.44	17.49	17.86	15.34	17.96
-7.48							77.92	79.65	77.95
-8.00	64.24	67.08	65.84	66.94	69.24	67.93	59.72	60.72	63.47
-8.48	79.45	88.08	81.35	86.19	81.49	79.31			
-9.00	87.15	86.18	83.27	98.85	99.53	98.08	78.27	80.69	78.01
-10.00	105.53	97.95	102.73	102.30	98.89	98.19	91.12	90.22	80.35

	Replicate 2	Replicate 3	Replicate 4
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-0.2111	0.8594	0.8676
TOP	97.38	101.2	76.52
LOGEC50	-7.730	-7.720	-7.071
HILLSLOPE	-1.005	-0.9771	-7.924
EC50	1.862e-008	1.905e-008	8.484e-008
Std. Error			
BOTTOM	1.579	0.9272	2.547
TOP	2.255	1.332	2.547
LOGEC50	0.05304	0.03066	0.8557
HILLSLOPE	0.09643	0.05272	91.87
95% Confidence Intervals			
BOTTOM	-3.504 to 3.082	-1.075 to 2.794	-4.445 to 6.180
TOP	92.68 to 102.1	98.44 to 104.0	71.21 to 81.83
LOGEC50	-7.841 to -7.619	-7.784 to -7.656	-8.856 to -5.286
HILLSLOPE	-1.206 to -0.8034	-1.087 to -0.8672	-199.6 to 183.7
EC50	1.443e-008 to 2.402e-008	1.644e-008 to 2.207e-008	1.392e-009 to 5.172e-006
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9907	0.9970	0.9641
Absolute Sum of Squares	382.4	129.5	1167
Sy.x	4.373	2.545	7.640
Data			
Number of X values	9	9	9
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	3	3	3

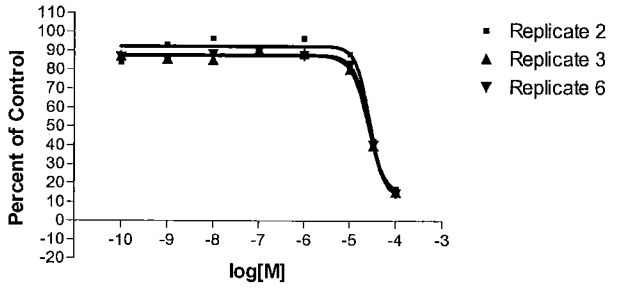
IVT 4-16 Task 7 Prochloraz Replicates 2, 3 and 4



log[Prochloraz]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	1.24	1.18	0.84	1.27	1.64	3.97	0.31	-0.05	-0.18
-5.00	-0.34	0.35	0.04	0.79	-0.75	0.18	0.37	0.23	0.51
-6.00	1.24	1.45	1.28	3.43	2.39	2.28	2.08	2.26	2.23
-7.00	10.66	10.26	18.23	15.92	17.44	17.49	17.86	15.34	17.96
-7.48							77.92	79.65	77.95
-8.00	64.24	67.08	65.84	66.94	69.24	67.93	59.72	60.72	63.47
-8.48	79.45	88.08	81.35	86.19	81.49	79.31			
-9.00	87.15	86.18	83.27	98.85	99.53	98.08	78.27	80.69	78.01

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.6911
TOP	92.54
LOGEC50	-7.468
HILLSLOPE	-0.9893
EC50	3.408e-008
Std. Error	
BOTTOM	1.942
TOP	2.477
LOGEC50	0.06196
HILLSLOPE	0.1154
95% Confidence Intervals	
BOTTOM	-4.571 to 3.189
TOP	87.59 to 97.48
LOGEC50	-7.591 to -7.344
HILLSLOPE	-1.220 to -0.7587
EC50	2.563e-008 to 4.531e-008
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9528
Absolute Sum of Squares	5509
Sy.x	9.001
Data	
Number of X values	9
Number of Y replicates	9
Total number of values	72
Number of missing values	9

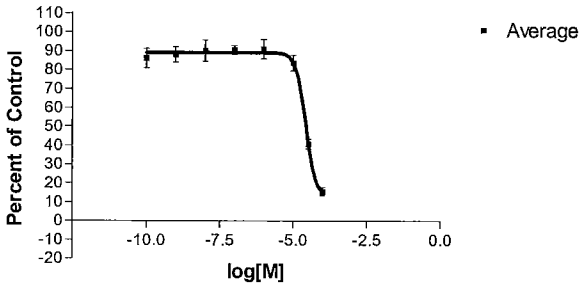
IVT 4-16 Task 7 4-Nonylphenol Replicate 2, 3 and 6



log[4-Nonylphenol]	Replicate 2			Replicate 3			Replicate 6		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	18.29	15.24	17.50	17.67	14.54	13.42	17.42	13.70	12.82
-4.48	41.28	40.09	45.70	41.26	38.96	39.43	43.74	38.90	38.27
-5.00	88.19	91.56	85.31	81.68	81.90	77.70	84.21	82.07	80.87
-6.00	98.95	97.56	93.34	85.37	87.75	92.85	84.59	89.03	89.04
-7.00	92.64	93.70	87.44	91.04	88.61	91.92	92.09	90.81	87.33
-8.00	95.49	100.33	94.44	87.77	85.53	82.78	90.08	88.18	86.26
-9.00	93.62	93.80	91.94	83.35	86.84	87.24	85.37	86.10	85.38
-10.00	82.40	93.78	76.37	89.26	87.37	85.68	83.57	91.16	85.48

	Replicate 2	Replicate 3	Replicate 6
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	15.44	12.03	12.20
TOP	92.38	87.56	87.63
LOGEC50	-4.572	-4.580	-4.567
HILLSLOPE	-2.939	-2.340	-2.601
EC50	2.682e-005	2.631e-005	2.710e-005
Std. Error			
BOTTOM	3.807	2.236	2.024
TOP	1.389	0.6874	0.6671
LOGEC50	0.03478	0.02110	0.01807
HILLSLOPE	0.7787	0.2442	0.2992
95% Confidence Intervals			
BOTTOM	7.494 to 23.38	7.364 to 16.69	7.979 to 16.42
TOP	89.49 to 95.28	86.13 to 89.00	86.24 to 89.03
LOGEC50	-4.644 to -4.499	-4.624 to -4.536	-4.605 to -4.529
HILLSLOPE	-4.564 to -1.315	-2.849 to -1.830	-3.225 to -1.976
EC50	2.269e-005 to 3.169e-005	2.378e-005 to 2.912e-005	2.485e-005 to 2.956e-005
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9694	0.9916	0.9922
Absolute Sum of Squares	578.1	140.9	133.1
Sy.x	5.376	2.655	2.580
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

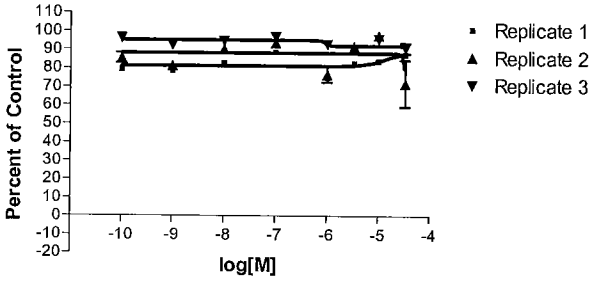
IVT 4-16 Task 7 4-Nonylphenol Replicate 2, 3 and 6



log[4-Nonylphenol]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	18.29	15.24	17.50	17.67	14.54	13.42	17.42	13.70	12.82
-4.48	41.28	40.09	45.70	41.26	38.96	39.43	43.74	38.90	38.27
-5.00	88.19	91.56	85.31	81.68	81.90	77.70	84.21	82.07	80.87
-6.00	98.95	97.56	93.34	85.37	87.75	92.85	84.59	89.03	89.04
-7.00	92.64	93.70	87.44	91.04	88.61	91.92	92.09	90.81	87.33
-8.00	95.49	100.33	94.44	87.77	85.53	82.78	90.08	88.18	86.26
-9.00	93.62	93.80	91.94	83.35	86.84	87.24	85.37	86.10	85.38
-10.00	82.40	93.78	76.37	89.26	87.37	85.68	83.57	91.16	85.48

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	13.27
TOP	89.19
LOGEC50	-4.574
HILLSLOPE	-2.606
EC50	2.669e-005
Std. Error	
BOTTOM	1.873
TOP	0.6282
LOGEC50	0.01704
HILLSLOPE	0.2727
95% Confidence Intervals	
BOTTOM	9.527 to 17.01
TOP	87.94 to 90.45
LOGEC50	-4.608 to -4.540
HILLSLOPE	-3.150 to -2.061
EC50	2.468e-005 to 2.887e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9773
Absolute Sum of Squares	1204
Sy.x	4.208
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

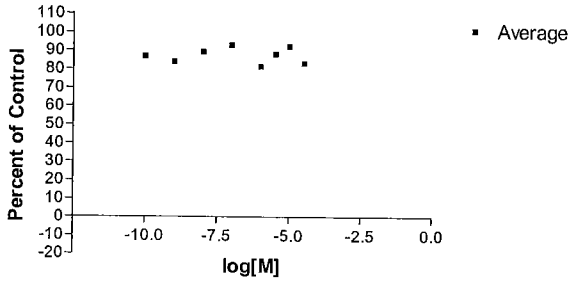
IVT 4-16 Task 7 dibenz[a,h]anthracene Replicates 1, 2 and 3



log[dibenz[a,h]anthracene]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.48	76.51	95.36	91.15	68.76	51.57	95.18	85.31	92.87	94.41
-5.00	85.73	86.77	78.92	98.73	97.43	97.30	96.49	96.75	94.07
-5.48	87.23	82.60	78.24	96.42	90.46	88.83	90.00	93.64	87.08
-6.00	68.41	64.99	91.84	82.64	79.36	68.34	88.02	95.07	95.60
-7.00	85.51	88.03	92.46	93.84	94.16	93.00	95.29	98.68	96.46
-8.00	85.74	85.01	78.16	88.40	93.36	89.28	97.89	92.91	93.25
-9.00	79.10	75.25	81.74	79.73	84.54	79.57	90.33	93.69	93.59
-10.00	77.42	80.12	80.75	89.21	87.41	80.27	99.29	94.19	95.26

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	81.16	-501.9	92.29
TOP	88.47	88.20	95.07
LOGEC50	-4.901	-4.385	-6.040
HILLSLOPE	2.133	-16.33	-13.99
EC50	1.255e-005	4.117e-005	9.127e-007
Std. Error			
BOTTOM	1.991	2.752e+010	1.415
TOP	16.60	2.519	1.001
LOGEC50	1.479	809920	137292
HILLSLOPE	12.32	1.118e+008	4.825e+007
95% Confidence Intervals			
BOTTOM	77.01 to 85.32	-57400000000 to 5.740e+010	89.34 to 95.24
TOP	53.85 to 123.1	82.95 to 93.46	92.98 to 97.16
LOGEC50	-7.986 to -1.817	-1689000 to 1.689e+006	-286400 to 286385
HILLSLOPE	-23.57 to 27.83	-233200000 to 2.332e+008	-100700000 to 1.007e+008
EC50	1.033e-008 to 0.01524		
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.09373	0.2453	0.1494
Absolute Sum of Squares	1109	2163	240.4
Sy.x	7.447	10.40	3.467
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

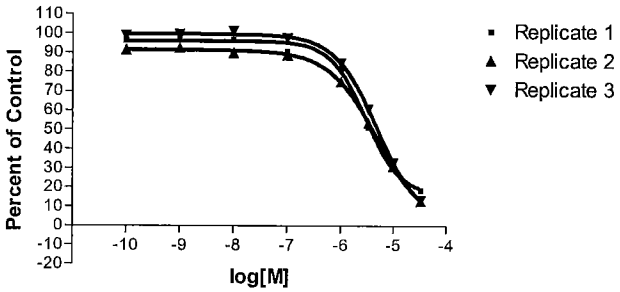
IVT 4-16 Task 7 dibenz[a,h]anthracene Replicates 1, 2 and 3



log[dibenz[a,h]anthracene]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.48	76.51	95.36	91.15	68.76	51.57	95.18	85.31	92.87	94.41
-5.00	85.73	86.77	78.92	98.73	97.43	97.30	96.49	96.75	94.07
-5.48	87.23	82.60	78.24	96.42	90.46	88.83	90.00	93.64	87.08
-6.00	68.41	64.99	91.84	82.64	79.36	68.34	88.02	95.07	95.60
-7.00	85.51	88.03	92.46	93.84	94.16	93.00	95.29	98.68	96.46
-8.00	85.74	85.01	78.16	88.40	93.36	89.28	97.89	92.91	93.25
-9.00	79.10	75.25	81.74	79.73	84.54	79.57	90.33	93.69	93.59
-10.00	77.42	80.12	80.75	89.21	87.41	80.27	99.29	94.19	95.26

	Average
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	

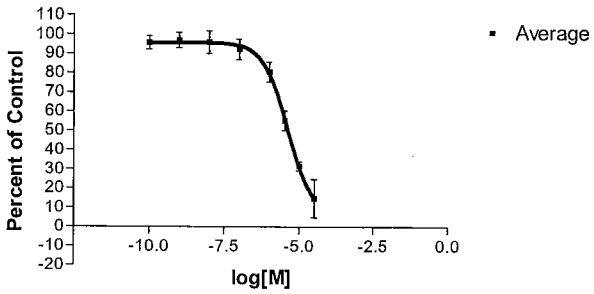
IVT 4-16 Task 7 Fenarimol Replicates 1, 2 and 3



Log[Fenarimol]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.48	3.91	11.54	40.20	13.53	12.39	12.68	12.50	14.69	12.26
-5.00	29.24	28.24	34.78	30.66	31.35	31.21	30.61	32.19	35.33
-5.48	51.73	50.61	51.45	50.09	57.38	53.41	63.04	62.88	56.53
-6.00	85.14	82.62	78.13	75.37	71.64	77.77	87.74	83.69	82.03
-7.00	90.76	91.59	88.97	91.90	90.28	84.13	103.54	93.42	95.63
-8.00	98.35	98.23	93.19	83.79	93.67	92.38	102.14	101.85	99.28
-9.00	101.21	97.52	97.29	94.52	92.51	90.70	100.53	95.71	101.99
-10.00	97.60	94.18	97.89	93.06	91.94	89.36	98.67	98.14	99.58

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	15.71	-0.3581	2.048
TOP	96.08	91.40	99.63
LOGEC50	-5.531	-5.307	-5.314
HILLSLOPE	-1.292	-0.9339	-1.072
EC50	2.946e-006	4.933e-006	4.855e-006
Std. Error			
BOTTOM	5.951	5.496	4.303
TOP	2.049	0.9069	0.8521
LOGEC50	0.08531	0.07228	0.04959
HILLSLOPE	0.2851	0.09861	0.09480
95% Confidence Intervals			
BOTTOM	3.300 to 28.13	-11.82 to 11.11	-6.927 to 11.02
TOP	91.80 to 100.4	89.51 to 93.29	97.85 to 101.4
LOGEC50	-5.709 to -5.353	-5.458 to -5.156	-5.417 to -5.210
HILLSLOPE	-1.886 to -0.6968	-1.140 to -0.7282	-1.270 to -0.8741
EC50	1.955e-006 to 4.438e-006	3.486e-006 to 6.981e-006	3.826e-006 to 6.160e-006
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9591	0.9920	0.9938
Absolute Sum of Squares	932.2	162.2	155.2
Sy.x	6.827	2.847	2.785
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

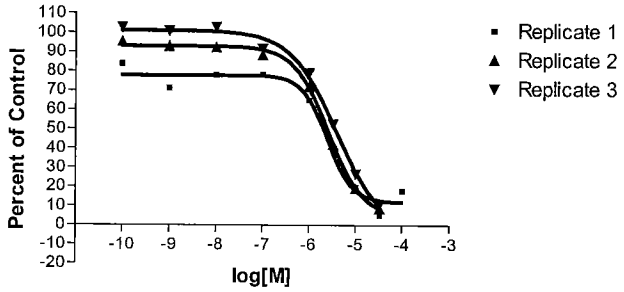
IVT 4-16 Task 7 Fenarimol Replicates 1, 2 and 3



Log[Fenarimol]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.48	3.91	11.54	40.20	13.53	12.39	12.68	12.50	14.69	12.26
-5.00	29.24	28.24	34.78	30.66	31.35	31.21	30.61	32.19	35.33
-5.48	51.73	50.61	51.45	50.09	57.38	53.41	63.04	62.88	56.53
-6.00	85.14	82.62	78.13	75.37	71.64	77.77	87.74	83.69	82.03
-7.00	90.76	91.59	88.97	91.90	90.28	84.13	103.54	93.42	95.63
-8.00	98.35	98.23	93.19	83.79	93.67	92.38	102.14	101.85	99.28
-9.00	101.21	97.52	97.29	94.52	92.51	90.70	100.53	95.71	101.99
-10.00	97.60	94.18	97.89	93.06	91.94	89.36	98.67	98.14	99.58

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	6.360
TOP	95.74
LOGEC50	-5.390
HILLSLOPE	-1.069
EC50	4.075e-006
Std. Error	
BOTTOM	4.333
TOP	0.9721
LOGEC50	0.05595
HILLSLOPE	0.1130
95% Confidence Intervals	
BOTTOM	-2.295 to 15.01
TOP	93.80 to 97.68
LOGEC50	-5.502 to -5.278
HILLSLOPE	-1.295 to -0.8431
EC50	3.151e-006 to 5.271e-006
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9704
Absolute Sum of Squares	2029
Sy.x	5.463
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

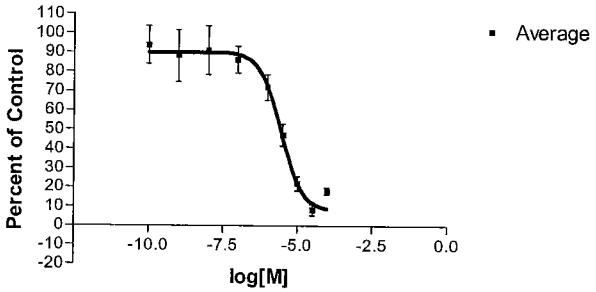
IVT 4-16 Task 7 Chrysin Replicates 1, 2 and 3



log[Chrysin]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	18.80	18.92	15.98						
-4.48	2.55	6.74	6.62	10.54	7.38	8.14	11.30	10.15	10.29
-5.00	18.91	20.93	19.70	20.74	19.74	17.72	25.00	25.78	28.98
-5.48				41.46	42.88	41.84	52.68	52.35	52.09
-6.00	66.24	69.72	58.94	73.57	70.70	71.84	78.55	79.41	78.91
-7.00	81.75	79.26	73.99	88.81	89.95	86.40	97.47	87.37	89.95
-8.00	87.18	84.22	62.87	90.77	93.34	92.88	102.22	103.69	102.23
-9.00	72.33	71.81	69.45	93.20	92.87	93.07	103.50	99.94	99.09

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	11.78	4.150	-4.555
TOP	77.89	92.93	101.0
LOGEC50	-5.607	-5.582	-5.401
HILLSLOPE	-1.599	-1.167	-0.8720
EC50	2.473e-006	2.617e-006	3.969e-006
Std. Error			
BOTTOM	3.548	1.762	7.843
TOP	2.138	0.5786	1.411
LOGEC50	0.1347	0.02391	0.09502
HILLSLOPE	0.4607	0.06700	0.1198
95% Confidence Intervals			
BOTTOM	4.377 to 19.18	0.4733 to 7.826	-20.92 to 11.80
TOP	73.43 to 82.35	91.72 to 94.13	98.09 to 104.0
LOGEC50	-5.888 to -5.326	-5.632 to -5.532	-5.600 to -5.203
HILLSLOPE	-2.560 to -0.6383	-1.307 to -1.027	-1.122 to -0.6221
EC50	1.295e-006 to 4.722e-006	2.333e-006 to 2.936e-006	2.514e-006 to 6.264e-006
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9527	0.9973	0.9871
Absolute Sum of Squares	1060	70.82	364.4
Sy.x	7.280	1.882	4.269
Data			
Number of X values	9	9	9
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	3	3	3

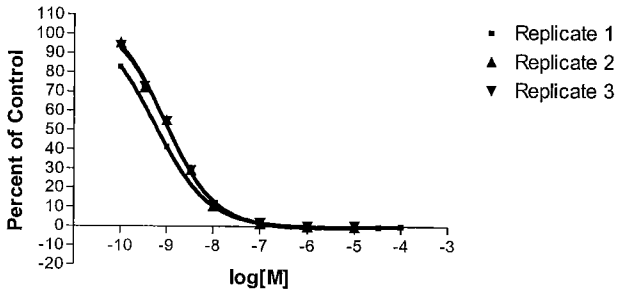
IVT 4-16 Task 7 Chrysin Replicates 1, 2 and 3



log[Chrysin]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	18.80	18.92	15.98						
-4.48	2.55	6.74	6.62	10.54	7.38	8.14	11.30	10.15	10.29
-5.00	18.91	20.93	19.70	20.74	19.74	17.72	25.00	25.78	28.98
-5.48				41.46	42.88	41.84	52.68	52.35	52.09
-6.00	66.24	69.72	58.94	73.57	70.70	71.84	78.55	79.41	78.91
-7.00	81.75	79.26	73.99	88.81	89.95	86.40	97.47	87.37	89.95
-8.00	87.18	84.22	62.87	90.77	93.34	92.88	102.22	103.69	102.23
-9.00	72.33	71.81	69.45	93.20	92.87	93.07	103.50	99.94	99.09

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	7.774
TOP	90.17
LOGEC50	-5.542
HILLSLOPE	-1.238
EC50	2.873e-006
Std. Error	
BOTTOM	3.699
TOP	1.518
LOGEC50	0.06280
HILLSLOPE	0.1841
95% Confidence Intervals	
BOTTOM	0.3861 to 15.16
TOP	87.14 to 93.21
LOGEC50	-5.667 to -5.416
HILLSLOPE	-1.606 to -0.8704
EC50	2.152e-006 to 3.834e-006
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9365
Absolute Sum of Squares	5165
Sy.x	8.715
Data	
Number of X values	9
Number of Y replicates	9
Total number of values	72
Number of missing values	9

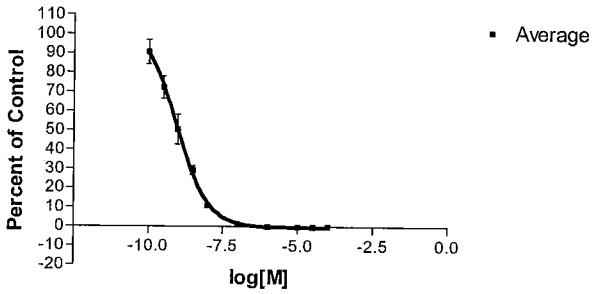
IVT 4-16 Task 7 Econazole Replicates 1, 2 and 3



log[Econazole]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	-0.43	-0.24	0.79						
-4.48	0.08	-0.86	-0.27						
-5.00	-0.51	-0.54	-0.45	0.08	-0.67	-0.28	0.65	-0.04	-0.17
-6.00	-0.46	-0.40	0.01	0.25	-0.27	-0.46	-0.11	-0.04	0.14
-7.00	0.56	1.50	1.19	1.58	1.55	1.04	0.94	2.49	2.46
-8.00	11.83	9.69	11.20	10.84	9.48	11.02	9.98	10.11	12.83
-8.48				28.07	29.21	30.57	30.75	25.17	31.10
-9.00	44.84	46.10	33.19	54.56	55.62	55.30	56.83	55.42	51.66

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-0.4892	-1.033	-0.4977
TOP	105.7	109.9	106.7
LOGEC50	-9.247	-9.039	-9.011
HILLSLOPE	-0.7479	-0.8011	-0.8255
EC50	5.659e-010	9.141e-010	9.751e-010
Std. Error			
BOTTOM	0.7689	1.143	1.215
TOP	9.106	5.606	5.528
LOGEC50	0.1288	0.07001	0.07082
HILLSLOPE	0.09158	0.06886	0.07624
95% Confidence Intervals			
BOTTOM	-2.093 to 1.115	-3.417 to 1.351	-3.031 to 2.036
TOP	86.66 to 124.7	98.21 to 121.6	95.13 to 118.2
LOGEC50	-9.516 to -8.979	-9.185 to -8.893	-9.159 to -8.863
HILLSLOPE	-0.9389 to -0.5568	-0.9447 to -0.6574	-0.9845 to -0.6665
EC50	3.048e-010 to 1.050e-009	6.531e-010 to 1.279e-009	6.940e-010 to 1.370e-009
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9928	0.9938	0.9926
Absolute Sum of Squares	139.7	181.2	209.4
Sy.x	2.643	3.010	3.236
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

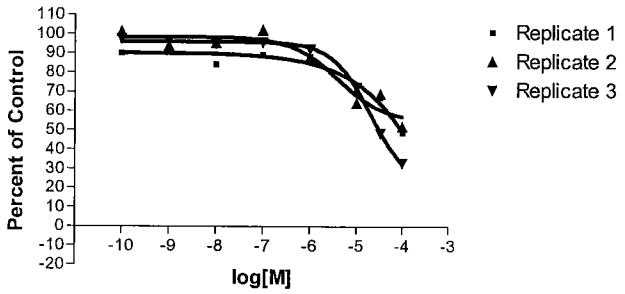
IVT 4-16 Task 7 Econazole Replicates 1, 2 and 3



log[Econazole]	Average								
	Y	X	S	W	5	8	Y	8	9
-4.00	-0.43	-0.24	0.79						
-4.48	0.08	-0.86	-0.27						
-5.00	-0.51	-0.54	-0.45	0.08	-0.67	-0.28	0.65	-0.04	-0.17
-6.00	-0.46	-0.40	0.01	0.25	-0.27	-0.46	-0.11	-0.04	0.14
-7.00	0.56	1.50	1.19	1.58	1.55	1.04	0.94	2.49	2.46
-8.00	11.83	9.69	11.20	10.84	9.48	11.02	9.98	10.11	12.83
-8.48				28.07	29.21	30.57	30.75	25.17	31.10
-9.00	44.84	46.10	33.19	54.56	55.62	55.30	56.83	55.42	51.66

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.5405
TOP	104.5
LOEC50	-9.029
HILLSLOPE	-0.8285
EC50	9.364e-010
Std. Error	
BOTTOM	0.7662
TOP	4.042
LOEC50	0.05372
HILLSLOPE	0.05798
95% Confidence Intervals	
BOTTOM	-2.071 to 0.9898
TOP	96.43 to 112.6
LOEC50	-9.136 to -8.921
HILLSLOPE	-0.9444 to -0.7127
EC50	7.314e-010 to 1.199e-009
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9866
Absolute Sum of Squares	1078
Sy.x	3.982
Data	
Number of X values	10
Number of Y replicates	9
Total number of values	72
Number of missing values	18

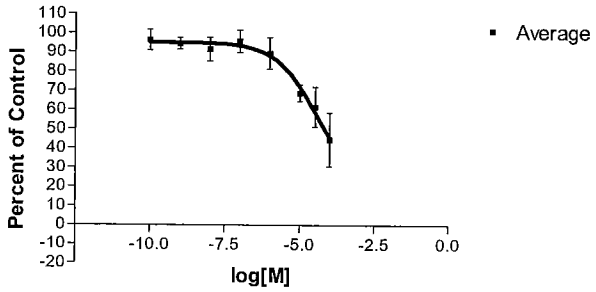
IVT 4-16 Task 7 Dicofol Replicates 1, 2 and 3



log[Dicofol]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	40.90	49.57	55.66	30.85	67.50	58.22	25.61	33.49	39.64
-4.48	70.36	66.71	63.66	70.71	72.09	64.56	49.88	52.21	43.47
-5.00	68.58	73.41	67.52	68.17	63.69	61.17	71.46	73.83	71.44
-6.00	87.96	89.84	86.92	70.27	97.25	96.30	89.80	89.78	96.65
-7.00	91.79	90.41	86.07	104.15	102.72	99.33	95.82	94.14	96.59
-8.00	89.25	82.66	80.99	97.75	97.07	91.07	96.31	92.87	95.90
-9.00	91.72	92.62	94.99	93.76	89.99	98.14	98.13	93.14	97.93
-10.00	90.48	90.70	89.43	102.54	103.11	98.70	99.92	94.48	97.76

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-99.52	54.76	20.56
TOP	90.29	98.46	96.03
LOGEC50	-2.830	-5.402	-4.690
HILLSLOPE	-0.4795	-0.8759	-1.038
EC50	0.001478	3.966e-006	2.042e-005
Std. Error			
BOTTOM	649.7	9.467	7.940
TOP	1.926	3.144	0.9745
LOGEC50	4.586	0.3843	0.1110
HILLSLOPE	0.2868	0.4966	0.1930
95% Confidence Intervals			
BOTTOM	-1455 to 1256	35.01 to 74.50	3.994 to 37.12
TOP	86.27 to 94.30	91.90 to 105.0	94.00 to 98.06
LOGEC50	-12.40 to 6.736	-6.203 to -4.600	-4.921 to -4.458
HILLSLOPE	-1.078 to 0.1187	-1.912 to 0.1600	-1.441 to -0.6358
EC50	4.008e-013 to 5.447e+006	6.261e-007 to 2.513e-005	1.198e-005 to 3.480e-005
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9121	0.7878	0.9831
Absolute Sum of Squares	466.0	1868	228.9
Sy.x	4.827	9.666	3.383
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

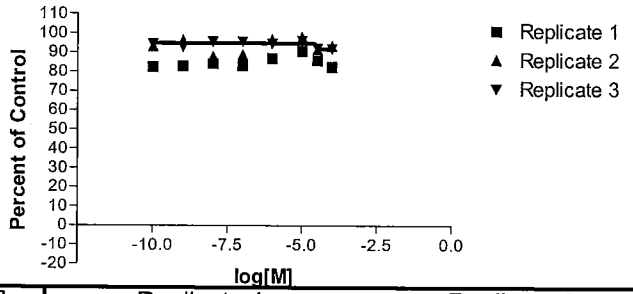
IVT 4-16 Task 7 Dicofol Replicates 1, 2 and 3



log[Dicofol]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	40.90	49.57	55.66	30.85	67.50	58.22	25.61	33.49	39.64
-4.48	70.36	66.71	63.66	70.71	72.09	64.56	49.88	52.21	43.47
-5.00	68.58	73.41	67.52	68.17	63.69	61.17	71.46	73.83	71.44
-6.00	87.96	89.84	86.92	70.27	97.25	96.30	89.80	89.78	96.65
-7.00	91.79	90.41	86.07	104.15	102.72	99.33	95.82	94.14	96.59
-8.00	89.25	82.66	80.99	97.75	97.07	91.07	96.31	92.87	95.90
-9.00	91.72	92.62	94.99	93.76	89.99	98.14	98.13	93.14	97.93
-10.00	90.48	90.70	89.43	102.54	103.11	98.70	99.92	94.48	97.76

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	18.42
TOP	95.15
LOGEC50	-4.417
HILLSLOPE	-0.6266
EC50	3.825e-005
Std. Error	
BOTTOM	30.41
TOP	1.613
LOGEC50	0.5848
HILLSLOPE	0.2132
95% Confidence Intervals	
BOTTOM	-42.32 to 79.16
TOP	91.93 to 98.37
LOGEC50	-5.585 to -3.249
HILLSLOPE	-1.052 to -0.2007
EC50	2.597e-006 to 0.0005633
Goodness of Fit	
Degrees of Freedom	68
R^2	0.8446
Absolute Sum of Squares	4345
Sy.x	7.994
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

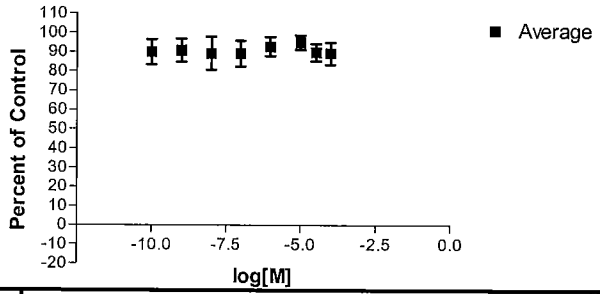
IVT 4-16 Task 7 Atrazine Replicates 1, 2 and 3



log[Atrazine]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	84.23	85.37	78.29	94.87	90.69	94.51	90.87	96.09	88.91
-4.48	78.76	87.76	91.02	91.94	91.26	91.24	95.41	91.03	90.09
-5.00	93.12	87.12	92.51	98.47	98.50	97.03	97.68	97.05	93.92
-6.00	90.33	84.14	86.48	95.36	99.46	95.66	93.64	94.62	95.54
-7.00	83.84	83.89	81.43	96.74	88.05	81.80	94.45	95.57	97.07
-8.00	88.81	80.93	83.02	92.49	98.13	73.32	92.99	98.06	95.43
-9.00	82.19	83.94	82.65	97.03	95.55	96.50	92.32	92.75	93.72
-10.00	81.93	82.45	82.79	94.06	92.26	93.37	87.62	95.51	99.84

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)	Does not converge.	Does not converge.	
Best-fit values			
BOTTOM			91.96
TOP			94.88
LOGEC50			-4.543
HILLSLOPE			-17.34
EC50			2.866e-005
Std. Error			
BOTTOM			1.678
TOP			0.7500
LOGEC50			156017
HILLSLOPE			4.293e+007
95% Confidence Intervals			
BOTTOM			88.46 to 95.46
TOP			93.31 to 96.44
LOGEC50			-325500 to 325447
HILLSLOPE			-89550000 to 8.955e+007
EC50			
Goodness of Fit			
Degrees of Freedom			20
R^2			0.1742
Absolute Sum of Squares			168.8
Sy.x			2.905
Data			
Number of X values			8
Number of Y replicates			3
Total number of values			24
Number of missing values			0

IVT 4-16 Task 7 Atrazine Replicates 1, 2 and 3



log[Atrazine]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	84.23	85.37	78.29	94.87	90.69	94.51	90.87	96.09	88.91
-4.48	78.76	87.76	91.02	91.94	91.26	91.24	95.41	91.03	90.09
-5.00	93.12	87.12	92.51	98.47	98.50	97.03	97.68	97.05	93.92
-6.00	90.33	84.14	86.48	95.36	99.46	95.66	93.64	94.62	95.54
-7.00	83.84	83.89	81.43	96.74	88.05	81.80	94.45	95.57	97.07
-8.00	88.81	80.93	83.02	92.49	98.13	73.32	92.99	98.06	95.43
-9.00	82.19	83.94	82.65	97.03	95.55	96.50	92.32	92.75	93.72
-10.00	81.93	82.45	82.79	94.06	92.26	93.37	87.62	95.51	99.84

	Average
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	

Appendix 6: Copy of Statistician's Report

DRAFT REPORT

On

PLACENTAL AROMATASE VALIDATION STUDY INTRALABORATORY STATISTICAL ANALYSIS OF IN VITRO TECHNOLOGIES DATA

CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-16, TASK 7

May 24, 2006

Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
ENDOCRINE DISRUPTOR SCREENING PROGRAM
WASHINGTON, D.C.

Prepared by

BATTELLE
505 King Avenue
Columbus, Ohio 43201

Placental Aromatase Validation Study

Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories
Intralaboratory Statistical Analysis of
In Vitro Technologies Data

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-16, TASK 7

Ying-Liang Chou, Author _____ Date _____

Paul I. Feder, Reviewer _____ Date _____

Offsite Quality Assurance Statement

Study Number:

This study was inspected by the Quality Assurance Unit and reports were submitted to the Study Director and Management as follows:

Phase Inspected	Inspection Date	Date Reported to Battelle Task Leader/ Battelle Management	Date Reported to Offsite Study Director/ Management
Audit study file	5/23/2006	5/23/2006	5/23/2006
Audit draft report	5/23/2006	5/23/2006	5/23/2006

Quality Assurance Unit **Date**

Hillary Flory 5 24 06

This report discusses the methods and results of the intralaboratory statistical analysis of the data collected at In Vitro Technologies with the placental aromatase assay for WA 4-16 Task 7: Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories.

Summary and Conclusions

Ten test substances were analyzed: 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). In Vitro Technologies (IVT) analyzed five chemicals with microsomes prepared at Battelle (prochloraz, dibenz[a,h]anthracene, fenarimol, econazole, chrysin) and five chemicals with microsomes prepared at IVT (aminoglutethimide, ketoconazole, 4-nonylphenol, dicofol, atrazine) and provided summary results from the concentration response curve fits from each chemical for statistical analysis. Three replicates were carried out for each chemical. For each chemical the statistical analyses were based on the data from all the replicates.

Two types of data were obtained: inhibition curve fit data and control activity data. Statistical analyses were carried out separately for these two types of data. The response variable was the "percent of control". Percent of control is defined as the ratio of the background adjusted aromatase activity in the tube under consideration to the average background adjusted aromatase activity among the four full enzyme activity control tubes within the replicate, times 100. Control activity data consists of four types of controls: full enzyme activity control, background activity control, positive control (4-OH ASDN), and negative control (lindane). Statistical analyses were performed separately for these four types of controls.

For the inhibition curve fit data separate analyses were carried out for each of the ten test substances. For each test substance concentration response curves were fitted within each replicate to describe the relation between test substance concentrations and extent of inhibition. The concentration response curves were summarized by four parameters: the baseline response (bottom), the maximum response (top), the concentration that corresponds to a response halfway between baseline and maximum (IC_{50}), and the slope. Results were compared across replicates within test substances. 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, differences among test substances, and differences among microsome sources.

The following results were observed:

1. Test substances V (dibenz[a,h]anthracene) and X (atrazine) are non-inhibitors. With only a few exceptions, the majority of concentration responses were close to or above 80% of control.

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

Chemical I (aminoglutethimide): The lower end of the concentration response curve for replicate 1 did not exhibit a threshold. This resulted in inflation in the estimate and variability of the average bottom parameter.

Chemical II (ketoconazole): The top threshold from replicate 1 differed somewhat from those of the other replicates. This inflated the variability of the average top threshold.

Chemical III (prochloraz): The concentration response curve for replicate 4 was well out of line with the response curves for the other replicates. The top threshold and the slope were particularly different.

Chemical IV (4-nonylphenol): The top threshold for replicate 2 was out of line with and higher than those from the other two replicates. This inflated the variability of the overall average estimate.

Chemical VII (econazole): The replicate 1 concentration response curve was out of line with those from the other replicates.

Chemical VIII (chrysin): The top, bottom, and slope parameters varied somewhat across replicates.

Chemical IX (dicofol): The bottom threshold was particularly variable among replicates and to a lesser extent the top threshold. This resulted in variation of the IC_{50} estimates across replicates since they estimated different percentiles [(B+T)/2] of the response distributions and introduced bias into their comparisons.

3. Chemical VII (econazole) is a strong aromatase inhibitor. The bottom thresholds of the concentration response distributions extend below concentration 10^{-7}. The concentration response distributions exhibit inhibition and do not attain their top thresholds even at concentration 10^{-10}.

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

5. Several sets of chemicals were analyzed on the same day with common controls. These are: Chemicals (I, II); (VII, VIII); and(IX, X).

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

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

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

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

Introduction and Background

In Task 7 of the Placental Aromatase Validation Study, each laboratory carried out multiple independent replicates of the placental aromatase assay with ten test substances according to a common test protocol: five chemicals with microsomal preparations made at Battelle and five chemicals with microsomal preparations made at In Vitro Technologies (IVT). For each test substance, concentration response curves were fitted to the results from each replicate. Graphical displays and analysis of variance summary comparisons of the concentration response curves were prepared separately for each inhibitor and for the full enzyme activity control, background activity control, positive, and negative controls combined across inhibitors.

This report discusses the methods and results of the statistical analyses of the data collected at IVT for the ten test substances: aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, dibenz[a,h]anthracene, fenarimol, econazole, chrysin, dicofol, and atrazine, which were coded: Chemicals I, II, III, IV, V, VI, VII, VIII, IX, and X, respectively. Four chemicals: aminoglutethimide, ketoconazole, econazole, and chrysin were previously analyzed and discussed in Task 5.

Data Used in the Analyses

Aromatase activity levels were determined for the full enzyme activity control, background activity control, positive control, and negative control, and for eight graded concentrations of each of the ten test substances. The specific concentration levels varied for each of the test substances.

Three replicates were carried out for each chemical. Within each replicate of each test substance three repetitions were run at each of the eight concentrations. In addition, two repeat tubes of the full enzyme activity, background activity, positive, and negative controls were run prior to the inhibition concentration runs and two repeat tubes of each the four control types were run following the inhibition concentration runs.

Statistical analyses were carried out on the "percent of control" responses. Percent of control is defined as the ratio of the background adjusted aromatase activity in the tube under consideration to the background adjusted average aromatase activity among the four full enzyme activity control tubes within the replicate, times 100. The average percent of control among the four full enzyme activity control tubes is necessarily 100 percent within each replicate. The average percent of control among the four background activity control tubes is necessarily 0 percent within each replicate.

Nominally for an inhibitor the percent of control activity values vary between approximately 0% near the high inhibition concentrations and approximately 100% near the low inhibition concentrations, but this may vary with the inhibitor. Chemical IX (dicofol) departs from this behavior with respect to the bottom threshold. Chemicals V (dibenz[a,h]anthracene) and X (atrazine) are apparent non-inhibitors.

The percent of control activity responses at varying inhibitor concentrations within each replicate are displayed in Tables A-1a through A-1j for Chemicals I through X, respectively. The full enzyme activity, background activity, positive, and negative controls background adjusted aromatase activity and the percent of control data within each replicate are displayed in Tables A-2a through A-2j.

Several sets of test substances were analyzed together on the same day with a common set of controls. Therefore, the control values were the same. They were: Chemical I and Chemical II; Chemical VII and Chemical VIII; Chemical IX and Chemical X, as seen in Tables A-2a and A-2b; Tables A-2g and A-2h; Tables A-2i and A-2j, respectively. It is also reflected in the common variance component values, shown in Table 11.

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 might be expected:

- Chemical I – Replicate 1 (assay date 7-27-05), level 1, Percent of Control values 87.10, 93.61, 59.51; level 4, Percent of Control value 63.90
- Chemical III – Replicate 4 (assay date 8-15-05), level 5, Percent of Control values 77.92, 79.65, 77.95
- Chemical IV – Replicate 2 (assay date 9-20-05), level 8, Percent of Control values 82.40, 76.37
- Chemical VI – Replicate 1 (assay date 8-3-05), level 1, Percent of Control value 40.20

- Chemical VIII – Replicate 1 (assay date 8-29-05), level 1, Percent of Control values 18.80, 18.92, 15.98; level 6, Percent of Control value 62.87; level 7 Percent of Control Values 72.33, 71.81, 69.45
- Chemical IX – Replicate 1 (assay date 9-8-05), level 6, Percent of Control values 82.66, 80.99; replicate 2 (assay date 9-12-05), level 1, Percent of Control values 67.50, 58.22; replicate 2, level 2, Percent of Control values 70.71, 72.09, 64.56; replicate 2, level 4, Percent of Control value 70.27.

There were no indications for any of these values of departures from protocol test procedures or of clerical errors. It was thus decided to include all these values in the statistical analyses.

Objectives

The primary objectives of the statistical analysis are:

1. Fit concentration curves within each replicate to describe the trend in the percent of control activity across varying inhibitor concentrations for each of the ten test substances.
2. Estimate baseline response, maximum response, the IC_{50} concentration, slope, and associated standard errors within each replicate of each test substance.
3. Combine results across replicates within test substances to determine the average baseline response, average maximum response, average IC_{50} concentration, average slope, and associated standard errors among replicates.
4. Determine whether there are differences between the full enzyme activity, background activity, positive, and negative controls obtained at the beginning and those obtained at the end of each replicate within each test substance.
5. Assess the consistency of test conditions within replicates, across replicates within test substances, and across test substances based on the full enzyme activity, background activity, positive, and negative control values.
6. Assess whether there are any differences in control responses between the microsomes prepared at Battelle and those prepared at In Vitro Technologies.

Statistical Analysis Methods

Concentration Response Trend Curves

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

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

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

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

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

where ϵ is the variation among repetitions, distributed with mean 0 and variance proportional to DAVG (based on Poisson distribution theory for radiation counts) and also approximately proportional to the response Y. This model directly corresponds to the model specified in the protocol. The slope parameter "β" is alternative notation for the hillslope parameter "H" specified in the protocol. The random variation term "ϵ" is not explicitly stated in the model in the protocol, but is tacitly understood there. The response curve was fitted by non-weighted least squares nonlinear regression analysis. Model fits were carried out using PRISM software (Version 4).

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 does not go below 80% of control, the test substance is a non-inhibitor and 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 replicate. The random effect was replicate within test substance. The within replicate variances were estimated as the squares of the standard errors for each replicate. The analysis of variance fits provide estimated averages effects (mean) across the replicates within test substances and their associated standard errors. These standard errors include both within replicate and between replicate components of variation. Degrees of freedom associated with the mean effects were calculated based on Satterthwaite's approximation.

The estimated IC_{50} for the test substance was estimated as 10 to the power mean $\log_{10}IC_{50}$.

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

Top (**T**), bottom (**B**), slope (β), and $\log_{10}IC_{50}$ (μ) were each compared across replicates within test substances based on 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) are 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 among the ten test substances. The control responses were expressed as percent of control. The full enzyme activity, background activity, positive, and negative controls percent of control responses were plotted across replicates, with plotting symbol distinguishing between beginning and end, and with reference line at 0% (background activity controls), at 100% (full

enzyme activity controls), or at 100% (negative controls). These plots indicate the extent of consistency across replicates with respect to average value and variability, and provide comparisons of beginning versus end of each replicate. Two separate plots (with the same vertical scale) were generated for chemicals with microsome preparations at each of the two laboratories. Chemicals III, V, VI, VII, and VIII were included in one plot (microsomes prepared at Battelle); Chemicals I, II, IV, IX, and X were included in another plot (microsomes prepared at IVT). Additional plots were prepared displaying the difference of the average of the first two percent of control values (i.e. those based on the "beginning" tubes) and the average of the last two percent of control values (i.e. those based on the "end" tubes) across replicates. Each plot of differences has a reference line at 0. Again, two separate plots (with the same vertical scale) were generated for chemicals with microsomes prepared at each of the two laboratories.

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

Roundoff

Some derived numbers in the results tables may differ from those in the computer printouts or from those obtained using hand calculations by several units in the least significant digit due to round off in intermediate numbers and in intermediate calculations.

Statistical Analysis Results

Concentration Response Trend Curves

Concentration response curves were fitted separately to the repeat tubes data within each replicate and to the averages of the repetitions within each replicate. Percent of control, the response variable for the statistical analyses, is displayed in Tables A-1a through A-1j for Chemicals I through X, respectively. The estimated parameters of the fitted concentration response curves are displayed in Tables 1-1, 2-1, 3-1, 4-1, 6-1, 7-1, 8-1, and 9-1 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively. The concentration response curves fitted to the averages of the three repetitions within each replicate for Chemicals I, II, III, IV, VI, VII, VIII, and IX are displayed in Figures 1-1, 2-1, 3-1, 4-1, 6-1, 7-1, 8-1, and 9-1.

The parameters of the average concentration response curves, based on random effects analysis of variance model fits with replicate as a random effect are displayed in Tables 1-1, 2-1, 3-1, 4-1, 6-1, 7-1, 8-1, and 9-1 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively.

("Mean" under "Replicate" column). The parameters within each replicate are also displayed. The average concentration response curves, along with the averages of three repetitions within each replicate are plotted together in Figures 1-2, 2-2, 3-2, 4-2, 6-2, 7-2, 8-2, and 9-2 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively.

The parameter estimates for each replicate and the average parameter estimates across replicates and their associated 95% confidence intervals are displayed in Tables 1-2, 2-2, 3-2, 4-2, 6-2, 7-2, 8-2, and 9-2 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively. The corresponding graphs for bottom parameter are presented in Figures 1-3, 2-3, 3-3, 4-3, 6-3, 7-3, 8-3, and 9-3; for top parameter, Figures 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.

With only a few exceptions, all the responses were above 75 percent of control for Chemical V (dibenz[a,h]anthracene) and all the responses were above 75 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 carried out for these two chemicals. Figures 5-1 and 10-1 display for Chemicals V and X respectively, averages of the percent of control of the three repetitions within each replicate. 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):

Figures 1-1 and 1-2 show that responses at log concentration -3 for replicate 1 were not in line with the fitted response curve. As shown in Table A-1a, replicate 1 had extremely high responses at the level 1 concentration ($\log_{10}$ concentration=-3). These outlying responses for replicate 1 resulted in high bottom estimate, large negative slope, and non-convergence of the model fitted to the average responses, as seen in Table 1-1. However, there were no indications for any of these outlying values of departures of from protocol test procedures or of clerical errors. Therefore, they were kept in the analysis. Replicates 2 and 3 were more in line with the fitted response curve, as seen in Figures 1-1 and 1-2. The parameter estimates and their 95% confidence intervals are displayed in Table 1-2 and are plotted in Figures 1-3 to 1-6. Essentially, the wide confidence intervals for the average bottom estimates, the top, $\log_{10}IC_{50}$, and slope estimates in replicate 1 were due to the outlying responses in replicate 1.

Table 1-3 displays the estimated variance components for the parameter estimates. Among

three replicates, replicate 1 had higher variances for each of the four parameters. The replicate-to-replicate variation was an order of magnitude higher than the within-replicate variation for the bottom parameter. For top parameter, there were some variations in the within-replicate effect for replicates 2 and 3. The replicate-to-replicate variation was zero for the top parameter. For $\log_{10}IC_{50}$ and slope, the within-replicate variations for replicates 2 and 3 and the replicate-to-replicate variation were close to zero.

Chemical II (ketoconazole):

Figures 2-1 and 2-2 show that replicates 2 and 3 were in line with one another. Replicate 1 had higher responses than replicates 2 and 3 which resulted in a higher top estimate, as seen in Table 2-1. Replicate 1 also had higher standard errors for each of the parameter estimates. Figure 2-4 shows that the top parameter estimate for replicate 1 was out of line with those for the other two replicates. Figures 2-5 and 2-6 show that the $\log_{10}IC_{50}$ and slope estimates vary somewhat among replicates.

Table 2-3 shows that for the bottom parameter, the replicate-to-replicate variation was zero and replicate 1's within replicate variation was nine times higher than replicate 2's within replicate variation. For the top parameter, the majority of the overall variation was from replicate-to-replicate variation. For $\log_{10}IC_{50}$ and slope, the individual replicate within-replicate variances and the replicate-to-replicate variations were all close to zero. The within replicate variation for replicate 1 was larger than the within replicate variation for replicates 2 and 3 and larger than the replicate-to-replicate variation.

Chemical III (prochloraz):

Figure 3-1 shows that replicate 2 and 3's fitted response curves were in line with one another. Three outlying responses for replicate 4 at the level 5 concentration ($\log_{10}$ concentration=-7.4815) were kept in the analysis since there were no indications for any of these outlying values departure of from protocol test procedures or of clerical errors (Table A-1c). These outlying responses resulted in a low top estimate and in a very steep slope estimate for replicate 4, as shown in Table 3-1. Table 3-1 also shows that the standard errors for $\log_{10}IC_{50}$ and slope for replicate 4 were extremely high (which resulted in the geometric standard error for IC_{50} was too high to be displayed in Table 3-1) because of the outlying responses. The top parameter is out of line with the other replicates, as seen in Figure 3-4. For the bottom parameter, replicates 3 and 4 were close to the average while replicate 2 was below the average, as seen in Figure 3-3. The large standard errors for the replicate 4 bottom, $\log_{10}IC_{50}$, and slope estimates are seen in Figures 3-3, 3-5, and 3-6.

Table 3-3 shows that the replicate-to-replicate variation was zero for the bottom, $\log_{10}IC_{50}$, and slope. For the top parameter, the majority of the overall variation was from the replicate-to-replicate variation. For $\log_{10}IC_{50}$ and slope, replicate 4's within-replicate variances were extremely high due to the outlying responses, while replicates 2 and 3's within-replicate variances were close to zero.

Chemical IV (4-Nonylphenol):

Figures 4-1 and 4-2 show that the data from replicates 3 and 6 were in line with one another. Replicate 2 had a higher top threshold. Two potential outlying responses for replicate 2 (log_{10} concentration= -10.0000) were kept in the analysis since there were no indications for any of these outlying values of departure from protocol test procedures or clerical errors. Figures 4-3 and 4-6 show that replicate 2 had relatively large within replicate standard errors for the bottom threshold and for the slope. As seen in Figure 4-5, the three replicates' $log_{10}IC_{50}$ estimates were very close to one other. Figure 4-6 shows that replicate 2 had steeper (more negative) slope than replicates 3 and 6.

Table 4-3 shows that for the bottom parameter, the replicate-to-replicate variation was zero and the individual replicate within-replicate variance for replicate 2 was nearly three or more times higher than the within-replicate variances for replicates 3 and 6. For the top parameter, the replicate-to-replicate variation was about three times the within-replicate variance for replicate 2 and more than ten times the individual replicate within-replicate variances for replicates 3 and 6. For $log_{10}IC_{50}$ and for slope, the replicate-to-replicate variation was zero. The within-replicate variance for replicate 2 was larger than for replicates 3 and 6.

Chemical V (dibenz[a,h]anthracene):

This is a non-inhibitor chemical. With four exceptions, all the responses were above 75 percent of control for replicates 1 and 2 combined. All responses were above 85 percent of control for replicate 3. Response curve analysis was not performed for this chemical since its parameters were not estimable. Averages percent of control of the three repetitions within each replicate are displayed in Figure 5-1. The percent of control values of the three repetitions for each of the three replicates are plotted in Figures 5-2, 5-3, and 5-4 and the values are shown in Tables A-1e.

Chemical VI (fenarimol):

Figures 6-1 and 6-2 show that the three replicates had some variation with respect to their fitted response curves. Table 6-1 and Figure 6-3 show that the bottom parameter estimate was somewhat higher for replicate 1 than for replicates 2 and 3. This is due to one potential outlying response at the level 1 concentration (log_{10} concentration=-4.4776). The bottom parameter was estimated as 15.8 for replicate 1. Table 6-1 and Figure 6-4 display variation in the top parameter among replicates. Table 6-1 and in Figure 6-5 show that replicate 1 had a slightly lower $log_{10}IC_{50}$ estimate than replicates 2 and 3. For slope replicate 1 had a steeper (more negative) and a more variable estimate than for replicates 2 and 3, as shown in Table 6-1 and Figure 6-6.

Table 6-3 shows that for the bottom parameter, the replicate-to-replicate variation and the within-replicate variances for replicates 1 and 2 were similar. For the top parameter, the replicate-to-replicate variation was much larger than the within-replicate variances for replicates 1, 2, and 3. For $log_{10}IC_{50}$ and slope, the replicate-to-replicate variations and the within-replicate variances for replicates 1, 2 and 3 were close to zero. For slope the within replicate variance for replicate 1 was more than eight times that for the other replicates.

Chemical VII (econazole):

Figures 7-1 and 7-2 show that replicates 2 and 3 were in line with one another and replicate 1 was a little lower. Econazole is a strong inhibitor. The bottom response plateau extends to lower than concentration 10^{-7}. The concentration response curves do not plateau, even at concentration 10^{-10}.

For the bottom parameter, the estimates were close to zero and consistent across the three replicates, as seen in Figure 7-3 and Table 7-1. Table 7-1 and Figure 7-4 show that the top estimates were also consistent across replicates.

The $\log_{10}IC_{50}$ estimates were consistent and close to the average for three replicates, as seen in Figure 7-5 and Table 7-1. The slope estimates were all close to the average for three replicates, as shown in Figure 7-6 and Table 7-1.

Table 7-3 shows that the replicate-to-replicate variation was zero for each of the four parameters. The within-replicate variances for the bottom parameter were low for each of the replicates. For the top parameter, within-replicate variance for replicate 1 was more than twice those for replicates 2 and 3. For $\log_{10}IC_{50}$ and slope, the individual replicate within-replicate variances for replicates 1, 2 and 3 were all close to zero.

Chemical VIII (chrysin):

Several potentially outlying responses were identified in the percent of control activity responses inhibitor concentrations data at the low and high levels of concentrations ($\log_{10}$ concentration=-9, -8, -4) for replicate 1. However, there were no indications for any of these values of departures from protocol test procedures or of clerical errors. Therefore, it was decided to include all these values in the statistical analyses. Figures 8-1 and 8-2 show that replicate 1 had a lower top threshold than replicates 2 and 3 The outlying values in replicate 1 ($\log_{10}$ concentration = -4) increased the estimate of the bottom parameter as seen in Table 8-1 and Figure 8-3. The relatively low top threshold in replicate 1 decreased the estimate of the top parameter in replicate 1 and resulted in a large standard error of the overall mean, as seen in Table 8-1 and Figure 8-4. Figures 8-5, 8-6, and Table 8-1 show that for $\log_{10}IC_{50}$ and slope, the replicate 1 estimate had a larger standard error than the estimates from the other replicates.

Table 8-3 shows that replicate 1 had larger within replicate variances for top, $\log_{10}IC_{50}$, and slope than the other replicates. The variation among the top thresholds for the three replicates, as shown in Figures 8-1 and 8-2, is reflected in the replicate-to-replicate variation for the top parameter being an order of magnitude greater than the within replicate variation for each of the replicates. For $\log_{10}IC_{50}$ and for slope the replicate-to-replicate variation was relatively small.

Chemical IX (dicofol):

Several potentially outlying responses were identified in the percent of control activity responses inhibitor concentrations data at level 6 concentration ($\log_{10}$ concentration=-8) for replicate 1 and at level 1 concentration ($\log_{10}$ concentration=-4), at level 2 concentration ($\log_{10}$ concentration=-4.4815), and level 4 concentration ($\log_{10}$ concentration=-6) for replicate 2. However, there were no indications for any of these values of departures from protocol test procedures or of clerical errors. Therefore, it was decided to include all these values in the

statistical analyses.

Figures 9-1 and 9-2 show that the bottom threshold was not attained for any of the replicates within the range of test concentrations. Table 9-1 shows that the bottom threshold exceeded 20 percent of control in both replicate 2 and 3 and was effectively not determined in replicate 1. Thus the bottom threshold is an extrapolation beyond the range of the data. Figure 9-3 displays the very large standard error for the bottom parameter for replicate 1. The top parameter estimates were close to 100 percent for replicates 2 and 3, as shown in Figure 9-1, Table 9-1 and Figure 9-4. Replicate 1's top parameter was estimated at about 90 percent. Figure 9-5 displays the relatively large variability in the $\log_{10}IC_{50}$ estimate for replicate 1. Figure 9-6 displays the relatively large variability for the slope estimate from replicate 2. Because of the wide variation in the bottom thresholds it may not be meaningful to compare the $\log_{10}IC_{50}$ across replicates for dicofol. The $\log_{10}IC_{50}$ estimate corresponds to the $[(B+T)/2]^{th}$ percentile of the concentration response distribution. When T = 100 and B = 0 the $\log_{10}IC_{50}$ corresponds to the 50^{th} percentile. For dicofol the estimates for the $\log_{10}IC_{50}$ for replicates 1, 2, and 3 correspond to the -0.8^{th}, 76.6^{th}, and the 58.4^{th} distribution percentiles respectively. Thus the parameter estimates are not directly comparable.

Table 9-3 shows that the within-replicate variance for the bottom parameter in replicate 1 was orders of magnitude higher than those for the other replicates. The replicate-to-replicate variation was also high. For the top parameter, the replicate-to-replicate variation was relatively high. For $\log_{10}IC_{50}$, replicate 1's within-replicate variance was orders of magnitude higher than the replicate-to-replicate variation and the within-replicate variances for replicates 2 and 3. For slope, the within-replicate variation for replicate 2 was relatively large.

Chemical X (atrazine):

This is a non-inhibitor chemical. With just one exception, all the responses were above 78% of control for replicates 1, 2, and 3. Response curve analysis was not performed for this chemical since its parameters were not estimable. Averages percent of control of the three repetitions within each replicate are displayed in Figure 10-1. The percent of control values of the three repetitions for each of the three replicates are plotted in Figures 10-2, 10-3, and 10-4 and the values are shown in Tables A-1j.

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

The control data were combined across the 30 replicates among the ten test substances. The control responses were expressed as percent of control. The full enzyme activity, background activity, positive, and negative control responses for each replicate are displayed in Tables A-2a through A-2j for Chemicals I through X, respectively. The percent of control data are plotted by replicate in Figures 11a, 11b, 12a, 12b, 13a, 13b, 14a, and 14b with plotting symbol distinguishing between beginning and end of the replicate. Figures 15a, 15b, 16a, 16b, 17a, 17b, 18a, and 18b show the differences between the averages at the beginning and at the end within each replicate (end minus beginning). In the horizontal axis of these figures, "I-1" stands for replicate 1 of

Chemical I, "V-2" stands for replicate 2 of Chemical V, and so on. The chemicals in these figures were grouped by the laboratory that prepared the microsomes. Figures 11a, 12a, 13a, 14a, 15a, 16a, 17a, and 18a are for chemicals whose microsomes were prepared at Battelle (Chemicals III, V, VI, VII, and VIII); Figures 11b, 12b, 13b, 14b, 15b, 16b, 17b, and 18b are for chemicals whose microsomes were prepared at IVT (Chemicals I, II, IV, IX, and X). For the same figure number, "a" plots and "b" plots have the same vertical scale.

Several sets of chemicals were analyzed on the same day, with common controls. Therefore, the control values were the same. The sets were: Chemicals I and II; Chemicals VII and VIII; Chemicals IX and X. The control results are plotted separately for each of the ten chemicals in Figures 11 to 18. However each pair was included just once in the analysis summaries displayed in Table 11.

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 source of microsomes, test substance, portion (beginning or end), and portion by test substance interaction. The random effects were replicate nested within test substance and portion by replicate within test substance interaction. The residual error variation was variation among repetitions within test substance, replicate, and portion. For the background and full enzyme activity control responses the component of variation due to replicate is constrained to be 0 by the definitions of these control responses. The analysis results for the four types of controls are displayed in Table 11. The left panel of the table displays the results of the tests for the differences between the responses collected at the beginning and at the end of a replicate. The right panel displays the estimated variance components across replicate within test substances and across portion by replicate interaction within test substances. The chemicals were grouped by the laboratory that prepared the microsomes.

The source of microsomes fixed effect and the chemical by portion interaction fixed effect were not significant for three types of control data: full enzyme activity, background activity, and positive. The source of microsomes fixed effect and the chemical by portion interaction fixed effect were significant for the negative control (p-values = 0.0179 and 0.0321, respectively). Test substances whose microsomes were made at IVT had significantly higher average negative control values than test substances whose microsomes were made at Battelle. Comparison of Figures 14a and 14b shows that the Battelle microsomes had lower percent of control at the beginnings of the replicates than did the IVT microsomes. However the percent of control at the ends of the replicates were about the same for the Battelle and IVT microsomes.

Figures 11a and 11b show that the full enzyme activity control responses were nearly always lower at the end of a replicate than at the beginning for the full enzyme activity. This is reflected in the curves in Figures 15a and 15b almost always lying below zero and in the significant portion effect for the full enzyme activity in Table 11 ($p<0.0001$).

Figures 14a and 14b show that for the negative controls the majority of control responses were higher at the end of a replicate than at the beginning. This is reflected in the curves in Figures 18a and 18b almost always lying above zero and in the significant portion effect for the negative control activity in Table 11 ($p=0.0022$).

Figures 12a and 12b show that background activity control were tightly distributed around 0 percent of control (within about ± 1 percent), except for replicate 1 for Chemicals I, II (IVT microsomes). Figures 16a and 16b show that for the Battelle microsomes the percent of control at the beginnings and at the ends of replicates were on average about the same. For the IVT microsomes except for Chemicals I, II the percent of control at ends of replicates were on average about the same as at the beginnings. For Chemicals I, II the percent of control at ends of replicates were higher than at the beginning.

Figures 13a and 17a show that for the positive controls with microsomes prepared at Battelle the percent of control at the ends of replicates tended to be greater than the beginnings of the replicates. For the positive controls with microsomes prepared at IVT the percent of control activity at the ends and beginnings of replicates were more tightly intermixed.

Figure 13a shows that two outlying positive control values on the low side for Chemical VI replicate 1 at the beginning resulted in large variances for the replicate and replicate by portion interaction effects for Chemical VI for positive controls (as seen in Table 11). Except for these two outlying values, other positive control values whose microsomes were prepared at Battelle were for the most part between 30 percent and 55 percent of control. Figure 13b shows that for positive controls whose microsomes were prepared at IVT the percent of control values were for the most part between 30 percent and 55 percent of control.

Figure 14a shows that for the negative controls whose microsomes were prepared at Battelle the percent of control at the ends of replicates were for the most part between 80 percent and 95 percent of control (except for Chemicals III and VI, where they were lower) (average 83 percent). The percent of control at the beginnings of replicates were for the most part between 50 percent and 80 percent of control (except for Chemicals III and VI, where they were between 30 percent and 50 percent) (average 60 percent).

Figure 14b shows that for the negative controls whose microsomes were prepared at IVT the percent of control at the ends of replicates were for the most part between 80 percent and 95 percent of control (average 88 percent). The percent of control at the beginnings of replicates were for the most part between 60 percent and 90 percent of control (average 79 percent).

It is interesting to note that the percent of control activity of the negative controls at the end of each replicate were similar for those with microsomes prepared at Battelle (83 percent) and for those with microsomes prepared at IVT (89 percent). However the percent of control activity of the negative controls at the beginning of each replicate differed between those with microsomes prepared at Battelle (60 percent) and those with microsomes prepared at IVT (79 percent). Thus the increase in activity was greater for the Battelle microsomes than for the IVT microsomes. In general, except for a few replicates in some test substances (e.g. Chemical VI, replicate 1), the test system appears to be consistent across all the replicates within all the chemicals, based on microsomes from the same source.

There were significant decreases in control activity between the beginning and the end for full enzyme activity controls and significant increases in control activity between the beginning

and the end for the negative controls. This 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 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 inhibition tubes (8 inhibitor concentrations × 3 repetitions per concentration) were tested in randomized order then the decrease in aromatase activity within the replicate would increase the residual variation about the fitted concentration response curves for each replicate but would avoid resulting in biased response curves.

Table 1-1. Reference Chemical I: Aminoglutethimide.
Microsomes Prepared by IVT.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	51.690 (6.735)	110.100 (5.128)	-4.980 (4646.000)	1.046E-05 (NA)	-7.565 (1750000)	I[e]
2	-1.140 (5.063)	101.300 (1.620)	-4.229 (0.069)	5.900E-05 (1.171)	-0.988 (0.137)	C
3	-9.097 (6.083)	103.300 (1.370)	-4.171 (0.083)	6.750E-05 (1.211)	-0.776 (0.091)	C
Mean [c]	13.668 (18.981)	102.771 (1.025)	-4.205 (0.053)	6.230E-05 (1.130)	-0.858 (0.103)	C
Average Values [b]						
1	NA (NA)	NA (NA)	NA (NA)	NA (NA)	NA (NA)	I[f]
2	-207 (4.706)	98.720 (1.485)	-4.201 (0.065)	6.292E-05 (1.161)	-1.005 (0.133)	C
3	-9.851 (6.397)	99.870 (1.342)	-4.119 (0.089)	7.609E-05 (1.228)	-0.782 (0.096)	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.
e. Incomplete Curve. Lower threshold is at about 50 percent.
f. Incomplete Curve. Dose-response model did not converge.

Table 1-2. Reference Chemical I: Aminoglutethimide.
Microsomes Prepared by IVT.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Estimate (95% CI)			
	Replicate 1[a]	Replicate 2[a]	Replicate 3[a]	Mean[b]
Bottom	51.690 (37.641,65.739)	-1.140 (-11.701,9.421)	-9.097 (-21.786,3.592)	13.668 (-68.780,96.116)
Top	110.100 (99.403,120.797)	101.300 (97.921,104.679)	103.300 (100.442,106.158)	102.771 (100.668,104.874)
$Log_{10}IC_{50}$	-4.980 (-9696.37,9686.406)	-4.229 (-4.372,-4.086)	-4.171 (-4.344,-3.998)	-4.205 (-4.316,-4.095)
Slope	-7.565 (-3650444,3650428)	-0.988 (-1.273,-0.703)	-0.776 (-0.966,-0.586)	-0.858 (-1.073,-0.643)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 1-3. Reference Chemical I: Aminoglutethimide.
Microsomes Prepared by IVT.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	45.36023 /df=20	25.63397 /df=20	37.00289 /df=20	1044.84314 /df=2 (p=0.168)	360.26054 /df=1.980
Top	26.29638 /df=20	2.62440 /df=20	1.87690 /df=20	0.00000 /df=2 (p=NA)	1.05057 /df=27.026
$Log_{10}IC_{50}$	21585316.0 /df=20	0.00471 /df=20	0.00691 /df=20	0.00000 /df=2 (p=NA)	0.00280 /df=20.000
Slope	3.0625E12[e] /df=20	0.01866 /df=20	0.00829 /df=20	0.00901 /df=2 (p=0.3884)	0.01064 /df=20.000

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.
e. 3.0625E12 is the square of the standard error of the slope parameter for replicate 1 (1750000), shown in Table 1-1. It differs from the value shown in the study file by a factor of 10^4 since we needed to adjust the standard error value downward by a factor of 100 in order to calculate the weighted average across replicates and its associated variability estimates, shown in Table 1-1 (Mean) and in the right-hand two columns for slope in Table 1-3. We then back adjusted the standard error upward by a factor of 100 to calculate the replicate 1 variance for slope shown in Table 1-3.

Table 2-1. Reference Chemical II: Ketoconazole.
Microsomes Prepared by IVT.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-1.233 (2.881)	108.000 (1.647)	-6.315 (0.063)	4.846E-07 (1.156)	-0.872 (0.094)	C
2	0.016 (0.947)	94.000 (0.600)	-6.404 (0.025)	3.946E-07 (1.058)	-1.014 (0.045)	C
3	0.703 (1.524)	95.930 (1.002)	-6.459 (0.040)	3.471E-07 (1.096)	-1.097 (0.079)	C
Mean [c]	0.103 (0.775)	99.203 (4.333)	-6.407 (0.030)	3.922E-07 (1.071)	-1.007 (0.047)	C
Average Values [b]						
1	-1.233 (2.796)	108.000 (1.599)	-6.315 (0.061)	4.846E-07 (1.151)	-0.872 (0.091)	C
2	0.016 (1.146)	94.000 (0.726)	-6.404 (0.030)	3.946E-07 (1.071)	-1.014 (0.054)	C
3	0.703 (2.206)	95.930 (1.450)	-6.459 (0.058)	3.471E-07 (1.142)	-1.097 (0.114)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 2-2. Reference Chemical II: Ketoconazole.
Microsomes Prepared by IVT.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Estimate (95% CI)			
	Replicate 1[a]	Replicate 2[a]	Replicate 3[a]	Mean[b]
Bottom	-1.233 (-7.243,4.777)	0.016 (-1.959,1.992)	0.703 (-2.477,3.882)	0.103 (-1.469,1.676)
Top	108.000 (104.564,111.436)	94.000 (92.749,95.251)	95.930 (93.840,98.020)	99.203 (80.165,118.241)
$Log_{10}IC_{50}$	-6.315 (-6.447,-6.183)	-6.404 (-6.456,-6.352)	-6.459 (-6.542,-6.376)	-6.407 (-6.648,-6.165)
Slope	-0.872 (-1.067,-0.676)	-1.014 (-1.108,-0.920)	-1.097 (-1.261,-0.933)	-1.007 (-1.645,-0.370)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 2-3. Reference Chemical II: Ketoconazole.
Microsomes Prepared by IVT.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	8.30016 /df=20	0.89700 /df=20	2.32258 /df=20	0.00000 /df=2 (p=NA)	0.60029 /df=35.345
Top	2.71261 /df=20	0.35988 /df=20	1.00400 /df=20	54.98419 /df=2 (p=0.167)	18.77523 /df=1.957
$Log_{10}IC_{50}$	0.00398 /df=20	0.00061 /df=20	0.00158 /df=20	0.00114 /df=2 (p=0.389)	0.00088 /df=1.240
Slope	0.00881 /df=20	0.00202 /df=20	0.00621 /df=20	0.00205 /df=2 (p=0.426)	0.00218 /df=0.970

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.
Microsomes Prepared by Battelle.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
2	-0.214 (1.579)	97.380 (2.255)	-7.730 (0.053)	1.862E-08 (1.130)	-1.004 (0.096)	C
3	0.856 (0.931)	101.200 (1.337)	-7.720 (0.031)	1.905E-08 (1.073)	-0.977 (0.053)	C
4	0.863 (2.546)	76.510 (2.546)	-7.038 (484.700)	9.162E-08 (NA)	-14.850 (183796.0)	C
Mean [c]	0.605 (0.765)	91.797 (7.642)	-7.723 (0.027)	1.894E-08 (1.063)	-0.983 (0.046)	C
Average Values [b]						
2	-0.214 (2.905)	97.380 (4.149)	-7.730 (0.098)	1.862E-08 (1.252)	-1.004 (0.177)	C
3	0.856 (1.674)	101.200 (2.405)	-7.720 (0.055)	1.905E-08 (1.136)	-0.977 (0.095)	C
4	0.868 (5.471)	76.510 (5.471)	-7.062 (1.986)	8.678E-08 (96.828)	-8.993 (216.700)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 3-2. Reference Chemical III: Prochloraz.
Microsomes Prepared by Battelle.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Estimate (95% CI)			
	Replicate 2[a]	Replicate 3[a]	Replicate 4[a]	Mean[b]
Bottom	-0.214 (-3.508,3.079)	0.856 (-1.086,2.797)	0.863 (-4.448,6.174)	0.605 (-0.941,2.152)
Top	97.380 (92.676,102.084)	101.200 (98.411,103.989)	76.510 (71.199,81.821)	91.797 (58.668,124.925)
$Log_{10}IC_{50}$	-7.730 (-7.841,-7.619)	-7.720 (-7.784,-7.656)	-7.038 (-1018.10,1004.028)	-7.723 (-7.778,-7.667)
Slope	-1.004 (-1.205,-0.803)	-0.977 (-1.087,-0.866)	-14.850 (-383407,383376.9)	-0.983 (-1.080,-0.886)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 3-3. Reference Chemical III: Prochloraz.
Microsomes Prepared by Battelle.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 2	Replicate 3	Replicate 4	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	2.49324 /df=20	0.86639 /df=20	6.48212 /df=20	0.00000 /df=2 (p=NA)	0.58494 /df=39.547
Top	5.08503 /df=20	1.78757 /df=20	6.48212 /df=20	170.77513 /df=2 (p=0.166)	58.40149 /df=1.984
$Log_{10}IC_{50}$	0.00282 /df=20	0.00095 /df=20	234934.090 /df=20	0.00000 /df=2 (p=NA)	0.00071 /df=20.000
Slope	0.00930 /df=20	0.00280 /df=20	3.3781E10 /df=20	0.00000 /df=2 (p=NA)	0.00215 /df=20.000

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.
Microsomes Prepared by IVT.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
2	15.470 (3.790)	92.380 (1.389)	-4.573 (0.035)	2.673E-05 (1.083)	-2.949 (0.781)	C
3	12.090 (2.222)	87.560 (0.688)	-4.581 (0.021)	2.622E-05 (1.050)	-2.347 (0.245)	C
6	12.250 (2.013)	87.630 (0.668)	-4.569 (0.018)	2.700E-05 (1.042)	-2.609 (0.300)	C
Mean [c]	12.620 (1.388)	88.969 (1.473)	-4.574 (0.013)	2.667E-05 (1.030)	-2.479 (0.184)	C
Average Values [b]						
2	15.470 (6.289)	92.380 (2.305)	-4.573 (0.058)	2.673E-05 (1.142)	-2.949 (1.296)	C
3	12.090 (3.073)	87.560 (0.951)	-4.581 (0.029)	2.622E-05 (1.069)	-2.347 (0.339)	C
6	12.250 (2.256)	87.630 (0.748)	-4.569 (0.020)	2.700E-05 (1.048)	-2.609 (0.336)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 4-2. Reference Chemical IV: 4-Nonylphenol.
Microsomes Prepared by IVT.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Estimate (95% CI)			
	Replicate 2 [a]	Replicate 3 [a]	Replicate 6 [a]	Mean [b]
Bottom	15.470 (7.564,23.376)	12.090 (7.455,16.725)	12.250 (8.051,16.449)	12.620 (9.822,15.417)
Top	92.380 (89.483,95.277)	87.560 (86.126,88.994)	87.630 (86.237,89.023)	88.969 (81.475,96.463)
$Log_{10}IC_{50}$	-4.573 (-4.645,-4.501)	-4.581 (-4.625,-4.537)	-4.569 (-4.607,-4.531)	-4.574 (-4.600,-4.548)
Slope	-2.949 (-4.578,-1.320)	-2.347 (-2.857,-1.837)	-2.609 (-3.235,-1.983)	-2.479 (-2.856,-2.103)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 4-3. Reference Chemical IV: 4-Nonylphenol.
Microsomes Prepared by IVT.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 2	Replicate 3	Replicate 6	Random Replicate (p-value) [d]	Variance of Mean
Bottom	14.36410 /df=20	4.93728 /df=20	4.05217 /df=20	0.00000 /df=2 (p=NA)	1.92701 /df=44.139
Top	1.92932 /df=20	0.47266 /df=20	0.44569 /df=20	5.62420 /df=2 (p=0.214)	2.16847 /df=1.708
$Log_{10}IC_{50}$	0.00120 /df=20	0.00044 /df=20	0.00032 /df=20	0.00000 /df=2 (p=NA)	0.00016 /df=44.349
Slope	0.60949 /df=20	0.05988 /df=20	0.09000 /df=20	0.00000 /df=2 (p=NA)	0.03395 /df=30.099

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.
Microsomes Prepared by Battelle.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay.

Only a few data points were below 80 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 data values.

Table 6-1. Reference Chemical VI: Fenarimol.
Microsomes Prepared by Battelle.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	15.790 (5.918)	96.080 (2.049)	-5.532 (0.085)	2.934E-06 (1.216)	-1.294 (0.285)	C
2	-0.401 (5.522)	91.400 (0.908)	-5.307 (0.073)	4.930E-06 (1.182)	-0.933 (0.099)	C
3	2.016 (4.320)	99.630 (0.852)	-5.314 (0.050)	4.850E-06 (1.121)	-1.071 (0.095)	C
Mean [c]	5.362 (4.711)	95.690 (2.519)	-5.373 (0.067)	4.236E-06 (1.168)	-1.021 (0.067)	C
Average Values [b]						
1	15.800 (4.550)	96.080 (1.579)	-5.533 (0.065)	2.934E-06 (1.163)	-1.294 (0.220)	C
2	-0.401 (3.039)	91.400 (0.500)	-5.307 (0.040)	4.930E-06 (1.096)	-0.933 (0.054)	C
3	2.016 (2.560)	99.630 (0.505)	-5.314 (0.030)	4.850E-06 (1.070)	-1.071 (0.056)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 6-2. Reference Chemical VI: Fenarimol.
Microsomes Prepared by Battelle.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Estimate (95% CI)			
	Replicate 1[a]	Replicate 2[a]	Replicate 3[a]	Mean[b]
Bottom	15.790 (3.445,28.135)	-0.401 (-11.919,11.118)	2.016 (-6.995,11.027)	5.362 (-17.879,28.603)
Top	96.080 (91.806,100.354)	91.400 (89.506,93.294)	99.630 (97.852,101.408)	95.690 (85.901,105.479)
$Log_{10}IC_{50}$	-5.532 (-5.709,-5.355)	-5.307 (-5.458,-5.156)	-5.314 (-5.418,-5.210)	-5.373 (-5.718,-5.028)
Slope	-1.294 (-1.889,-0.699)	-0.933 (-1.139,-0.727)	-1.071 (-1.269,-0.873)	-1.021 (-1.156,-0.885)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 6-3. Reference Chemical VI: Fenarimol.
Microsomes Prepared by Battelle.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	35.02272 /df=20	30.49248 /df=20	18.66240 /df=20	39.26917 /df=2 (p=0.292)	22.19427 /df=1.755
Top	4.19840 /df=20	0.82410 /df=20	0.72659 /df=20	17.24753 /df=2 (p=0.170)	6.34518 /df=2.242
$Log_{10}IC_{50}$	0.00723 /df=20	0.00527 /df=20	0.00248 /df=20	0.00894 /df=2 (p=0.277)	0.00455 /df=1.700
Slope	0.08145 /df=20	0.00975 /df=20	0.00899 /df=20	0.00000 /df=2 (p=NA)	0.00442 /df=29.479

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.
Microsomes Prepared by Battelle.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-0.488 (0.769)	105.600 (9.108)	-9.247 (0.129)	5.659E-10 (1.345)	-0.748 (0.092)	C
2	-1.033 (1.146)	109.900 (5.634)	-9.040 (0.070)	9.124E-10 (1.176)	-0.801 (0.069)	C
3	-0.496 (1.217)	106.700 (5.551)	-9.012 (0.071)	9.733E-10 (1.178)	-0.825 (0.077)	C
Mean [c]	-0.623 (0.566)	107.852 (3.627)	-9.055 (0.047)	8.809E-10 (1.113)	-0.797 (0.045)	C
Average Values [b]						
1	-0.488 (0.222)	105.600 (2.623)	-9.247 (0.037)	5.659E-10 (1.089)	-0.748 (0.026)	C
2	-1.033 (1.889)	109.900 (9.286)	-9.040 (0.116)	9.124E-10 (1.306)	-0.801 (0.114)	C
3	-0.496 (1.478)	106.700 (6.739)	-9.012 (0.086)	9.733E-10 (1.220)	-0.825 (0.093)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 7-2. Reference Chemical VII: Econazole.
Microsomes Prepared by Battelle.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Estimate (95% CI)			
	Replicate 1[a]	Replicate 2[a]	Replicate 3[a]	Mean[b]
Bottom	-0.488 (-2.093,1.117)	-1.033 (-3.424,1.358)	-0.496 (-3.035,2.042)	-0.623 (-1.757,0.511)
Top	105.600 (86.601,124.599)	109.900 (98.148,121.652)	106.700 (95.121,118.279)	107.852 (100.559,115.145)
$Log_{10}IC_{50}$	-9.247 (-9.516,-8.978)	-9.040 (-9.187,-8.893)	-9.012 (-9.160,-8.864)	-9.055 (-9.149,-8.961)
Slope	-0.748 (-0.939,-0.557)	-0.801 (-0.945,-0.657)	-0.825 (-0.985,-0.666)	-0.797 (-0.886,-0.707)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 7-3. Reference Chemical VII: Econazole.
Microsomes Prepared by Battelle.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	0.59182 /df=20	1.31332 /df=20	1.48109 /df=20	0.00000 /df=2 (p=NA)	0.31987 /df=53.724
Top	82.95566 /df=20	31.74196 /df=20	30.81360 /df=20	0.00000 /df=2 (p=NA)	13.15584 /df=47.911
$Log_{10}IC_{50}$	0.01659 /df=20	0.00495 /df=20	0.00506 /df=20	0.00000 /df=2 (p=NA)	0.00217 /df=43.487
Slope	0.00840 /df=20	0.00478 /df=20	0.00585 /df=20	0.00000 /df=2 (p=NA)	0.00200 /df=56.754

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.
Microsomes Prepared by Battelle.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	11.780 (3.550)	77.890 (2.138)	-5.607 (0.135)	2.472E-06 (1.364)	-1.600 (0.461)	C
2	4.177 (1.764)	92.930 (0.579)	-5.583 (0.024)	2.611E-06 (1.057)	-1.169 (0.067)	C
3	-4.581 (7.862)	101.000 (1.412)	-5.402 (0.095)	3.964E-06 (1.245)	-0.872 (0.120)	C
Mean [c]	5.588 (3.577)	90.696 (6.712)	-5.543 (0.058)	2.865E-06 (1.143)	-1.082 (0.140)	C
Average Values [b]						
1	11.790 (5.585)	77.890 (3.362)	-5.607 (0.212)	2.471E-06 (1.628)	-1.601 (0.723)	C
2	4.177 (3.031)	92.930 (0.996)	-5.583 (0.041)	2.611E-06 (1.099)	-1.169 (0.116)	C
3	-4.583 (10.610)	101.000 (1.905)	-5.402 (0.129)	3.964E-06 (1.345)	-0.872 (0.162)	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.
Microsomes Prepared by Battelle.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Estimate (95% CI)			
	Replicate 1	Replicate 2	Replicate 3	Mean [b]
Bottom	11.780 (4.375,19.185)	4.177 (0.497,7.857)	-4.581 (-20.981,11.819)	5.588 (-8.752,19.927)
Top	77.890 (73.430,82.350)	92.930 (91.722,94.138)	101.000 (98.055,103.945)	90.696 (61.331,120.061)
$Log_{10}IC_{50}$	-5.607 (-5.888,-5.326)	-5.583 (-5.633,-5.533)	-5.402 (-5.601,-5.203)	-5.543 (-5.724,-5.361)
Slope	-1.600 (-2.562,-0.638)	-1.169 (-1.309,-1.029)	-0.872 (-1.122,-0.622)	-1.082 (-1.430,-0.735)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 8-3. Reference Chemical VIII: Chrysin.
Microsomes Prepared by Battelle.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	12.60250 /df=20	3.11170 /df=20	61.81104 /df=20	23.00068 /df=2 (p=0.310)	12.79194 /df=2.161
Top	4.57104 /df=20	0.33559 /df=20	1.99374 /df=20	132.87770 /df=2 (p=0.165)	45.05181 /df=1.966
$Log_{10}IC_{50}$	0.01814 /df=20	0.00057 /df=20	0.00907 /df=20	0.00496 /df=2 (p=0.329)	0.00339 /df=3.115
Slope	0.21271 /df=20	0.00452 /df=20	0.01438 /df=20	0.03354 /df=2 (p=0.294)	0.01953 /df=5.627

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.
Microsomes Prepared by IVT.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-91.880 (587.200)	90.280 (1.916)	-2.885 (4.350)	1.304E-03 (22387.21)	-0.483 (0.287)	I [e]
2	54.720 (9.467)	98.460 (3.144)	-5.400 (0.384)	3.979E-06 (2.422)	-0.875 (0.496)	C
3	20.710 (7.851)	96.020 (0.974)	-4.693 (0.110)	2.029E-05 (1.287)	-1.043 (0.194)	C
Mean [c]	37.195 (16.970)	94.652 (2.287)	-4.935 (0.332)	1.161E-05 (2.149)	-0.830 (0.208)	C
Average Values [b]						
1	-92.090 (962.500)	90.280 (3.117)	-2.883 (7.101)	1.308E-03 (12618275)	-0.482 (0.467)	I [e]
2	54.740 (11.250)	98.460 (3.749)	-5.401 (0.457)	3.971E-06 (2.867)	-0.876 (0.593)	C
3	20.720 (4.165)	96.020 (0.517)	-4.693 (0.058)	2.029E-05 (1.143)	-1.043 (0.103)	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.
e. Incomplete Curve. $Log_{10}IC_{50}$ was interpolated.

Table 9-2. Reference Chemical IX: Dicofol.
Microsomes Prepared by IVT.
Parameter Estimates of the Concentration Response Curves and Associated 95% Confidence Intervals.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Estimate (95% CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	-91.880 (-1316.76,1132.998)	54.720 (34.972,74.468)	20.710 (4.333,37.087)	37.195 (1.816,72.573)
Top	90.280 (86.283,94.277)	98.460 (91.902,105.018)	96.020 (93.988,98.052)	94.652 (84.849,104.456)
$Log_{10}IC_{50}$	-2.885 (-11.959,6.189)	-5.400 (-6.201,-4.599)	-4.693 (-4.922,-4.464)	-4.935 (-5.627,-4.244)
Slope	-0.483 (-1.081,0.116)	-0.875 (-1.910,0.160)	-1.043 (-1.447,-0.639)	-0.830 (-1.403,-0.257)

a. Parameter estimates and their associated 95% confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95% confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 9-3. Reference Chemical IX: Dicofol.
Microsomes Prepared by IVT.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Placental Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	344803.840 /df=20	89.62409 /df=20	61.63820 /df=20	501.15354 /df=2 (p=0.269)	287.98223 /df=20.182
Top	3.67106 /df=20	9.88474 /df=20	0.94907 /df=20	11.64486 /df=2 (p=0.239)	5.23169 /df=2.008
$Log_{10}IC_{50}$	18.92250 /df=20	0.14761 /df=20	0.01203 /df=20	0.16129 /df=2 (p=0.316)	0.11038 /df=20.792
Slope	0.08237 /df=20	0.24621 /df=20	0.03756 /df=20	0.04584 /df=2 (p=0.345)	0.04308 /df=4.057

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.
Microsomes Prepared by IVT.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Placental Aromatase Assay.

Only a few data points were below 80 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)		Variance Components								
	Estimate (%) (Std. Error)	p-Value / Degree of Freedom		Chemical							Residual (Repetition)
				Battelle Microsomes				IVT Microsomes			
				III	V	VI	VII VIII	I II	IV	IX X	
Full Enzyme Activity Control	-10.016 (1.371)	<.0001 /df=22.72	Replicate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.61
			Replicate * Portion	6.33	26.35	0.00	23.12	2.71	0.00	18.06	
Background Activity Control	-0.407 (0.228)	0.1061 /df=9.25	Replicate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44
			Replicate * Portion	0.00	0.00	0.00	0.00	2.26	0.00	0.00	
Positive Control	3.581 (1.636)	0.0638 /df=7.17	Replicate	0.00	14.00	271.43	6.40	22.61	0.92	0.00	6.46
			Replicate * Portion	17.30	10.28	73.99	2.95	62.62	0.00	6.85	
Negative Control	17.384 (4.263)	0.0022 /df=10.10	Replicate	118.89	4.70	0.00	0.00	0.00	0.00	0.00	46.95
			Replicate * Portion	289.56	4.96	665.52	0.00	82.26	0.00	129.01	

Note: The source of microsomes fixed effect and the chemical by portion interaction fixed effect were not significant for three types of control activity data: full enzyme activity control, background activity control, and positive control. However, the source of microsomes fixed effect and the chemical by portion interaction fixed effect were significant for negative control (p-values = 0.0179 and,0.0321, respectively).

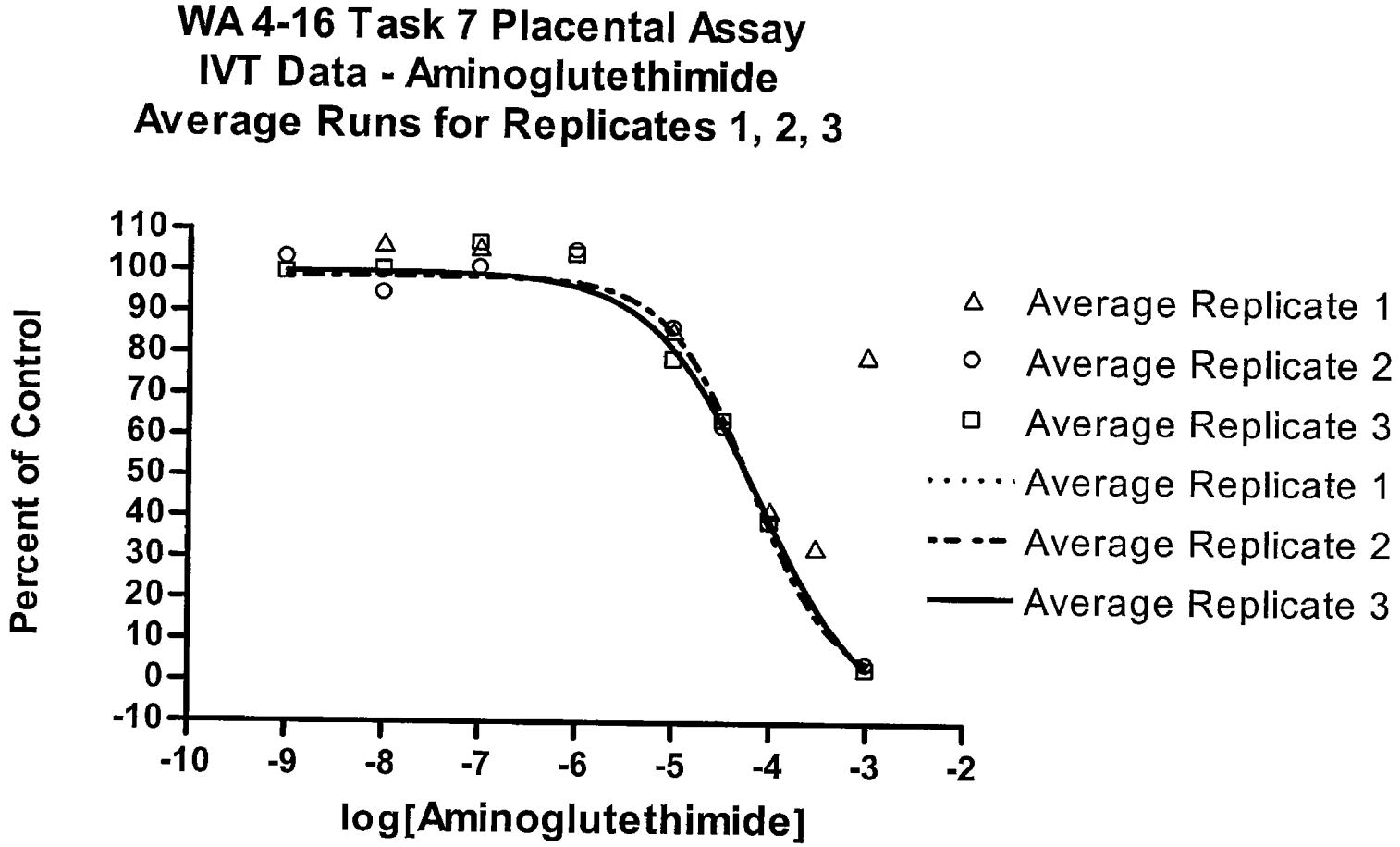


Figure 1-1. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by IVT. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

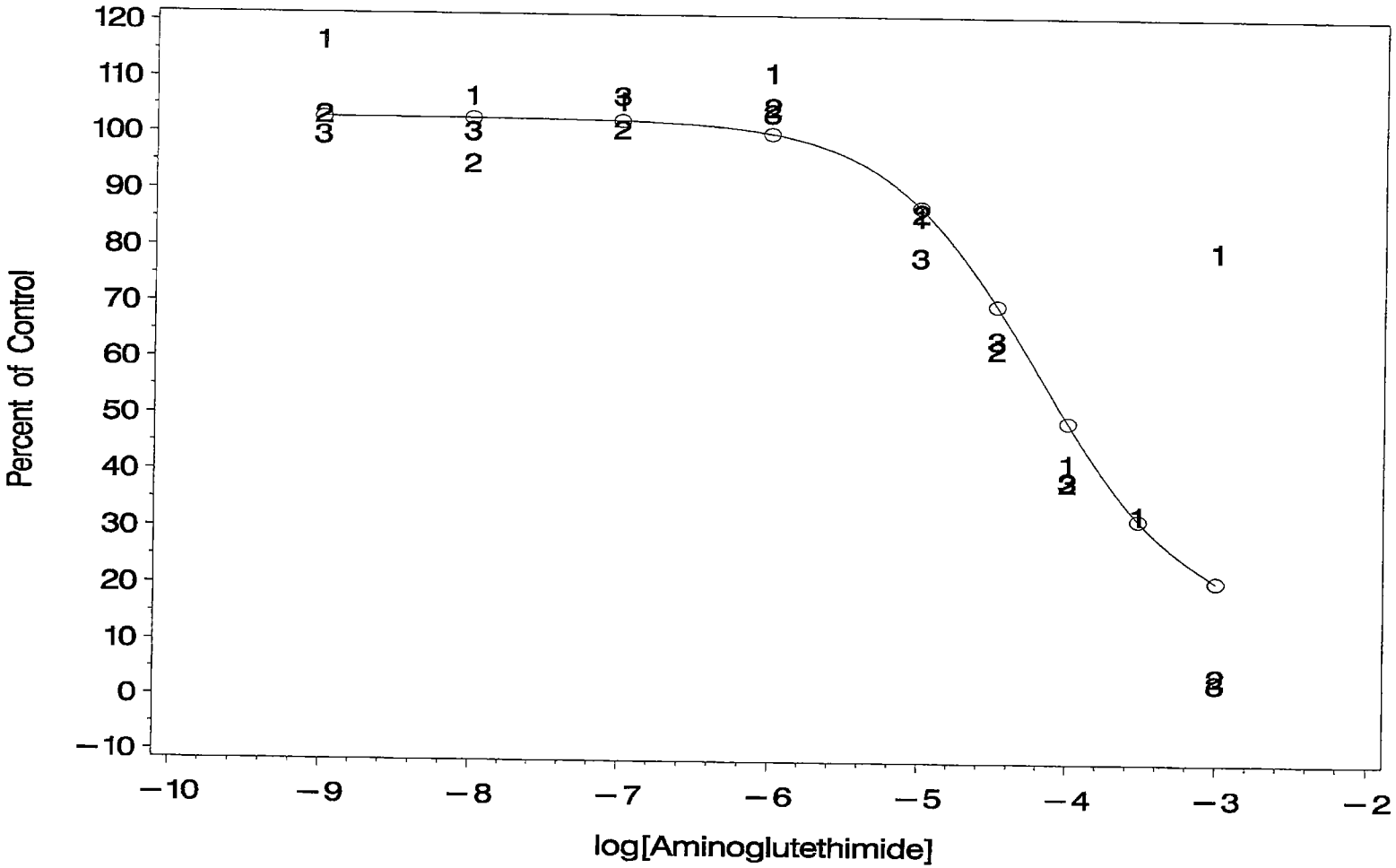


Figure 1-2. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by IVT. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

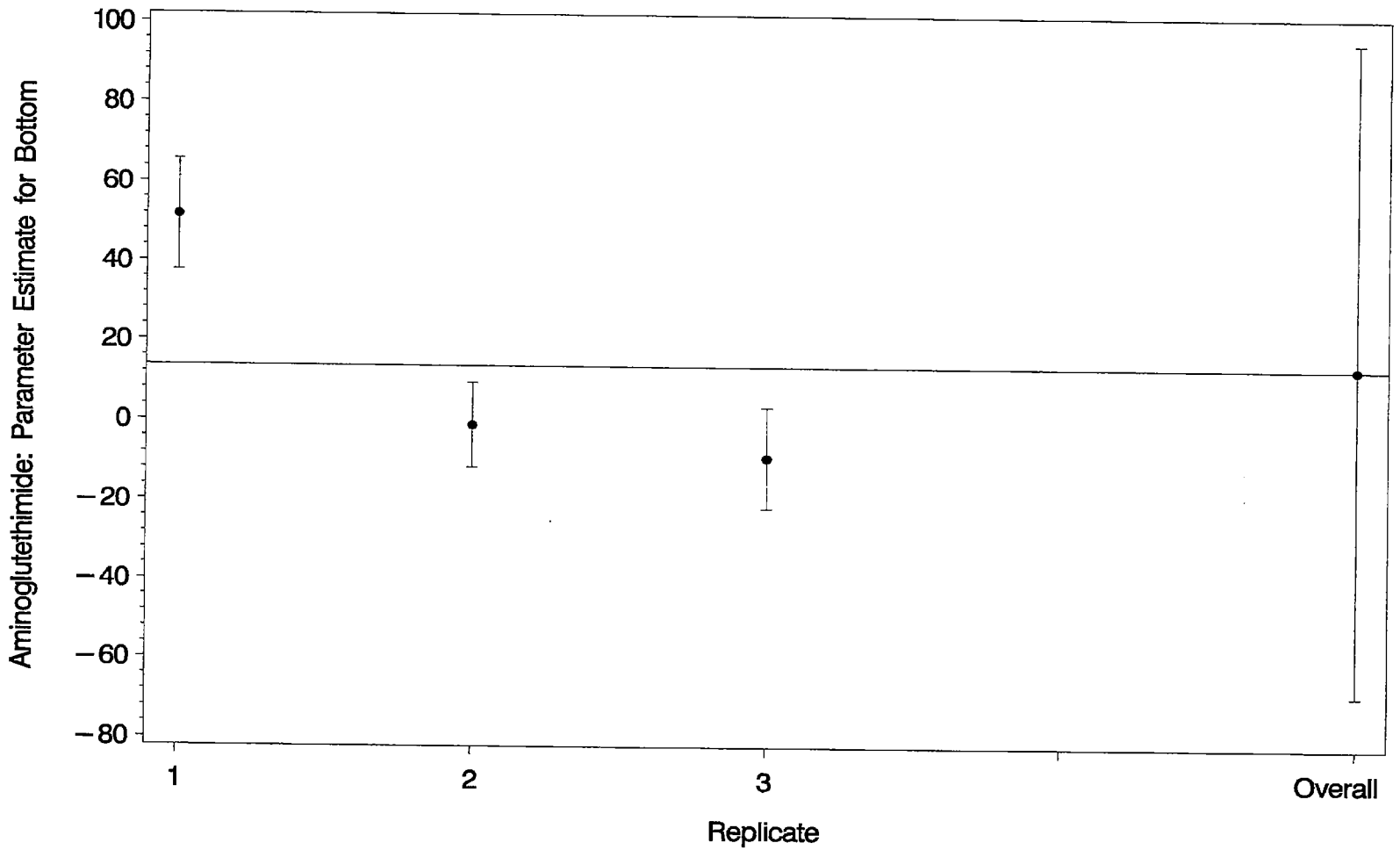


Figure 1-3. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by IVT. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

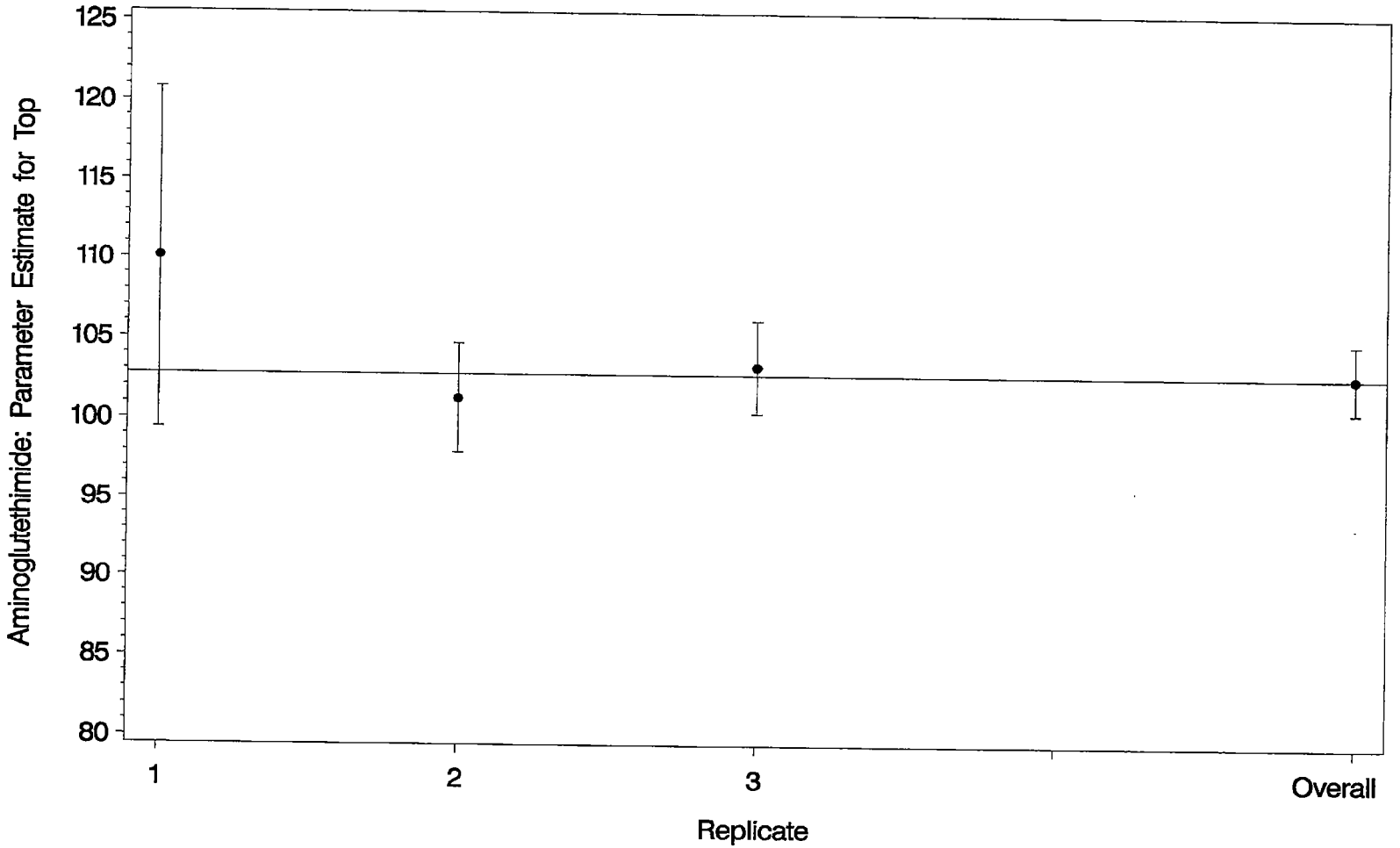


Figure 1-4. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by IVT. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

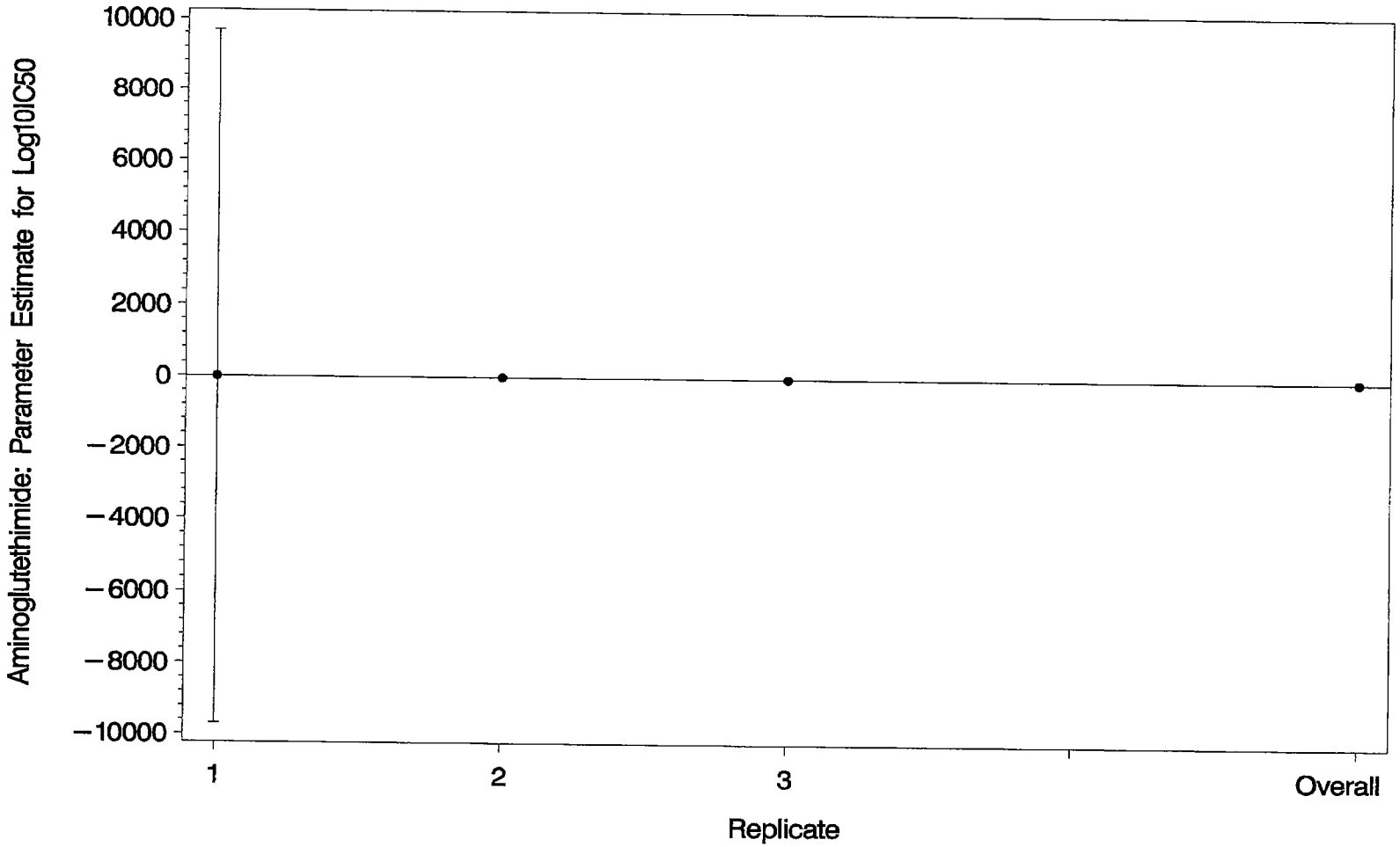


Figure 1-5. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by IVT. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

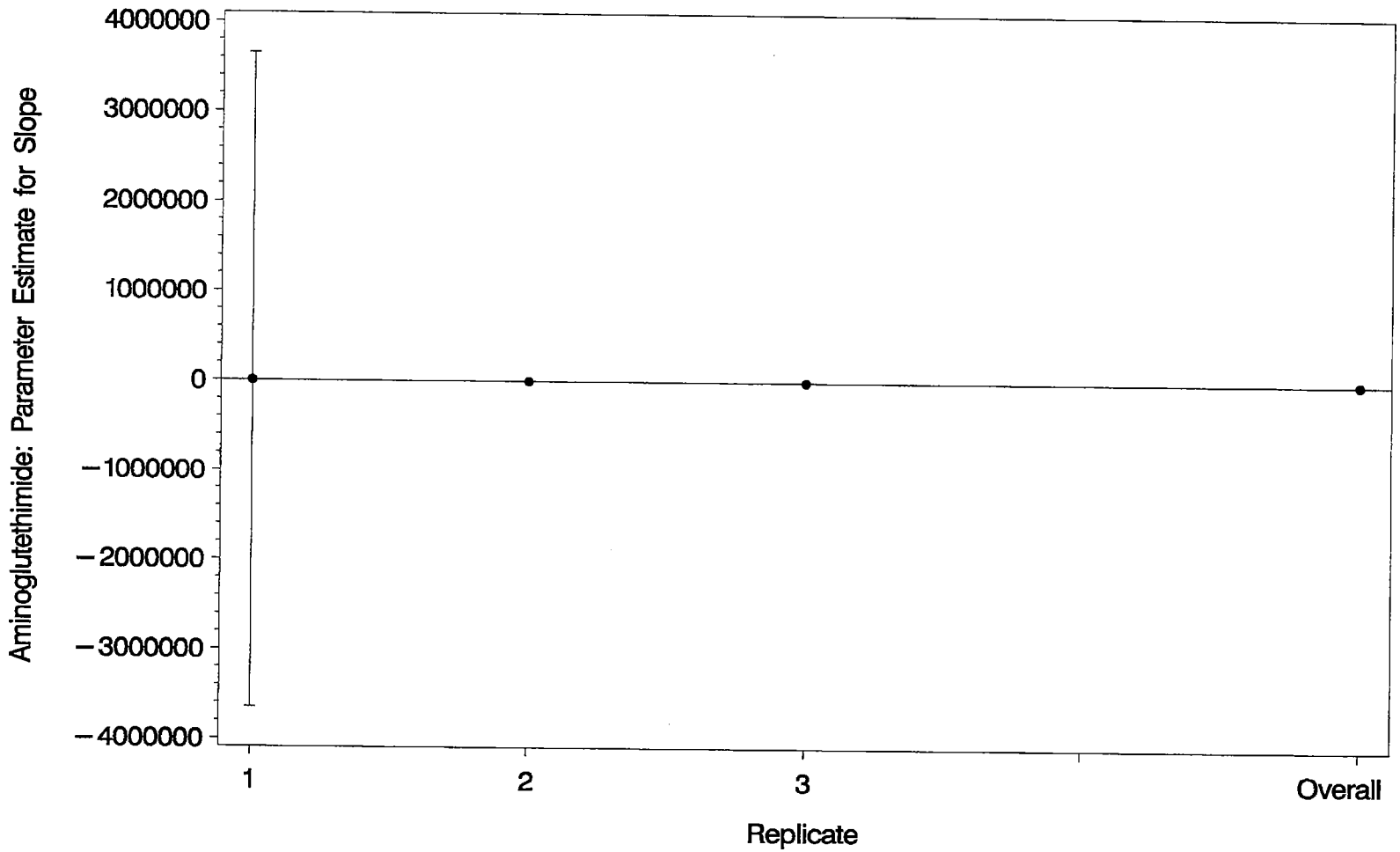


Figure 1-6. **Reference Chemical I: Aminoglutethimide. Microsomes Prepared by IVT. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

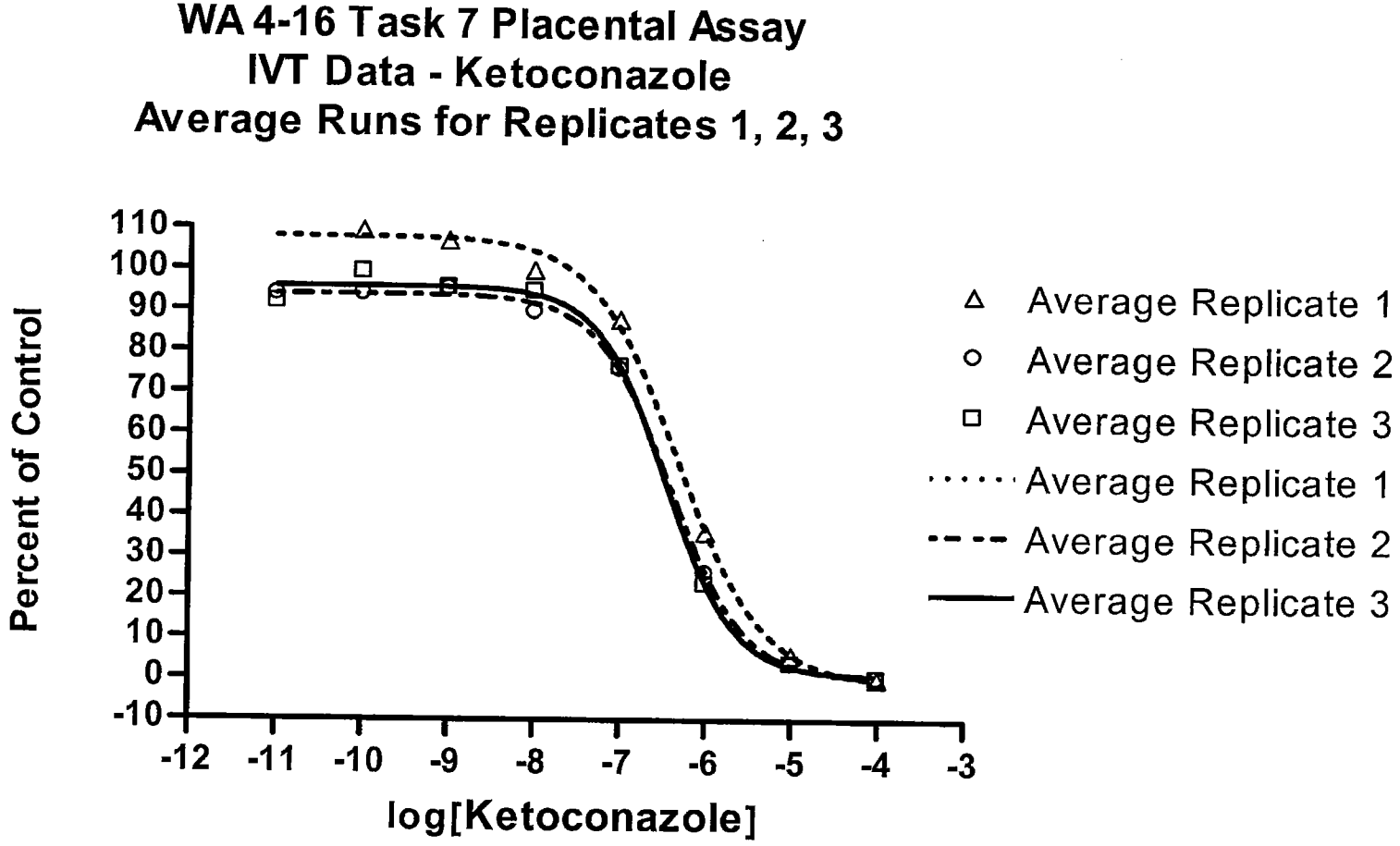


Figure 2-1. **Reference Chemical II: Ketoconazole. Microsomes Prepared by IVT Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

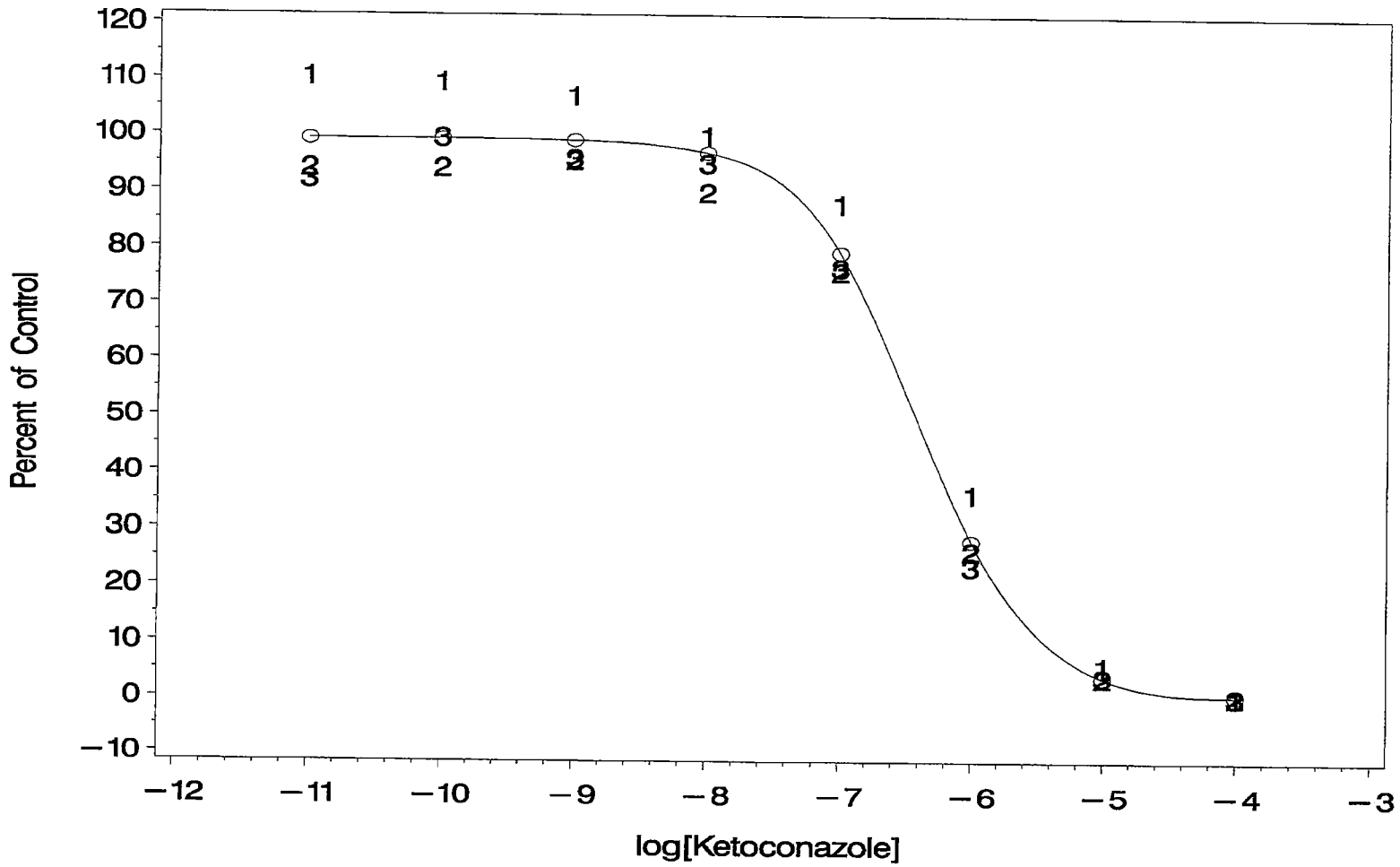


Figure 2-2. **Reference Chemical II: Ketoconazole. Microsomes Prepared by IVT. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

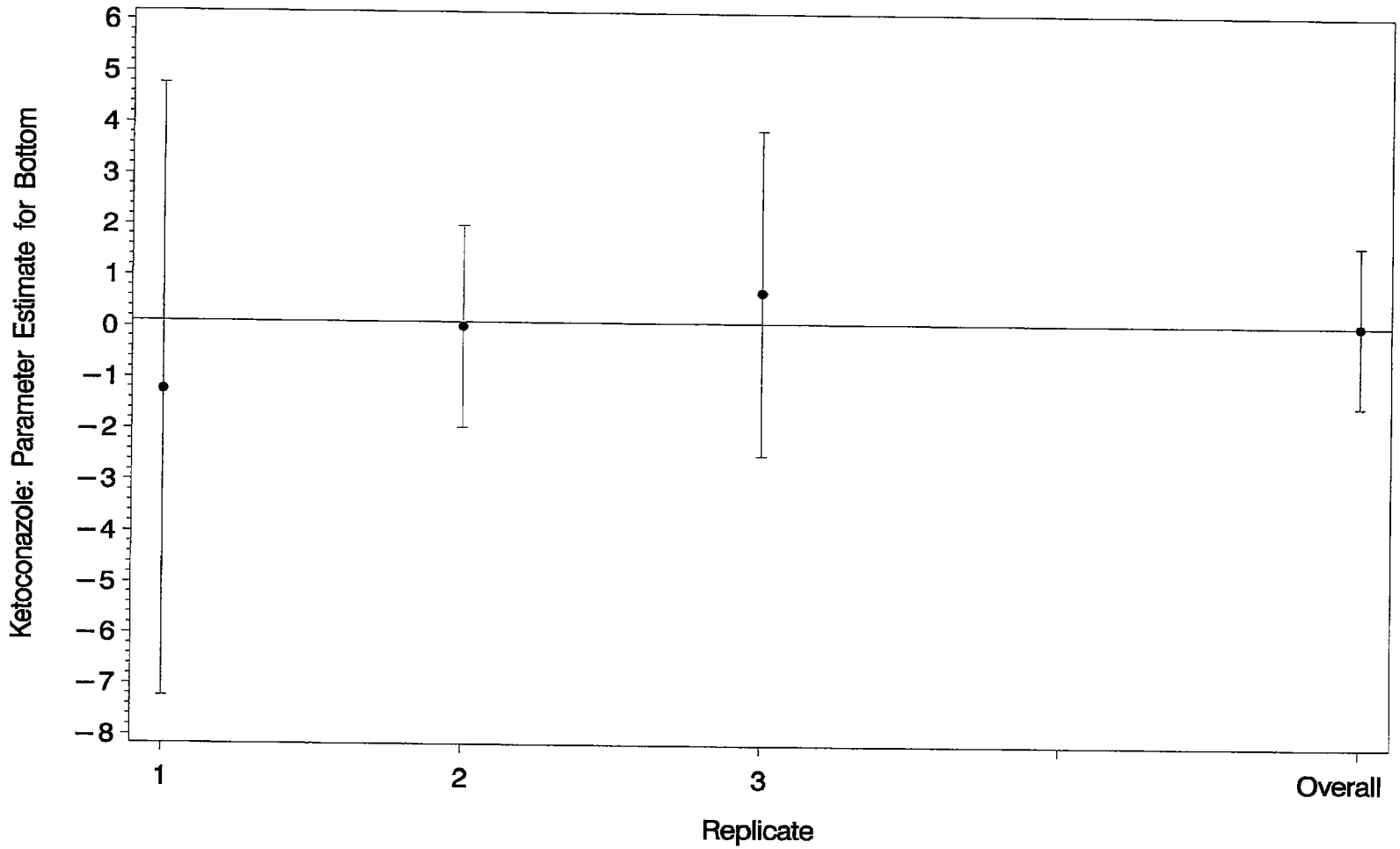


Figure 2-3. **Reference Chemical II: Ketoconazole. Microsomes Prepared by IVT. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

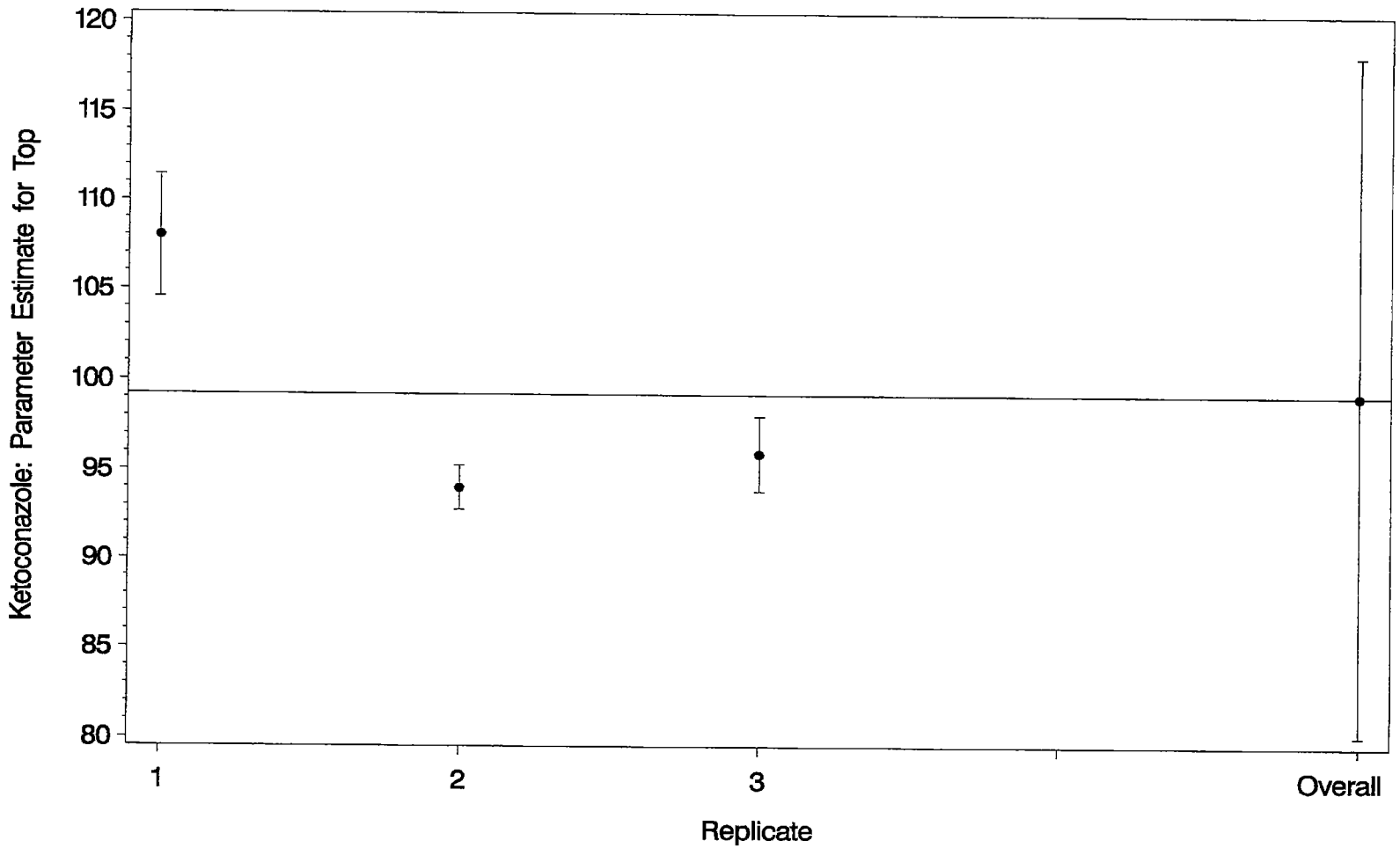


Figure 2-4. **Reference Chemical II: Ketoconazole. Microsomes Prepared by IVT. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

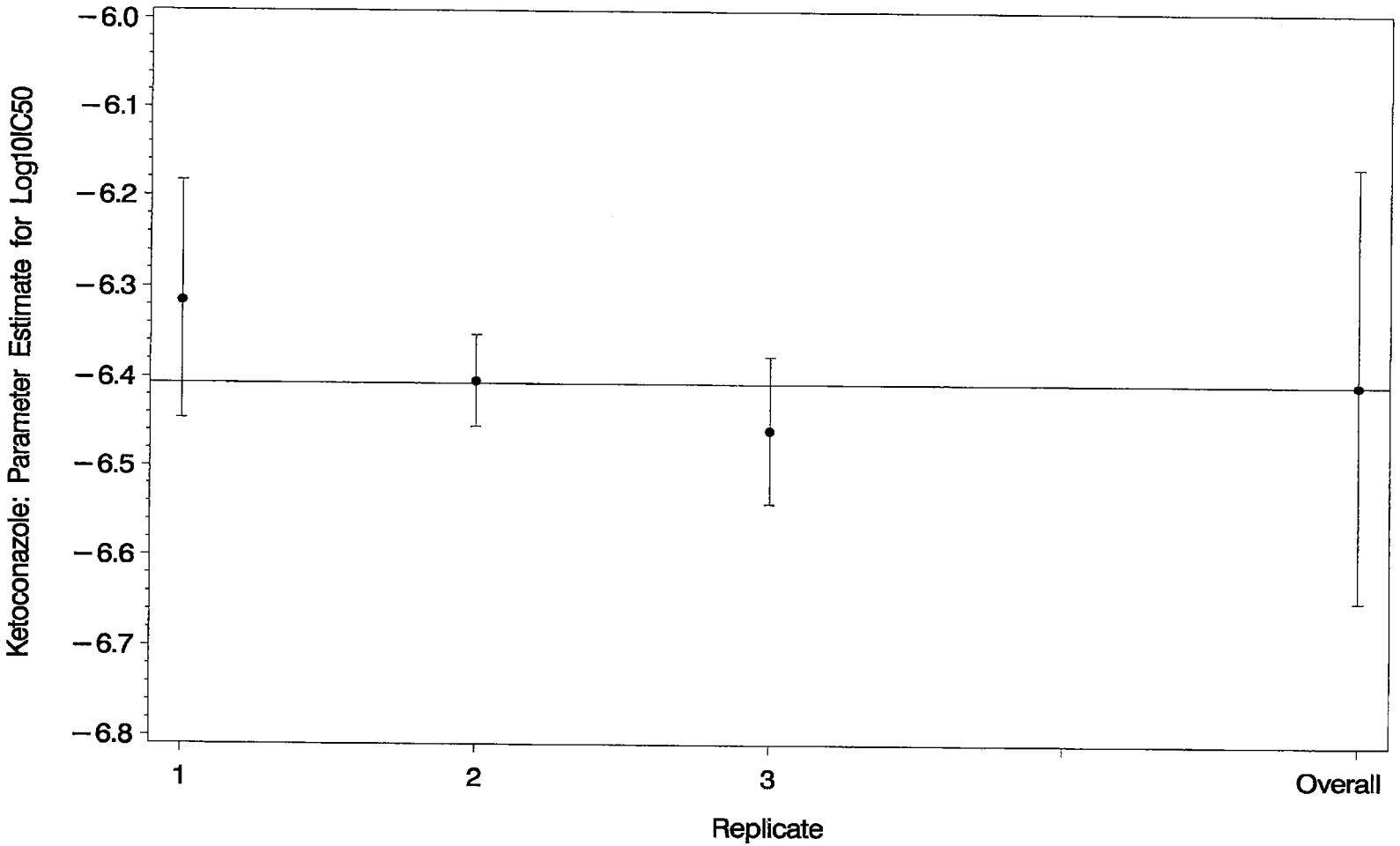


Figure 2-5. **Reference Chemical II: Ketoconazole. Microsomes Prepared by IVT. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

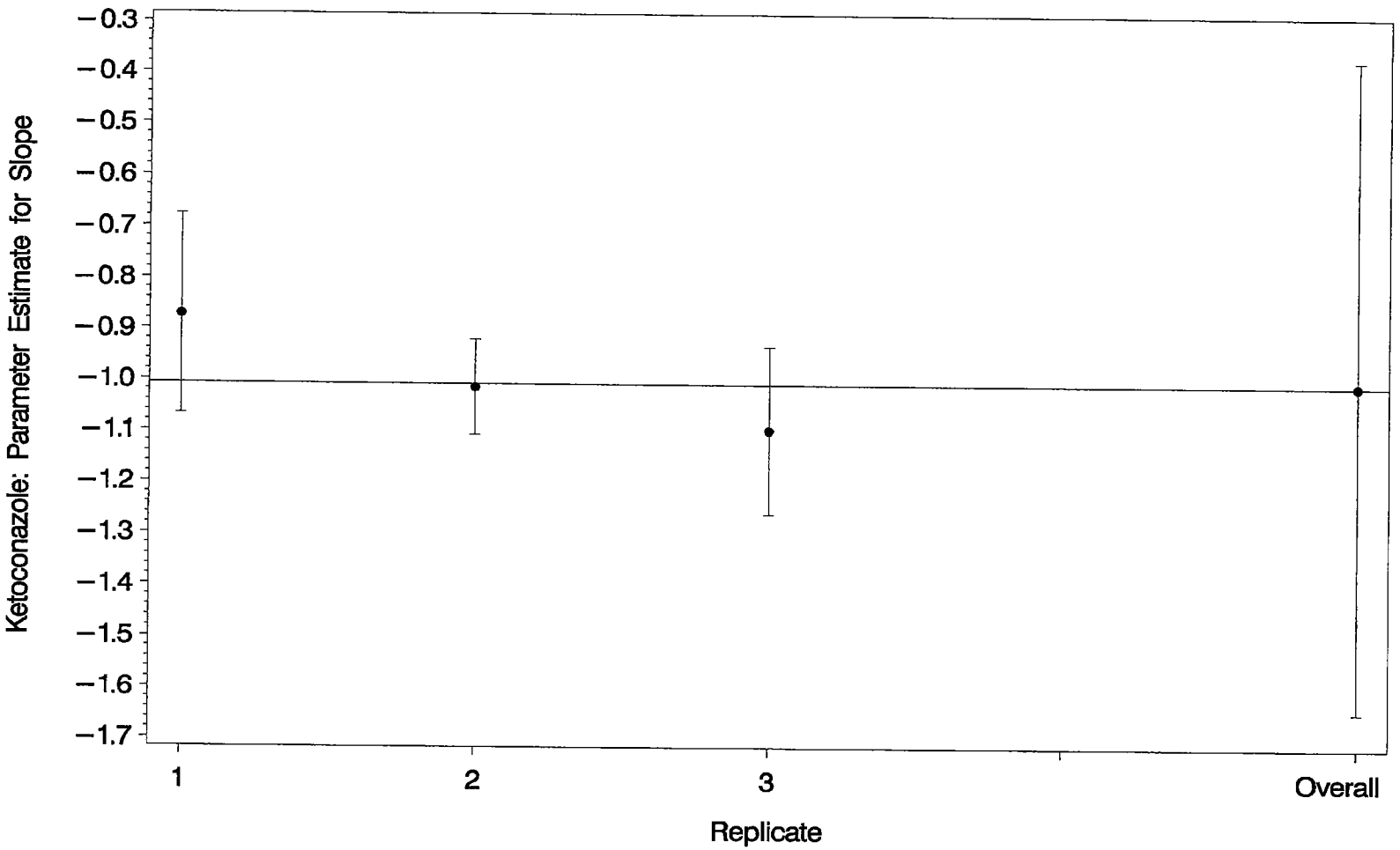


Figure 2-6. **Reference Chemical II: Ketoconazole. Microsomes Prepared by IVT. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates**

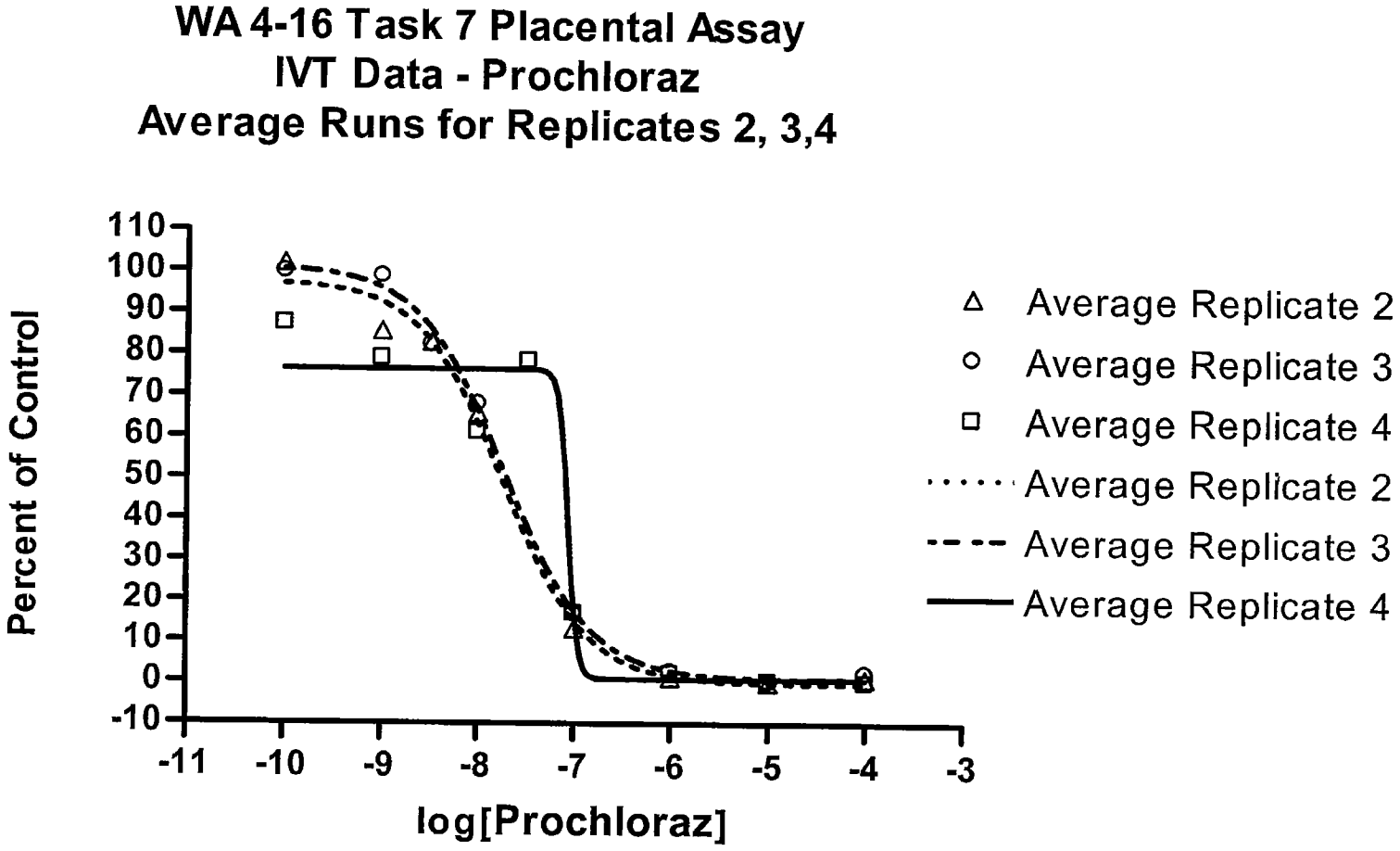


Figure 3-1. **Reference Chemical III: Prochloraz. Microsomes Prepared by Battelle. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

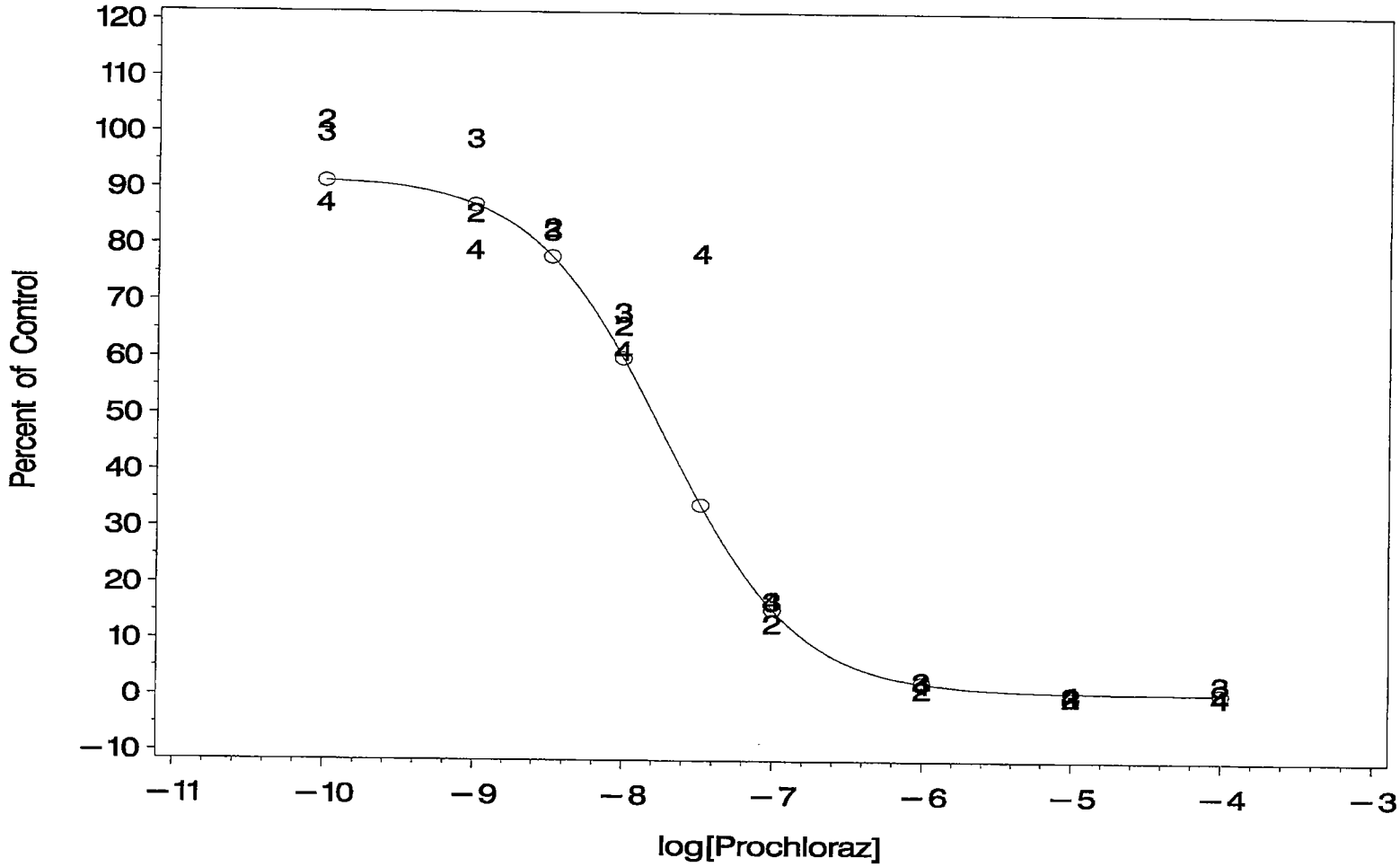


Figure 3-2. **Reference Chemical III: Prochloraz. Microsomes Prepared by Battelle. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

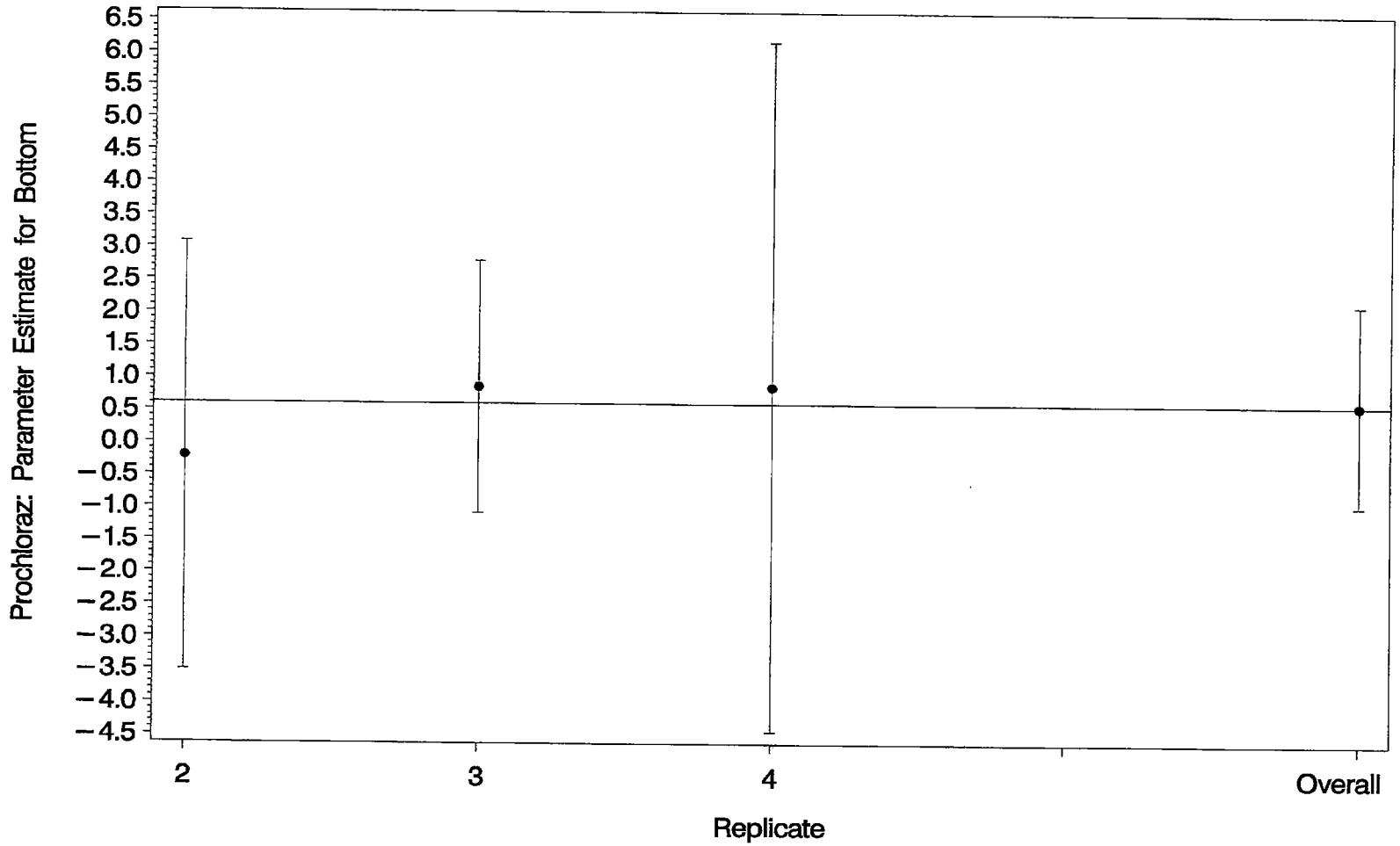


Figure 3-3. **Reference Chemical III: Prochloraz. Microsomes Prepared by Battelle. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

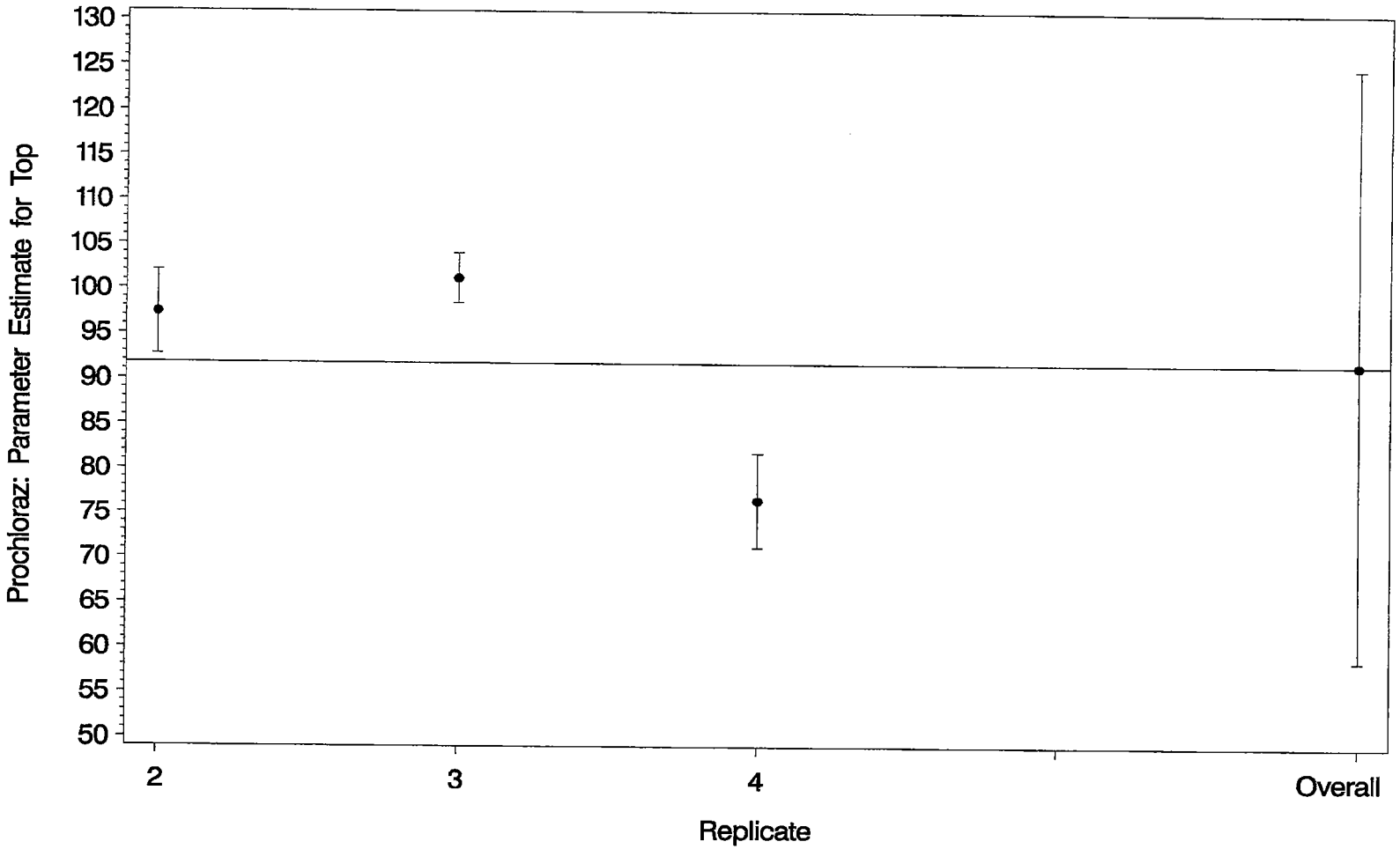


Figure 3-4. **Reference Chemical III: Prochloraz. Microsomes Prepared by Battelle. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

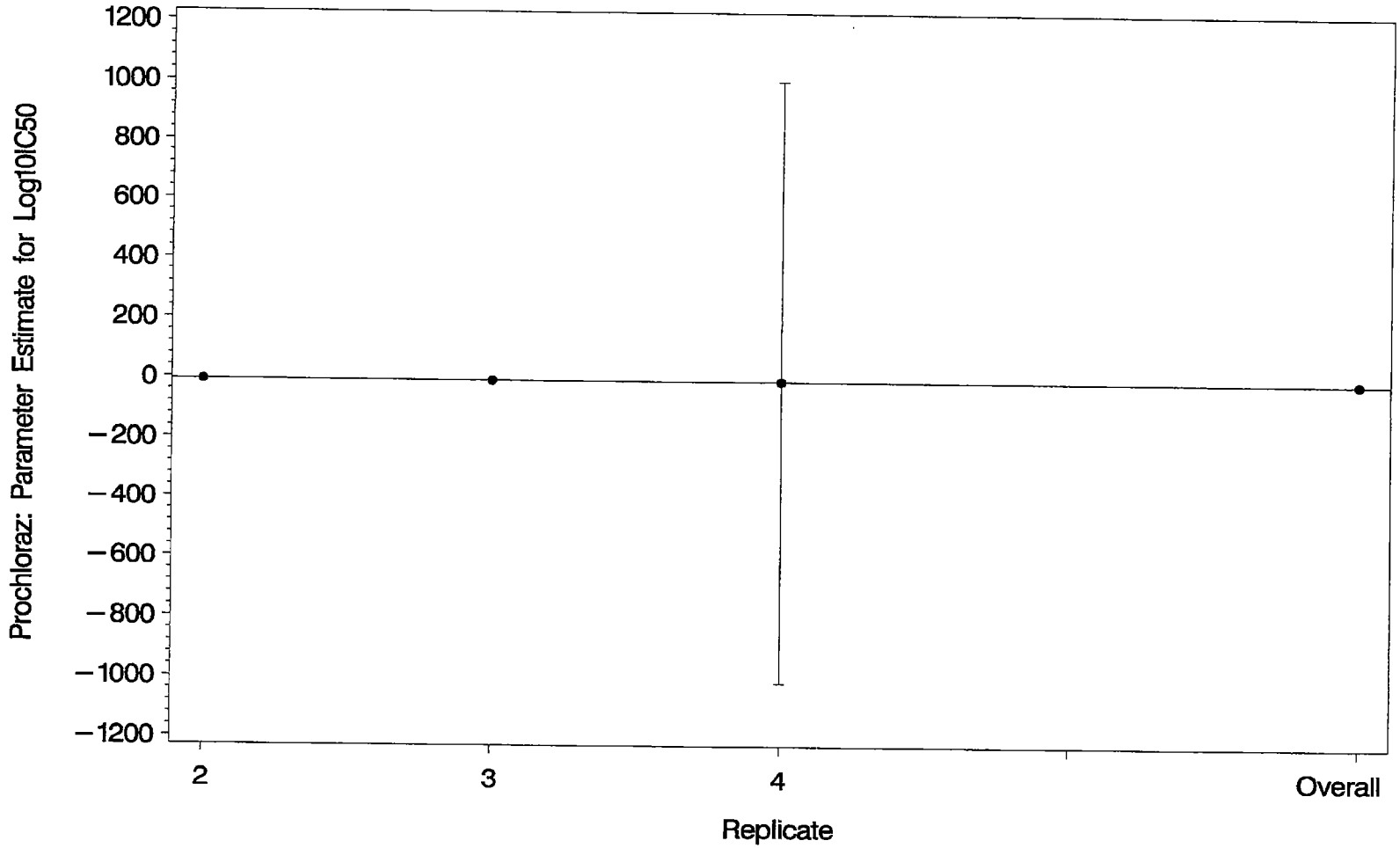


Figure 3-5. **Reference Chemical III: Prochloraz. Microsomes Prepared by Battelle. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

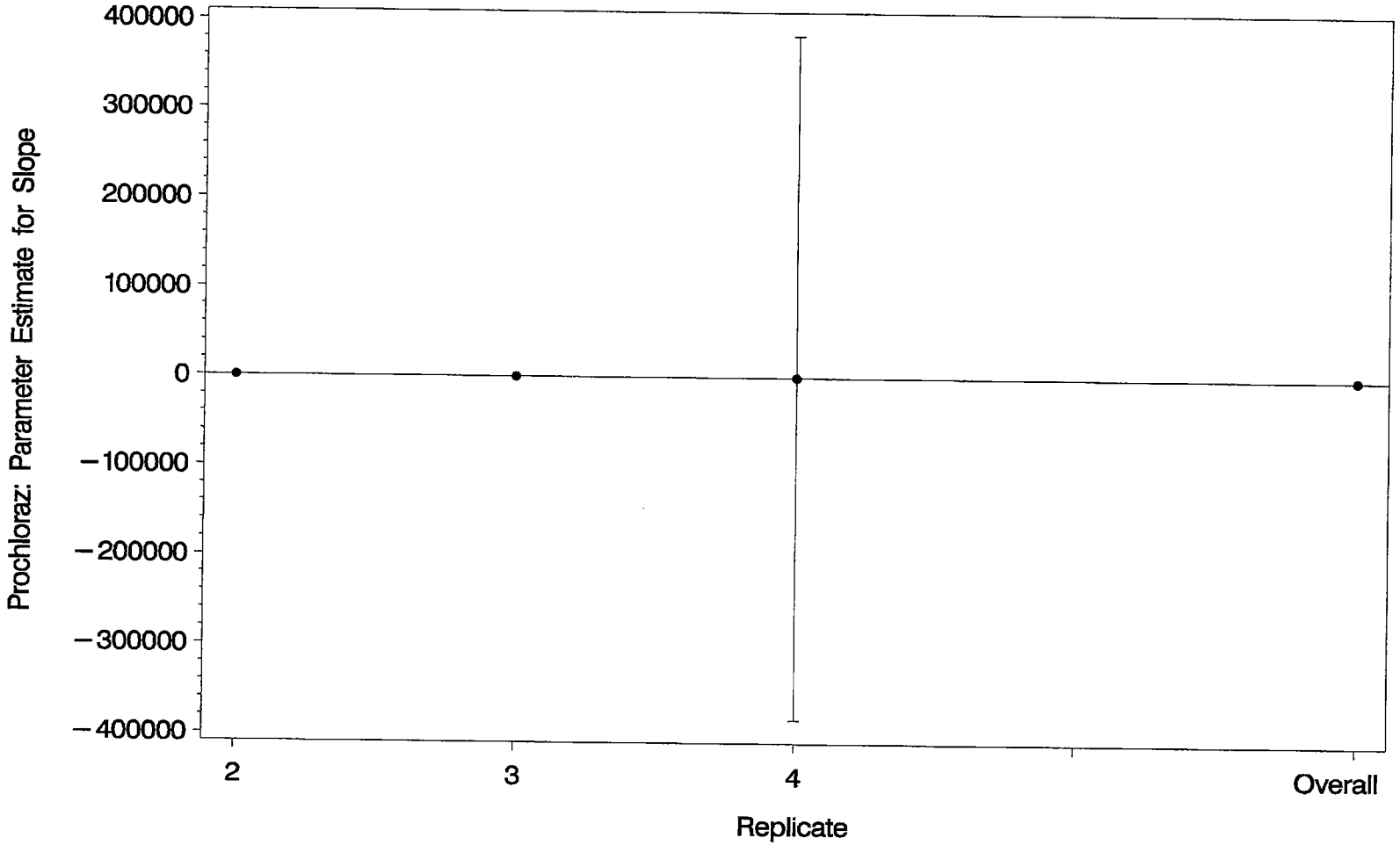


Figure 3-6. **Reference Chemical III: Prochloraz. Microsomes Prepared by Battelle. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

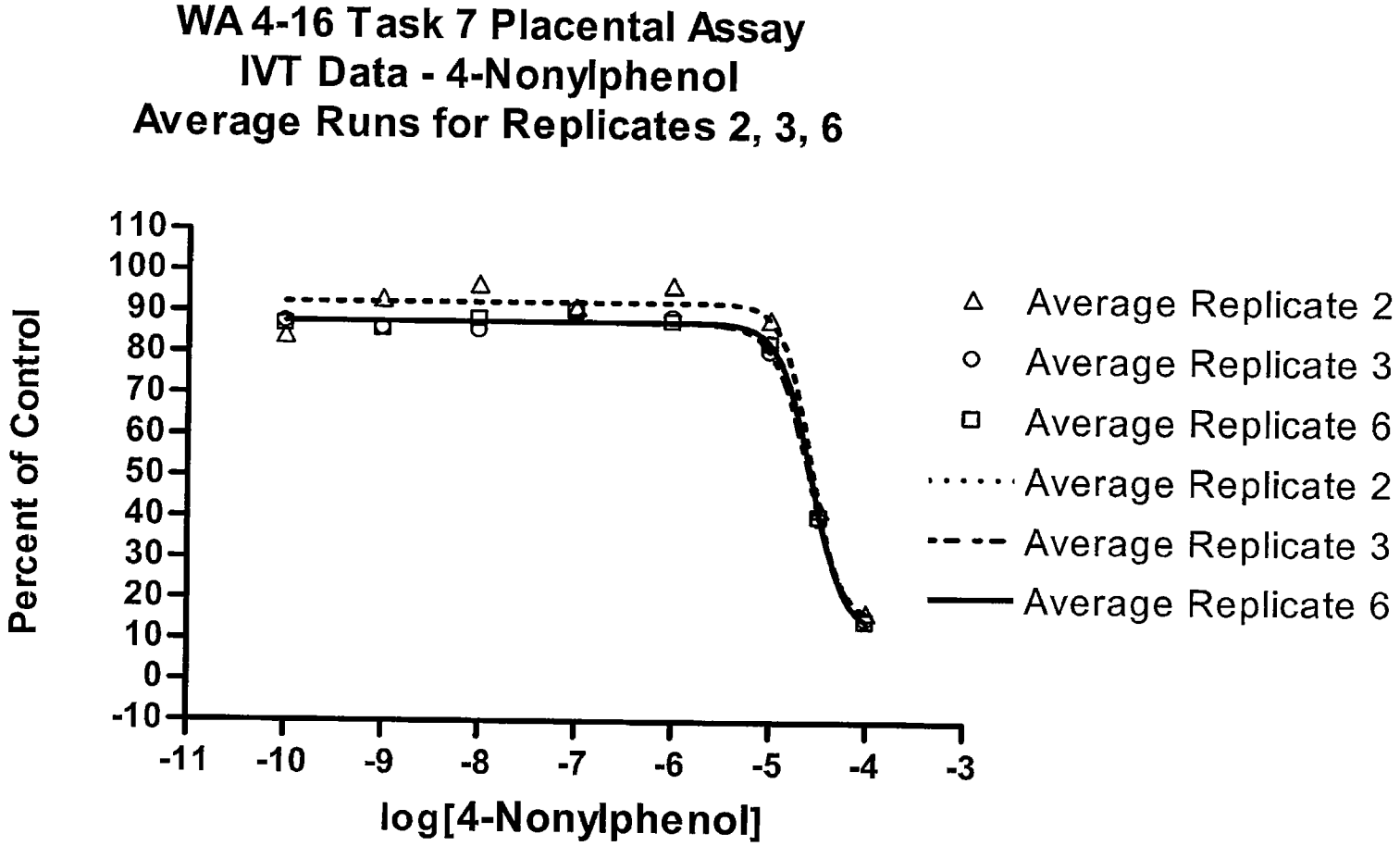


Figure 4-1. **Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

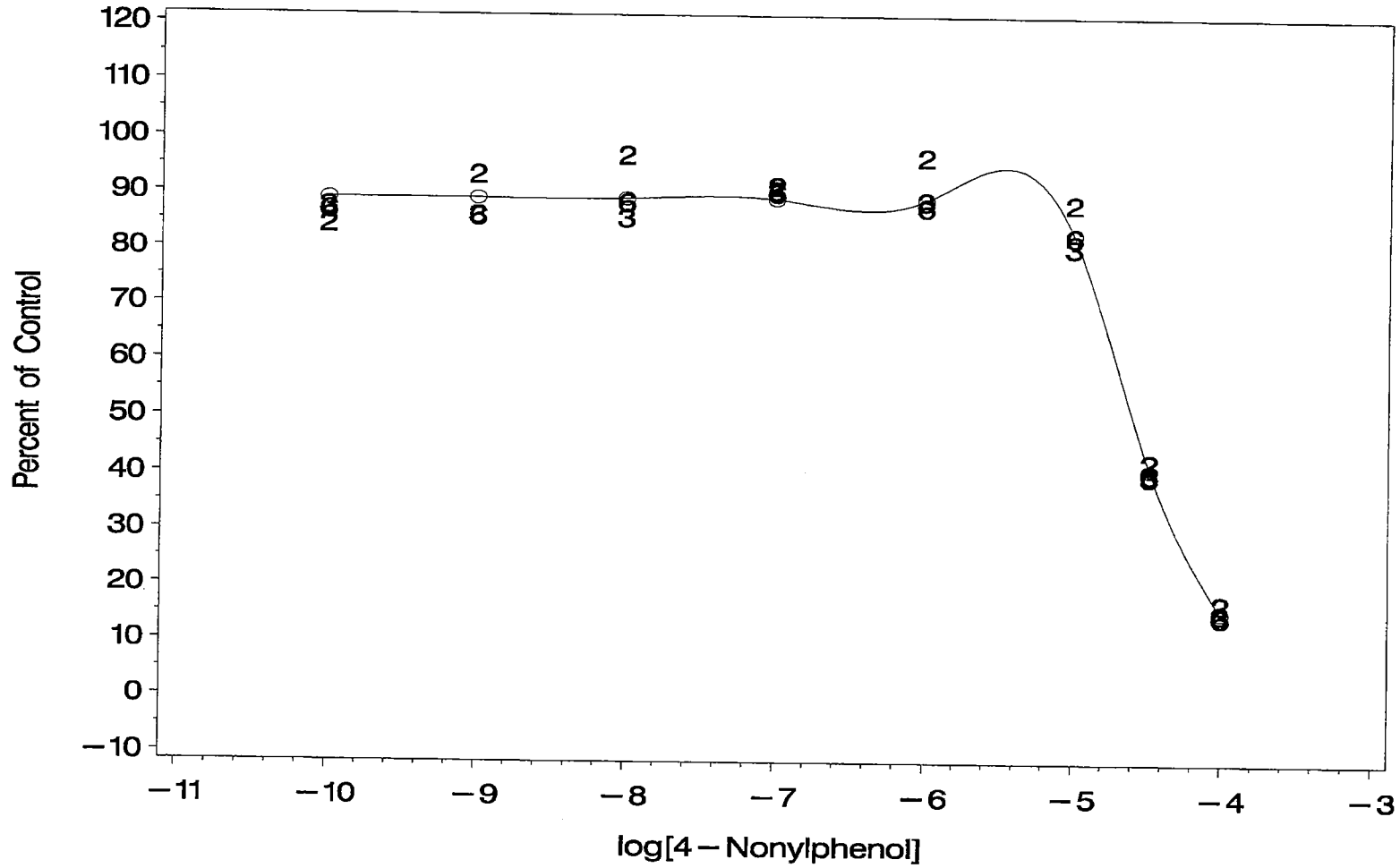


Figure 4-2. **Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

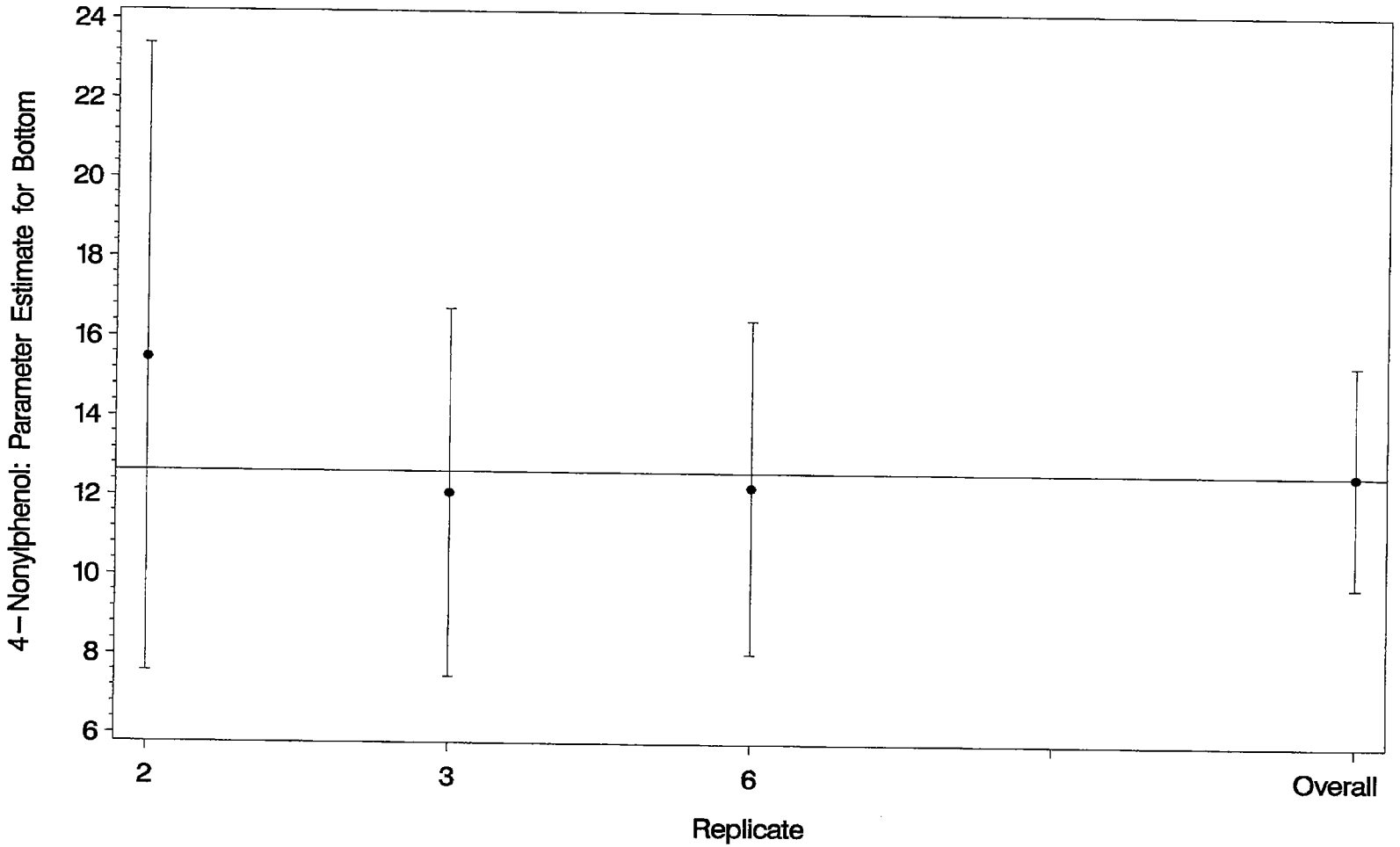


Figure 4-3. **Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

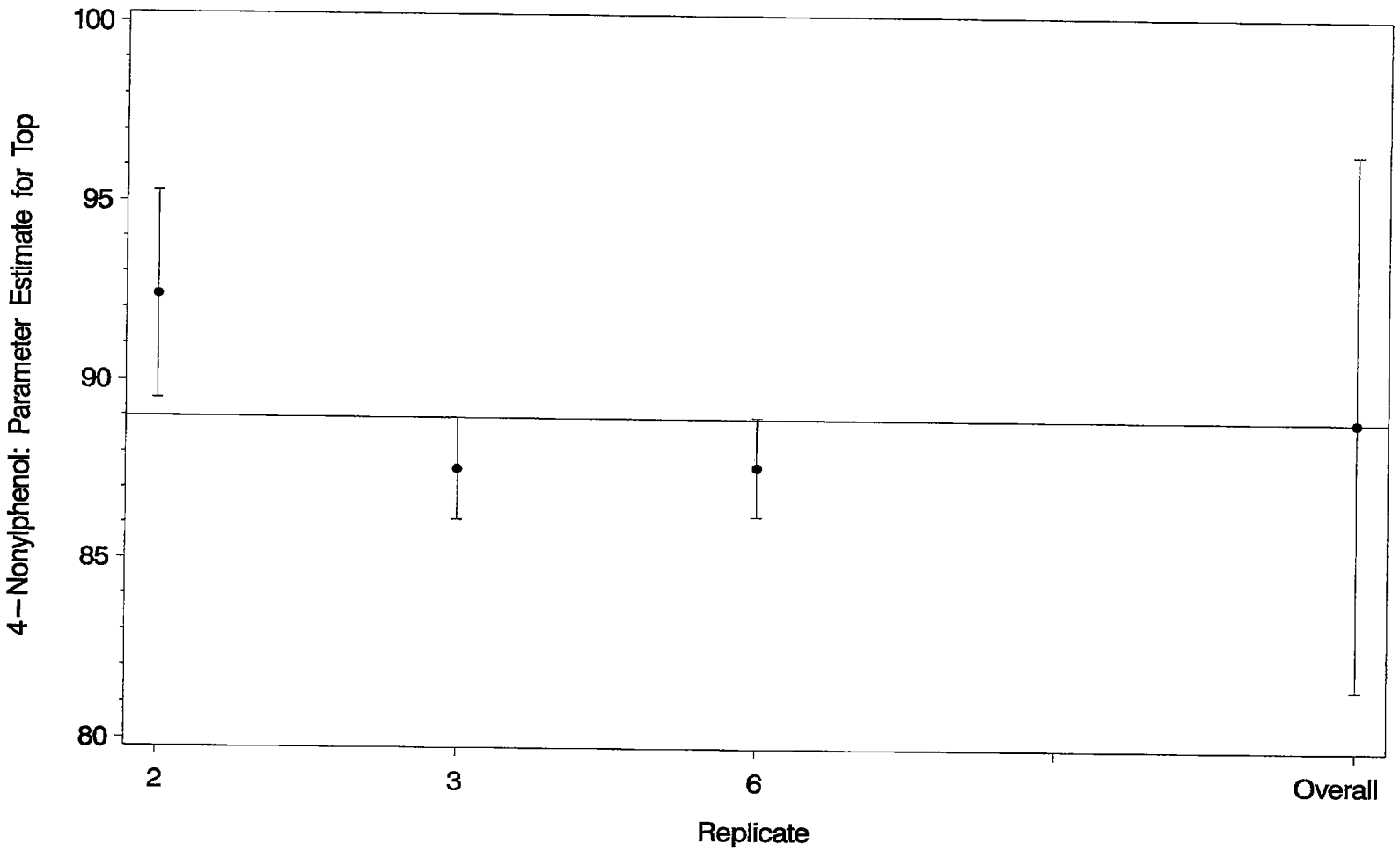


Figure 4-4. **Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

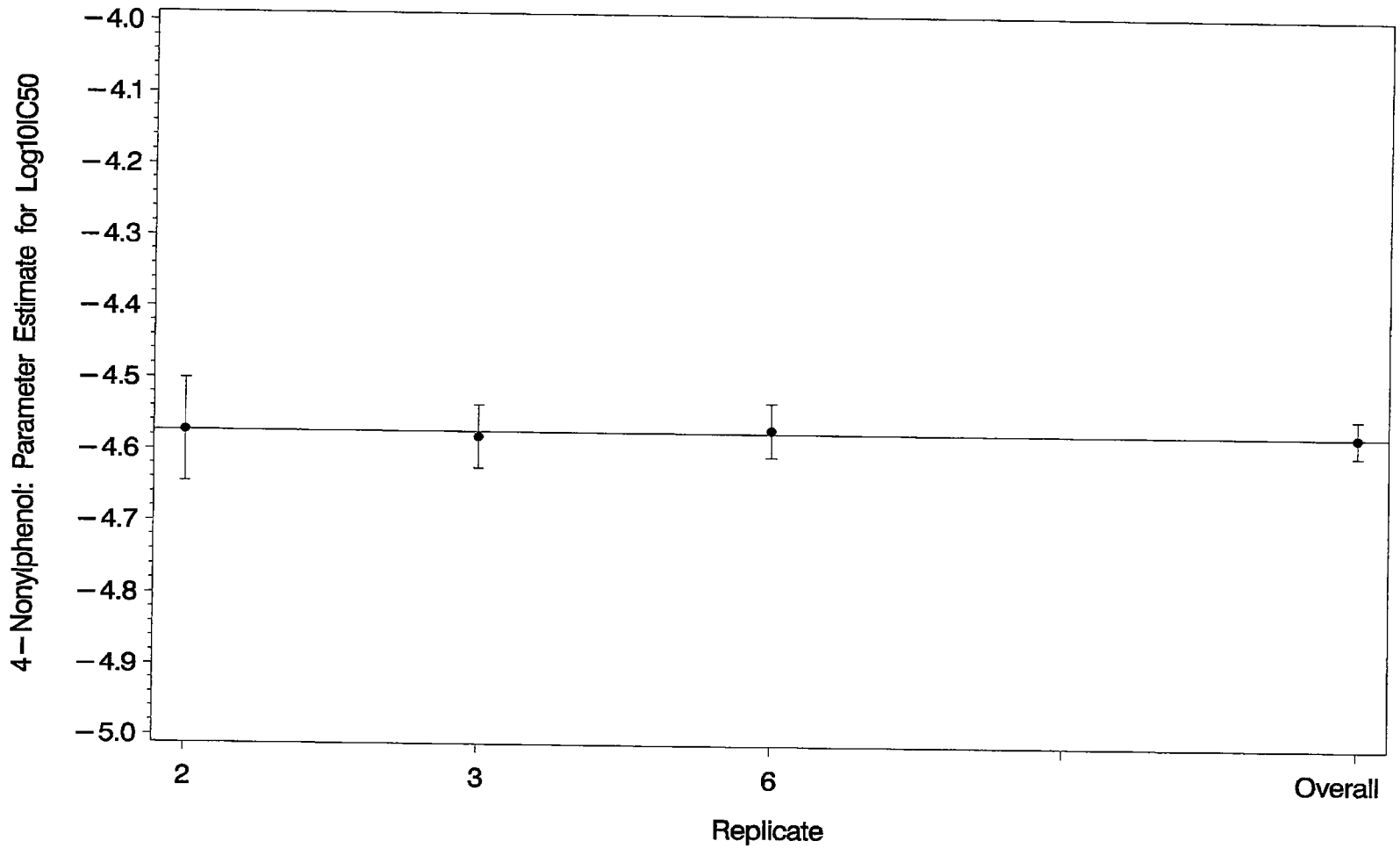


Figure 4-5. **Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

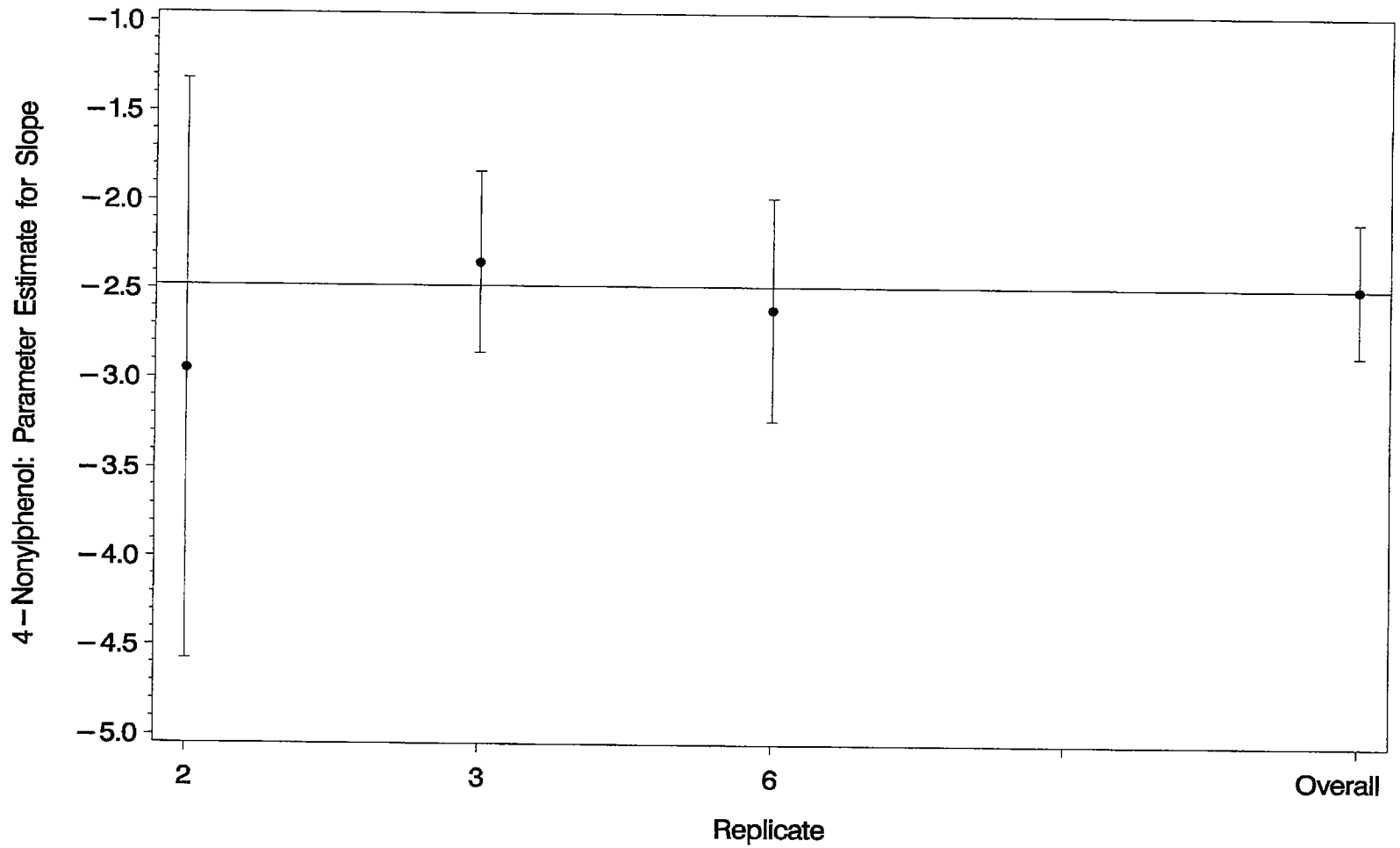


Figure 4-6. **Reference Chemical IV: 4-Nonylphenol. Microsomes Prepared by IVT. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

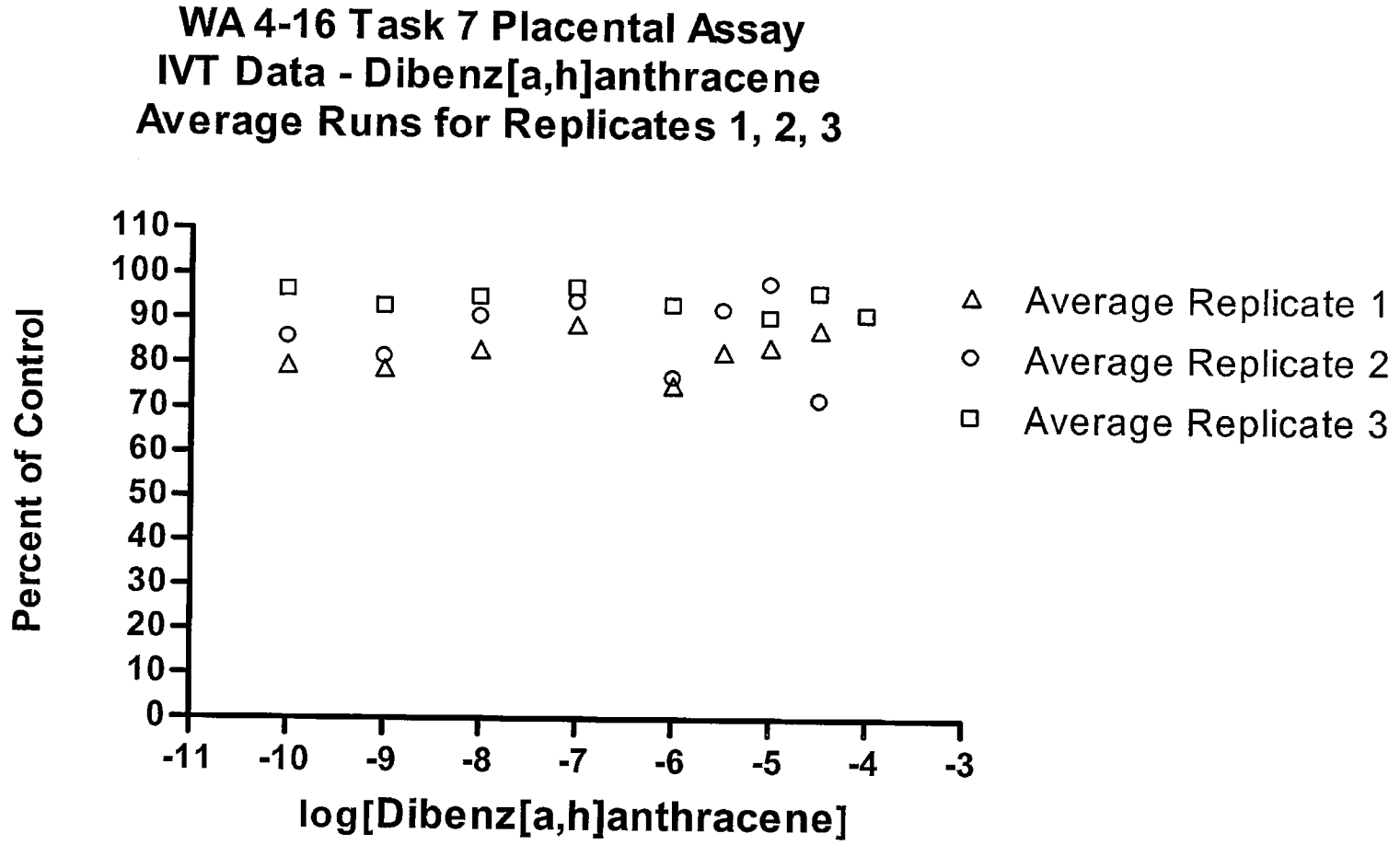


Figure 5-1. **Reference Chemical V: Dibenz[a, h]anthracene. Microsomes Prepared by Battelle. Scatter Plot for Averages of Repetitions within Concentration for Each Replicate. Placental Aromatase Assay.**

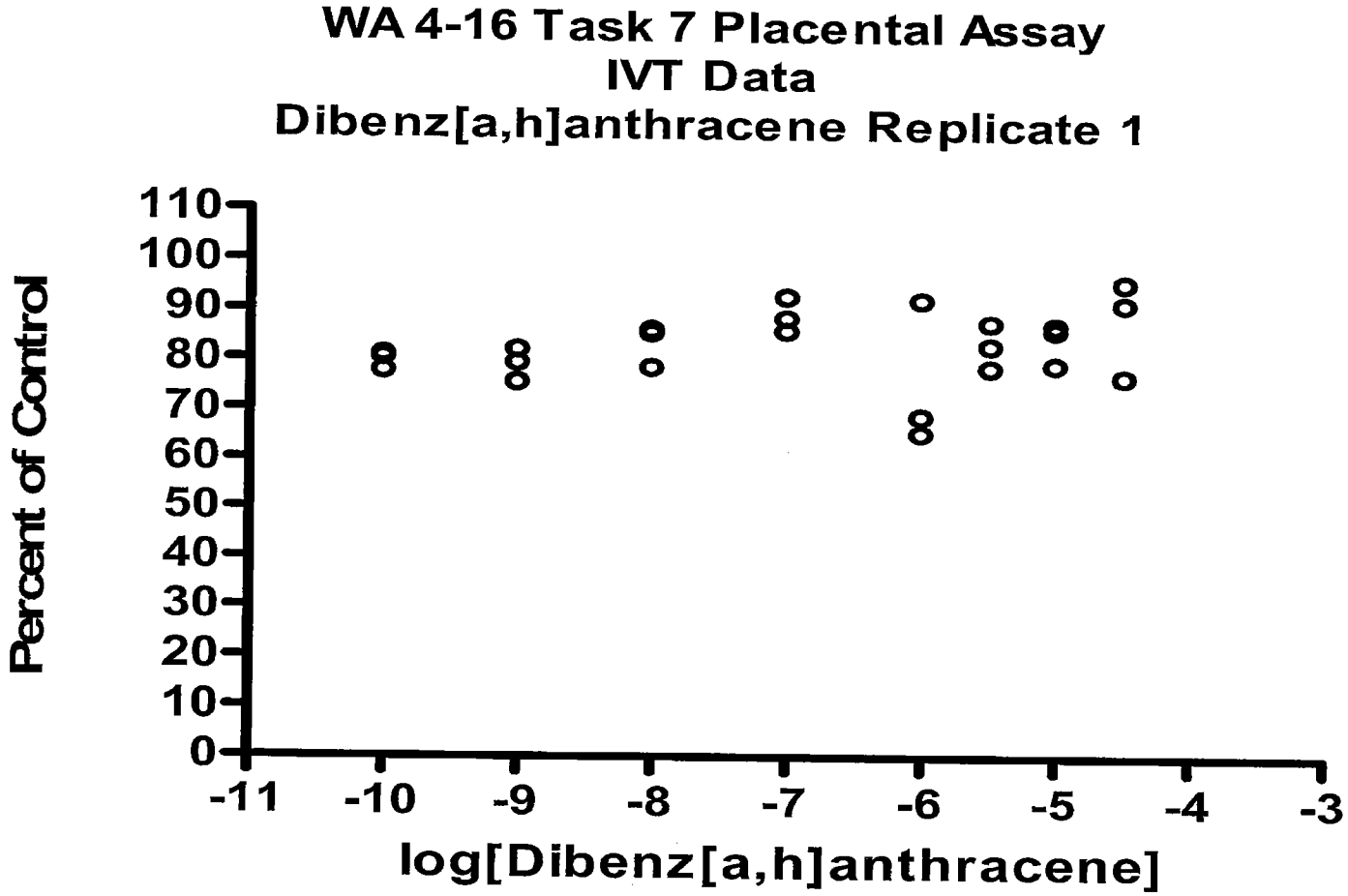


Figure 5-2. **Reference Chemical V: Dibenz[a, h]anthracene. Microsomes Prepared by Battelle. Scatter Plot for Replicate 1 Data. Placental Aromatase Assay.**

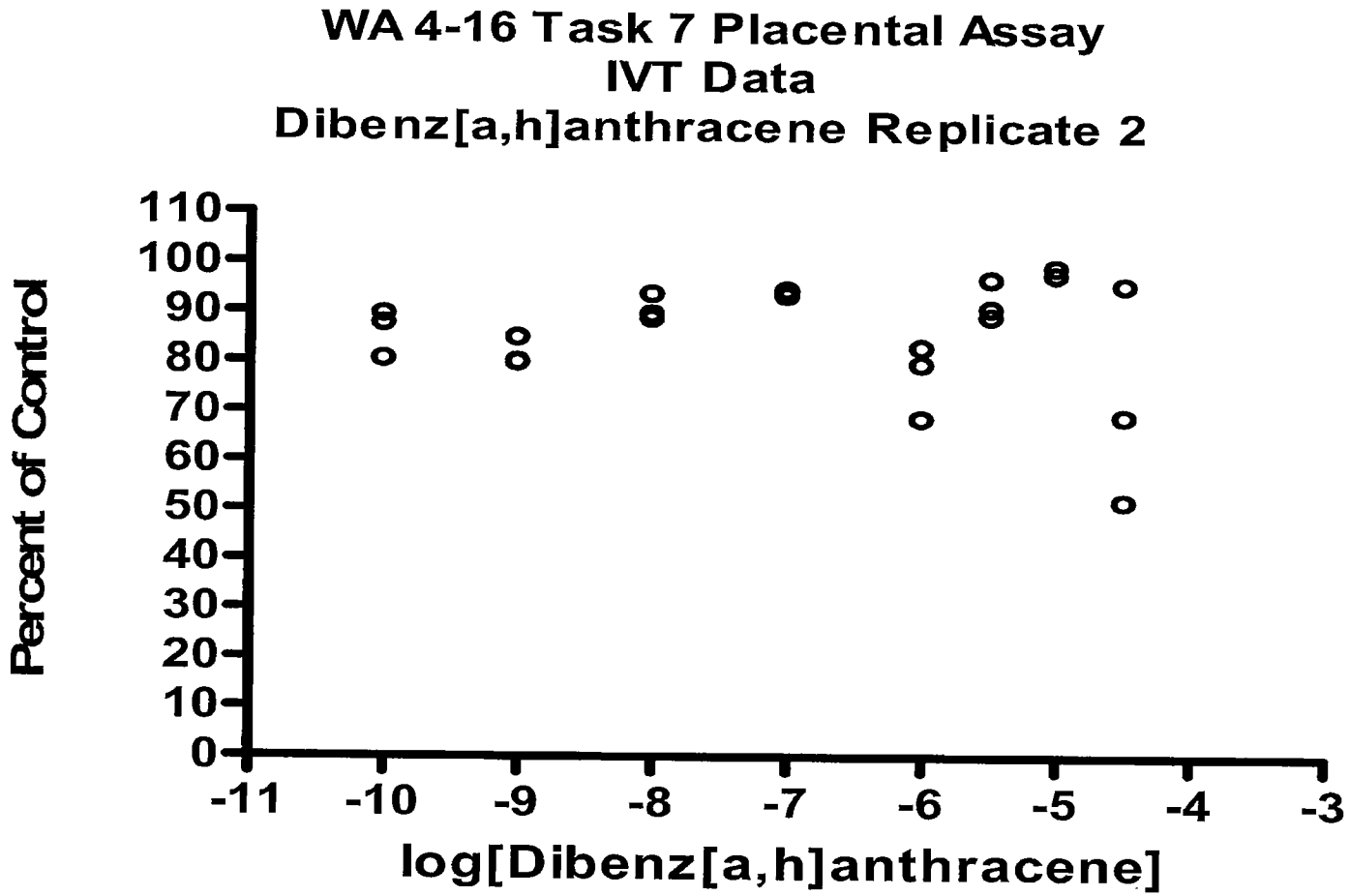


Figure 5-3. **Reference Chemical V: Dibenz[a, h]anthracene. Microsomes Prepared by Battelle. Scatter Plot for Replicate 2 Data. Placental Aromatase Assay.**

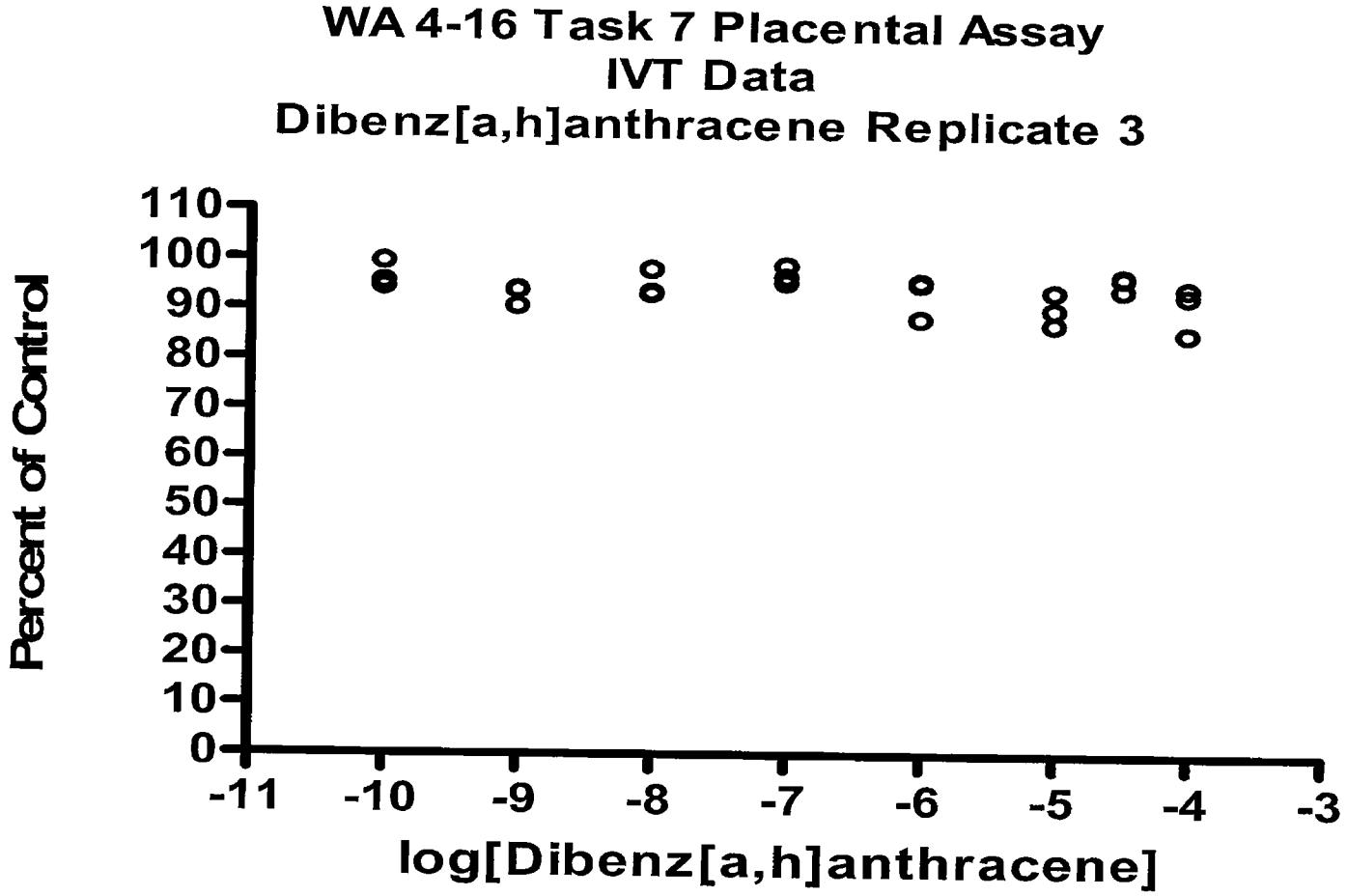


Figure 5-4. **Reference Chemical V: Dibenz[a, h]anthracene. Microsomes Prepared by Battelle. Scatter Plot for Replicate 3 Data. Placental Aromatase Assay.**

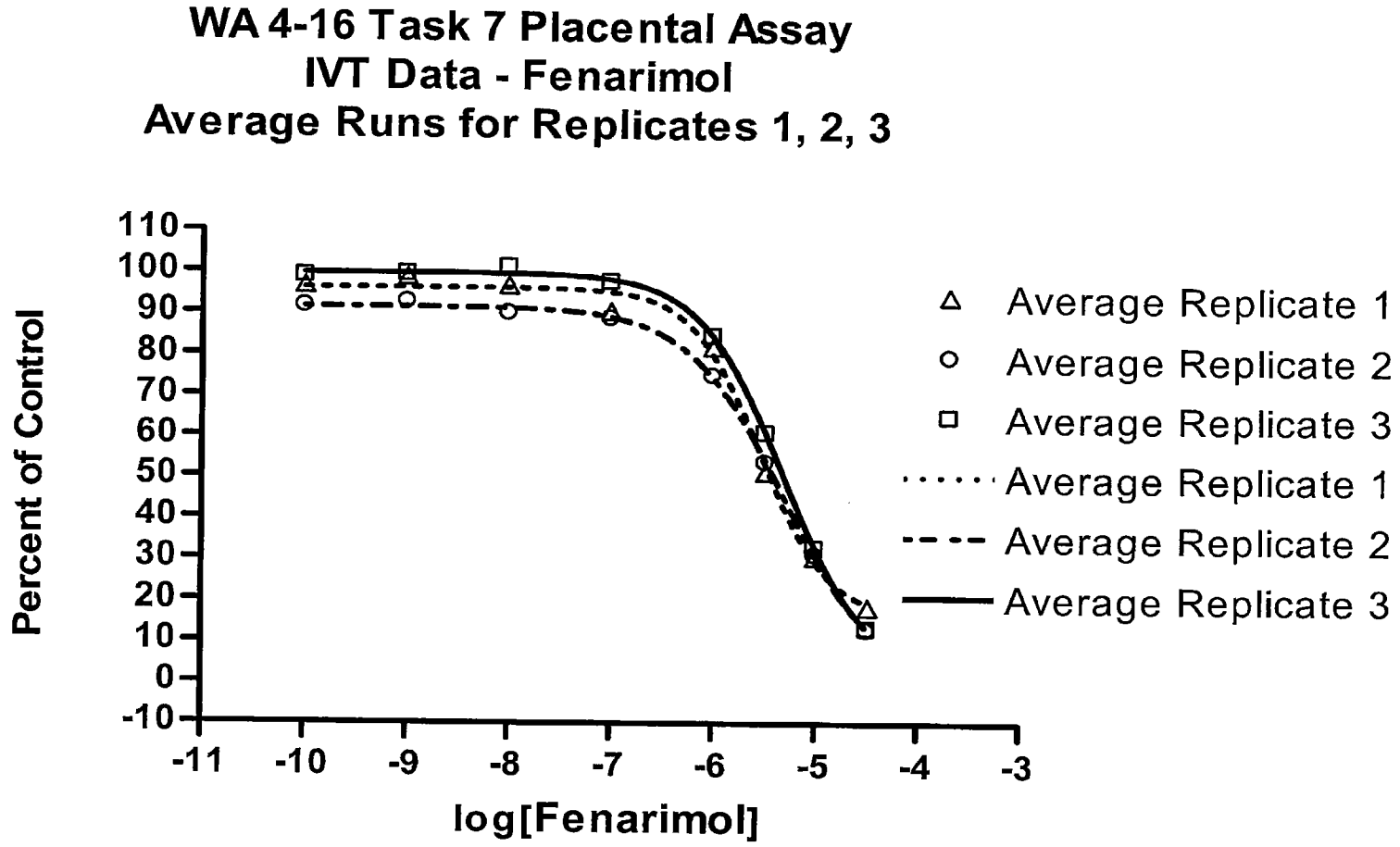


Figure 6-1. **Reference Chemical VI: Fenarimol. Microsomes Prepared by Battelle. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

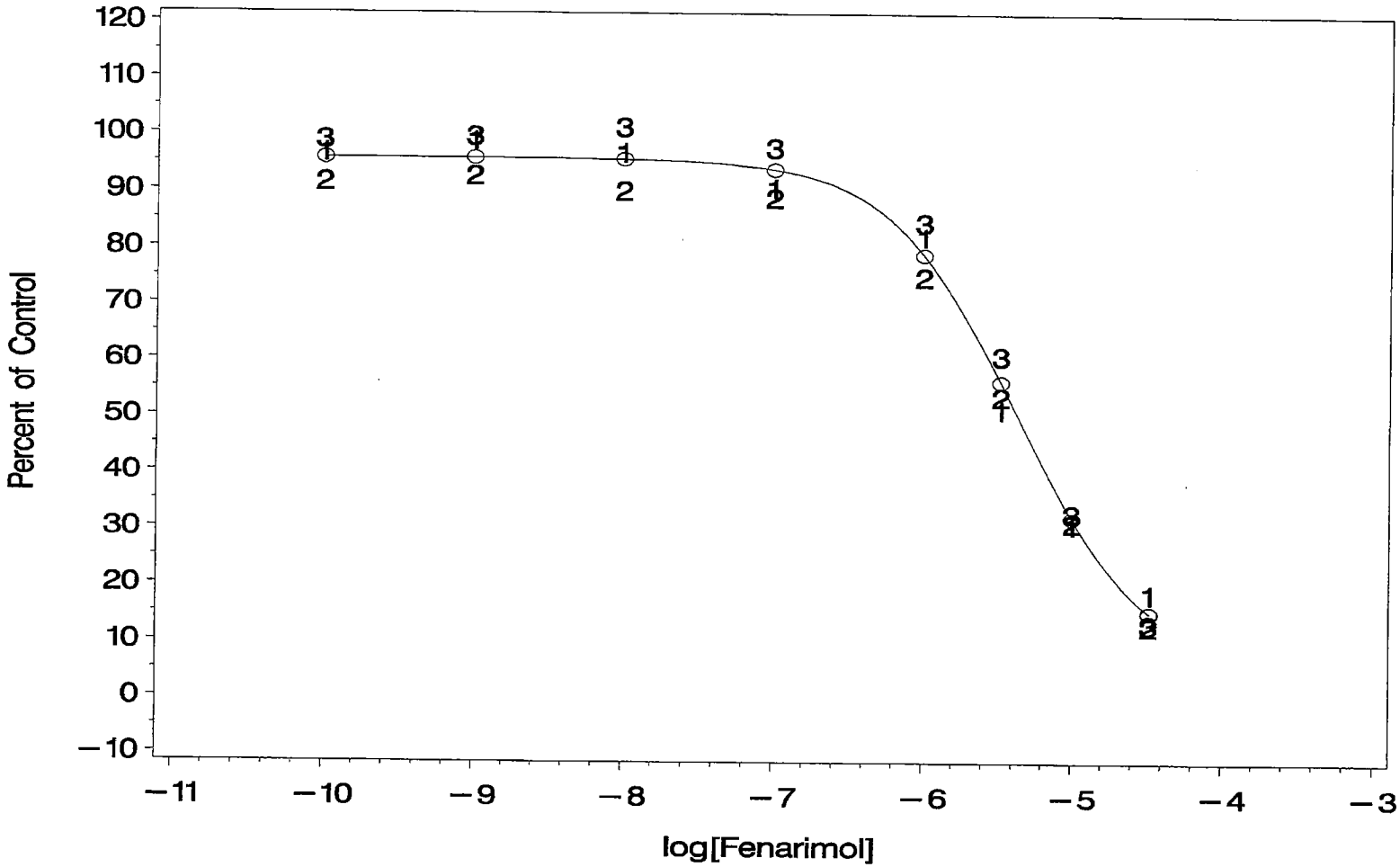


Figure 6-2. **Reference Chemical VI: Fenarimol. Microsomes Prepared by Battelle. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

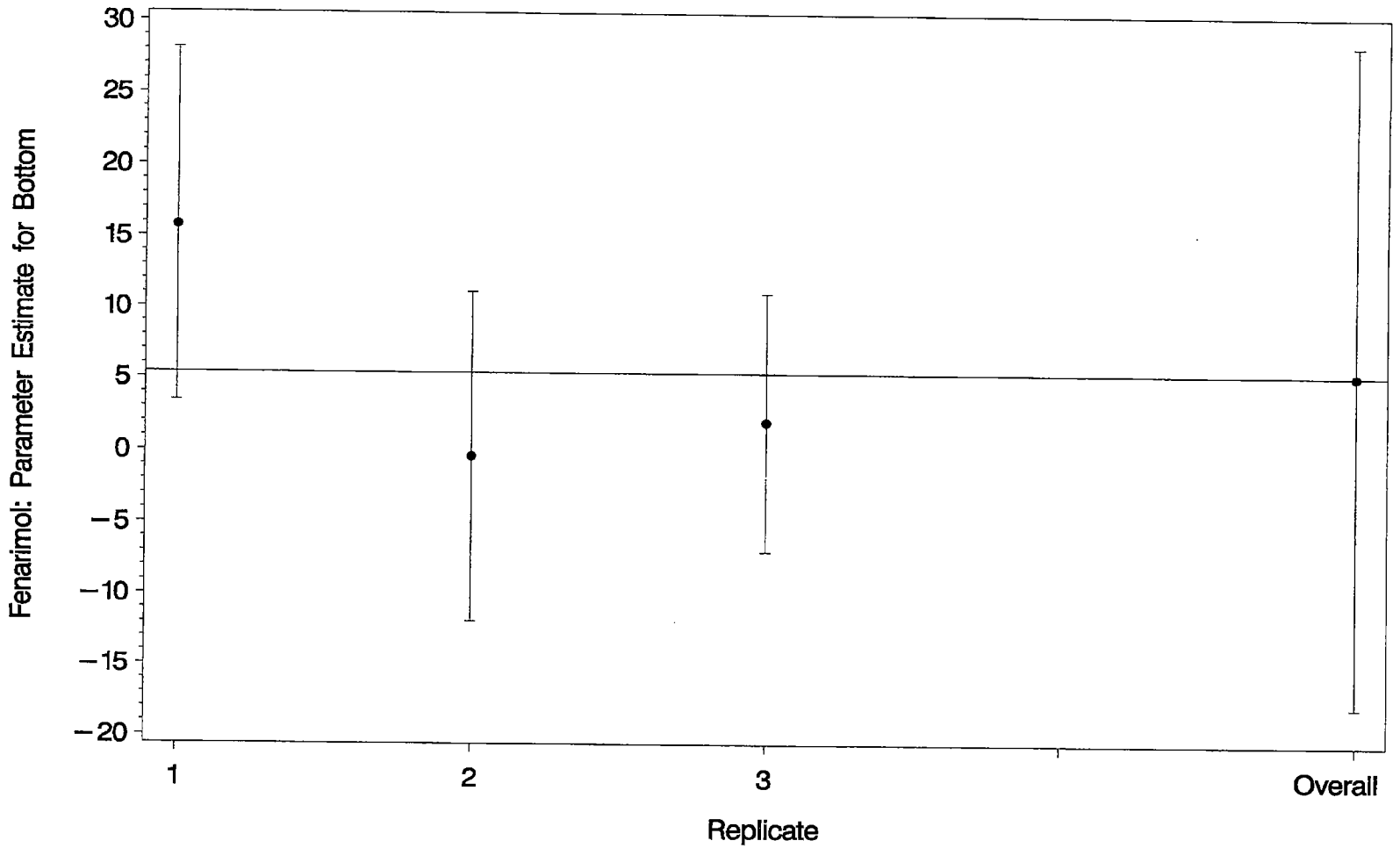


Figure 6-3. **Reference Chemical VI: Fenarimol. Microsomes Prepared by Battelle. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

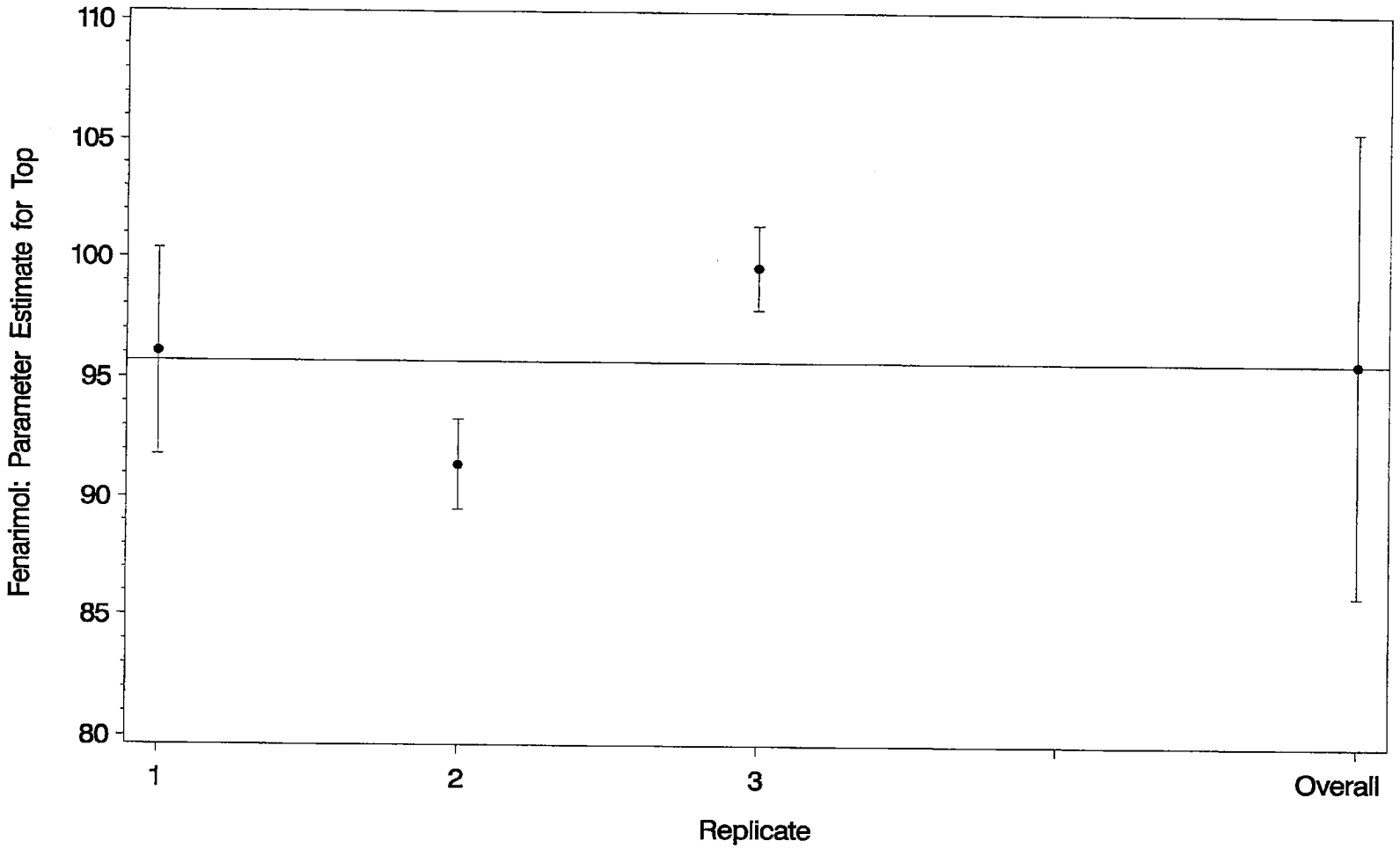


Figure 6-4. **Reference Chemical VI: Fenarimol. Microsomes Prepared by Battelle. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

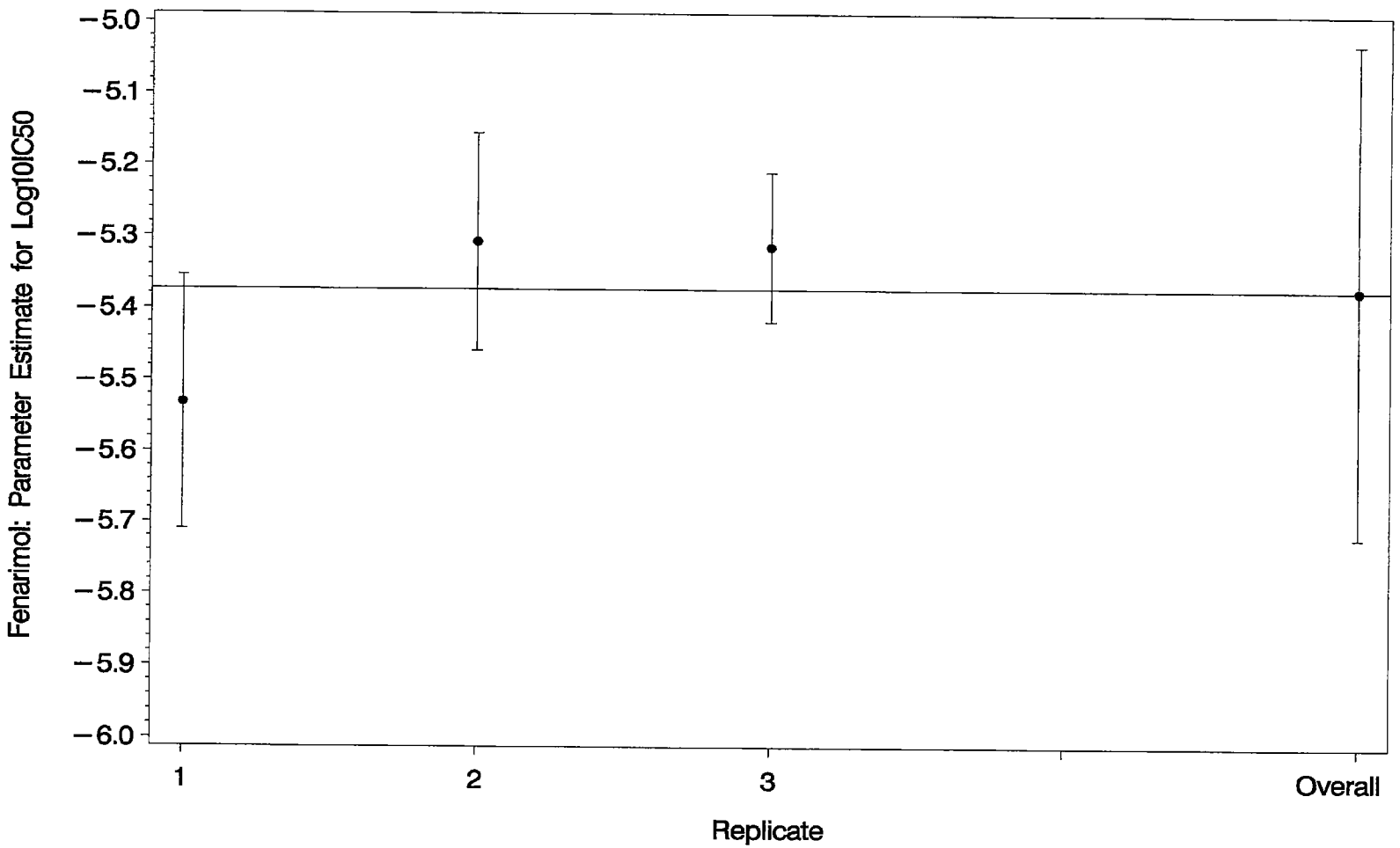


Figure 6-5. **Reference Chemical VI: Fenarimol. Microsomes Prepared by Battelle. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

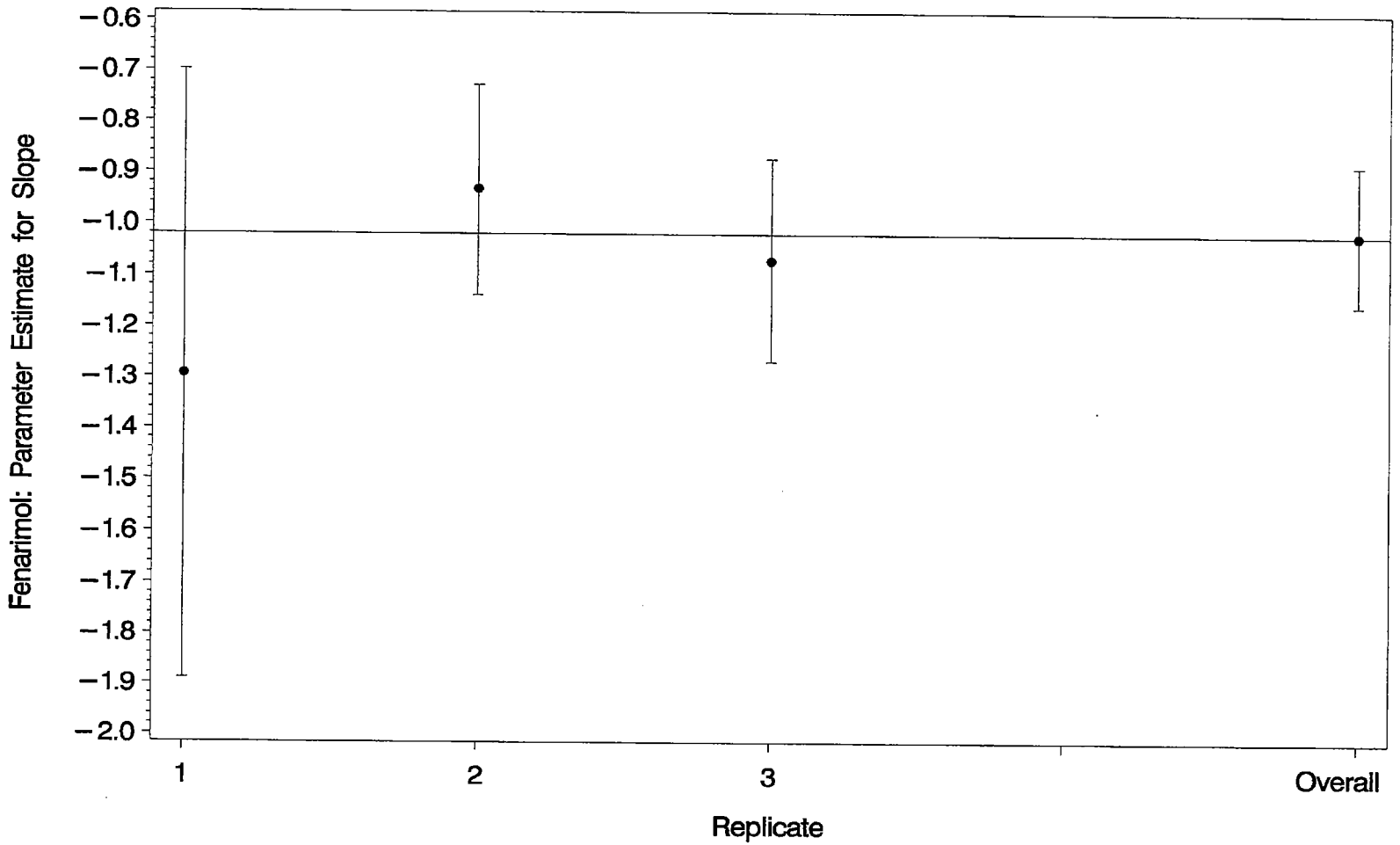


Figure 6-6. **Reference Chemical VI: Fenarimol. Microsomes Prepared by Battelle. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

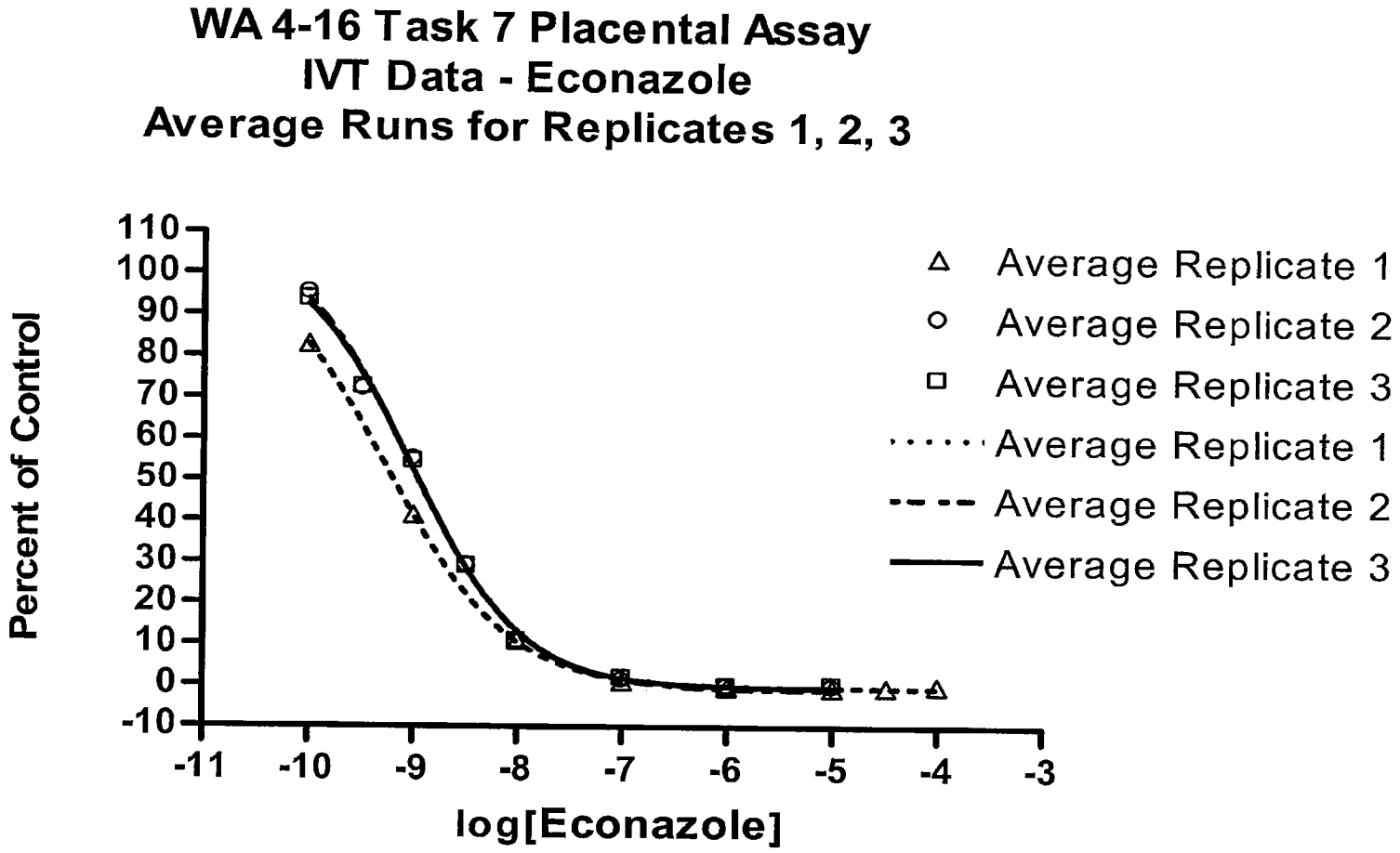


Figure 7-1. **Reference Chemical VII: Econazole. Microsomes Prepared by Battelle. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

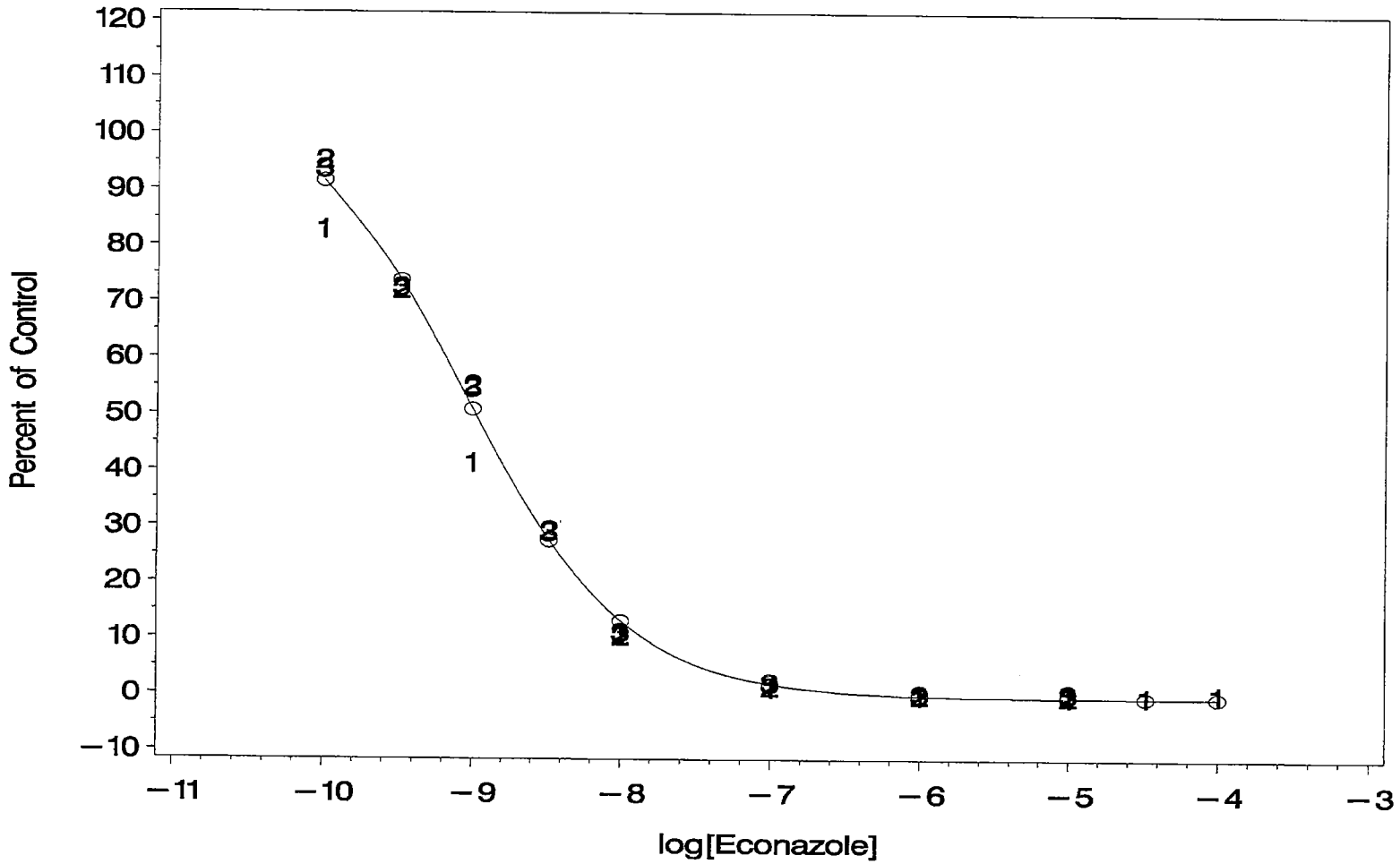


Figure 7-2. **Reference Chemical VII: Econazole. Microsomes Prepared by Battelle. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

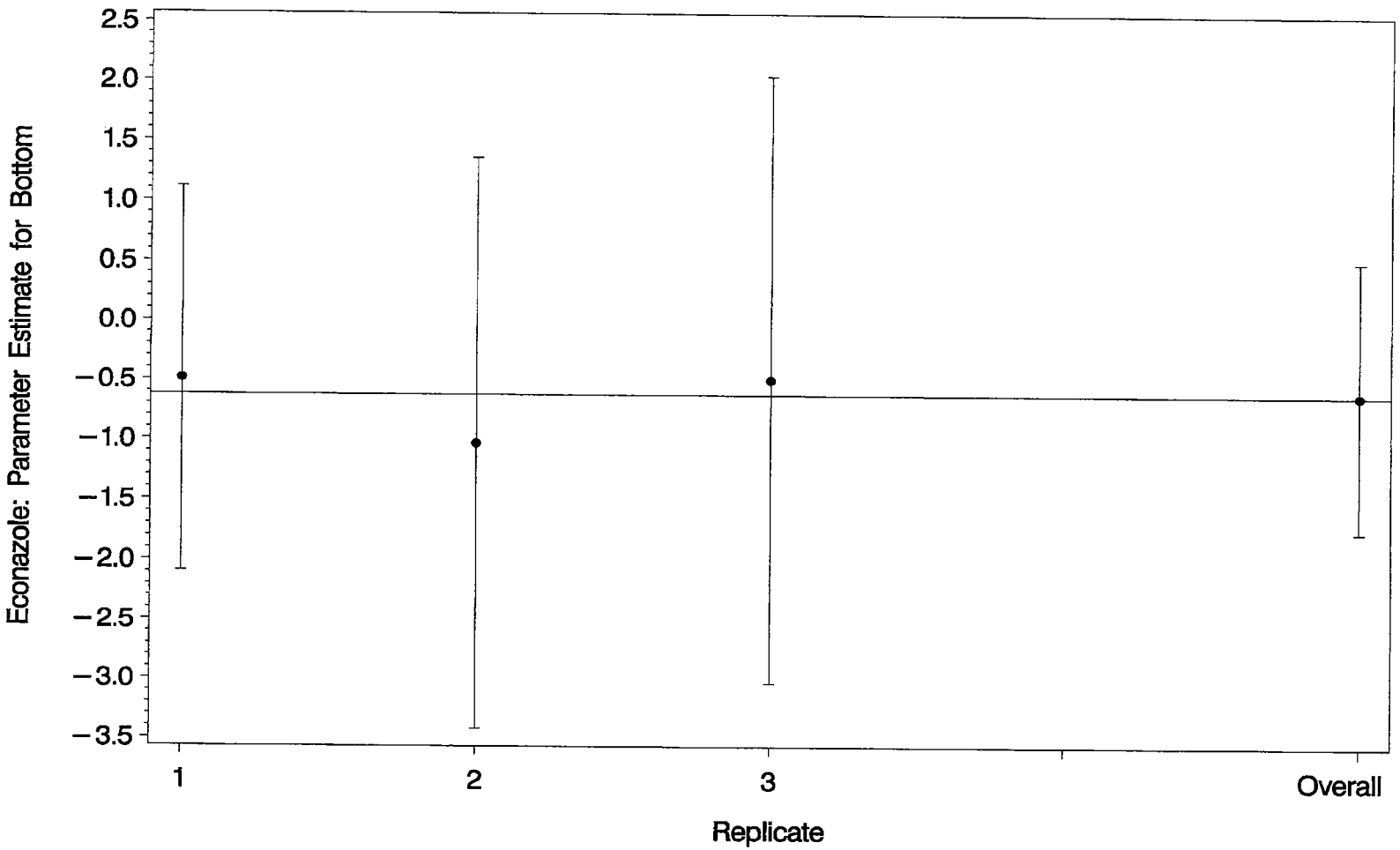


Figure 7-3. **Reference Chemical VII: Econazole. Microsomes Prepared by Battelle. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

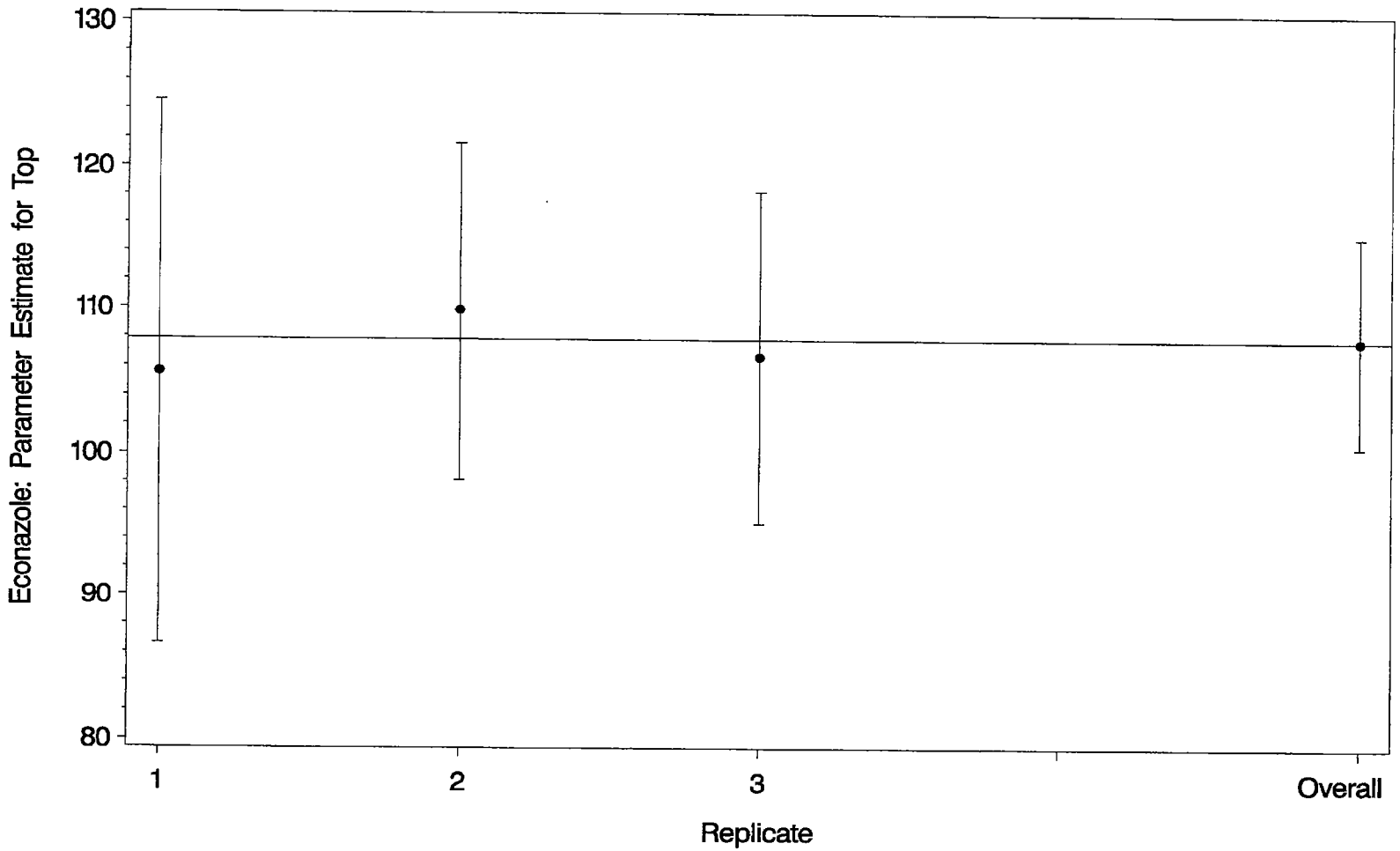


Figure 7-4. **Reference Chemical VII: Econazole. Microsomes Prepared by Battelle. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

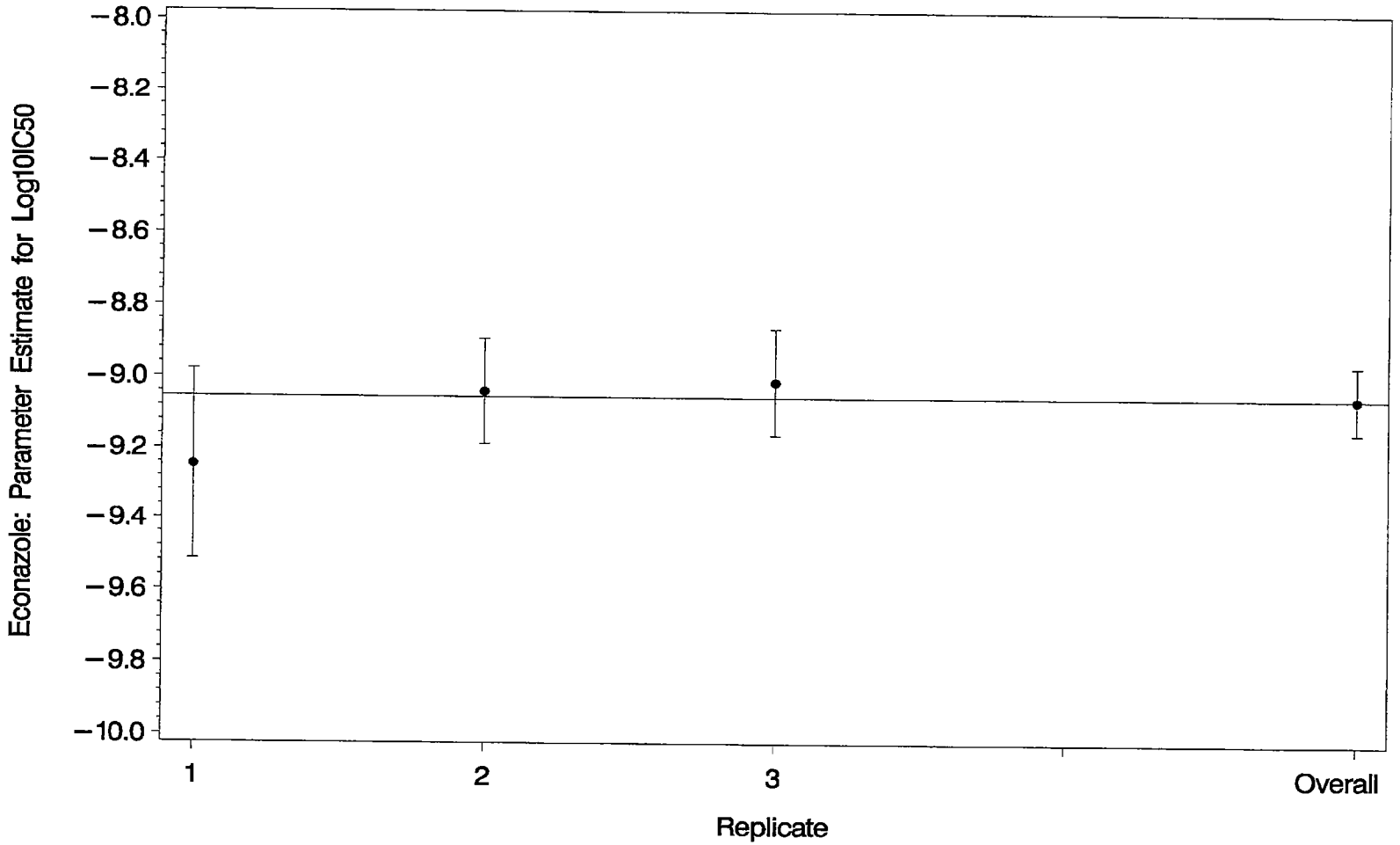


Figure 7-5. **Reference Chemical VII: Econazole. Microsomes Prepared by Battelle. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

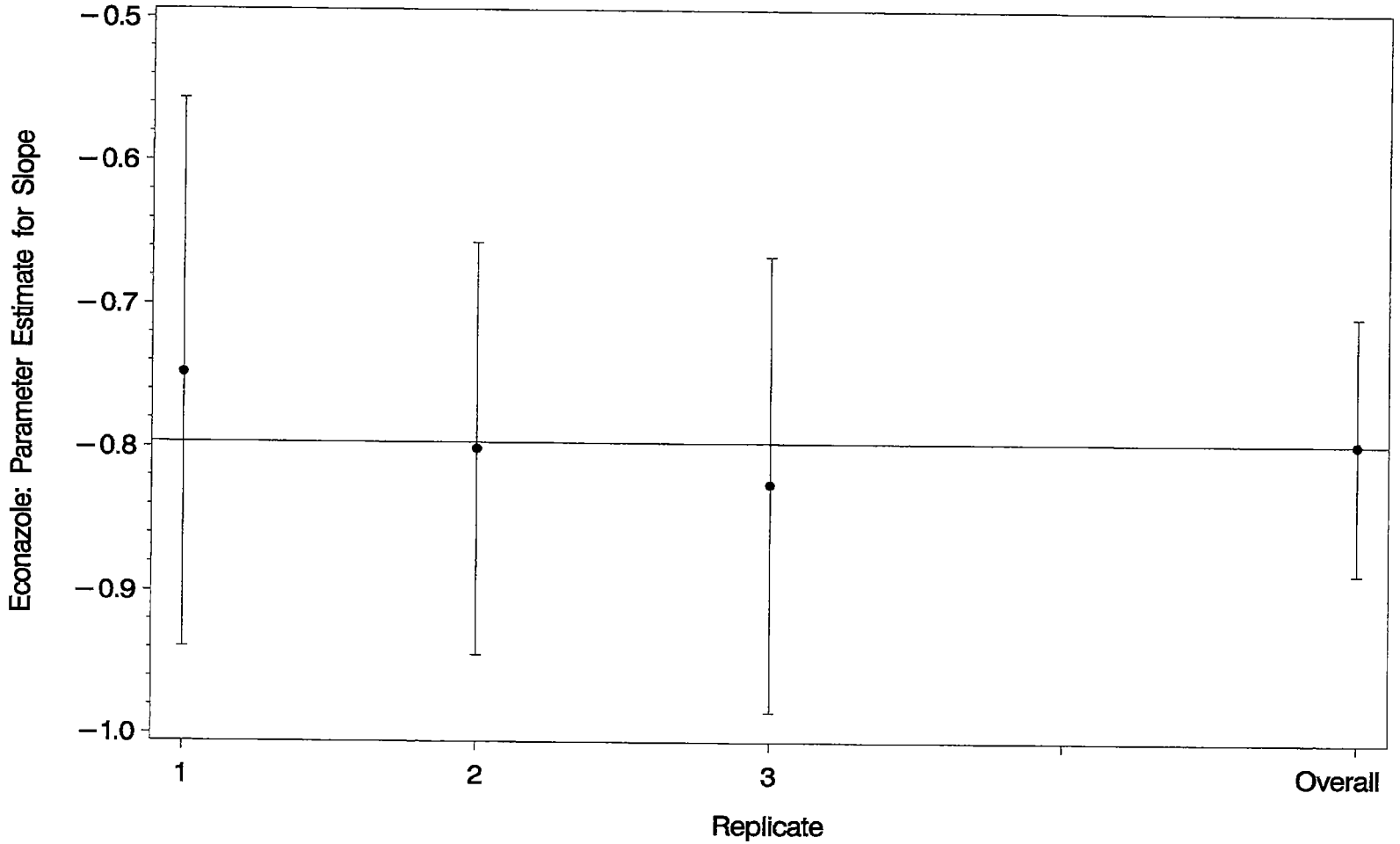


Figure 7-6. **Reference Chemical VII: Econazole. Microsomes Prepared by Battelle. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

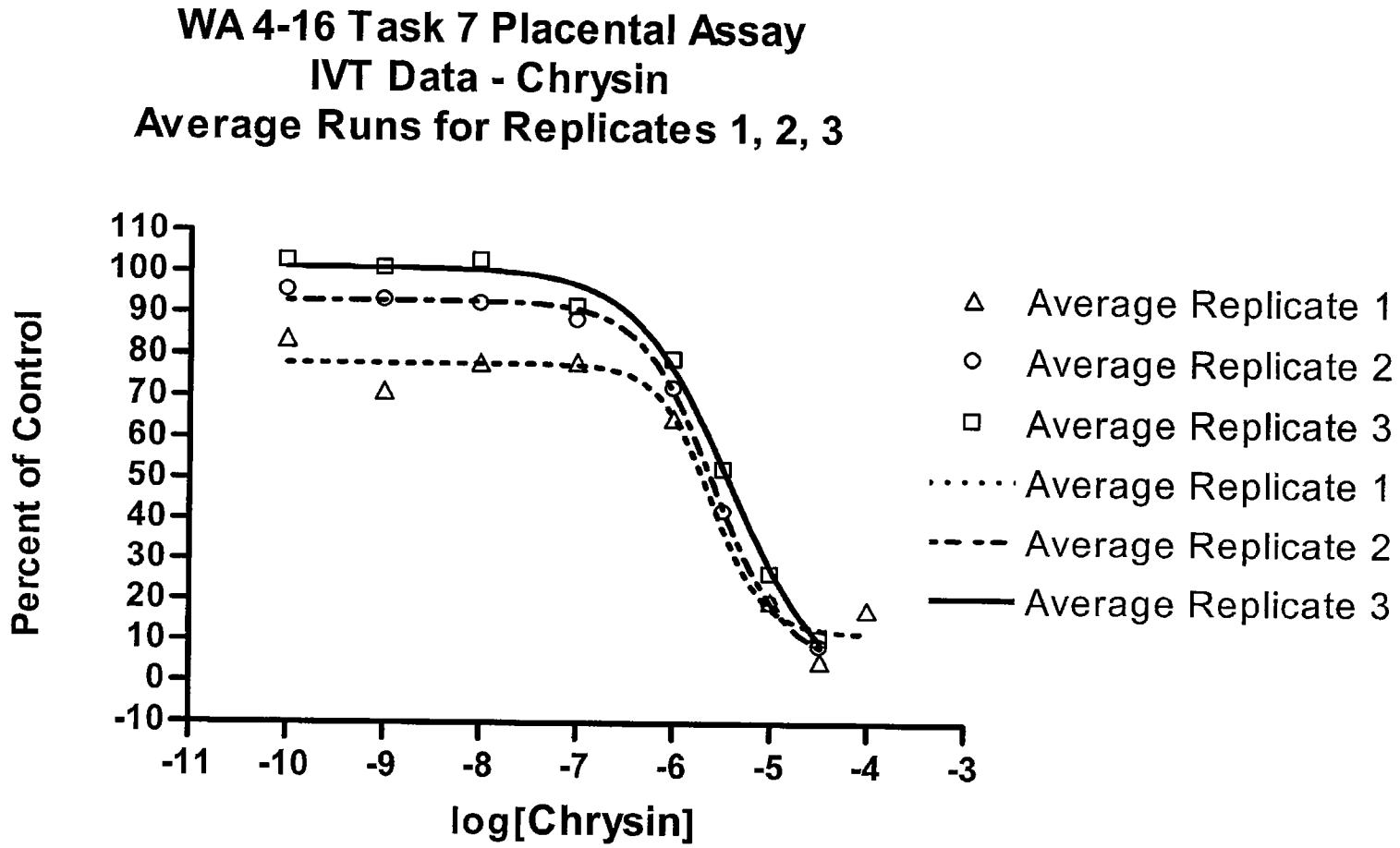


Figure 8-1. **Reference Chemical VIII: Chrysin. Microsomes Prepared by Battelle. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

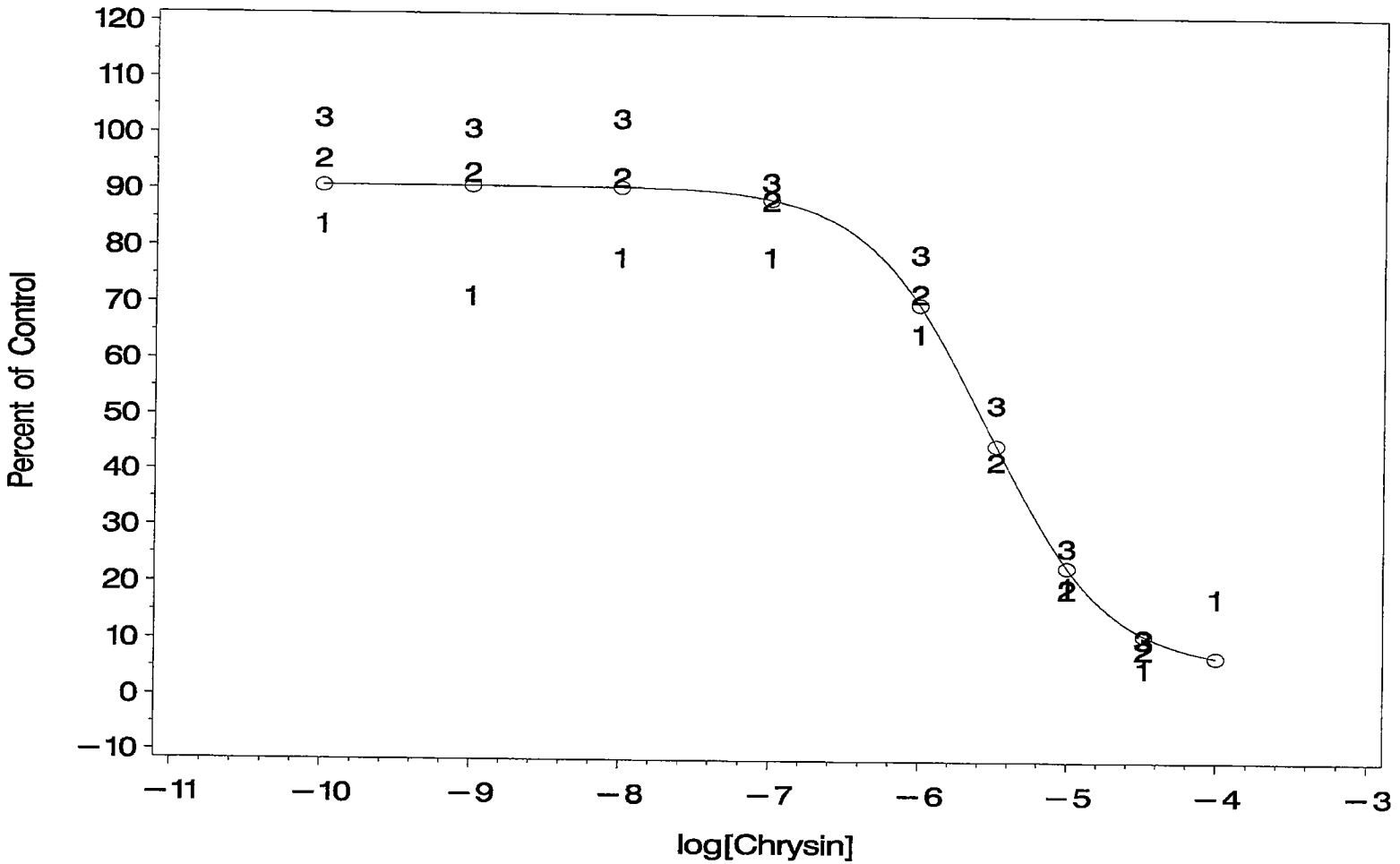


Figure 8-2. **Reference Chemical VIII: Chrysin. Microsomes Prepared by Battelle. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

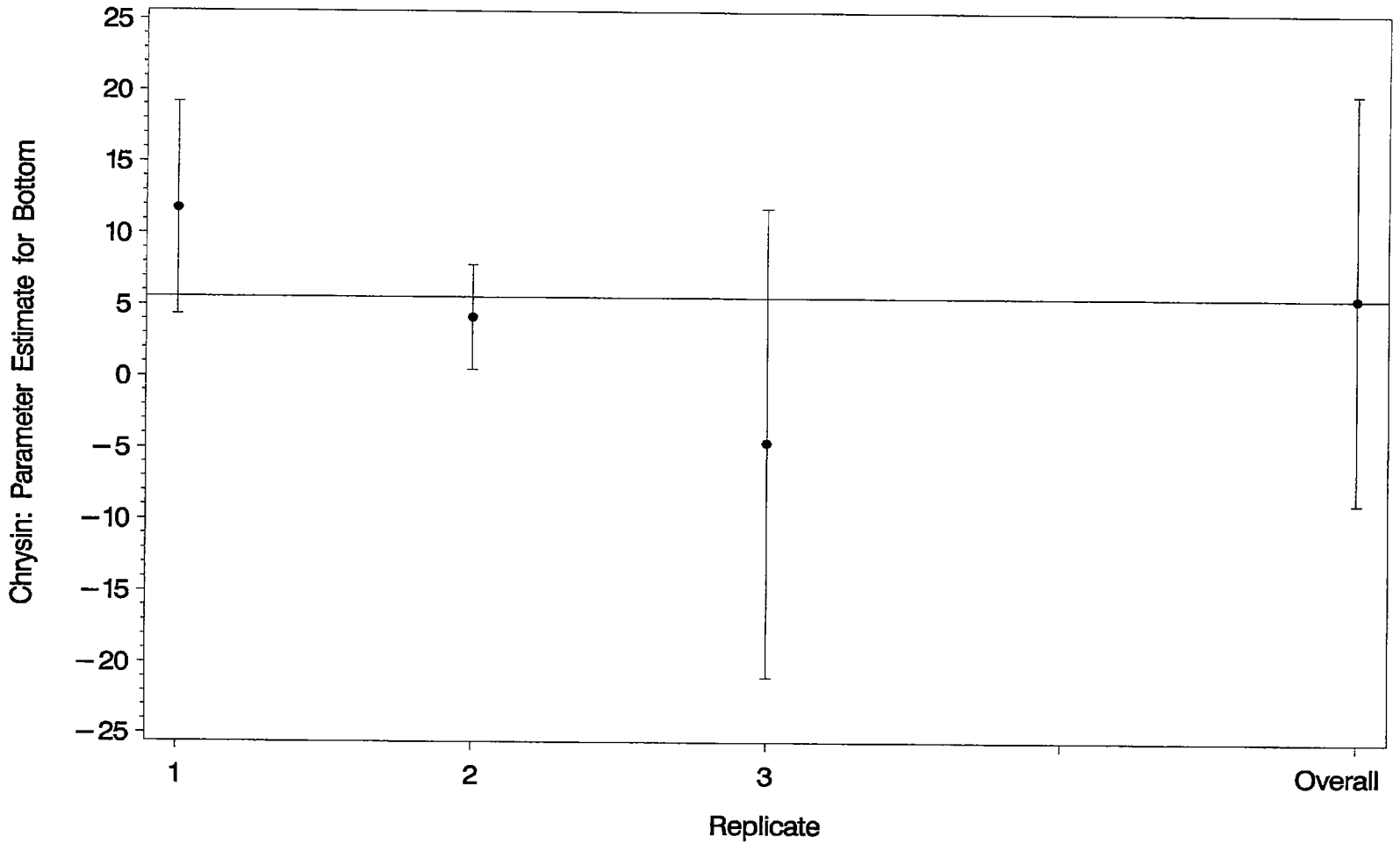


Figure 8-3. **Reference Chemical VIII: Chrysin. Microsomes Prepared by Battelle. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

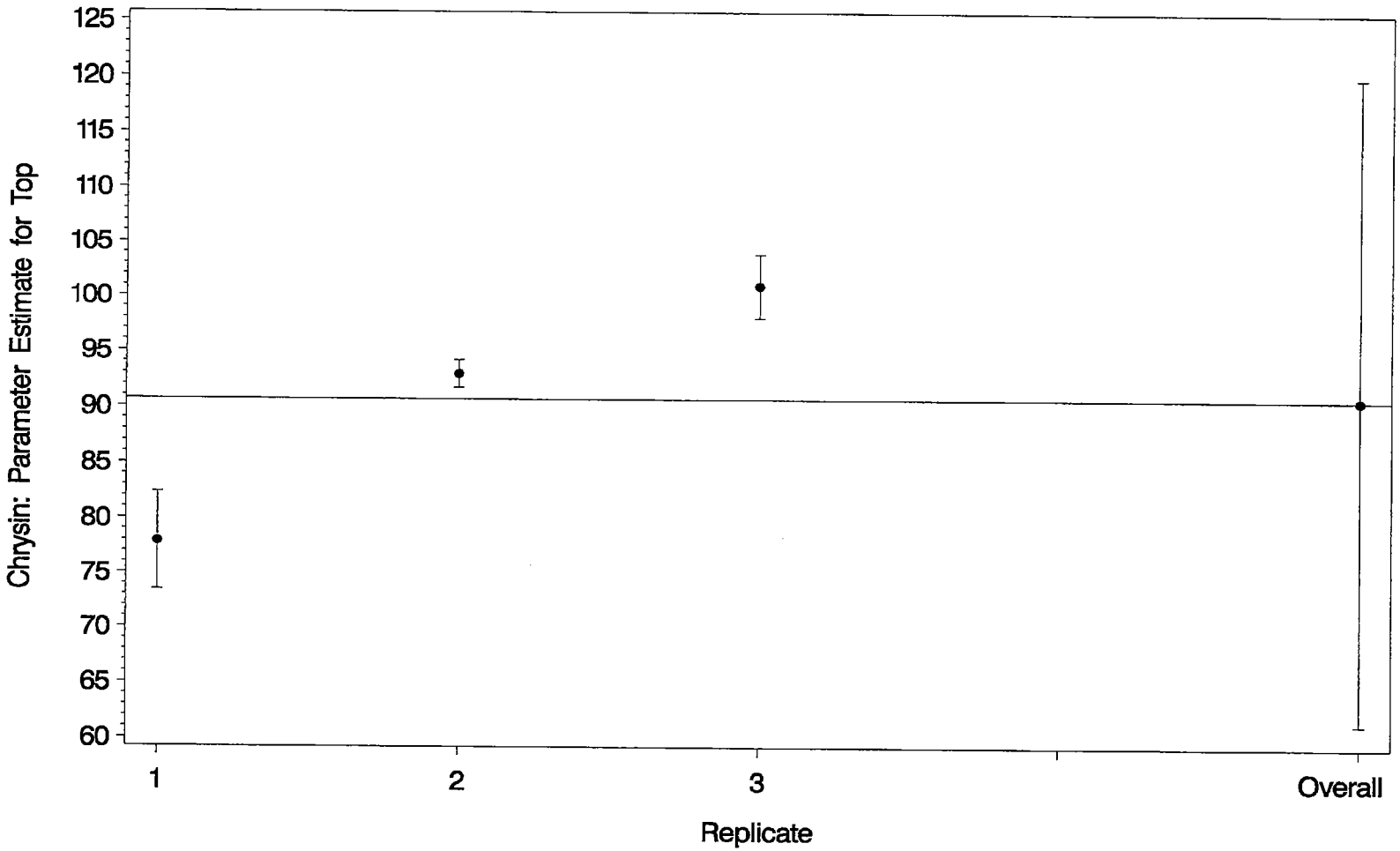


Figure 8-4. **Reference Chemical VIII: Chrysin. Microsomes Prepared by Battelle. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

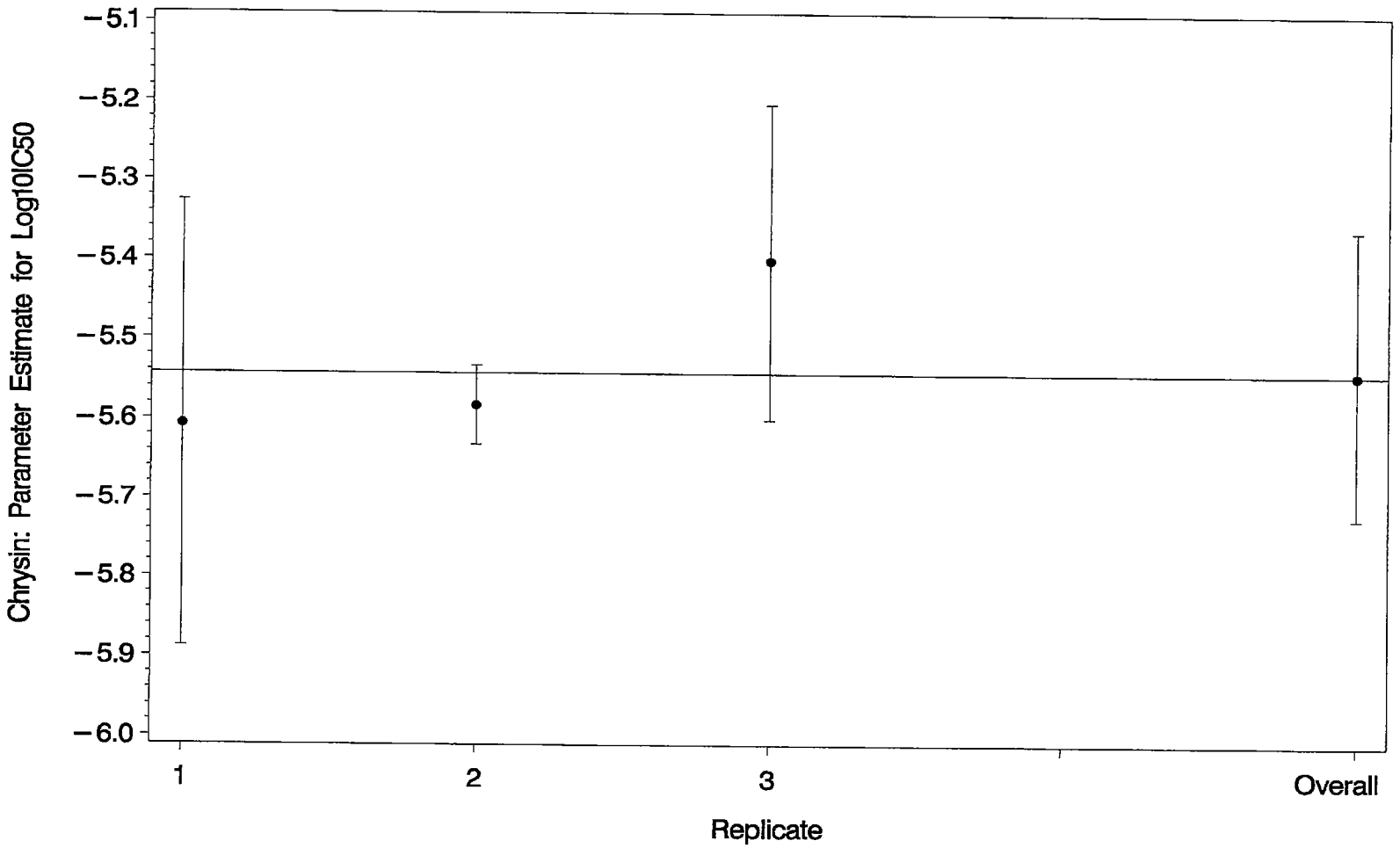


Figure 8-5. **Reference Chemical VIII: Chrysin. Microsomes Prepared by Battelle. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

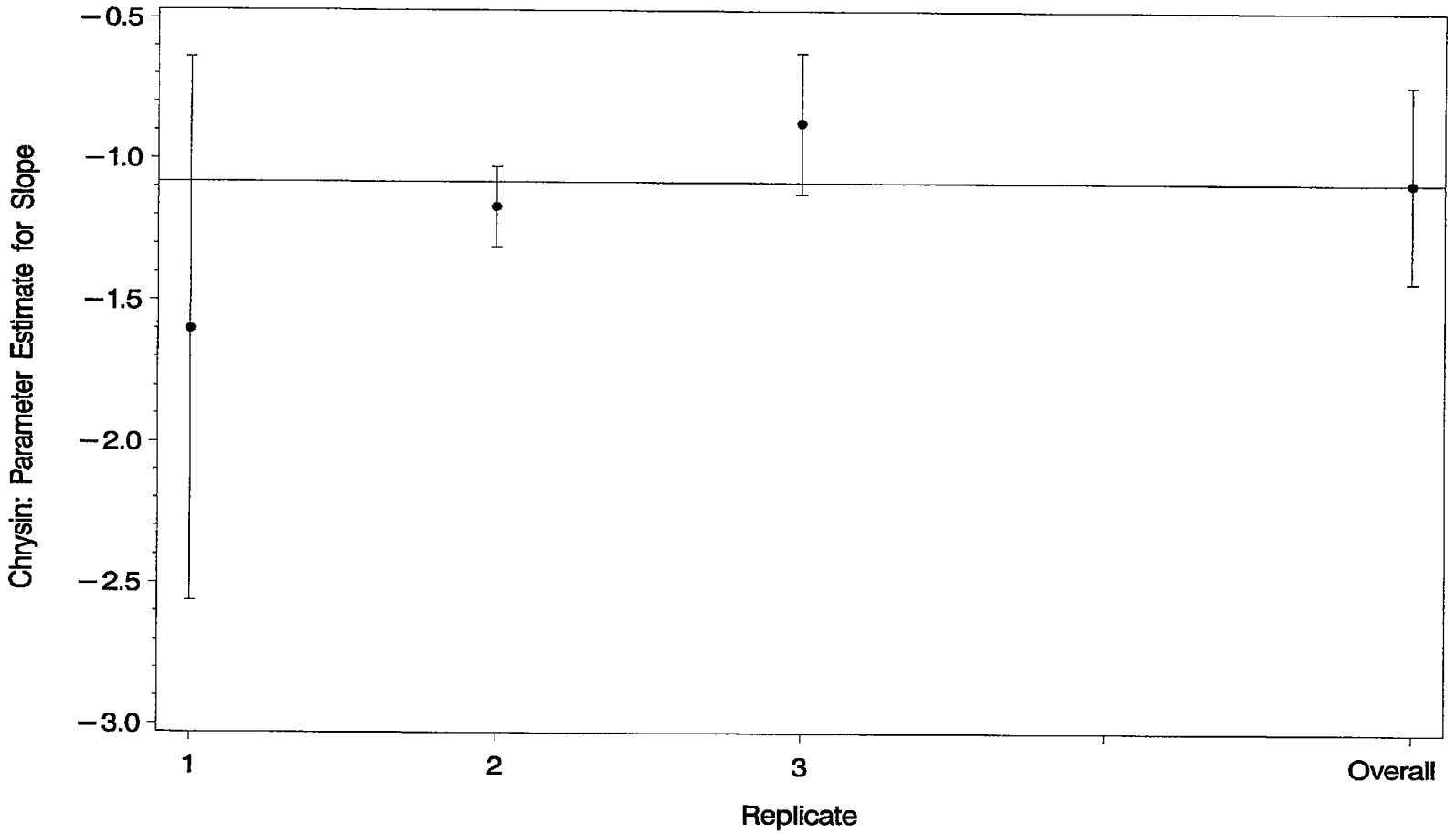


Figure 8-6. **Reference Chemical VIII: Chrysin. Microsomes Prepared by Battelle. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

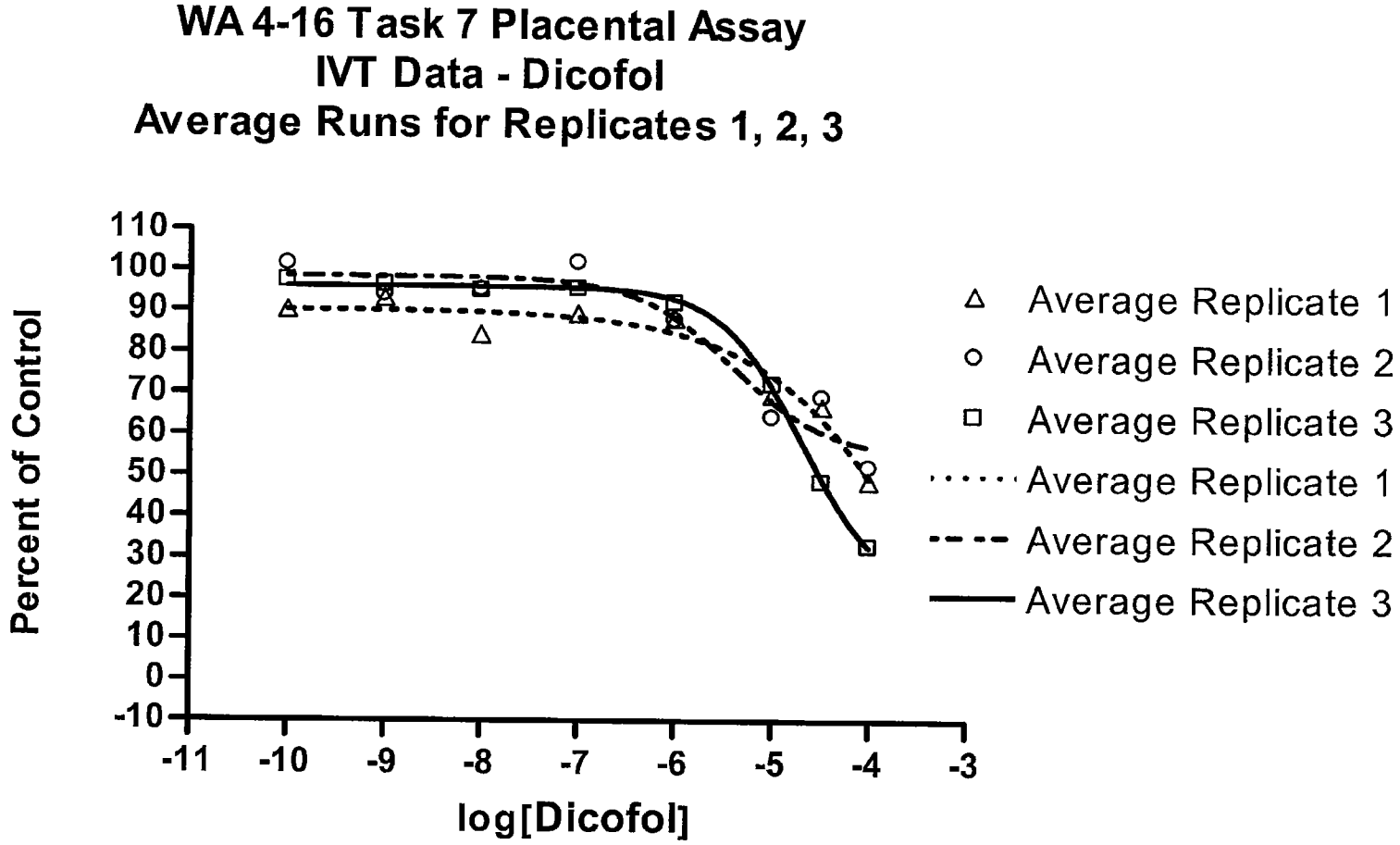


Figure 9-1. **Reference Chemical IX: Dicofol. Microsomes Prepared by IVT. Concentration Response Curves and Averages of Repetitions within Each Concentration. Placental Aromatase Assay.**

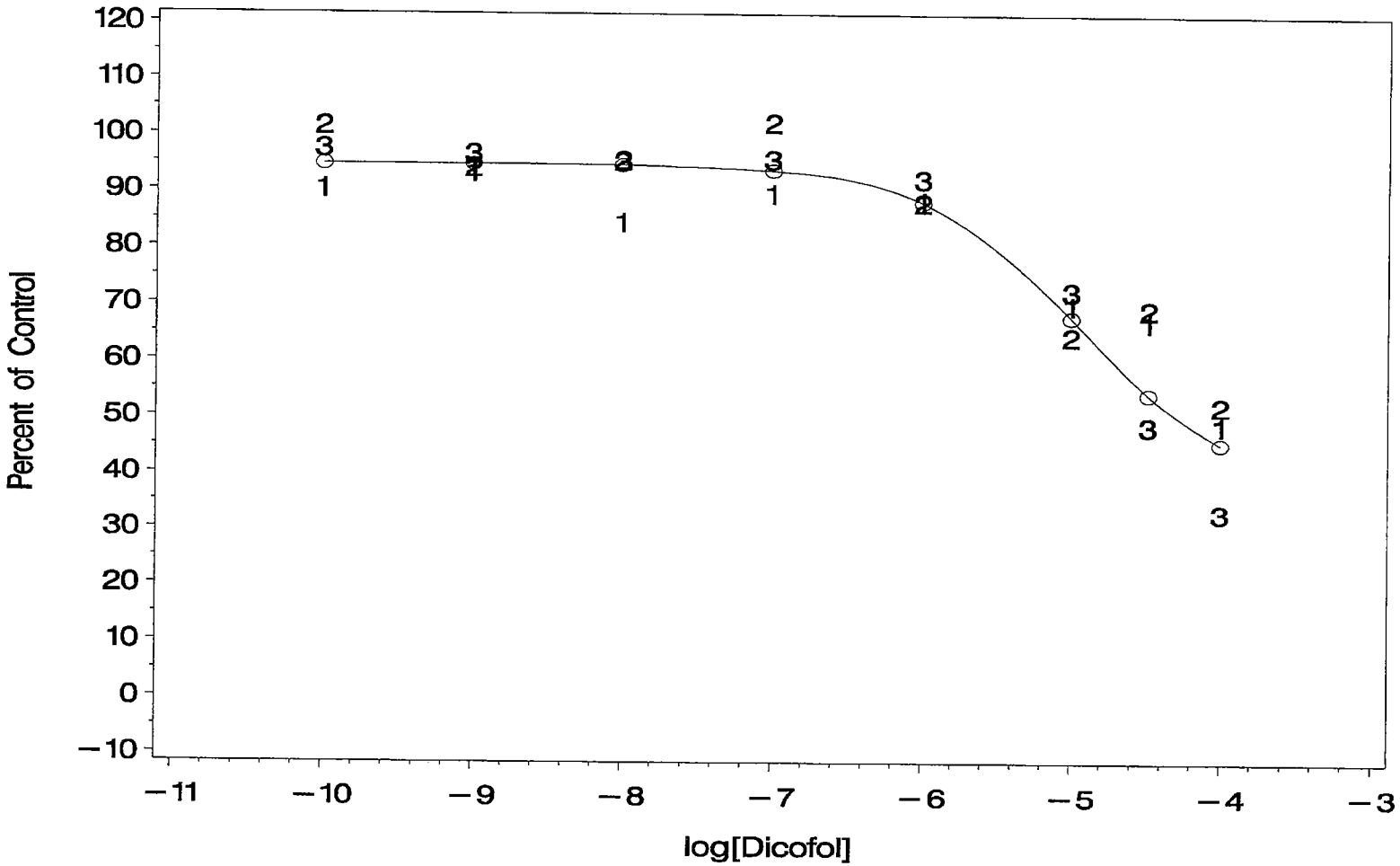


Figure 9-2. **Reference Chemical IX: Dicofol. Microsomes Prepared by IVT. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Placental Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

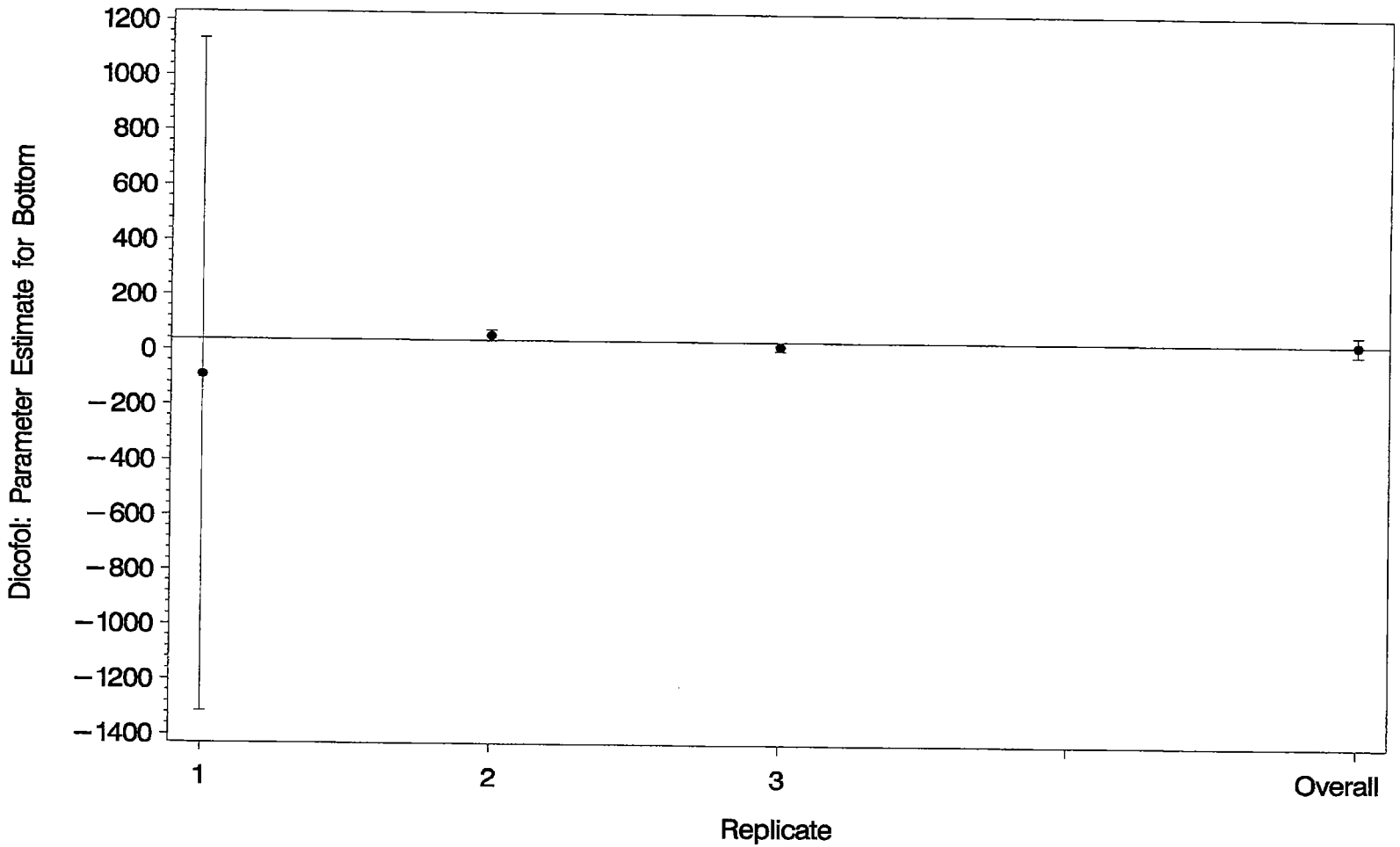


Figure 9-3. **Reference Chemical IX: Dicofol. Microsomes Prepared by IVT. Bottom Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

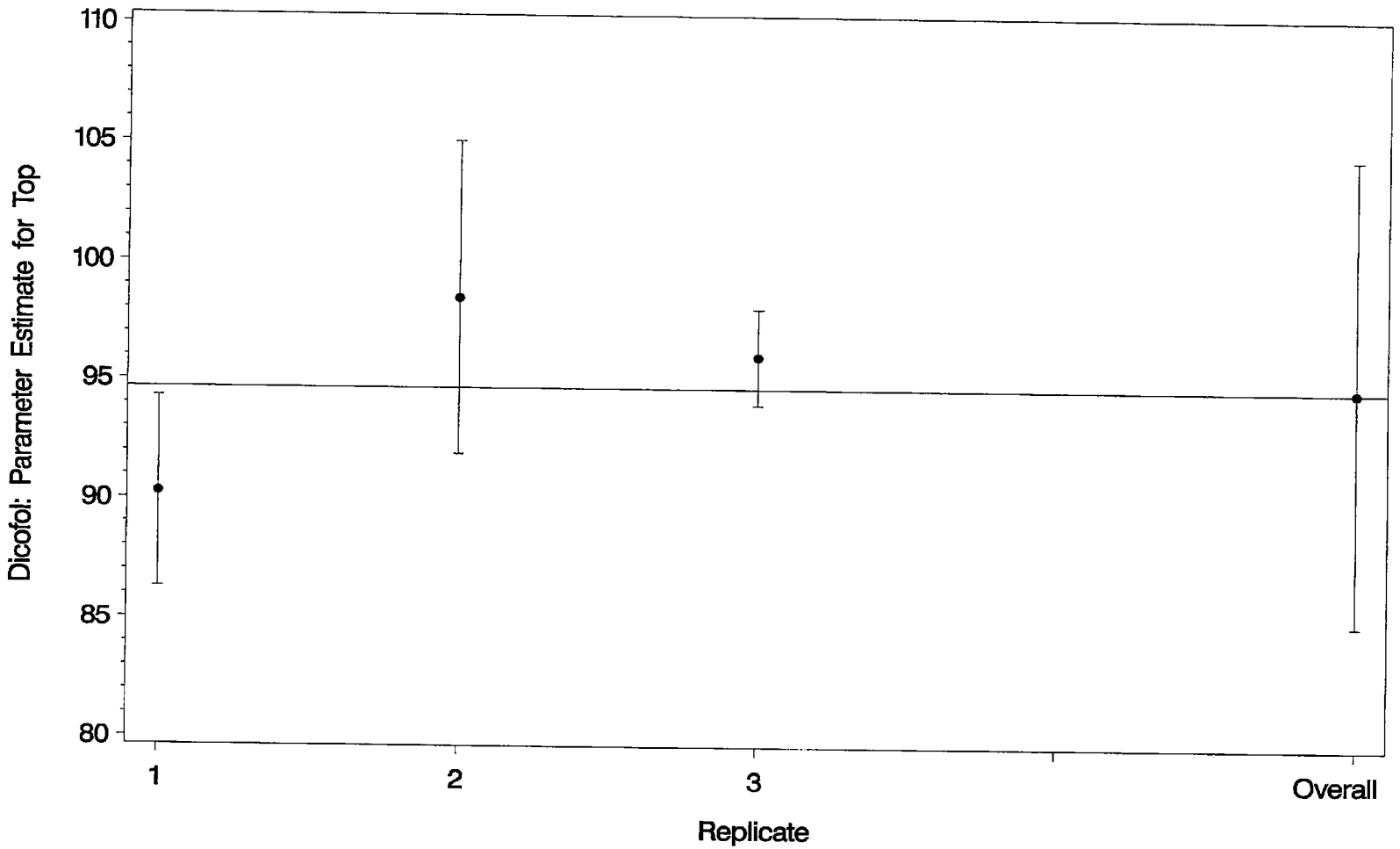


Figure 9-4. **Reference Chemical IX: Dicofol. Microsomes Prepared by IVT. Top Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

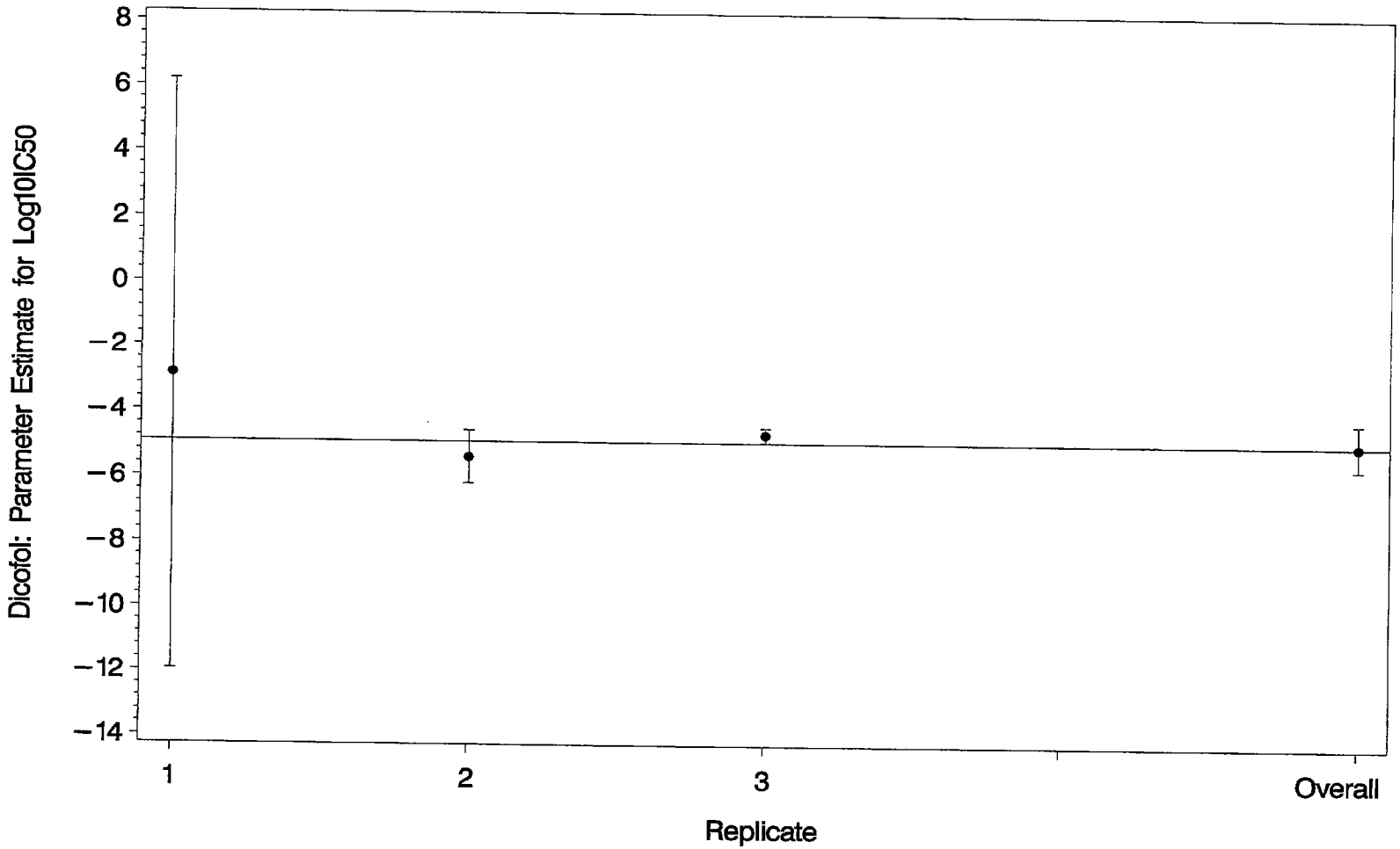


Figure 9-5. **Reference Chemical IX: Dicofol. Microsomes Prepared by IVT. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

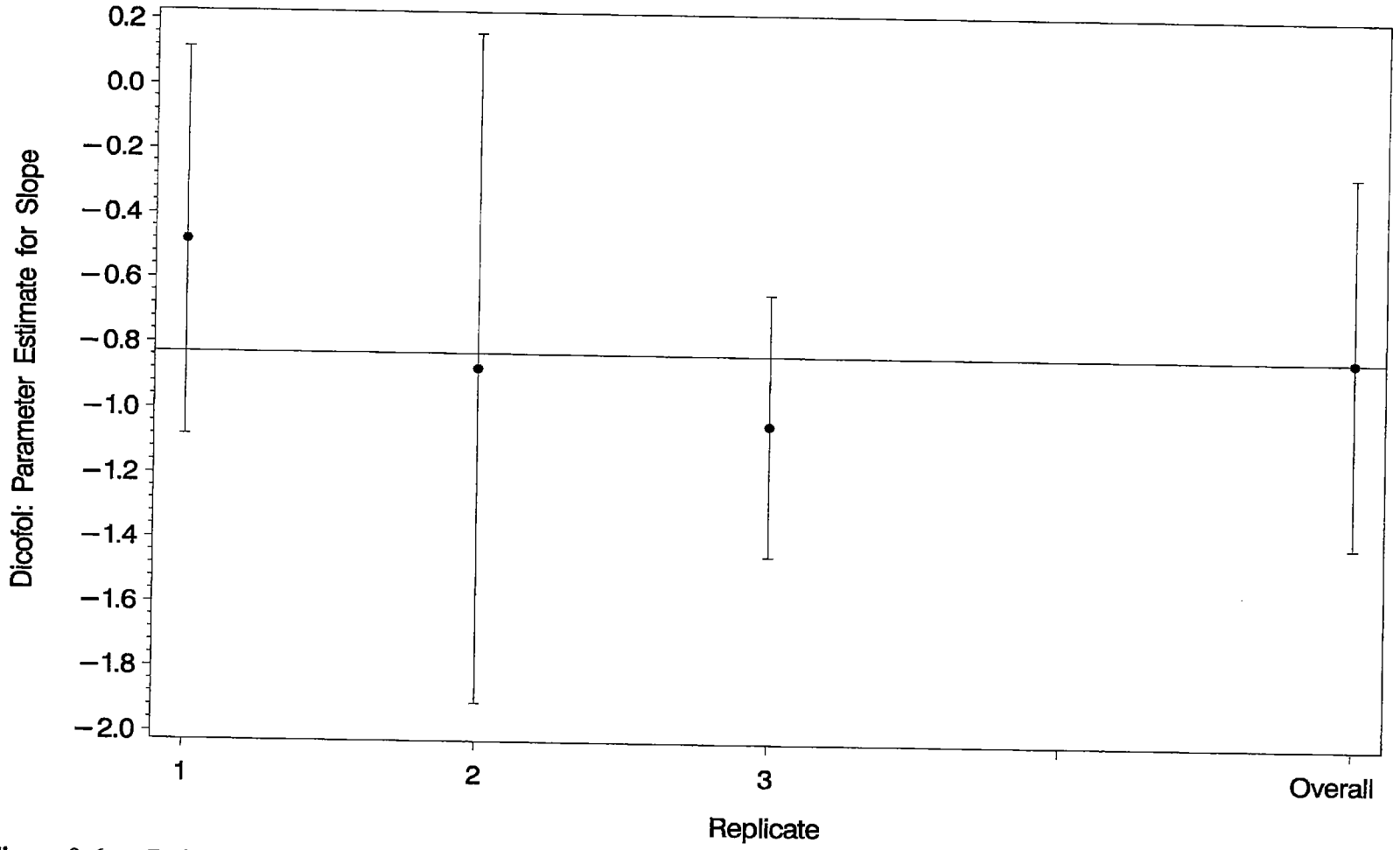


Figure 9-6. **Reference Chemical IX: Dicofol. Microsomes Prepared by IVT. Slope Parameter Estimates and Their Associated 95% Confidence Intervals for Each Replicate and Across Replicates. Placental Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

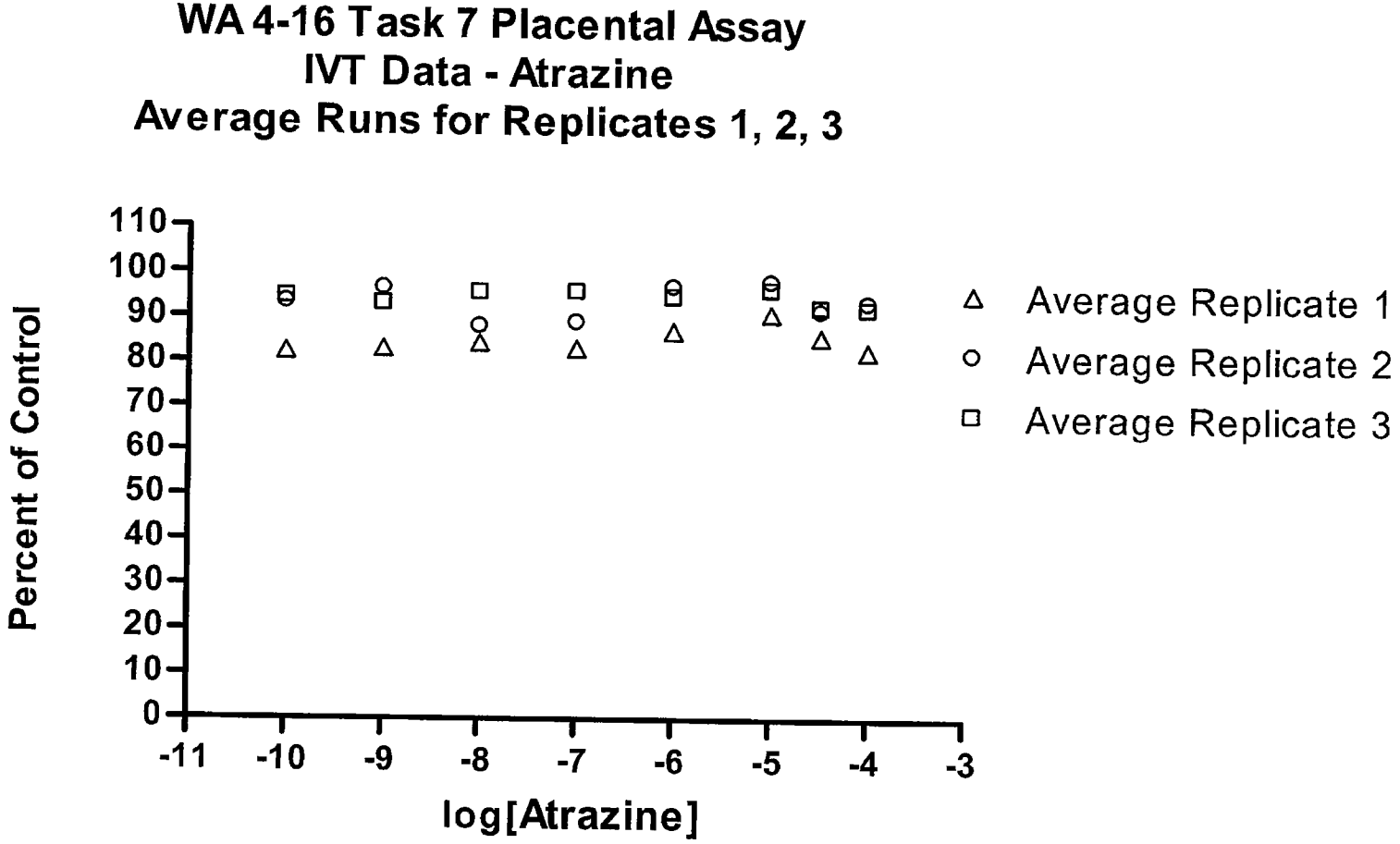


Figure 10-1. **Reference Chemical X: Atrazine. Microsomes Prepared by IVT. Scatter Plot for Averages of Repetitions within Concentration for Each Replicate. Placental Aromatase Assay.**

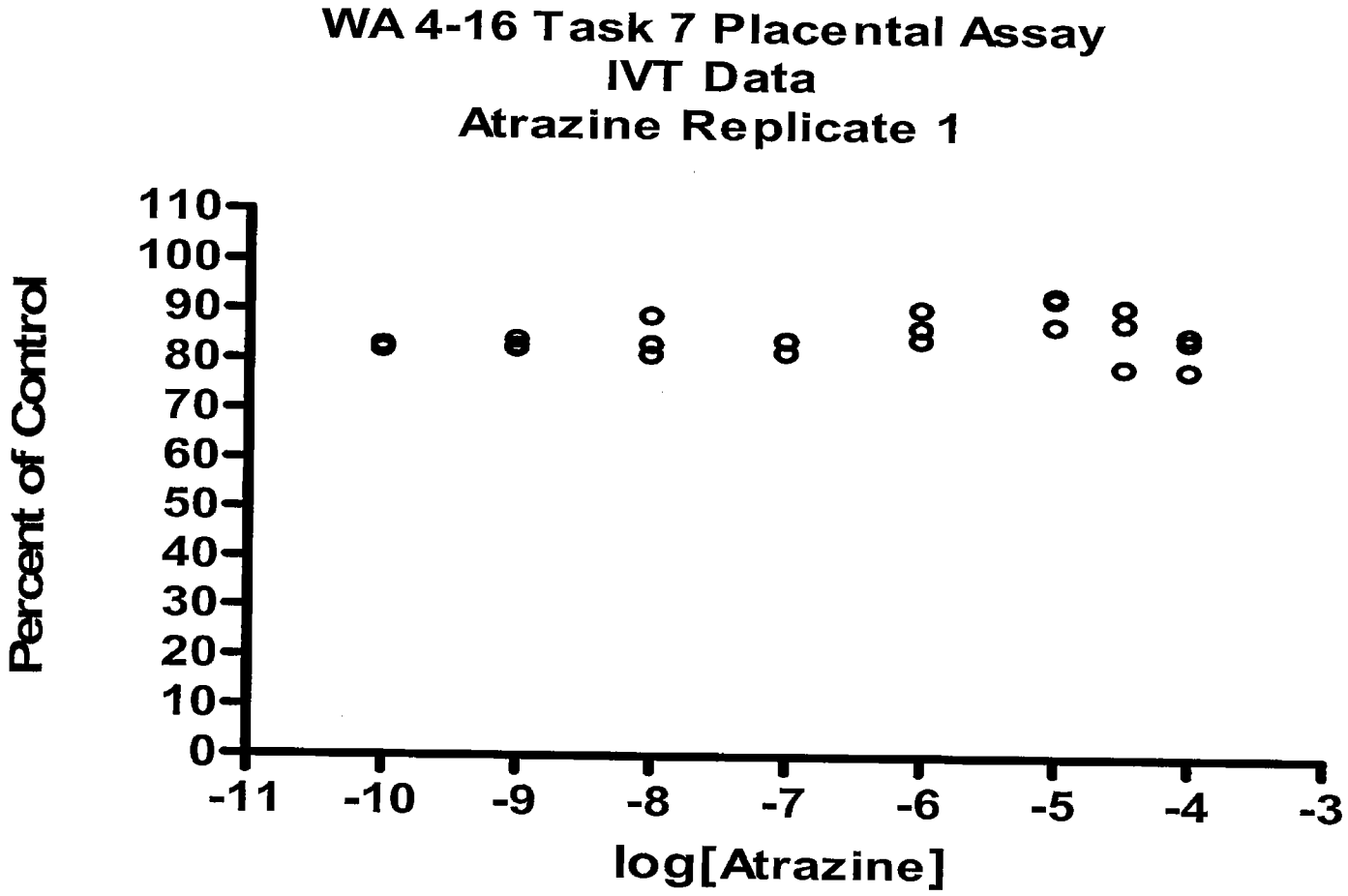


Figure 10-2. **Reference Chemical X: Atrazine. Microsomes Prepared by IVT. Scatter Plot for Replicate 1 Data. Placental Aromatase Assay.**

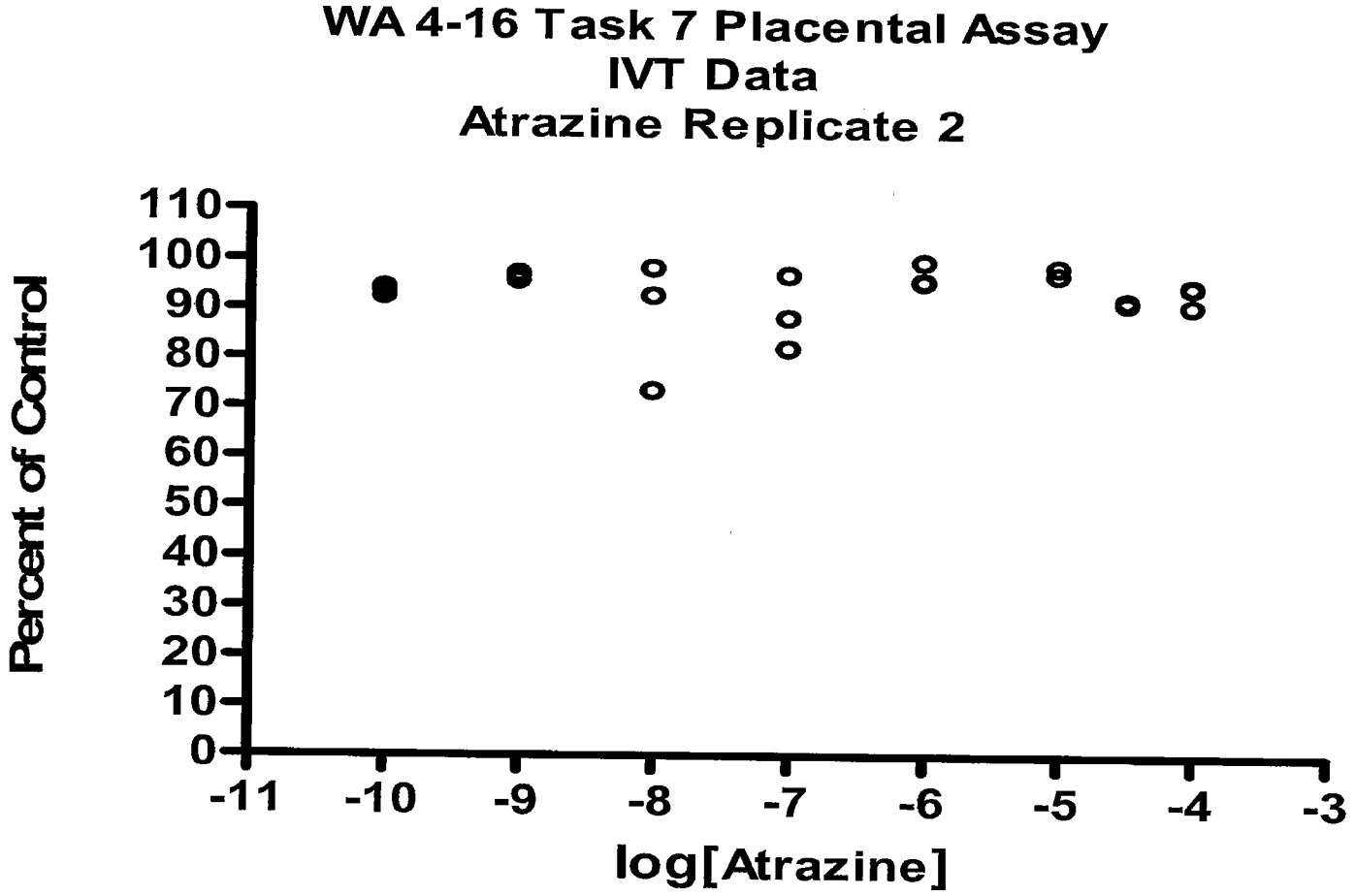


Figure 10-3. Reference Chemical X: Atrazine. Microsomes Prepared by IVT. Scatter Plot for Replicate 2 Data. Placental Aromatase Assay.

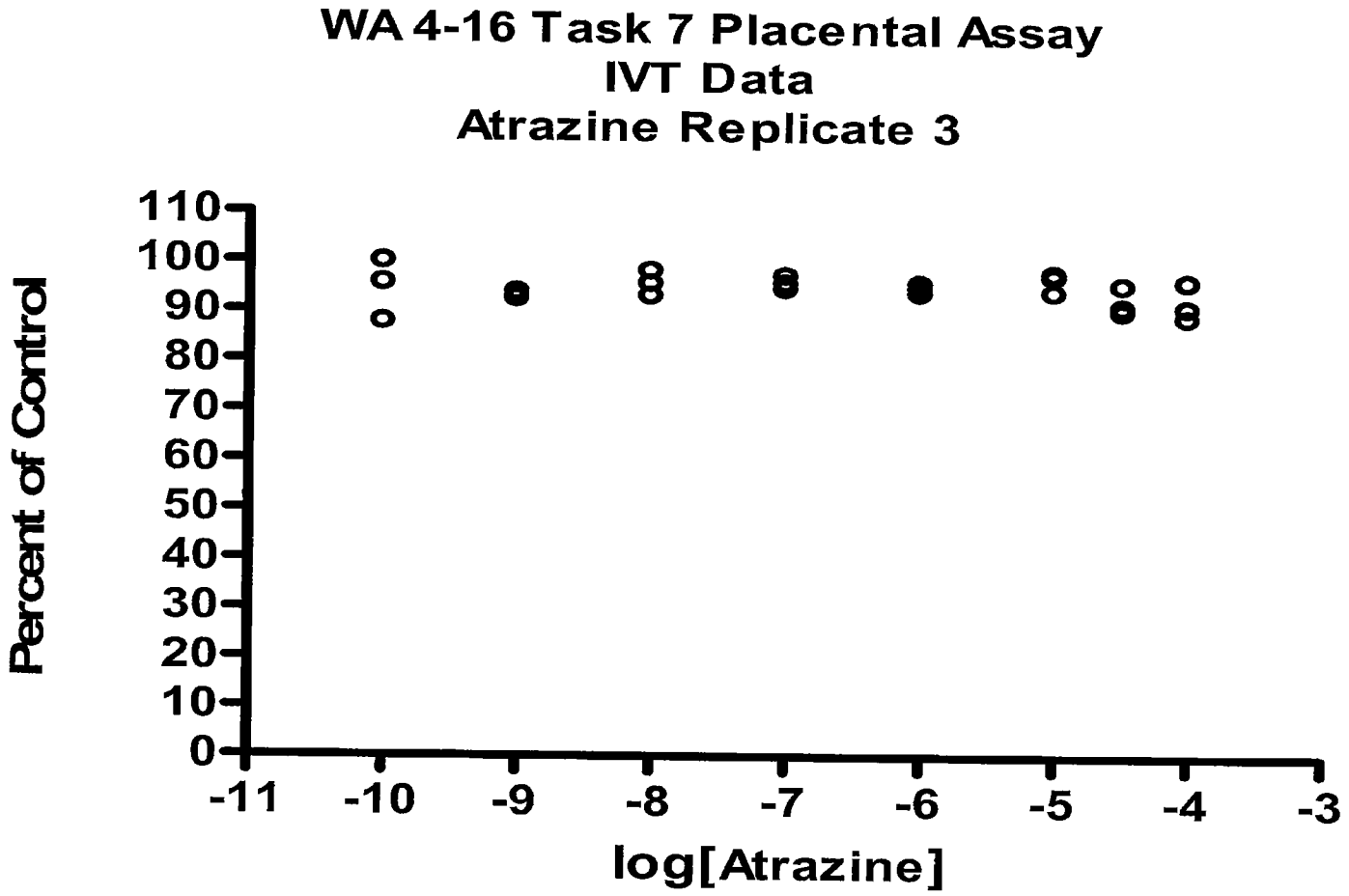


Figure 10-4. Reference Chemical X: Atrazine. Microsomes Prepared by IVT. Scatter Plot for Replicate 3 Data. Placental Aromatase Assay.

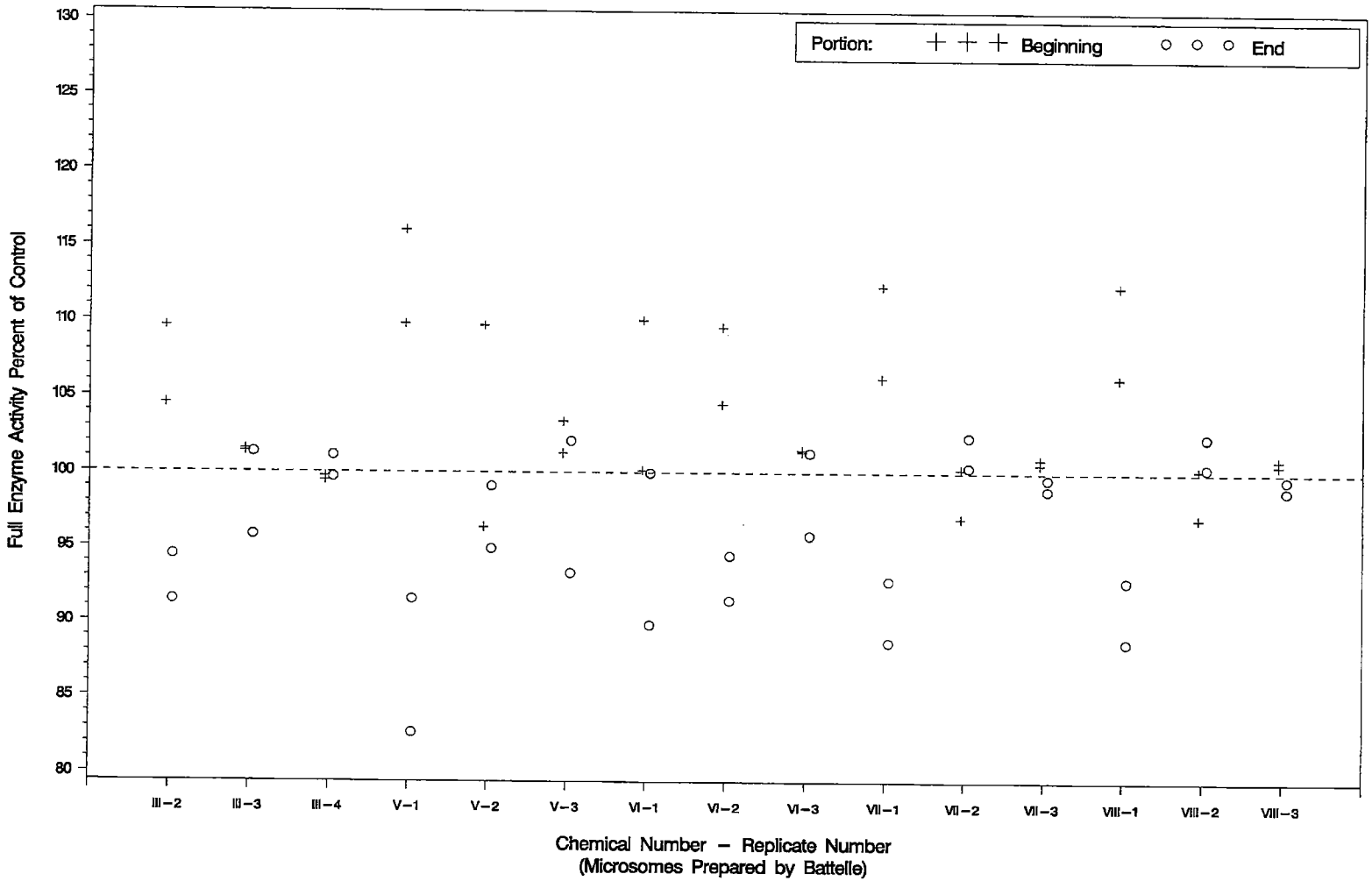


Figure 11a. **Full Enzyme Activity Control Data. Microsomes Prepared by Battelle. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 100%.**

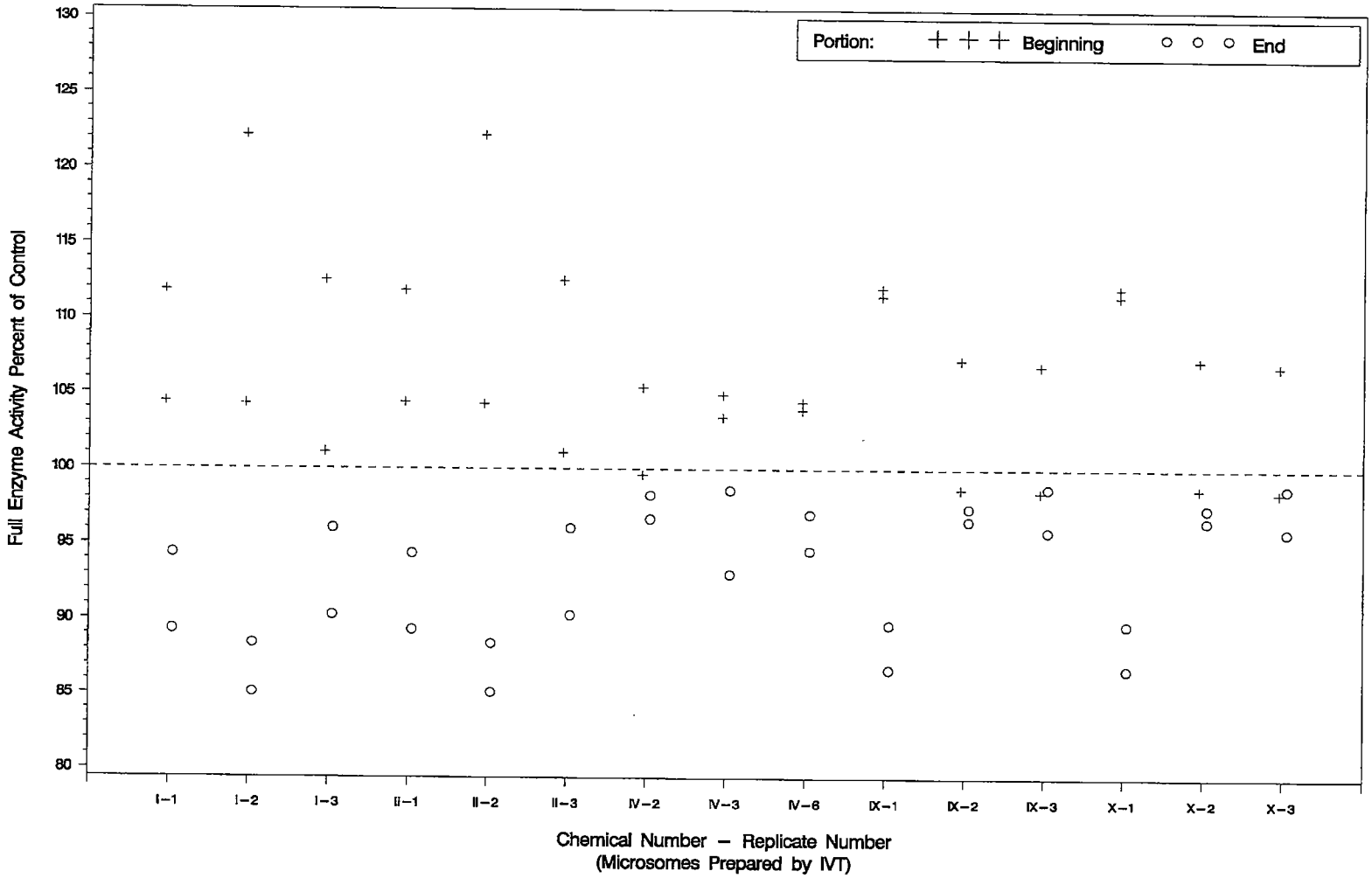


Figure 11b. **Full Enzyme Activity Control Data. Microsomes Prepared by IVT. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 100%.**

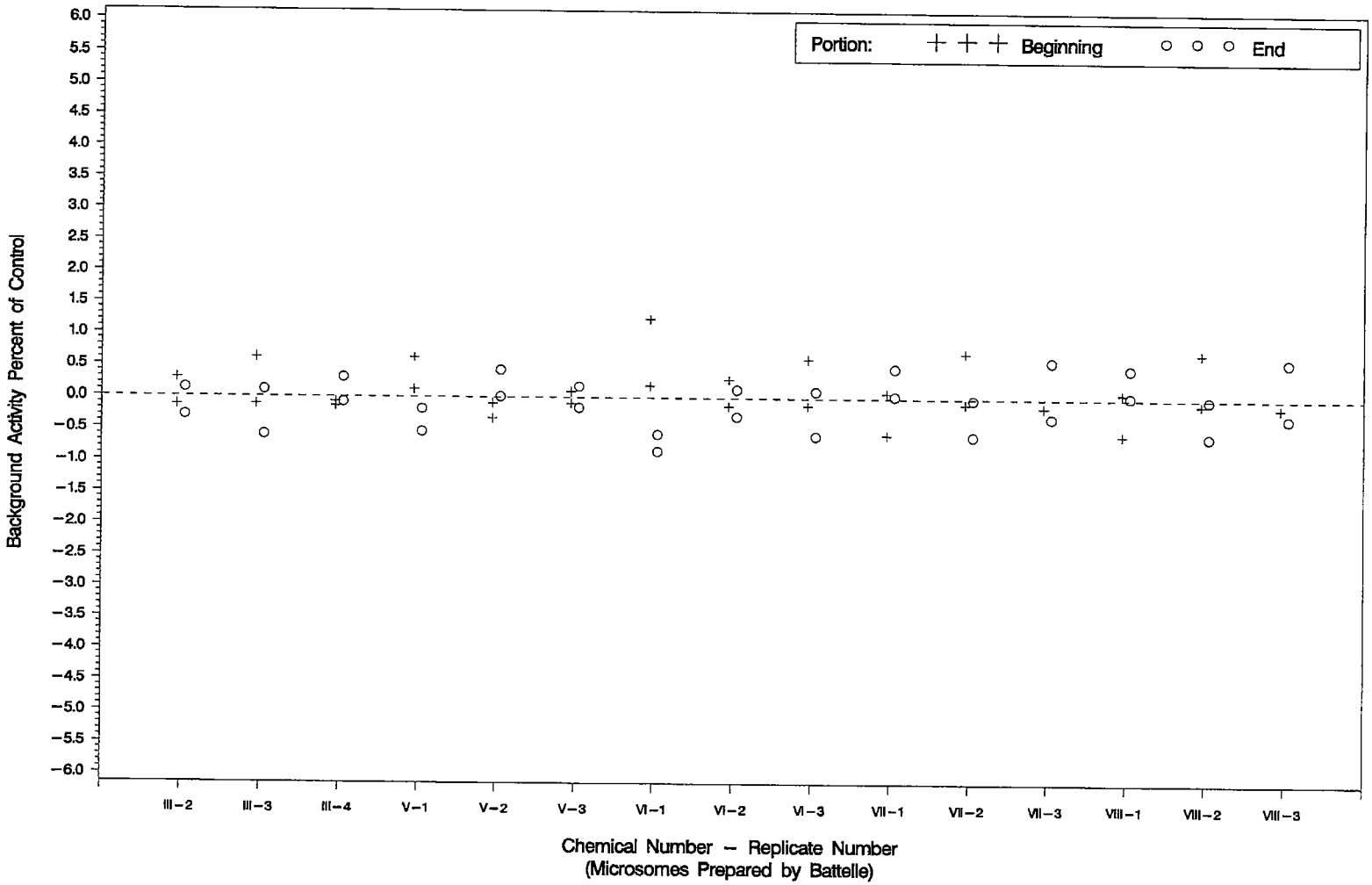


Figure 12a. **Background Activity Control Data. Microsomes Prepared by Battelle. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 0%.**

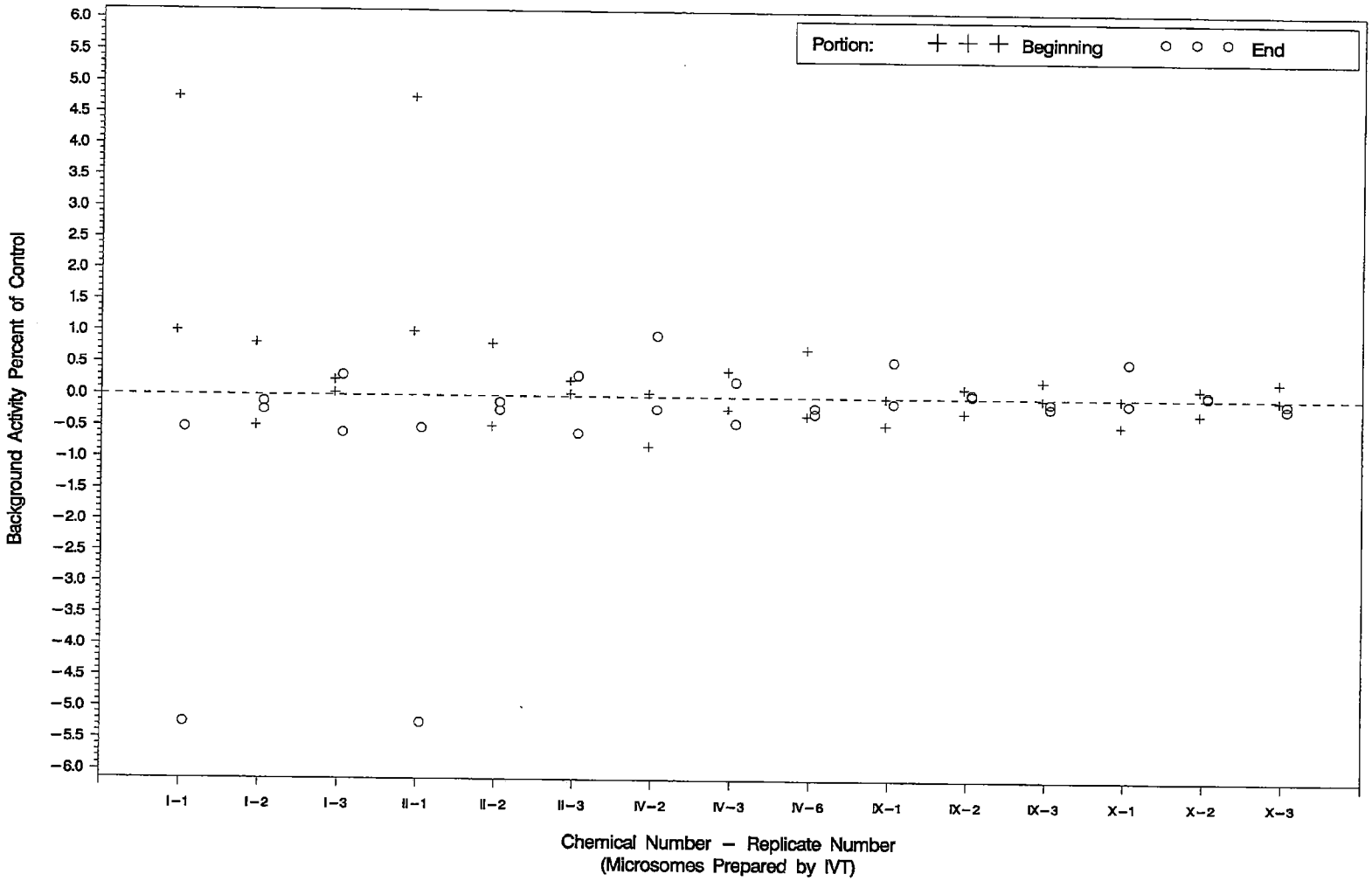


Figure 12b. **Background Activity Control Data. Microsomes Prepared by IVT. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 0%.**

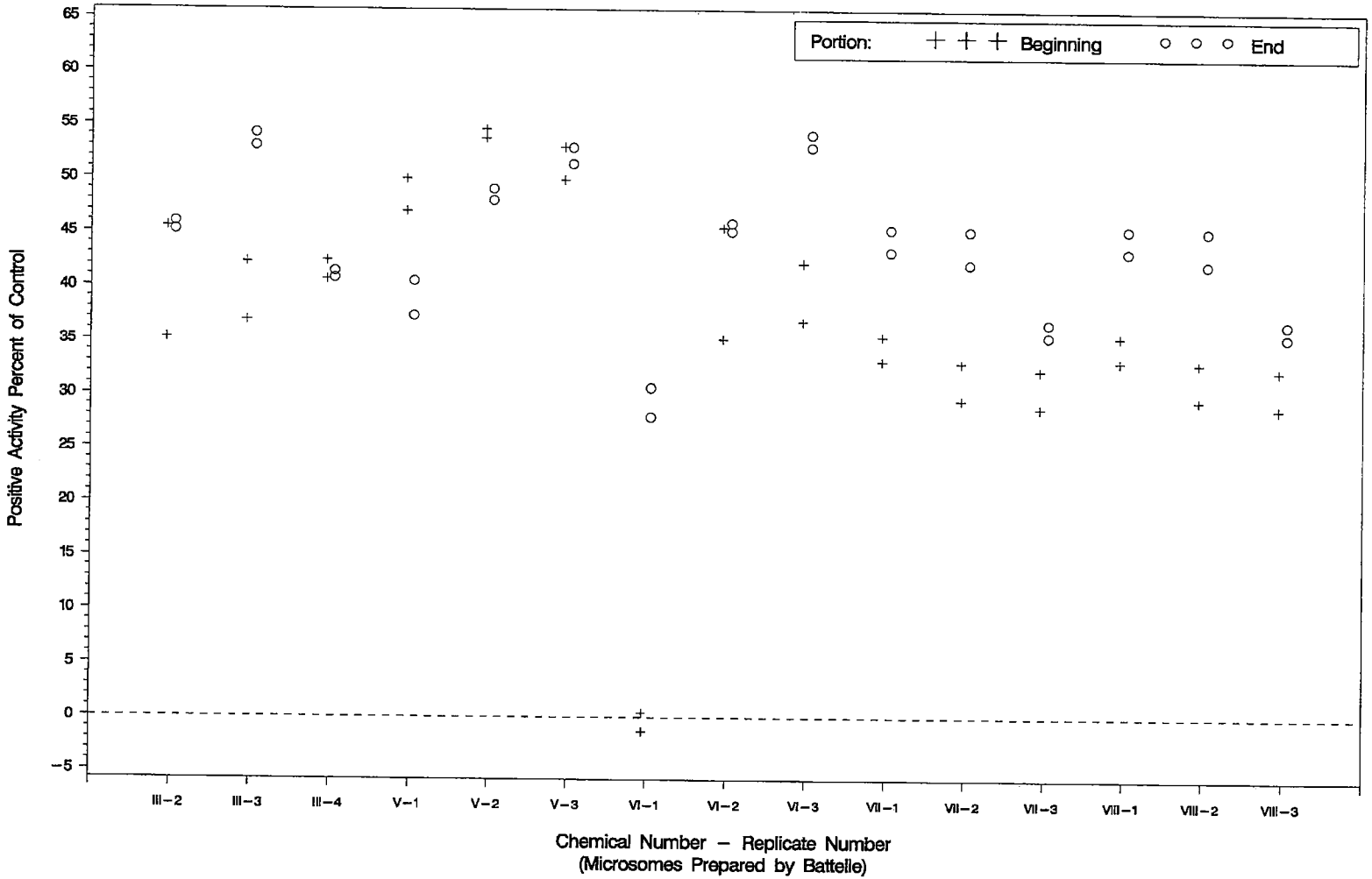


Figure 13a. **Positive Control Data. Microsomes Prepared by Battelle. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay.**

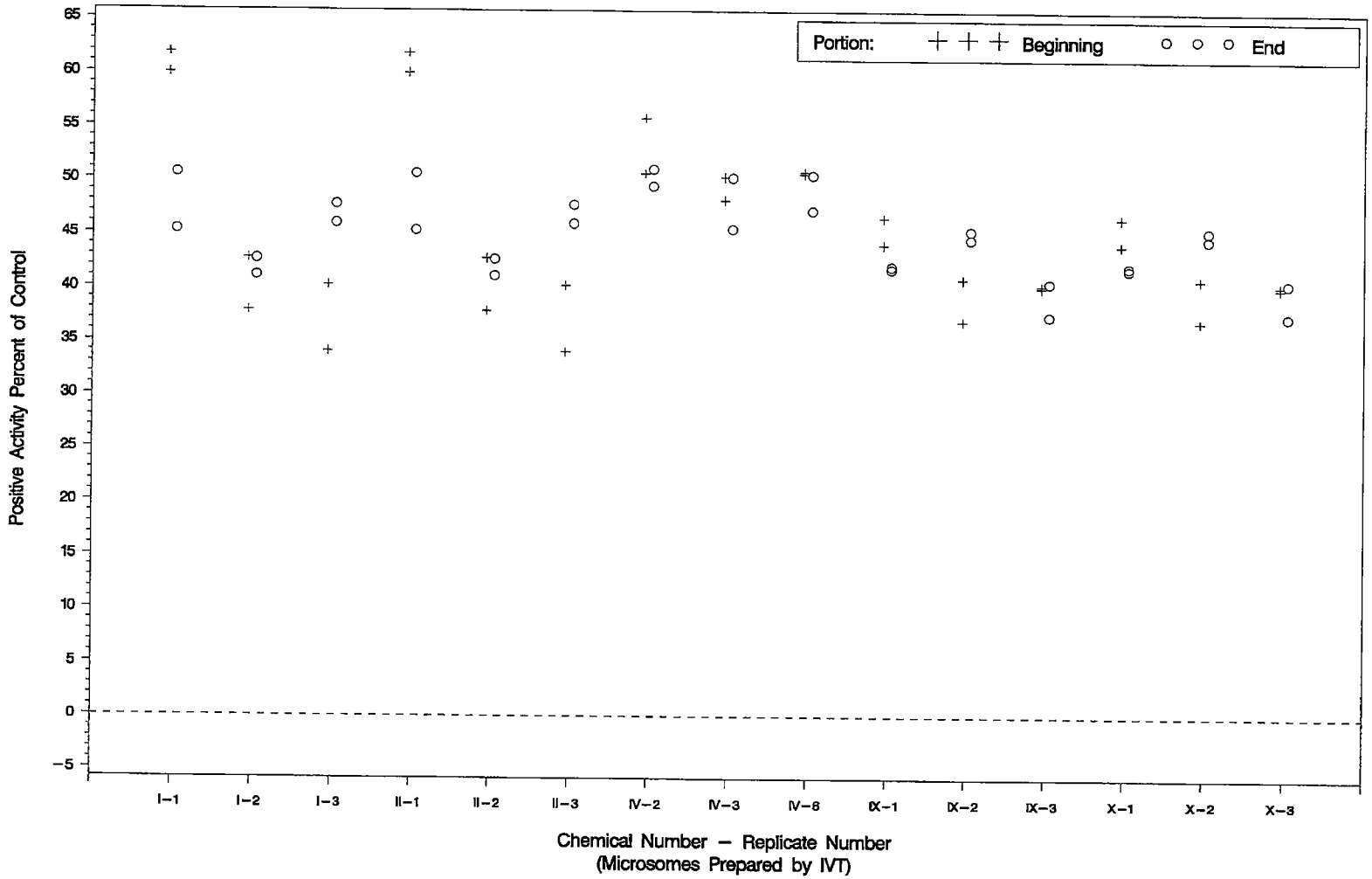


Figure 13b. **Positive Control Data. Microsomes Prepared by IVT. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay.**

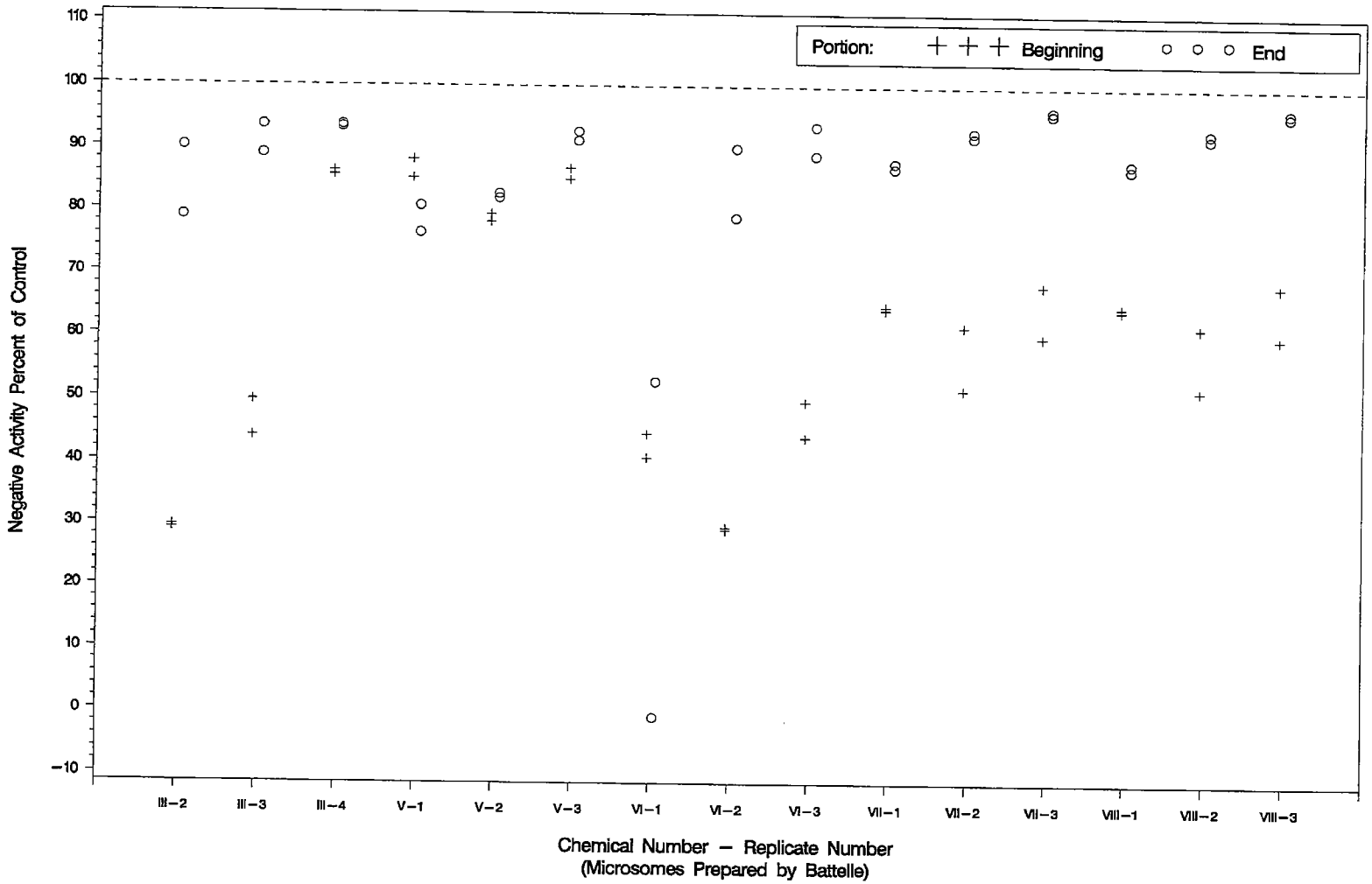


Figure 14a. **Negative Control Data. Microsomes Prepared by Battelle. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 100%.**

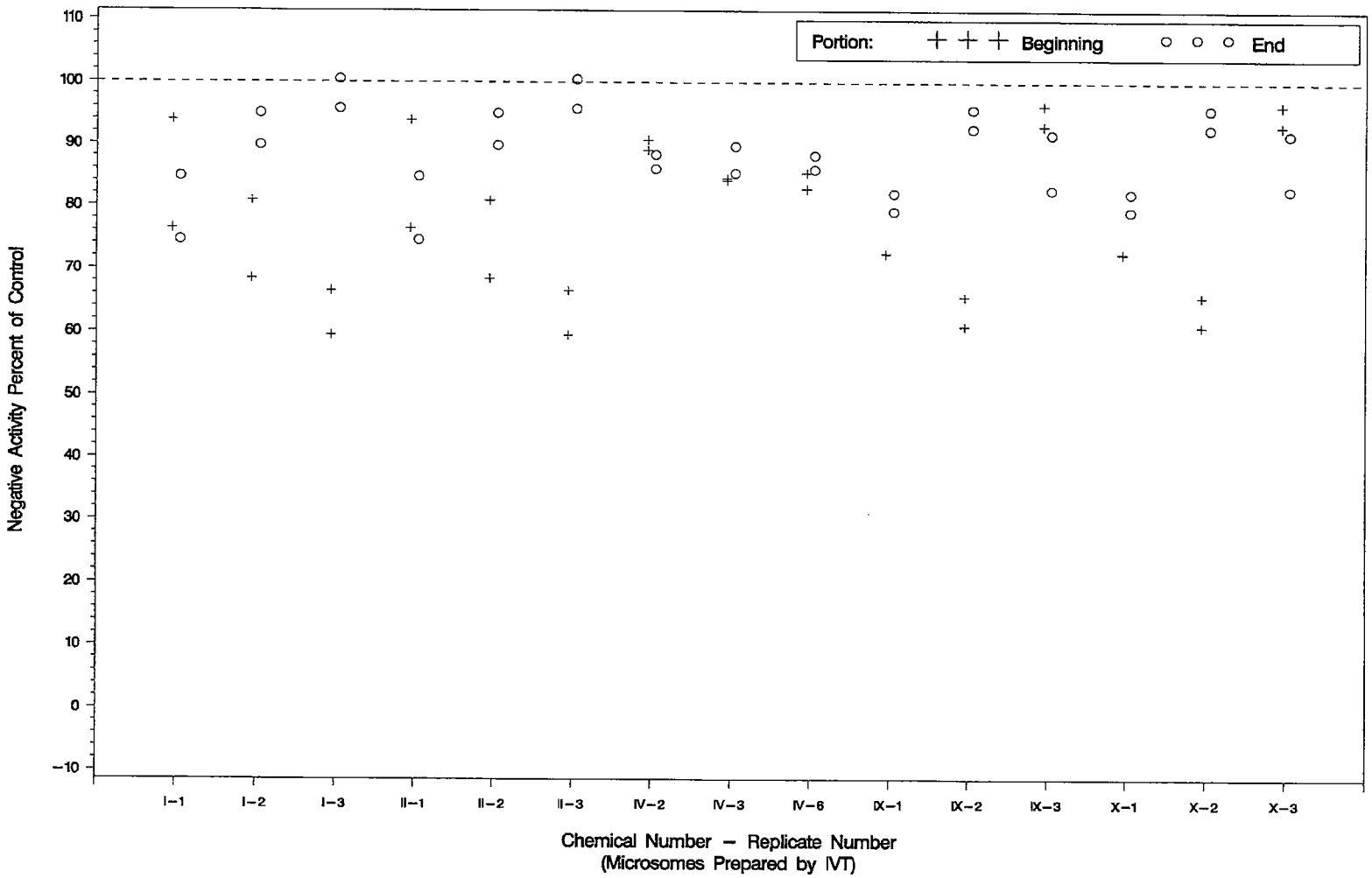


Figure 14b. **Negative Control Data. Microsomes Prepared by IVT. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Placental Aromatase Assay. Reference Line at 100%.**

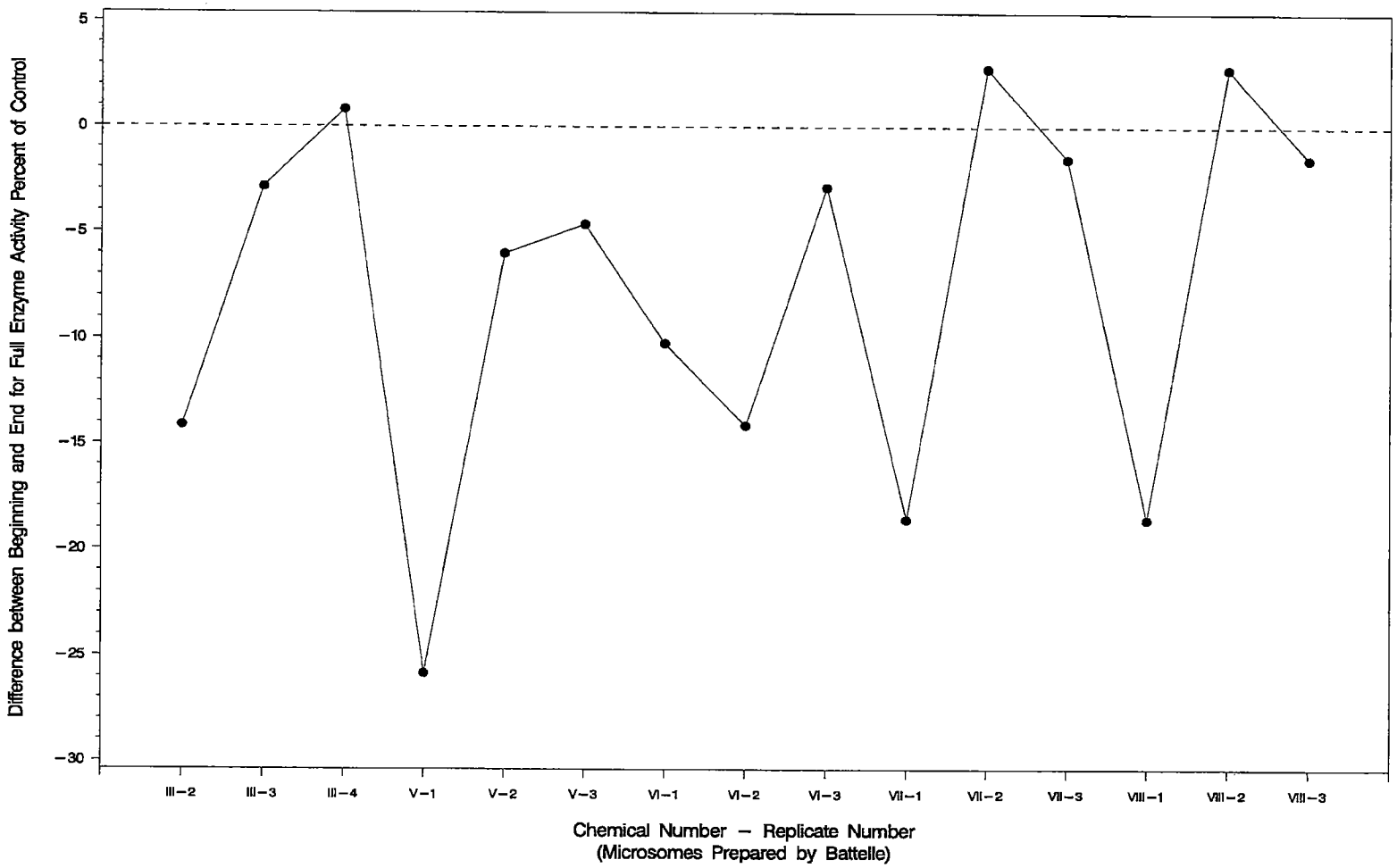


Figure 15a. **Full Enzyme Activity Control Data. Microsomes Prepared by Battelle. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

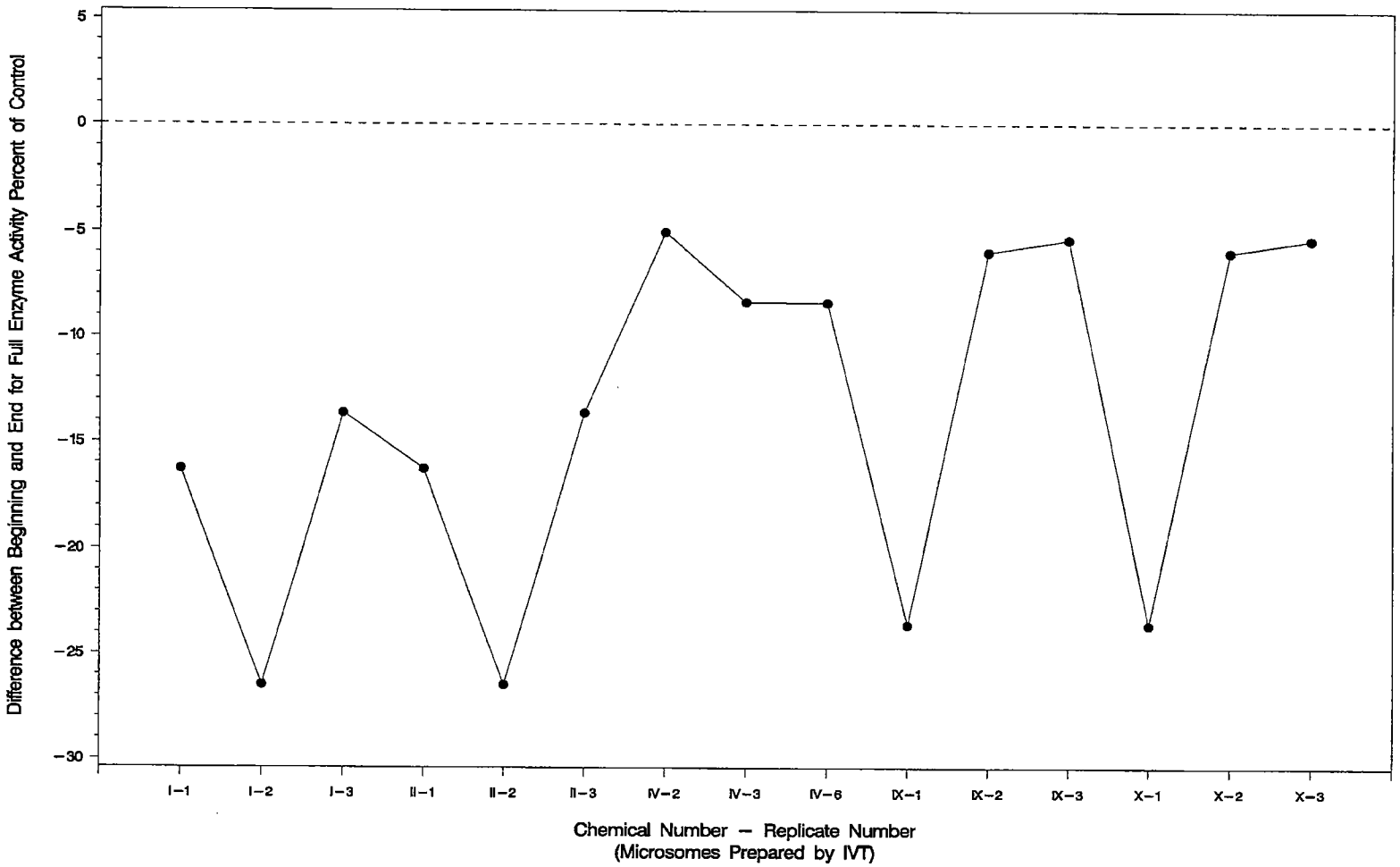


Figure 15b. **Full Enzyme Activity Control Data. Microsomes Prepared by IVT. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

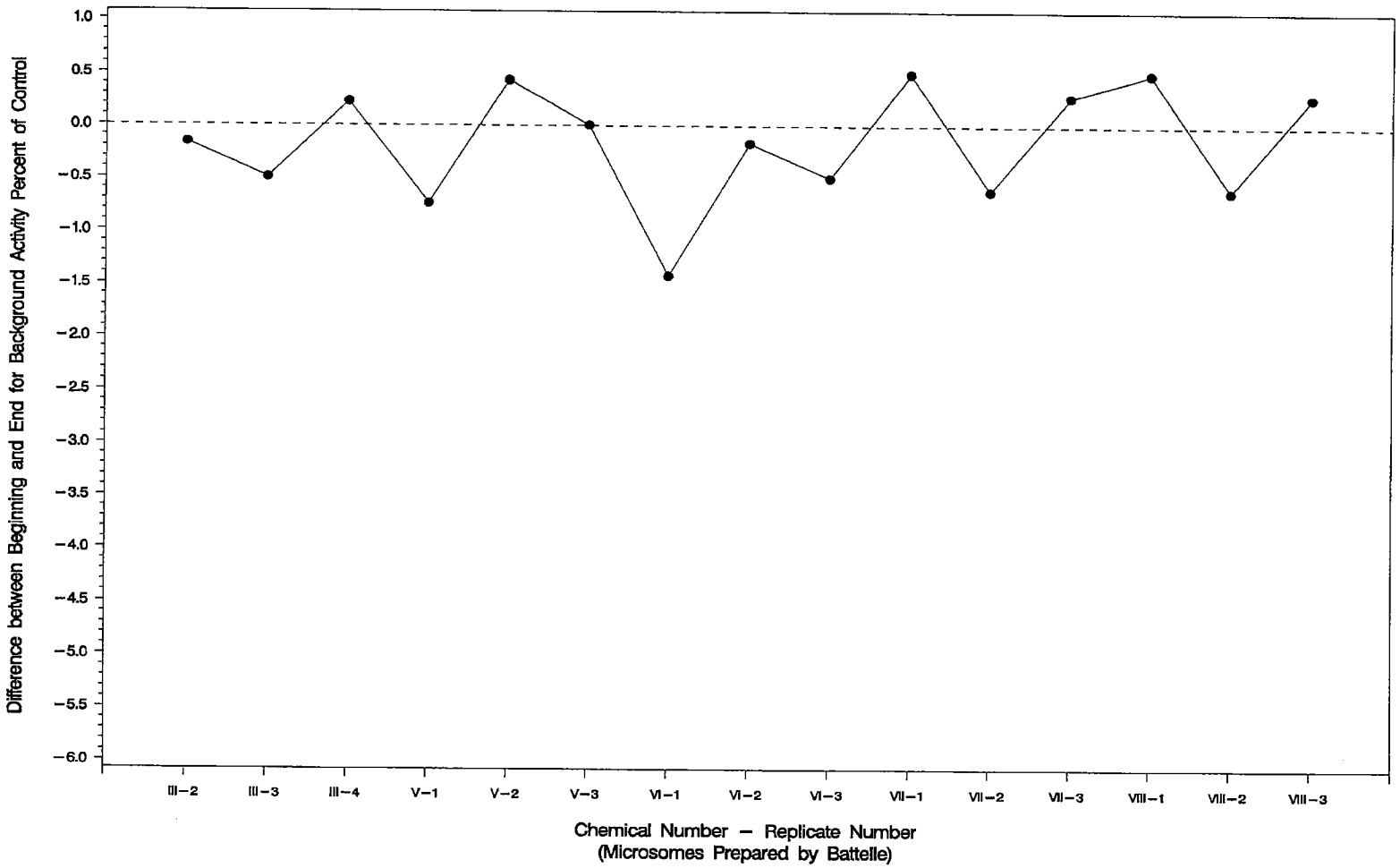


Figure 16a. **Background Activity Control Data. Microsomes Prepared by Battelle. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

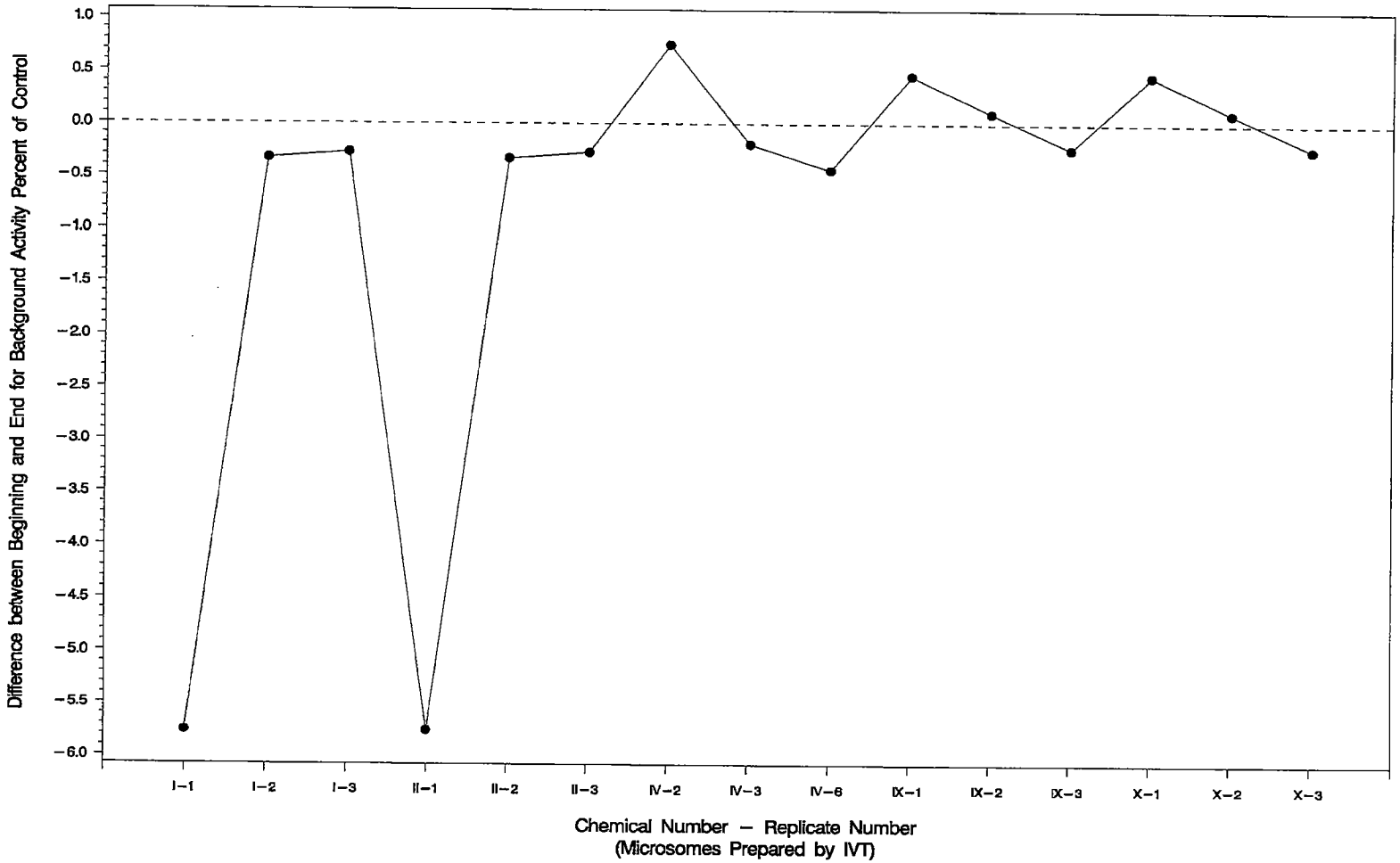


Figure 16b. **Background Activity Control Data. Microsomes Prepared by IVT. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

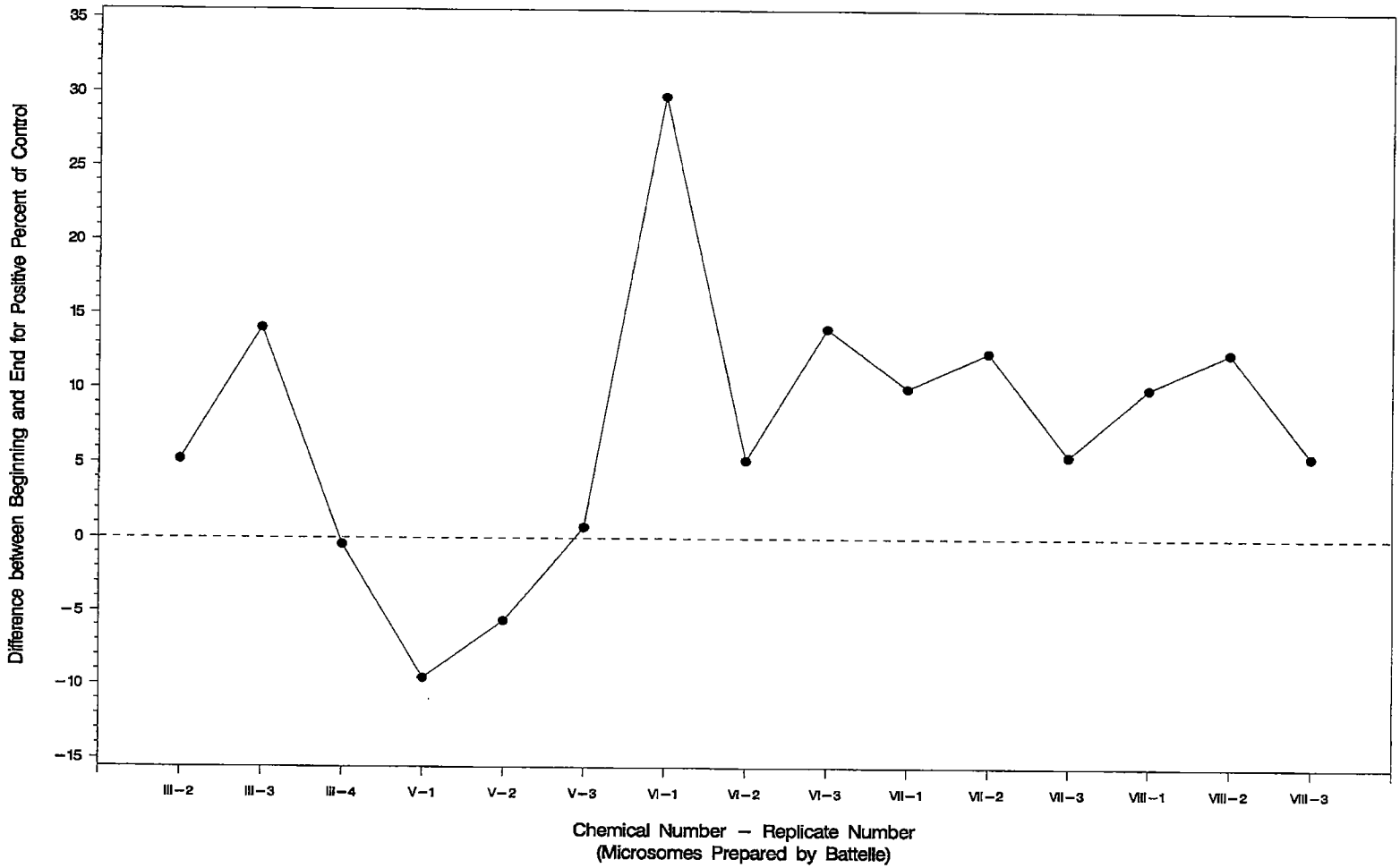


Figure 17a. **Positive Control Data. Microsomes Prepared by Battelle. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

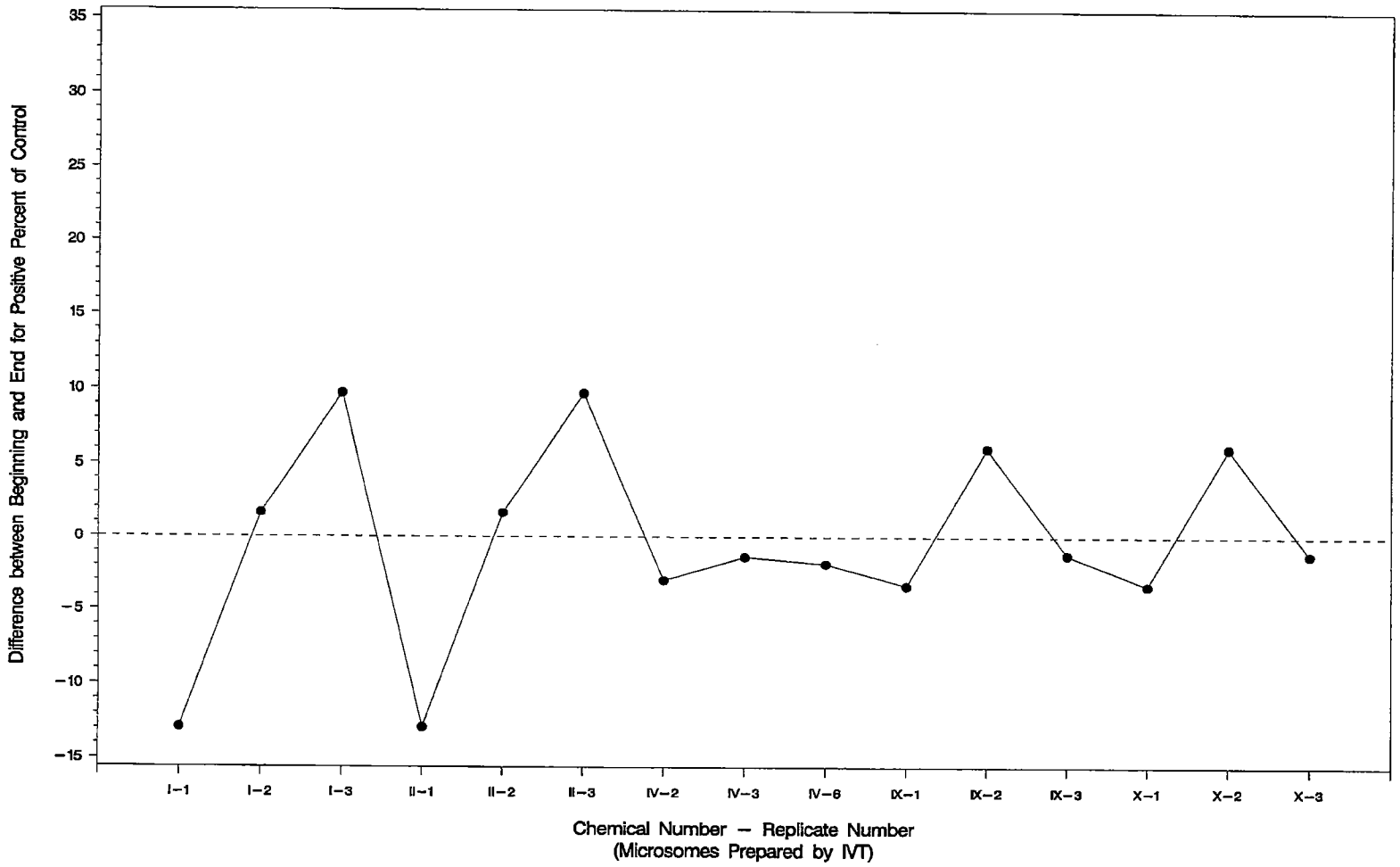


Figure 17b. **Positive Control Data. Microsomes Prepared by IVT. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

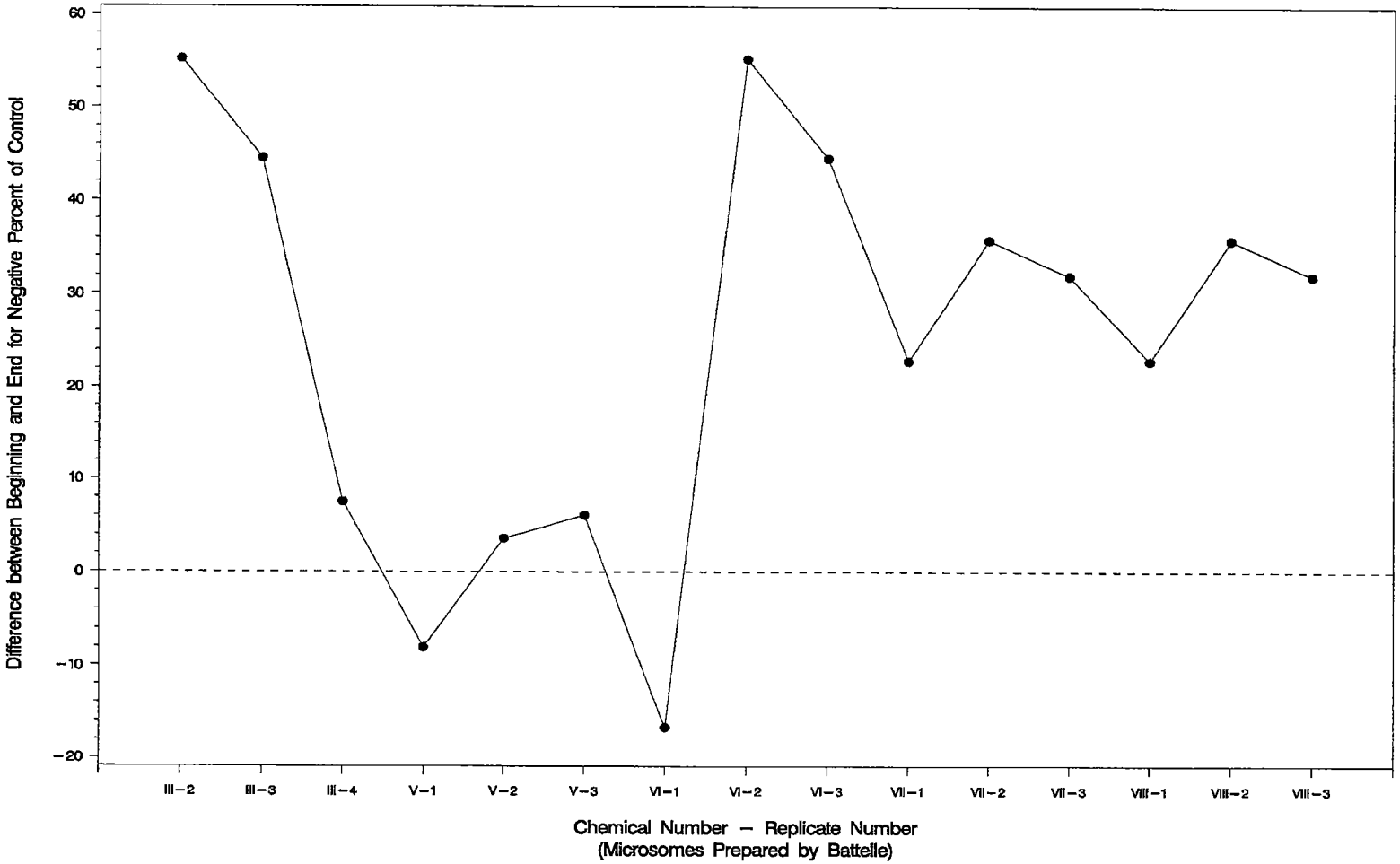


Figure 18a. **Negative Control Data. Microsomes Prepared by Battelle. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Placental Aromatase Assay. Reference Line at 0%.**

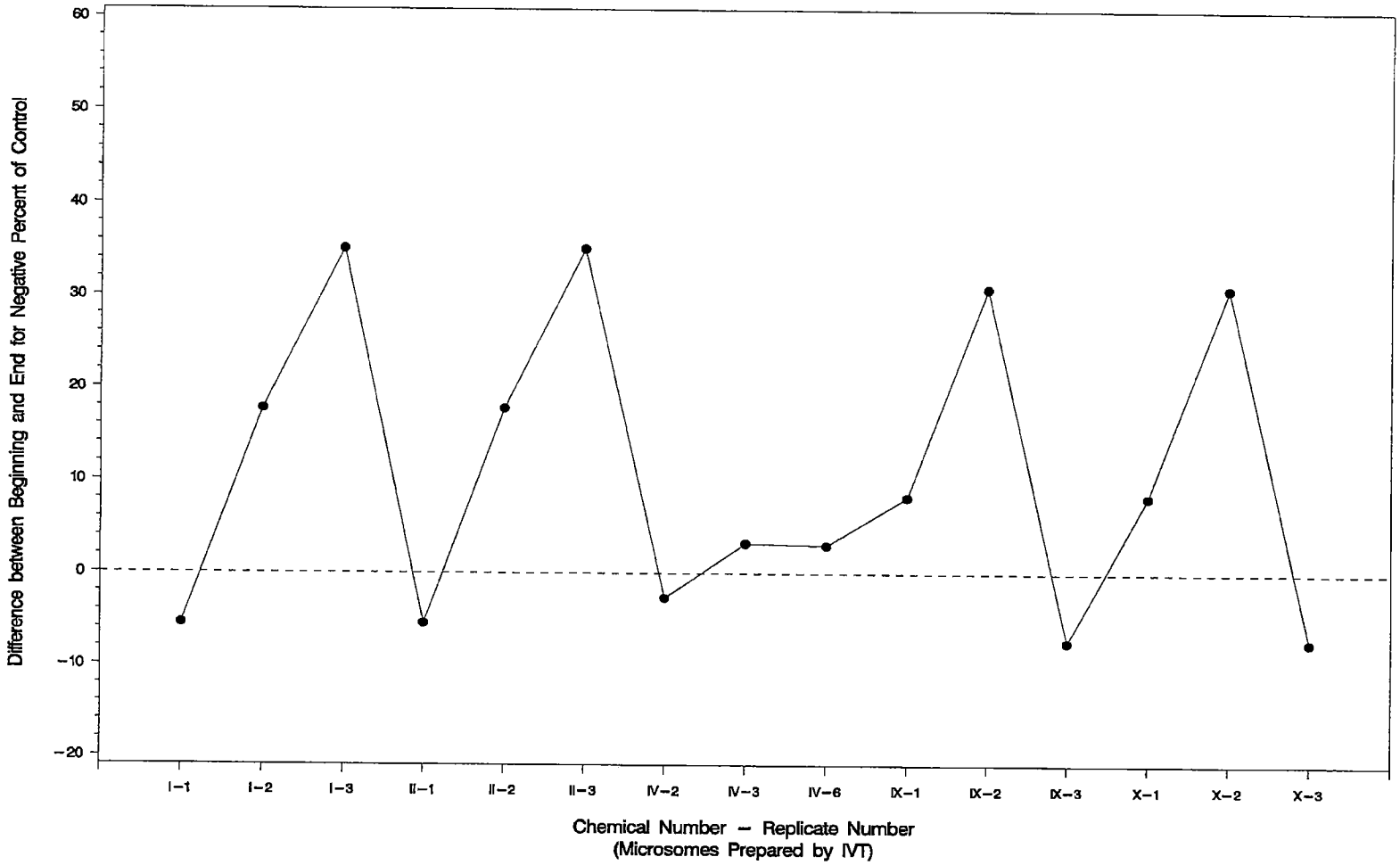


Figure 18b. **Negative Control Data. Microsomes Prepared by IVT.**
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Placental Aromatase Assay. Reference Line at 0%.

Table A-1a. Reference Chemical I: Aminoglutethimide
Microsomes Prepared by: In Vitro Technologies

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Aminoglutethimide]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-3.0000	87.096	93.608	59.515
	-3.5229	27.709	42.161	29.16
	-4.0000	42.045	49.298	34.628
	-5.0000	63.905	99.623	94.083
	-6.0000	111.552	109.741	112.519
	-7.0000	113.392	100.884	103.415
	-8.0000	99.669	113.77	106.623
	-9.0000	115.451	110.566	123.878
2	-3.0000	2.832	5.525	4.545
	-4.0000	40.745	37.58	37.796
	-4.4815	59.96	53.858	72.865
	-5.0000	87.502	80.803	90.908
	-6.0000	103.207	106.027	105.925
	-7.0000	103.863	99.134	99.542
	-8.0000	96.581	92.651	94.706
	-9.0000	106.739	101.78	101.122
3	-3.0000	2.179	1.653	5.293
	-4.0000	40.056	37.394	39.112
	-4.4815	64.901	65.289	61.441
	-5.0000	80.297	77.602	77.889
	-6.0000	105.662	104.419	101.635
	-7.0000	111.613	106.392	102.497
	-8.0000	102.373	101.618	97.015
	-9.0000	98.261	101.323	98.983

Table A-1b. Reference Chemical II: Ketoconazole
Microsomes Prepared by: In Vitro Technology

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Ketoconazole]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	-1.453	-0.384	2.355
	-5.0000	3.694	4.512	9.353
	-6.0000	36.806	43.59	27.931
	-7.0000	90.245	94.354	78.777
	-8.0000	99.933	93.447	106.013
	-9.0000	102.554	110.525	107.813
	-10.0000	110.674	112.327	105.226
	-11.0000	108.684	109.505	112.897
2	-4.0000	0.944	0.024	-0.205
	-5.0000	4.274	2.775	3.973
	-6.0000	26.072	25.175	27.008
	-7.0000	72.773	77.768	77.028
	-8.0000	86.918	91.996	90.27
	-9.0000	95.433	95.702	95.02
	-10.0000	92.687	93.586	95.736
	-11.0000	97.152	91.644	93.172
3	-4.0000	0.41	0.644	-0.48
	-5.0000	2.942	4.71	3.806
	-6.0000	18.75	24.281	26.932
	-7.0000	77.233	73.51	78.632
	-8.0000	93.885	94.304	96.437
	-9.0000	97.26	99.516	90.093
	-10.0000	102.499	99.516	96.039
	-11.0000	91.886	92.22	91.602

Table A-1c. Reference Chemical III: Prochloraz
Microsomes Prepared by: Battelle

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Prochloraz]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
2	-4.0000	1.237	1.177	0.844
	-5.0000	-0.34	0.352	0.037
	-6.0000	1.237	1.45	1.284
	-7.0000	10.655	10.259	18.235
	-8.0000	64.243	67.083	65.843
	-8.4815	79.446	88.081	81.348
	-9.0000	87.154	86.182	83.272
	-10.0000	105.531	97.948	102.728
3	-4.0000	1.267	1.643	3.97
	-5.0000	0.786	-0.754	0.183
	-6.0000	3.43	2.388	2.281
	-7.0000	15.92	17.438	17.495
	-8.0000	66.94	69.243	67.93
	-8.4815	86.193	81.491	79.307
	-9.0000	98.848	99.531	98.076
	-10.0000	102.302	98.886	98.195
4	-4.0000	0.306	-0.052	-0.181
	-5.0000	0.374	0.232	0.515
	-6.0000	2.081	2.263	2.229
	-7.0000	17.865	15.337	17.959
	-7.4815	77.918	79.655	77.953
	-8.0000	59.721	60.719	63.475
	-9.0000	78.269	80.692	78.012
	-10.0000	91.119	90.22	80.351

Table A-1d. Reference Chemical IV: 4-Nonylphenol
Microsomes Prepared by: In Vitro Technologies

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [4-Nonylphenol]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
2	-4.0000	18.292	15.245	17.503
	-4.4815	41.284	40.091	45.698
	-5.0000	88.195	91.559	85.312
	-6.0000	98.955	97.555	93.343
	-7.0000	92.643	93.704	87.437
	-8.0000	95.49	100.328	94.444
	-9.0000	93.616	93.797	91.944
	-10.0000	82.40	93.777	76.368
3	-4.0000	17.675	14.543	13.416
	-4.4815	41.264	38.959	39.434
	-5.0000	81.681	81.901	77.697
	-6.0000	85.367	87.754	92.846
	-7.0000	91.042	88.615	91.922
	-8.0000	87.769	85.531	82.781
	-9.0000	83.352	86.839	87.244
	-10.0000	89.265	87.372	85.683
6	-4.0000	17.425	13.695	12.818
	-4.4815	43.743	38.898	38.265
	-5.0000	84.209	82.068	80.866
	-6.0000	84.588	89.035	89.044
	-7.0000	92.093	90.812	87.333
	-8.0000	90.078	88.181	86.26
	-9.0000	85.371	86.097	85.378
	-10.0000	83.567	91.155	85.478

Table A-1e. Reference Chemical V: Dibenz[a,h]anthracene
Microsomes Prepared by: Battelle

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Dibenze[a,h]anthracene]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.4815	76.509	95.36	91.147
	-5.0000	85.725	86.766	78.918
	-5.4815	87.23	82.604	78.238
	-6.0000	68.406	64.992	91.845
	-7.0000	85.508	88.025	92.457
	-8.0000	85.742	85.012	78.158
	-9.0000	79.102	75.247	81.738
	-10.0000	77.424	80.116	80.747
2	-4.4815	68.756	51.565	95.18
	-5.0000	98.732	97.433	97.305
	-5.4815	96.421	90.456	88.826
	-6.0000	82.64	79.356	68.343
	-7.0000	93.839	94.157	92.997
	-8.0000	88.40	93.363	89.284
	-9.0000	79.731	84.537	79.568
	-10.0000	89.208	87.41	80.266
3	-4.0000	85.31	92.873	94.407
	-4.4815	96.492	96.755	94.074
	-5.0000	89.996	93.64	87.081
	-6.0000	88.018	95.072	95.595
	-7.0000	95.291	98.68	96.456
	-8.0000	97.885	92.907	93.253
	-9.0000	90.331	93.693	93.594
	-10.0000	99.295	94.188	95.256

Table A-1f. Reference Chemical VI: Fenarimol
Microsomes Prepared by: Battelle

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Fenarimol]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.4776	3.909	11.544	40.196
	-5.0000	29.243	28.237	34.782
	-5.4815	51.728	50.611	51.445
	-6.0000	85.138	82.617	78.129
	-7.0000	90.762	91.592	88.971
	-8.0000	98.351	98.227	93.187
	-9.0000	101.207	97.521	97.289
	-10.0000	97.60	94.18	97.892
2	-4.4815	13.526	12.389	12.676
	-5.0000	30.665	31.348	31.208
	-5.4815	50.088	57.378	53.409
	-6.0000	75.374	71.636	77.766
	-7.0000	91.902	90.279	84.129
	-8.0000	83.79	93.672	92.383
	-9.0000	94.524	92.51	90.702
	-10.0000	93.061	91.937	89.364
3	-4.4815	12.501	14.688	12.264
	-5.0000	30.61	32.192	35.33
	-5.4815	63.041	62.884	56.527
	-6.0000	87.742	83.689	82.026
	-7.0000	103.539	93.418	95.63
	-8.0000	102.14	101.85	99.278
	-9.0000	100.535	95.709	101.989
	-10.0000	98.669	98.137	99.582

Table A-1g. Reference Chemical VII: Econazole
Microsomes Prepared by: Battelle

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Econazole]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	-0.433	-0.237	0.794
	-4.4815	0.079	-0.858	-0.267
	-5.0000	-0.506	-0.543	-0.448
	-6.0000	-0.462	-0.402	0.014
	-7.0000	0.56	1.504	1.188
	-8.0000	11.831	9.685	11.199
	-9.0000	44.836	46.104	33.186
	-10.0000	87.379	79.836	81.388
2	-5.0000	0.085	-0.673	-0.283
	-6.0000	0.251	-0.271	-0.458
	-7.0000	1.579	1.553	1.035
	-8.0000	10.836	9.481	11.022
	-8.4815	28.073	29.207	30.573
	-9.0000	54.561	55.618	55.299
	-9.4815	69.134	68.324	79.04
	-10.0000	97.303	94.379	94.207
3	-5.0000	0.653	-0.035	-0.174
	-6.0000	-0.106	-0.039	0.138
	-7.0000	0.942	2.495	2.462
	-8.0000	9.978	10.106	12.833
	-8.4815	30.754	25.17	31.10
	-9.0000	56.833	55.419	51.663
	-9.4815	71.331	66.229	80.257
	-10.0000	92.459	95.183	93.421

Table A-1h. Reference Chemical VIII: Chrysin
Microsomes Prepared by: Battelle

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Chrysin]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	18.80	18.92	15.98
	-4.4815	2.554	6.74	6.618
	-5.0000	18.91	20.929	19.701
	-6.0000	66.24	69.716	58.937
	-7.0000	81.747	79.258	73.993
	-8.0000	87.184	84.216	62.869
	-9.0000	72.327	71.809	69.446
	-10.0000	85.239	88.753	77.303
2	-4.4815	10.538	7.376	8.137
	-5.0000	20.738	19.742	17.721
	-5.4815	41.461	42.876	41.837
	-6.0000	73.57	70.698	71.839
	-7.0000	88.814	89.946	86.40
	-8.0000	90.766	93.343	92.879
	-9.0000	93.197	92.871	93.066
	-10.0000	96.379	95.707	93.915
3	-4.4815	11.299	10.149	10.288
	-5.0000	25.002	25.778	28.975
	-5.4815	52.68	52.349	52.087
	-6.0000	78.554	79.409	78.906
	-7.0000	97.472	87.365	89.949
	-8.0000	102.225	103.691	102.233
	-9.0000	103.504	99.939	99.092
	-10.0000	112.204	100.636	94.992

Table A-1i. Reference Chemical IX: Dicofol
Microsomes Prepared by: In Vitro Technologies

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Dicofol]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	40.897	49.573	55.663
	-4.4815	70.357	66.708	63.66
	-5.0000	68.579	73.405	67.516
	-6.0000	87.957	89.844	86.919
	-7.0000	91.794	90.413	86.071
	-8.0000	89.252	82.658	80.994
	-9.0000	91.721	92.616	94.987
	-10.0000	90.484	90.703	89.429
2	-4.0000	30.847	67.495	58.22
	-4.4815	70.709	72.087	64.564
	-5.0000	68.172	63.69	61.174
	-6.0000	70.265	97.252	96.298
	-7.0000	104.147	102.721	99.33
	-8.0000	97.747	97.074	91.072
	-9.0000	93.757	89.989	98.135
	-10.0000	102.539	103.114	98.705
3	-4.0000	25.611	33.49	39.64
	-4.4815	49.879	52.214	43.468
	-5.0000	71.46	73.826	71.44
	-6.0000	89.796	89.778	96.653
	-7.0000	95.815	94.135	96.585
	-8.0000	96.306	92.868	95.899
	-9.0000	98.134	93.144	97.935
	-10.0000	99.92	94.481	97.762

Table A-1j. Reference Chemical X: Atrazine
Microsomes Prepared by: In Vitro Technologies

Percent of Control Activity in Placental Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Atrazine]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	84.225	85.374	78.294
	-4.4815	78.759	87.76	91.02
	-5.0000	93.116	87.119	92.51
	-6.0000	90.333	84.144	86.478
	-7.0000	83.838	83.892	81.435
	-8.0000	88.808	80.928	83.021
	-9.0000	82.189	83.938	82.65
	-10.0000	81.93	82.451	82.791
2	-4.0000	94.875	90.689	94.511
	-4.4815	91.939	91.26	91.244
	-5.0000	98.468	98.498	97.03
	-6.0000	95.362	99.461	95.657
	-7.0000	96.743	88.05	81.801
	-8.0000	92.495	98.133	73.317
	-9.0000	97.027	95.547	96.502
	-10.0000	94.055	92.258	93.375
3	-4.0000	90.873	96.088	88.907
	-4.4815	95.409	91.026	90.093
	-5.0000	97.685	97.055	93.917
	-6.0000	93.642	94.624	95.541
	-7.0000	94.453	95.573	97.072
	-8.0000	92.988	98.058	95.432
	-9.0000	92.317	92.748	93.715
	-10.0000	87.619	95.506	99.836

Table A-2a. Reference Chemical I: Aminoglutethimide
Microsomes Prepared by: In Vitro Technologies

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.03425	104.405
		Beginning	0.03670	111.881
		End	0.02930	89.324
		End	0.03097	94.391
	2	Beginning	0.06068	122.201
		Beginning	0.05178	104.284
		End	0.04390	88.415
		End	0.04226	85.1
	3	Beginning	0.05933	101.08
		Beginning	0.06607	112.549
		End	0.05640	96.079
		End	0.05300	90.293
Background Control	1	Beginning	0.00033	1.005
		Beginning	0.00156	4.756
		End	-0.00172	-5.244
		End	-0.00017	-0.517
	2	Beginning	0.00041	0.82
		Beginning	-0.00024	-0.488
		End	-0.00005	-0.099
		End	-0.00012	-0.232
	3	Beginning	0.00002	0.037
		Beginning	0.00014	0.239
		End	-0.00035	-0.593
		End	0.00019	0.317
Positive Control	1	Beginning	0.02028	61.828
		Beginning	0.01966	59.937
		End	0.01487	45.339
		End	0.01660	50.587
	2	Beginning	0.02121	42.719
		Beginning	0.01879	37.831
		End	0.02043	41.145
		End	0.02119	42.669
	3	Beginning	0.02362	40.233
		Beginning	0.01996	34.011
		End	0.02699	45.979
		End	0.02801	47.722
Negative Control	1	Beginning	0.03078	93.826
		Beginning	0.02507	76.405
		End	0.02781	84.756
		End	0.02445	74.529
	2	Beginning	0.04014	80.83
		Beginning	0.03398	68.428
		End	0.04460	89.812
		End	0.04715	94.948
	3	Beginning	0.03905	66.532
		Beginning	0.03492	59.486
		End	0.05897	100.465
		End	0.05618	95.711

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2b. Reference Chemical II: Ketoconazole
Microsomes Prepared by: In Vitro Technologies

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.03425	104.405
		Beginning	0.03670	111.881
		End	0.02930	89.324
		End	0.03097	94.391
	2	Beginning	0.06068	122.201
		Beginning	0.05178	104.284
		End	0.04390	88.415
		End	0.04226	85.1
	3	Beginning	0.05933	101.08
		Beginning	0.06607	112.549
		End	0.05640	96.079
		End	0.05300	90.293
Background Control	1	Beginning	0.00033	1.005
		Beginning	0.00156	4.756
		End	-0.00172	-5.244
		End	-0.00017	-0.517
	2	Beginning	0.00041	0.82
		Beginning	-0.00024	-0.488
		End	-0.00005	-0.099
		End	-0.00012	-0.232
	3	Beginning	0.00002	0.037
		Beginning	0.00014	0.239
		End	-0.00035	-0.593
		End	0.00019	0.317
Positive Control	1	Beginning	0.02028	61.828
		Beginning	0.01966	59.937
		End	0.01487	45.339
		End	0.01660	50.587
	2	Beginning	0.02121	42.719
		Beginning	0.01879	37.831
		End	0.02043	41.145
		End	0.02119	42.669
	3	Beginning	0.02362	40.233
		Beginning	0.01996	34.011
		End	0.02699	45.979
		End	0.02801	47.722
Negative Control	1	Beginning	0.03078	93.826
		Beginning	0.02507	76.405
		End	0.02781	84.756
		End	0.02445	74.529
	2	Beginning	0.04014	80.83
		Beginning	0.03398	68.428
		End	0.04460	89.812
		End	0.04715	94.948
	3	Beginning	0.03905	66.532
		Beginning	0.03492	59.486
		End	0.05897	100.465
		End	0.05618	95.711

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2c. Reference Chemical III: Prochloraz
Microsomes Prepared by: Battelle

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	2	Beginning	0.04657	104.521
		Beginning	0.04884	109.602
		End	0.04208	94.446
		End	0.04074	91.431
	3	Beginning	0.04335	101.504
		Beginning	0.04329	101.372
		End	0.04091	95.804
		End	0.04327	101.32
	4	Beginning	0.10805	99.724
		Beginning	0.10777	99.462
		End	0.10803	99.697
		End	0.10956	101.117
Background Control	2	Beginning	0.00013	0.291
		Beginning	-0.00006	-0.128
		End	-0.00013	-0.293
		End	0.00006	0.13
	3	Beginning	0.00026	0.613
		Beginning	-0.00005	-0.12
		End	0.00004	0.105
		End	-0.00026	-0.598
	4	Beginning	-0.00008	-0.076
		Beginning	-0.00016	-0.15
		End	0.00033	0.307
		End	-0.00009	-0.081
Positive Control	2	Beginning	0.02028	45.504
		Beginning	0.01568	35.184
		End	0.02013	45.184
		End	0.02047	45.944
	3	Beginning	0.01804	42.251
		Beginning	0.01573	36.824
		End	0.02263	52.997
		End	0.02313	54.173
	4	Beginning	0.04593	42.392
		Beginning	0.04403	40.633
		End	0.04487	41.407
		End	0.04418	40.776
Negative Control	2	Beginning	0.01316	29.541
		Beginning	0.01297	29.098
		End	0.03519	78.976
		End	0.04016	90.116
	3	Beginning	0.02119	49.631
		Beginning	0.01877	43.942
		End	0.03999	93.632
		End	0.03802	89.021
	4	Beginning	0.09356	86.346
		Beginning	0.09278	85.629
		End	0.10115	93.351
		End	0.10153	93.701

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2d. Reference Chemical IV: 4-Nonylphenol
Microsomes Prepared by: In Vitro Technologies

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	2	Beginning	0.04932	105.402
		Beginning	0.04661	99.623
		End	0.04525	96.706
		End	0.04598	98.27
	3	Beginning	0.05826	103.427
		Beginning	0.05910	104.922
		End	0.05555	98.611
		End	0.05241	93.041
	6	Beginning	0.10394	103.924
		Beginning	0.10447	104.458
		End	0.09704	97.02
		End	0.09461	94.598
Background Control	2	Beginning	-0.00037	-0.798
		Beginning	0.00002	0.046
		End	-0.00010	-0.205
		End	0.00045	0.957
	3	Beginning	-0.00012	-0.205
		Beginning	0.00022	0.399
		End	0.00013	0.232
		End	-0.00024	-0.426
	6	Beginning	0.00075	0.745
		Beginning	-0.00031	-0.308
		End	-0.00027	-0.266
		End	-0.00017	-0.171
Positive Control	2	Beginning	0.02612	55.826
		Beginning	0.02368	50.618
		End	0.02315	49.486
		End	0.02387	51.021
	3	Beginning	0.02835	50.321
		Beginning	0.02715	48.192
		End	0.02565	45.528
		End	0.02832	50.27
	6	Beginning	0.05063	50.626
		Beginning	0.05083	50.819
		End	0.04725	47.24
		End	0.05054	50.529
Negative Control	2	Beginning	0.04167	89.054
		Beginning	0.04244	90.698
		End	0.04134	88.354
		End	0.04027	86.07
	3	Beginning	0.04757	84.452
		Beginning	0.04741	84.169
		End	0.04809	85.371
		End	0.05053	89.701
	6	Beginning	0.08280	82.785
		Beginning	0.08544	85.431
		End	0.08826	88.247
		End	0.08598	85.964

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2e. Reference Chemical V: Dibenz[a,h]anthracene
Microsomes Prepared by: Battelle

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.02315	109.788
		Beginning	0.02447	116.045
		End	0.01743	82.653
		End	0.01930	91.514
	2	Beginning	0.05350	96.342
		Beginning	0.06091	109.672
		End	0.05270	94.89
		End	0.05503	99.097
	3	Beginning	0.05670	103.375
		Beginning	0.05554	101.267
		End	0.05599	102.084
		End	0.05116	93.273
Background Control	1	Beginning	0.00013	0.619
		Beginning	0.00002	0.117
		End	-0.00012	-0.547
		End	-0.00004	-0.189
	2	Beginning	-0.00006	-0.101
		Beginning	-0.00019	-0.335
		End	0	0.007
		End	0.00024	0.429
	3	Beginning	0.00005	0.089
		Beginning	-0.00005	-0.097
		End	-0.00009	-0.162
		End	0.00009	0.169
Positive Control	1	Beginning	0.00990	46.958
		Beginning	0.01053	49.949
		End	0.00786	37.275
		End	0.00854	40.513
	2	Beginning	0.03030	54.556
		Beginning	0.02985	53.748
		End	0.02724	49.052
		End	0.02664	47.968
	3	Beginning	0.02736	49.885
		Beginning	0.02903	52.933
		End	0.02819	51.397
		End	0.02901	52.903
Negative Control	1	Beginning	0.01859	88.169
		Beginning	0.01796	85.176
		End	0.01703	80.762
		End	0.01612	76.455
	2	Beginning	0.04409	79.384
		Beginning	0.04343	78.194
		End	0.04555	82.019
		End	0.04595	82.743
	3	Beginning	0.04661	84.993
		Beginning	0.04762	86.818
		End	0.05003	91.225
		End	0.05084	92.699

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2f. Reference Chemical VI: Fenarimol
Microsomes Prepared by: Battelle

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.05398	110.064
		Beginning	0.04913	100.171
		End	0.04403	89.782
		End	0.04904	99.983
	2	Beginning	0.04657	104.521
		Beginning	0.04884	109.602
		End	0.04208	94.446
		End	0.04074	91.431
	3	Beginning	0.04335	101.504
		Beginning	0.04329	101.372
		End	0.04091	95.804
		End	0.04327	101.32
Background Control	1	Beginning	0.00061	1.238
		Beginning	0.00009	0.183
		End	-0.00028	-0.576
		End	-0.00041	-0.845
	2	Beginning	0.00013	0.291
		Beginning	-0.00006	-0.128
		End	-0.00013	-0.293
		End	0.00006	0.13
	3	Beginning	0.00026	0.613
		Beginning	-0.00005	-0.12
		End	0.00004	0.105
		End	-0.00026	-0.598
Positive Control	1	Beginning	-0.00066	-1.341
		Beginning	0.00021	0.426
		End	0.01503	30.651
		End	0.01370	27.944
	2	Beginning	0.02028	45.504
		Beginning	0.01568	35.184
		End	0.02013	45.184
		End	0.02047	45.944
	3	Beginning	0.01804	42.251
		Beginning	0.01573	36.824
		End	0.02263	52.997
		End	0.02313	54.173
Negative Control	1	Beginning	0.02182	44.488
		Beginning	0.01995	40.68
		End	-0.00050	-1.019
		End	0.02589	52.795
	2	Beginning	0.01316	29.541
		Beginning	0.01297	29.098
		End	0.03519	78.976
		End	0.04016	90.116
	3	Beginning	0.02119	49.631
		Beginning	0.01877	43.942
		End	0.03999	93.632
		End	0.03802	89.021

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2g. Reference Chemical VII: Econazole
Microsomes Prepared by: Battelle

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.05690	112.345
		Beginning	0.05381	106.247
		End	0.04698	92.768
		End	0.04489	88.64
	2	Beginning	0.06201	96.973
		Beginning	0.06411	100.25
		End	0.06547	102.384
		End	0.06420	100.393
	3	Beginning	0.09192	100.6
		Beginning	0.09223	100.93
		End	0.09103	99.616
		End	0.09033	98.854
Background Control	1	Beginning	0.00004	0.077
		Beginning	-0.00029	-0.577
		End	0.00024	0.469
		End	0.00002	0.032
	2	Beginning	-0.00006	-0.093
		Beginning	0.00045	0.711
		End	-0.00001	-0.02
		End	-0.00038	-0.599
	3	Beginning	-0.00013	-0.137
		Beginning	-0.00013	-0.143
		End	0.00053	0.583
		End	-0.00028	-0.303
Positive Control	1	Beginning	0.01679	33.153
		Beginning	0.01796	35.459
		End	0.02193	43.308
		End	0.02299	45.387
	2	Beginning	0.02111	33.018
		Beginning	0.01890	29.559
		End	0.02698	42.195
		End	0.02895	45.263
	3	Beginning	0.02954	32.325
		Beginning	0.02631	28.795
		End	0.03350	36.662
		End	0.03243	35.489
Negative Control	1	Beginning	0.03258	64.323
		Beginning	0.03287	64.893
		End	0.04453	87.923
		End	0.04408	87.03
	2	Beginning	0.03943	61.66
		Beginning	0.03304	51.669
		End	0.05887	92.055
		End	0.05938	92.85
	3	Beginning	0.06231	68.192
		Beginning	0.05479	59.964
		End	0.08802	96.324
		End	0.08752	95.781

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2h. Reference Chemical VIII: Chrysin
Microsomes Prepared by: Battelle

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.05690	112.345
		Beginning	0.05381	106.247
		End	0.04698	92.768
		End	0.04489	88.64
	2	Beginning	0.06201	96.973
		Beginning	0.06411	100.25
		End	0.06547	102.384
		End	0.06420	100.393
	3	Beginning	0.09192	100.6
		Beginning	0.09223	100.93
		End	0.09103	99.616
		End	0.09033	98.854
Background Control	1	Beginning	0.00004	0.077
		Beginning	-0.00029	-0.577
		End	0.00024	0.469
		End	0.00002	0.032
	2	Beginning	-0.00006	-0.093
		Beginning	0.00045	0.711
		End	-0.00001	-0.02
		End	-0.00038	-0.599
	3	Beginning	-0.00013	-0.137
		Beginning	-0.00013	-0.143
		End	0.00053	0.583
		End	-0.00028	-0.303
Positive Control	1	Beginning	0.01679	33.153
		Beginning	0.01796	35.459
		End	0.02193	43.308
		End	0.02299	45.387
	2	Beginning	0.02111	33.018
		Beginning	0.01890	29.559
		End	0.02698	42.195
		End	0.02895	45.263
	3	Beginning	0.02954	32.325
		Beginning	0.02631	28.795
		End	0.03350	36.662
		End	0.03243	35.489
Negative Control	1	Beginning	0.03258	64.323
		Beginning	0.03287	64.893
		End	0.04453	87.923
		End	0.04408	87.03
	2	Beginning	0.03943	61.66
		Beginning	0.03304	51.669
		End	0.05887	92.055
		End	0.05938	92.85
	3	Beginning	0.06231	68.192
		Beginning	0.05479	59.964
		End	0.08802	96.324
		End	0.08752	95.781

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2i. Reference Chemical IX: Dicofol
Microsomes Prepared by: In Vitro Technologies

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.10719	112.074
		Beginning	0.10667	111.539
		End	0.08293	86.707
		End	0.08577	89.68
	2	Beginning	0.13225	107.269
		Beginning	0.12168	98.694
		End	0.12013	97.434
		End	0.11910	96.603
	3	Beginning	0.10730	98.475
		Beginning	0.11646	106.888
		End	0.10759	98.742
		End	0.10449	95.896
Background Control	1	Beginning	-0.00002	-0.018
		Beginning	-0.00043	-0.445
		End	0.00054	0.56
		End	-0.00009	-0.097
	2	Beginning	0.00018	0.145
		Beginning	-0.00031	-0.251
		End	0.00005	0.038
		End	0.00008	0.068
	3	Beginning	0.00029	0.262
		Beginning	-0.00003	-0.026
		End	-0.00017	-0.158
		End	-0.00009	-0.079
Positive Control	1	Beginning	0.04453	46.56
		Beginning	0.04214	44.061
		End	0.04027	42.101
		End	0.04003	41.853
	2	Beginning	0.05046	40.925
		Beginning	0.04560	36.983
		End	0.05503	44.632
		End	0.05598	45.409
	3	Beginning	0.04399	40.375
		Beginning	0.04376	40.164
		End	0.04086	37.502
		End	0.04424	40.604
Negative Control	1	Beginning	0.06928	72.437
		Beginning	0.06932	72.483
		End	0.07851	82.094
		End	0.07575	79.207
	2	Beginning	0.08093	65.643
		Beginning	0.07512	60.927
		End	0.11791	95.64
		End	0.11415	92.585
	3	Beginning	0.10489	96.263
		Beginning	0.10134	93.006
		End	0.09987	91.658
		End	0.09009	82.686

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

Table A-2j. Reference Chemical X: Atrazine
Microsomes Prepared by: In Vitro Technologies

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Placental Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	% of Control[a]
Full Activity Control	1	Beginning	0.10719	112.074
		Beginning	0.10667	111.539
		End	0.08293	86.707
		End	0.08577	89.68
	2	Beginning	0.13225	107.269
		Beginning	0.12168	98.694
		End	0.12013	97.434
		End	0.11910	96.603
	3	Beginning	0.10730	98.475
		Beginning	0.11646	106.888
		End	0.10759	98.742
		End	0.10449	95.896
Background Control	1	Beginning	-0.00002	-0.018
		Beginning	-0.00043	-0.445
		End	0.00054	0.56
		End	-0.00009	-0.097
	2	Beginning	0.00018	0.145
		Beginning	-0.00031	-0.251
		End	0.00005	0.038
		End	0.00008	0.068
	3	Beginning	0.00029	0.262
		Beginning	-0.00003	-0.026
		End	-0.00017	-0.158
		End	-0.00009	-0.079
Positive Control	1	Beginning	0.04453	46.56
		Beginning	0.04214	44.061
		End	0.04027	42.101
		End	0.04003	41.853
	2	Beginning	0.05046	40.925
		Beginning	0.04560	36.983
		End	0.05503	44.632
		End	0.05598	45.409
	3	Beginning	0.04399	40.375
		Beginning	0.04376	40.164
		End	0.04086	37.502
		End	0.04424	40.604
Negative Control	1	Beginning	0.06928	72.437
		Beginning	0.06932	72.483
		End	0.07851	82.094
		End	0.07575	79.207
	2	Beginning	0.08093	65.643
		Beginning	0.07512	60.927
		End	0.11791	95.64
		End	0.11415	92.585
	3	Beginning	0.10489	96.263
		Beginning	0.10134	93.006
		End	0.09987	91.658
		End	0.09009	82.686

a. Percent of control values were calculated by dividing the corrected aromatase activity values by the average of the four full enzyme activity control values within the same replicate and multiplied by 100 percent.

APPENDIX D

WIL RESEARCH LABORATORIES REPORT

ERRATA

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

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

AUDITED TASK REPORT

VALIDATION OF THE PLACENTAL AROMATASE ASSAY FOR ENDOCRINE DISRUPTOR SCREENING USING PREPARED MICROSOMES (WA 4-16, TASK 7)

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

EPA Contract Number 68-W-01-023
Work Assignment 4-16
WIL-431009

Sponsor:

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

Performing Laboratory:

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-9281

AUDITED REPORT

Title:	VALIDATION OF THE PLACENTAL AROMATASE ASSAY FOR ENDOCRINE DISRUPTOR SCREENING USING PREPARED MICROSOMES (WA 4-16, TASK 7)
Task 7:	Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories
Authors:	Jennifer A. Thomas-Wohlever, PhD Justin Godsey, BS, LATG
Performing Laboratory:	WIL Research Laboratories, LLC 1407 George Road Ashland, OH 44805-9281
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 7, 2005
Experimental Start Date:	July 20, 2005
Experimental Termination Date:	October 4, 2005
Audited Task Report Date	January 18, 2006

Quality Assurance Statements

The study was inspected in compliance and audited in accordance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 160), October 16, 1989; the United States EPA Good Laboratory Practice Standards (40 CFR Part 792), September 18, 1989; the standard operating procedures of WIL Research Laboratories, LLC, and the protocol and protocol amendments as approved by the Sponsor, with the following exceptions. The Sponsor has assured GLP compliance of the initial chemical analyses of the bulk chemicals for identity and purity and the preparation of stock formulations.

Intra-laboratory data requiring statistical analysis were analyzed by BioSTAT Consultants, Inc., following the current procedural guidelines of BioSTAT Consultants, Inc. BioSTAT Consultants, Inc. provided a statistical analysis report, which is included as Appendix E. Quality Assurance auditing of the statistical report (for internal consistency with the study report) was conducted under the direction of the Quality Assurance Unit of WIL Research Laboratories, LLC.

Quality Assurance findings, derived from the inspections during the conduct of the study and from the inspections of the raw data and draft report are documented and have been reported to the Study Director. A status report is submitted to management monthly. This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments and the standard operating procedures of WIL Research Laboratories, LLC.

The raw data and draft report were audited by the WIL Quality Assurance Unit prior to submission to the Sponsor to assure that the Final Report accurately describes the conduct and the findings of the study. Quality control (QC) and quality assurance (QA) procedures followed those outlined in the Quality Assurance Project Plan (QAPP) that was prepared for this study.

Phases Inspected

Date of Inspection	Phases Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditors
26-Jul-05	Test & Control Articles & Analytical Chemistry / (Aromatase Assay of 26 July 2005)	26-Jul-05	25-Aug-05	E.Crawford
22-Dec-2005, 27-Dec-2005, 28-Dec-2005, 04-Jan-2006, 05-Jan-2006	Reports / Draft Report, without BioSTAT Appendix	5-Jan-06	Feb-06	J.House
6-Jan-06	Reports / Draft Report, BioSTAT Appendix	6-Jan-06	Feb-06	J.House
02-Nov-2005, 07-Nov-2005	Study Records / (A-1)	7-Nov-05	21-Dec-05	J.House / L.Goodrich
02-Nov-2005, 03-Nov-2005, 04-Nov-2005, 07-Nov-2005	Study Records / (A-2)	7-Nov-05	21-Dec-05	J.House / L.Goodrich
02-Nov-2005, 07-Nov-2005	Study Records / (A-3)	7-Nov-05	21-Dec-05	J.House / L.Goodrich
03-Nov-2005, 04-Nov-2005, 07-Nov-2005	Study Records / (A-4)	7-Nov-05	21-Dec-05	J.House
29-Nov-2005, 30-Nov-2005	Study Records / (Spreadsheet Data for Statistical Analysis)	30-Nov-05	21-Dec-05	J.House
29-Jun-05	Protocol / Protocol Amendment Review / Protocol Review	29-Jun-05	25-Jul-05	L.Goodrich

Approval

This study was inspected according to the criteria discussed above.

Report Audited By:

_____ _____
Janet M. House, BS, RQAP-GLP Date
Compliance Specialist

Report Released By:

_____ _____
Heather L. Osborn, BS, RQAP-GLP Date
Manager, Quality Assurance

Compliance Statement

This study, designated WIL-431009, was conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 160), October 16, 1989; the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 792), September 18, 1989; the standard operating procedures of WIL Research Laboratories, LLC, and the protocol and amendments as approved by the sponsor.

_____ _____

Jennifer A. Thomas-Wohlever, Ph.D. Date
Research Scientist, Metabolism
Study Director

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1.0 Executive Summary

The placental aromatase assay combines human placental microsomes, substrate, appropriate cofactors and reference chemicals in a common reaction vessel under optimized conditions for the enzyme. The effect of the reference chemicals on microsomal enzyme activity is evaluated by measuring the amount of product formed by the enzyme-catalyzed substrate oxidation. The aromatase assay is conducted over a range of reference chemical concentrations such that a dose response curve can be developed and an IC_{50} calculated to quantify the concentration of reference chemical required to inhibit aromatase activity by 50%. The general purpose of this assay is to screen chemicals for their ability to inhibit aromatase activity, an indication of the chemical's potential to disrupt endocrine function. This specific study was undertaken to demonstrate the conduct and responsiveness of the placental microsome aromatase assay at WIL Research Laboratories, LLC (a participating laboratory in the inter-laboratory validation) using the known aromatase inhibitor 4-hydroxyandrostenedione as a Positive control, lindane as a Negative control and ten reference chemicals whose IC_{50} will be determined in the course of this Task
(WA 4-16, Task 7).

Complete dose response curves were achieved consistently with the reference chemicals prochloraz, 4-nonylphenol, fenarimol, dicofol, econazole, chrysin, aminoglutethimide and ketoconazole. Generally, reference chemical concentrations ranging from $1x10^{-10}$M to $1x10^{-3}$M resulted in sigmoidal dose response curves ranging from no inhibition (100 percent of control) to almost full inhibition (approximately 0-5% percent of control), respectively. As expected, no inhibition of aromatase activity was detected in the presence of any tested concentration of atrazine or dibenz[a,h]anthracene. The inclusion of Full Enzyme and Background Activity controls at the beginning and end of each assay ensured that the conditions were constant throughout each successful replicate test and that there was no background activity that might interfere with the interpretation of the results. The inclusion of Positive and Negative controls at the beginning and end of each assay demonstrated the reliable and reproducible response of the enzyme to a known inhibitor and a known non-inhibitor of aromatase activity.

2.0 Introduction

2.1 Background

The Food Quality Protection Act of 1996 was enacted by Congress to authorize the 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 definitive *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. Battelle Memorial Institute is administering the aromatase validation for the EPA.

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. 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 by the EDSP and encompassed (1) searching the literature databases, (2) contacting individuals to obtain information on unpublished research and (3) evaluating the literature and personal communications (WA 2-7).

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, respectively. 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 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 1970s and have expanded greatly in the past three decades.

An *in vitro* aromatase assay could easily be used as a 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 recombinant tissues, have been used as *in vitro* systems for measuring the effects of compounds on aromatase activity. These cell lines are also utilized for investigation 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 mM to greater than 50 mM (Battelle, 2005).

The human placental microsomal aromatase assay has been recommended as an *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 placental 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 to the performance of a recombinant microsomal assay system. Concerns with this initial work involving high variability in some runs and partial inhibition curves were addressed in a supplemental prevalidation study (WA 4-10). The placental microsome aromatase assay was previously validated at WIL Research Laboratories using the known aromatase inhibitor 4-hydroxyandrostenedione (4-OH ADSN) as a Positive control (WIL-431006, WA 4-16, Task 4). In addition, the microsomes used in this Task underwent preliminary validation for activity and inhibition response (WIL-431008, WA 4-16, Task 6) prior to acceptance for this Task.

2.2 Task Description And Objectives

WIL Research Laboratories, LLC (as one of the participating laboratories in the inter-laboratory evaluation) has demonstrated the responsiveness of the human placental microsomal aromatase assay to a series of 10 reference chemicals: aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, dibenz[a,h]anthracene, econazole, fenarimol, chrysin, dicofol and atrazine. For inter- and intra-laboratory variability assessment, each chemical was tested via three repetitions on each of three days (replicates). Each replicate included Full Enzyme Activity controls, Background Activity controls, and Positive and Negative controls to support the validation of the human placental microsomal aromatase assay. This report describes the intra-laboratory data for WIL Research, LLC.

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. It had a reported purity of 100%. The radiolabeled androstenedione ([1β-^{3}H]-androstenedione, [^{3}H]ASDN, Lot # 3538-496), was obtained from Perkin Elmer Life Science (Boston, MA) and had a reported specific activity of 25.30 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 found to be 97%. The results of this analysis are presented in the report contained in Appendix C.

A mixture of ASDN and [^{3}H]ASDN was made such that the final concentration of ASDN in the assay was 100 nM, and each assay contained approximately 0.1 µCi of tritium. This was accomplished by preparing a 100-fold dilution of the radiolabeled stock in buffer. In addition, a 1 mg/mL solution of ASDN in ethanol was prepared, and then dilutions of stock ASDN were made in buffer to a final concentration of 1 µg/mL. To make 8 mL of substrate solution (enough for 80 tubes), 4.5 mL of the 1 µg/mL solution of ASDN, 800 µL of the [^{3}H]ASDN dilution and 2.7 mL buffer were combined. For accuracy, the weight of each component added to the substrate solution was recorded. To determine the specific activity of the ASDN substrate, aliquots of substrate solution (ca 20 µL, weighed) were combined with scintillation cocktail for radiochemical content analysis.

3.2 Reference And Control Chemicals

Reference chemicals were selected by the EPA to represent a range of aromatase inhibitors and known non-inhibitors, as described in the following table. The Sponsor's Chemical Repository (CR) was responsible for the 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. These chemistry activities and results are described in the Sponsor's Chemistry report which is submitted in conjunction with this document.

Reference Chemical	Basis for Selection
aminoglutethimide	Non-steroidal aromatase inhibitor
chrysin	Potent flavonoid
dicofol	Organochlorine
econazole (nitrate)	Potent imidazole anti-fungal
ketoconazole	Weak imidazole anti-fungal
atrazine	Affects aromatase gene expression; no aromatase inhibition
fenarimol	pyrimidine fungicide
4-nonylphenol	Affects Androgen Receptor/Estrogen Receptor; no aromatase inhibition
prochloraz	conazole fungicide
dibenz [a,h] anthracene	Known non-aromatase inhibitor; Ah receptor agonist

When reference chemicals arrived at WIL, they were assigned a unique identification number (e.g. MET-XXXXY) which was recorded and dated on the log-in sheet as specified in WIL standard operating procedures. Also recorded on the log-in sheet were the label identification information, quantity received, storage conditions, storage location and a physical description of the material. Any documents accompanying the shipment were filed with the log-in sheet. The following table summarizes reference chemical information, including the WIL identification number assigned. The reference chemicals were numbered 1-10 by the CR and these same numeric designations were used when the samples were coded prior to distribution to the participating laboratories. This ensured that, for example, Chemical 1 was always the same chemical in each laboratory. This is important for the proper balancing of the study as outlined in Section 3.3.

Each replicate tested the response of aromatase activity to the presence of eight concentrations of a reference chemical. The reference chemicals were identified by the code assigned by the CR prior to distribution to the assaying technicians to ensure that the replicates were conducted blind for reference chemical identity. Each reference chemical in this task was tested via three independent replicates. All three replicates for a given reference chemical were conducted by the same technician. However, the same technician was not required to perform the three replicates for all ten reference chemicals. Multiple reference chemicals were conducted on some days. Each replicate for a given reference chemical was conducted entirely independently of the other replicates for that reference chemical. Thus, when multiple assays were conducted on a given day by a single technician, those assays used different reference chemicals. Reference chemicals were unblinded for data analysis and report writing.

Reference Chemical Information								
Chemical name	Chemical Code	Mfr. Purity	CAS No.	Molecular formula	M.W	Stock Solution (WIL ID)	Vehicle (used for dilution at WIL)	Storage
4-Androsten-4-ol-3,17-dione	4-OH ASDN (Positive Control)	99%	566-48-3	$C_{19}H_{26}O_3$	302.4	MET-0300A	95% Ethanol Lot # SW0045	~5°C
Lindane	Negative Control	99.6%	58-89-9	$C_6H_6Cl_6$	290.8	MET-0299A	DMSO Lot # 2969A4 6428	~5°C
Aminoglute-thimide	Reference Chemical 1	99%	125-84-8	$C_{13}H_{16}N_2O_2$	232.3	MET-0301A	DMSO Lot # 2969A4 6428	~5°C

Reference Chemical Information								
Chemical name	Chemical Code	Mfr. Purity	CAS No.	Molecular formula	M.W	Stock Solution (WIL ID)	Vehicle (used for dilution at WIL)	Storage
Ketoconazole	Reference Chemical 2	> 99%	65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	531.43	MET-0302A	DMSO Lot # 2969A46428	~5°C
Prochloraz	Reference Chemical 3	99.5%	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$	376.7	MET-0308A	DMSO Lot # 2969A46428	RT
4-Nonylphenol	Reference Chemical 4	>98.5%	104-40-5	$C_{15}H_{24}O$	220.4	MET-0319A	DMSO Lot # 2969A46428	~5°C
Dibenz[a,h]-anthracene	Reference Chemical 5	97%	53-70-3	$C_{22}H_{14}$	278.35	MET-0320A	DMSO Lot # 2969A46428	~5°C
Fenarimol	Reference Chemical 6	99%	60168-88-9	$C_{17}H_{12}Cl_2N_2O$	331.2	MET-0309A	DMSO Lot # 2969A46428	RT
Econazole	Reference Chemical 7	98%	24169-02-6	$C_{18}H_{15}Cl_3N_2O$-HNO_3	444.7	MET-0313A	DMSO Lot # 2969A46428	~5°C
Chrysin	Reference Chemical 8	98.2-101.4%	480-40-0	$C_{15}H_{10}O_4$	254.2	MET-0314A	DMSO Lot # 44155	2-8°C
Dicofol	Reference Chemical 9	96.5%	115-32-2	$C_{14}H_9Cl_5O$	370.47	MET-0317A	DMSO Lot # 2969A46428	~5°C
Atrazine	Reference Chemical 10	98%	1912-24-9	$C_8H_{14}ClN_5$	215.69	MET-0318A	DMSO Lot # 2969A46428	~5°C

The Positive control (4-OH ASDN) was received as a 0.01 M stock solution in 95% ethanol. This solution was used to create a 5 X 10^{-8} M working stock solution by first diluting 10 µL to 1 mL in 95% ethanol, then diluting again by adding 50 µL of that solution to an additional 950 µL of 95% ethanol. Lindane was received as a 0.1 M stock solution in DMSO. The stock solution was diluted 100-fold (10 µL in 1 mL DMSO) and then ten-fold (100 µL in 1 mL DMSO) to create a 1 x 10^{-4} M solution. The concentration of 4-OH ASDN was determined from previous work assignments (WA 4-16 Task 4, WIL-431006) and published values (reviewed in WA 4-10). The concentration of lindane was chosen because it was in the range of the concentration of reference chemicals, although no concentration was expected to inhibit aromatase.

The reference chemicals were received as 0.01 M or 0.1 M stock solutions in DMSO. The reference chemicals were diluted in DMSO according to the following schemes.

Reference Chemical Dilutions – 0.01 M Stock Solution (Chrysin, Ketoconazole, Dibenz[a,h]anthracene)					
	Reference Chemical Stock solution		Diluent (µL DMSO)	Solution Concentration (M)	Target Concentration in Assay (M)
	µL	M			
Concentration 1	200	$1 X 10^{-2}$	0	$1 x 10^{-2}$	$1 x 10^{-4}$
Concentration 2	100	$1 X 10^{-2}$	900	$1 x 10^{-3}$	$1 x 10^{-5}$
Concentration 3	500	$1 X 10^{-3}$	500	$5 x 10^{-4}$	$5 x 10^{-6}$
Concentration 4	100	$1 X 10^{-3}$	900	$1 x 10^{-4}$	$1 x 10^{-6}$
Concentration 5	100	$1 x 10^{-4}$	900	$1 x 10^{-5}$	$1 x 10^{-7}$
Concentration 6	100	$1 x 10^{-5}$	900	$1 x 10^{-6}$	$1 x 10^{-8}$
Concentration 7	100	$1 x 10^{-6}$	900	$1 x 10^{-7}$	$1 x 10^{-9}$
Concentration 8	100	$1 x 10^{-7}$	900	$1 x 10^{-8}$	$1 x 10^{-10}$

Reference Chemical Dilutions – 0.1 M Stock Solution (Aminoglutethimide, Prochloraz, 4-Nonylphenol, Fenarimol, Econazole, Dicofol, Atrazine)					
	Reference Chemical Stock solution		Diluent (µL DMSO)	Solution Concentration (M)	Target Concentration in Assay (M)
	µL	M			
Concentration 1	200	$1 X 10^{-1}$	0	$1 x 10^{-1}$	$1 x 10^{-3}$
Concentration 2	100	$1 X 10^{-1}$	900	$1 x 10^{-2}$	$1 x 10^{-4}$
Concentration 3	100	$1 X 10^{-2}$	900	$1 x 10^{-3}$	$1 x 10^{-5}$
Concentration 4	100	$1 X 10^{-3}$	900	$1 x 10^{-4}$	$1 x 10^{-6}$
Concentration 5	100	$1 x 10^{-4}$	900	$1 x 10^{-5}$	$1 x 10^{-7}$
Concentration 6	100	$1 x 10^{-5}$	900	$1 x 10^{-6}$	$1 x 10^{-8}$
Concentration 7	100	$1 x 10^{-6}$	900	$1 x 10^{-7}$	$1 x 10^{-9}$
Concentration 8	100	$1 x 10^{-7}$	900	$1 x 10^{-8}$	$1 x 10^{-10}$

After completion of the first replicate for each reference chemical, the data were reviewed and the concentration of reference chemical used in the second and third replicates were adjusted to better define the dose-response curve. The decision whether to adjust test concentrations was made by the Study Director. The decision was based on the results from the first replicate with the following guidelines in mind:

- If insolubility was observed at the high concentration (10^{-3} M), then the highest concentration for the second and third replicates was set at the highest concentration that appeared to be soluble (limited to 10^{-4} or 10^{-5} M). The highest concentration tested was never lower than 10^{-5} M.

- When the highest concentration to be tested was lowered to 10^{-4} M, then mid-log concentration(s) were added 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 was 10^{-10} M.

3.3 Microsomes

Human placental microsomes were received as multiple frozen aliquots from participating laboratories. Upon receipt, the sample code numbers MET-0297A and MET-0298A were assigned to the microsomes from Laboratory 4 (In Vitro Technologies) and Laboratory 2 (Battelle), respectively. The microsomes were stored between approximately -70 to -80°C. On the day of the assay, microsomes were thawed rapidly in a 37 ± 1°C water bath, re-homogenized by briefly vortex mixing and then kept on ice until used. Separate aliquots of microsomes were used for each assay. At no point were microsome aliquots thawed, re-frozen and thawed again for use. As determined in WA 4-16 Task 6 (WIL-431008), the concentration of the stock microsomes differed depending on the source. Therefore, the dilution factors were adjusted to ensure that the final protein concentration of the microsomes in the assay was approximately 0.0125 mg/mL. The final concentration of the microsomes in the stock solution was determined on each assay day regardless of assay source.

For each chemical (1 to 10), Laboratory 2 (Battelle) supplied two of laboratories with microsomes and Laboratory 4 (In Vitro Technologies) supplied the other two. The distribution of microsomal preparations differed for different chemicals. M_2 denotes the microsomal preparation from Laboratory 2 and M_4 denotes the microsomal preparation from Laboratory 4. The four laboratories used microsomal preparations from the laboratories as shown in the following table.

Microsome Distribution and Testing Matrix				
	Analysis Laboratory			
Chemical	Laboratory 1 RTI	Laboratory 2 Battelle	Laboratory 3 WIL	Laboratory 4 In Vitro Technologies
Chemical 1	M_2	M_2	M_4	M_4
Chemical 2	M_2	M_2	M_4	M_4
Chemical 3	M_4	M_4	M_2	M_2
Chemical 4	M_2	M_4	M_2	M_4
Chemical 5	M_4	M_2	M_4	M_2
Chemical 6	M_4	M_4	M_2	M_2
Chemical 7	M_2	M_4	M_4	M_2
Chemical 8	M_2	M_4	M_4	M_2
Chemical 9	M_4	M_2	M_2	M_4
Chemical 10	M_4	M_2	M_2	M_4

Each test laboratory analyzed five chemicals with a microsomal preparation made in Laboratory 2 and five chemicals with a microsomal preparation made in Laboratory 4. Across the 10 chemicals, the laboratories were approximately balanced for comparison of M_2 and M_4. Thus if there was no interaction between laboratory or microsome source and chemical, the laboratory effects were excluded from the comparison between microsomal preparations.

3.4 Other Assay Components

Assay Reagents – Information			
Chemical	Supplier	WIL ID	Lot Number
NADPH	Sigma-Aldrich	CP#05-077A,B	074K7057
Propylene glycol	J.T. Baker	CP#04-163	Y41659
Sodium phosphate dibasic	J.T. Baker	CP#04-090 CP#05-066	A11H37 B14H25
Sodium phosphate monobasic	J.T. Baker	CP#04-164	A28H21
Test/control vehicle A – Ethanol, 95%	Sponsor	MET-0304A #1	SW0045
Test/control vehicle B – Dimethylsulfoxide (DMSO)	Sponsor	MET-0303A#1 MET-0316A#1	2969A46428 2969A44155

3.4.1 NADPH

Nicotinamide adenine dinucleotide phosphate (NADPH, reduced form tetrasodium salt) is a required cofactor for aromatase activity. As such, it was included in excess in the aromatase assay. First, 0.05 g NADPH was weighed and transferred into a 10-mL volumetric flask to make a 5 mg/mL solution in phosphate buffer (see Section 3.4.2). Adding 100 µL of this NADPH solution to the reaction mixture resulted in a final assay concentration of 0.3 mM. NADPH was prepared fresh every assay day and was stored on ice until adding to the reaction mixture.

3.4.2 Assay Buffer

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

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. Six standards were prepared ranging from approximately 5 to 250 µg/mL. Five of the six standards (5–125 µg protein/mL) were used to generate the standard curve as the response of the assay was non-linear at 250 µg protein/mL. The protein standards were commercially made bovine serum albumin (BSA) standards obtained from BioRad (Hercules, CA). QC standards (nominal protein concentrations of 10 and 100 µg/mL) were prepared by the lead laboratory and distributed to each participating laboratory. Each of these QC standards was run in duplicate with each run of the protein assay. Total protein in standards, quality controls, and samples of the microsome preparation were analyzed using a DC Protein Assay kit also purchased from BioRad. To a 200-µL aliquot of unknown or standard, 100 µL of BioRad DC Protein Kit Reagent A was added and vortex mixed. Next, 800 µL of BioRad DC Protein Kit Reagent B was added to each standard or unknown and the samples were vortex mixed again. The samples were allowed to sit at room temperature for at least 15 minutes to allow for color development. The absorbance values were stable for about 1 hour. Each sample (unknown and standard) was transferred to an appropriately labeled cuvette and the

absorbance at 750 nm was measured using a spectrophotometer. The protein concentration of the microsomal sample was determined by extrapolation of the absorbance value using the curve developed from the protein standards.

3.6 Cytochrome P450 Aromatase (CYP19) Activity

Aromatase activity was determined via an in vitro screening assay utilizing human placental microsomes supplied by either Laboratory 2 or 4. The assays were performed in 13 x 100 mm test tubes. Each test tube was uniquely identified with replicate, repetition, and group information summarized in the table below as necessary to differentiate the tubes. In addition to tubes containing inhibitor, Full Enzyme Activity controls (includes vehicle but no inhibitor) and Background Activity controls (tubes contain no NADPH cofactor) were utilized to determine 100% and 0% activity. Positive controls utilizing the known aromatase inhibitor 4-hydroxyandrostenedione and Negative controls containing the known non-inhibitor lindane were also included in the assay design. The following table demonstrates the assay design with a 0.1 M stock of reference chemical. The reference chemical concentrations to be tested were adjusted for 0.01 M reference chemical stocks, as shown in Section 3.2. Following the first replicate, reference chemical concentrations were adjusted at the discretion of the Study Director, as described in Section 3.2.

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

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

[a] = Complete assay contains buffer, propylene glycol, microsomal protein, substrate and NADPH.
N/A = Not Applicable

Propylene glycol (100 µL), ^{3}H-ASDN substrate solution (100 µL), NADPH (100 µL, excluded from Background Activity control), and vehicle or Reference Chemical (20 µL) were added to the appropriate test tube with buffer to make 1 mL total volume. Microsomes were diluted to the appropriate concentration as detailed in Section 3.3. Both the reaction mixture and the microsomes were incubated at 37 ± 1 °C independently for at least 5 minutes with shaking. After the addition of 1 mL microsomes to the first test tube containing the reaction mixture, the remaining assays were initiated at 10 second intervals. Each assay was incubated at 37 ± 1°C in shaking water bath for 15 minutes. At the conclusion of the reaction time, tubes were quenched with 2.0 mL of methylene chloride in the order in which microsomes had been added, one every 10 seconds. The tubes were vortex-mixed for approximately 5 seconds and placed on ice until all tubes were quenched. The tubes were then vortex-mixed an additional 20-25 seconds. The tubes were centrifuged for 10 minutes at approximately 162 x g to facilitate separation of the organic and aqueous layers. 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. The aqueous layers were transferred to vials and duplicate aliquots (0.5-mL) were transferred to liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Perkin Elmer, approximately 10 mL) was added to each counting vial and shaken to mix the solution. Analysis of the samples was performed using a Beckman Coulter LS6500 liquid scintillation counter (LSC).

Radiolabel found in the aqueous fractions represented ^3H_2O formed. One ^3H_2O molecule was released per molecule of radiolabeled 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 (in dpm) 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 normalized per mg protein. The activity of the enzyme reaction is expressed in nmol/mg protein/min.

Each assay replicate was performed on the day shown in the following table. The same technician performed each replicate for a given reference chemical. As discussed in Section 2.2, study design and objectives were to be met by completing three repetitions with each reference chemical on each of three replicate days.

Assay Dates by Technician		
Replicate	Date	Technician
Aminoglutethimide Rep 1	7/20/2005	JG
Aminoglutethimide Rep 2	7/22/2005	JG
Aminoglutethimide Rep 3	7/26/2005	JG
Ketoconazole Rep 1	7/20/2005	JG
Ketoconazole Rep 2	7/22/2005	JG
Ketoconazole Rep 3	7/26/2005	JG
Prochloraz Rep 1	8/3/2005	TNB
Prochloraz Rep 2	8/5/2005	TNB
Prochloraz Rep 3	8/9/2005	TNB
4-Nonylphenol Rep 1	9/19/2005	JG
4-Nonylphenol Rep 2	9/22/2005	JG
4-Nonylphenol Rep 3	9/26/2005	JG
Dibenz[a,h]anthracene Rep 1	9/28/2005	TNB
Dibenz[a,h]anthracene Rep 2	9/30/2005	TNB
Dibenz[a,h]anthracene Rep 3	10/4/2005	TNB
Fenarimol Rep 1	8/3/2005	TNB
Fenarimol Rep 2	8/5/2005	TNB
Fenarimol Rep 3	8/9/2005	TNB
Econazole Rep 1	8/15/2005	JG
Econazole Rep 2	8/22/2005	JG
Econazole Rep 3	8/30/2005	JG
Chrysin Rep 1	8/15/2005	JG
Chrysin Rep 2	8/22/2005	JG
Chrysin Rep 3	8/30/2005	JG
Dicofol Rep 1	9/1/2005	TNB
Dicofol Rep 2	9/6/2005	TNB
Dicofol Rep 3	9/8/2005	TNB

Assay Dates by Technician		
Replicate	Date	Technician
Atrazine Rep 1	9/1/2005	TNB
Atrazine Rep 2	9/6/2005	TNB
Atrazine Rep 3	9/8/2005	TNB

3.7 Data Analysis

Relevant data were entered into the latest version of the Microsoft® Excel spreadsheet Aromatase_Master_Versionx.y.xls (where x and y denote version number designation) supplied by the Sponsor for calculation of aromatase activity and percent of control. Once the data was entered, the spreadsheet was saved as a new document where the name reflects the study number, reference chemical number and replicate. For each reference chemical, three independent replicates of the concentration response curve fit were performed.

For each replicate, two repeat tubes of the Full Enzyme Activity controls, the Background Activity controls and the Positive and Negative controls were run prior to the varying concentrations of the reference chemical. Also, two repeat tubes of each control were run following the reference chemical tubes. Three repetitions (tubes) were prepared for each concentration of the reference chemical.

The data recorded for each replicate included assay date, reference chemical ID, replicate, number of concentrations tested, technician, reference chemical concentration, total DPM minus background DPM and calculated percent of Full Enzyme Activity. 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. Total initial DPM was calculated by the multiplication of the volume of substrate solution added to the incubation by the substrate solution radiochemical content (average DPM/mL) and yields the total DPM present in the assay tube at initiation. Background DPM was calculated as the average of the DPM present in the aqueous portion for the Background Activity control tubes, and was subtracted from actual DPM for all samples to provide DPM for calculating aromatase activity. This corrected DPM is then converted to nmol product formed by dividing by the substrate specific activity (DPM/nmol). The activity of the enzyme reaction is expressed in nmol/mg protein/min and was calculated by dividing the amount of estrogen formed (nmol) by the product of mg microsomal protein used and the incubation time.

The average activity in the four background-corrected Full Enzyme Activity control samples for a given Replicate was calculated. Percent of Full Enzyme Activity remaining in the presence of various Reference Chemical (inhibitor) concentrations was calculated by dividing the aromatase activity at a given concentration by the average Full Enzyme Activity control and multiplying by 100. Thus, the average percent activity across the four Background Activity repeat tubes must necessarily equal 0 within each replicate and the average percent activity across the four Full Enzyme Activity repeat tubes must necessarily equal 100 within each replicate. Although percent of control values ideally vary between 0% near high inhibitor concentrations and 100% near low inhibitor concentrations, individual experimental percent of control activity values will sometimes extend below 0% or above 100%.

In several instances, the percent of Full Enzyme Activity control values within a replicate appeared to have an outlier. These values were subjected to a Q-test as described by Dean and Dixon (1951). Briefly, with an n of 3, if the quotient of the difference between the outlier (X_1) and the next closest value (X_2) and the range of values (W) exceed 0.94, the value could be rejected with 90% confidence, as shown in the following equation:

$$\frac{(X_2 - X_1)}{W} = Q$$

Percent of control activity data was exported to Prism (GraphPad, San Diego) for curve fitting. IC_{50} (output as EC_{50} in Prism and used interchangeably in this report) was calculated using Prism (v. 4.02) software to fit the percent of control activity versus log concentration data to a curve using the following equation:

$$Y = B + \frac{T - B}{1 + 10^{(\mu - X)*H}}$$

The response curve was fitted by non-weighted least squares nonlinear regression analysis with

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, ß
μ = $\log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to percent of control activity equal to 50%).

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 average values and standard errors of T, B, $\log_{10}IC_{50}$, or β and the replicate-to-replicate components of variation were calculated based on one-way random effects analysis of variance model fits. The estimated T, B, $\log_{10}IC_{50}$, and β for a reference chemical were (weighted) means across the replicates. The estimated overall standard errors were based on the standard errors within each replicate and the replicate-to-replicate variability. The "Status" of each replicate of each response curve was classified as:

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

Microsoft® Excel using full floating decimal point calculations was used to determine mathematical averages, standard deviations (sd), percent coefficient of variation (%CV) and standard errors of the mean (sem) in order to assess the arithmetic variation between repetitions (within a single replicate) and between replicates. Calculations were performed on the data as collected or as displayed or output by instruments, with no censoring for quantitation limits or significant figures. Slightly different results can be expected if calculations are based on the values as presented in the tables because some numbers have been rounded for display.

Within each replicate of each reference chemical, quadruplicate repetitions were made of the Full Enzyme Activity control, Background Activity control and Negative and Positive control tubes. Half the repetitions were performed at the beginning of the replicate and half at the end. If the conditions were consistent throughout the replicate test, the activity in the control tubes at the beginning of the sample set are equivalent to those at the end of the sample set. To assess whether this was the case, the control responses were adjusted for background DPM, divided by

the average of the (background adjusted) Full Enzyme Activity control values, and expressed as percent of control.

Three-factor mixed effects analysis of variance models were 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 were:

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

The random effects were:

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

The residual error variation corresponded to repetition within reference chemical, replicate, and portion. Statistical analysis was performed on the data reported as 'percent of Full Enzyme Activity control.' Because the average of repetitions for the Background Activity and Full Enzyme Activity controls within a reference chemical and replicate are constrained to be 0 and 100 respectively, and the way in which "percent of control" is defined, the variation associated with the reference chemical effect and the replication within reference chemical effect were both necessarily constrained to be 0.

If the daily replicates were in control, then the portion main effect, the portion by reference chemical interaction and the portion by replicate within reference chemical interaction would be non-significant. If the portion by reference chemical interaction was significant, the nature of the effect was 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 was significant, the nature of the effect was 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. Simultaneity of inference was adjusted for by Bonferroni's method.

3.8 Data Retention

The Sponsor has title to all documentation records, raw data, specimens or other work product generated during the performance of the study. All work product generated by WIL Research Laboratories, LLC, including raw paper data and pertinent electronic storage media, are retained in the Archives at WIL Research Laboratories, LLC, as specified in the study protocol. Data generated by BioSTAT Consultants, Inc. will be maintained in the archives at WIL Research Laboratories, LLC. Data generated by the Sponsor will be maintained as defined in the Sponsor's applicable standard operating procedures. Pertinent electronic storage media and the original final report are retained in the Archives at WIL Research Laboratories, LLC, in compliance with regulatory requirements.

4.0 Results

4.1 Radiochemical Purity

Purity Report: Appendix C

The radiochemical purity of the [^{3}H]ASDN was determined by RTI International to be 97%. The concentration and specific activity of the substrate was used to calculate the

aromatase activity in the assay following daily analysis of the assay substrate solution as detailed in the following table.

Assay Substrate Analysis Results					
Replicate	Radio-chemical Code	Radiochemical ID	Radiochemical Stock Concentration (µCi/g)	Assay Substrate Final Concentration (µg/g) (ASDN + 3H ASDN)	Substrate sol'n Specific Activity (dpm/nmol)
Aminoglutethimide /Ketoconazole Rep 1	[^{3}H]-ASDN	CP# 05-079	0.674	0.579	741060
Aminoglutethimide /Ketoconazole Rep 2	[^{3}H]-ASDN	CP# 05-079	0.634	0.589	684281
Aminoglutethimide /Ketoconazole Rep 3	[^{3}H]-ASDN	CP# 05-079	0.689	0.581	753259
Prochloraz/ Fenarimol Rep 1	[^{3}H]-ASDN	CP# 05-079	0.792	0.593	849956
Prochloraz/ Fenarimol Rep 2	[^{3}H]-ASDN	CP# 05-079	0.726	0.583	792035
Prochloraz/ Fenarimol Rep 3	[^{3}H]-ASDN	CP# 05-079	0.725	0.575	801061
4-Nonylphenol Rep 1	[^{3}H]-ASDN	CP# 05-079	0.793	0.582	866645
4-Nonylphenol Rep 2	[^{3}H]-ASDN	CP# 05-079	0.781	0.570	871364
4-Nonylphenol Rep 3	[^{3}H]-ASDN	CP# 05-079	0.775	0.593	831192
Dibenz[a,h]anthracene Rep 1	[^{3}H]-ASDN	CP# 05-079	0.799	0.586	866086
Dibenz[a,h]anthracene Rep 2	[^{3}H]-ASDN	CP# 05-079	0.805	0.570	897856
Dibenz[a,h]anthracene Rep 3	[^{3}H]-ASDN	CP# 05-079	0.836	0.591	900004
Econazole/ Chrysin Rep 1	[^{3}H]-ASDN	CP# 05-079	0.718	0.575	793187
Econazole/ Chrysin Rep 2	[^{3}H]-ASDN	CP# 05-079	0.693	0.585	753721
Econazole/ Chrysin Rep 3	[^{3}H]-ASDN	CP# 05-079	0.729	0.587	789640
Dicofol/ Atrazine Rep 1	[^{3}H]-ASDN	CP# 05-079	0.905	0.588	978424
Dicofol/ Atrazine Rep 2	[^{3}H]-ASDN	CP# 05-079	0.744	0.572	827457
Dicofol/ Atrazine Rep 3	[^{3}H]-ASDN	CP# 05-079	0.790	0.585	858574

4.2 Stock Formulation Analysis

Stock formulation analysis was performed by the CR. Briefly, solubility and formulation analyses showed that the stock formulations provided to the laboratories for this study were within the acceptance criteria for both average concentration and percent relative standard deviation between analyses. The Stock Formulation Analysis Report will be included in the Final Report submitted to the EPA which includes the reports from each lab involved in the inter-laboratory validations.

Test or Control Chemical	Reference Chemical Abbreviation	Reference Chemical Code	Reference Chemical ID	Stock Solution Concentration	Stock Solution Expiration Date
4-Hydroxy Androstenedione	4-OH ASDN	NA	MET-0300A	3.02 mg/mL 0.01M	12-17-05
Lindane	(None)	NA	MET-0299A	29.08 mg/mL 0.1M	12/16/05
Aminoglutethimide	AG	1	MET-0301A	23.2 mg/mL 0.1M	8-28-05
Ketoconazole	KCZ	2	MET-0302A	5.31 mg/mL 0.01M	8-28-05
Prochloraz	PCZ	3	MET-0308A	37.67 mg/mL 0.1M	9-26-05
4-Nonylphenol	NYP	4	MET-0319A	22.04 mg/mL 0.1M	10-03-05
Dibenz[a,h]an-thracene	DBA	5	MET-320A	2.78 mg/mL 0.01M	10-03-05
Fenarimol	FRM	6	MET-0309A	33.13mg/mL 0.1M	8-24-05
Econazole	ECZ	7	MET-0313A	44.5 mg/mL 0.1M	10-3-05
Chrysin	CYN	8	MET-0314A	2.54 mg/mL 0.01M	11-17-05
Dicofol	DCF	9	MET-0317A	37.05 mg/mL 0.1M	9-20-05
Atrazine	ATZ	10	MET-0318A	21.57 mg/mL 0.1M	9-19-05

4.3 Protein Analysis (Microsomes)

The protein concentration of each microsomal preparation was determined on the day that the microsomes were used in the assay. Variation in the handling of microsomes during dilution (e.g. homogenization procedure, see Section 3.3) was likely the source of the variability in the protein concentrations shown in the following table when from the same source.

Protein Analysis Results			
Replicate	Assay Date	Source	Protein stock concentration (mg/mL, measured)
Aminoglutethimide /Ketoconazole Rep 1	7/20/2005	Laboratory 4	8.089
Aminoglutethimide /Ketoconazole Rep 2	7/22/2005	Laboratory 4	8.442
Aminoglutethimide /Ketoconazole Rep 3	7/26/2005	Laboratory 4	8.436
Prochloraz/ Fenarimol Rep 1	8/3/2005	Laboratory 2	22.309
Prochloraz/ Fenarimol Rep 2	8/5/2005	Laboratory 2	19.426
Prochloraz/ Fenarimol Rep 3	8/9/2005	Laboratory 2	23.498
4-Nonylphenol Rep 1	9/19/2005	Laboratory 2	27.902
4-Nonylphenol Rep 2	9/22/2005	Laboratory 2	23.861
4-Nonylphenol Rep 3	9/26/2005	Laboratory 2	23.477
Dibenz[a,h]anthracene Rep 1	9/28/2005	Laboratory 4	9.081
Dibenz[a,h]anthracene Rep 2	9/30/2005	Laboratory 4	10.457
Dibenz[a,h]anthracene Rep 3	10/3/2005	Laboratory 4	9.838
Econazole/ Chrysin Rep 1	8/15/2005	Laboratory 4	7.288
Econazole/ Chrysin Rep 2	8/22/2005	Laboratory 4	6.634
Econazole/ Chrysin Rep 3	8/30/2005	Laboratory 4	8.444
Dicofol/ Atrazine Rep 1	9/1/2005	Laboratory 2	23.249
Dicofol/ Atrazine Rep 2	9/6/2005	Laboratory 2	24.471
Dicofol/ Atrazine Rep 3	9/8/2005	Laboratory 2	27.349

Quality Control standards for protein assay were included. Inter-laboratory differences in protein concentration could be a factor in varying aromatase activity between labs. The results from the determination of protein concentration for 10 and 100 µg/mL BSA standards are summarized as follows.

Replicate	Experimental µg/mL BSA (10 µg/mL target)	Experimental µg/mL BSA (100 µg/mL target)
Aminoglutethimide /Ketoconazole Rep 1	8	107
Aminoglutethimide /Ketoconazole Rep 2	10	101
Aminoglutethimide /Ketoconazole Rep 3	10	92

Replicate	Experimental µg/mL BSA (10 µg/mL target)	Experimental µg/mL BSA (100 µg/mL target)
Prochloraz/ Fenarimol Rep 1	15	104
Prochloraz/ Fenarimol Rep 2	7	95
Prochloraz/ Fenarimol Rep 3	11	100
4-Nonylphenol Rep 1	13	93
4-Nonylphenol Rep 2	10	103
4-Nonylphenol Rep 3	10	100
Dibenz[a,h]anthracene Rep 1	11	105
Dibenz[a,h]anthracene Rep 2	13	101
Dibenz[a,h]anthracene Rep 3	11	101
Econazole/ Chrysin Rep 1	2	96
Econazole/ Chrysin Rep 2	1	98
Econazole/ Chrysin Rep 3	10	107
Dicofol/ Atrazine Rep 1	15	98
Dicofol/ Atrazine Rep 2	11	106
Dicofol/ Atrazine Rep 3	11	101
AVERAGE	9.94	100.71
Standard Deviation	3.69	4.51
%CV	37.06	4.48
Percent of Expected Value	99.44	100.71

4.4 Control Aromatase Activity

Figure 1a. Full Enzyme Activity Controls by Portion
Figure 1b. Full Enzyme Activity Controls – Difference by Portion (Beginning Minus End)
Figure 2a. Background Enzyme Activity Controls by Portion
Figure 2b. Background Enzyme Activity Controls – Difference by Portion (Beginning Minus End)
Figure 3a. Positive Controls by Portion
Figure 3b. Positive Controls – Difference by Portion (Beginning Minus End)
Figure 4a. Negative Controls by Portion
Figure 4b. Negative Controls – Difference by Portion (Beginning Minus End)

Appendix D – Individual Replicate Spreadsheets

Full Enzyme Activity controls were conducted at the beginning and end of each replicate. The average of the four Background Activity controls within a replicate were necessarily 0 percent and the average of the four Full Enzyme Activity controls within a replicate were necessarily 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 were plotted across reference chemical and replicate within reference chemical, with plotting symbols distinguishing between beginning and end, and with reference line 0% (Background Activity control) or 100% (Full Enzyme Activity control) respectively. These plots display the extent of consistency across reference chemicals and 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) (beginning minus end) across reference chemicals and replicates within reference chemicals. Each plot has a reference line of 0.

As shown in the following table, the percent coefficient of variation (%CV) within each replicate of all ten Reference Chemicals was generally less than 10%. Therefore, the enzymatic activity was consistent throughout the assay. By contrast, the inter-assay variability within a reference chemical was greater, but still averaged approximately 16% over all chemicals and enzyme preparations. Full Enzyme Activity by Portion and the difference in Full Enzyme Activity by Portion are illustrated in Figures 1a and 1b.

Examining plots of Full Enzyme Activity across chemicals (Figure 1a) revealed that the beginning values were frequently higher than the ending enzyme activity values. Plotting the difference between the beginning end ending portion (Figure 1b) showed that in fourteen of eighteen cases the activity in the beginning portion was higher than the activity in the ending portion. This will be evaluated for statistical significance in Section 4.7.

Full Enzyme Activity Control Aromatase Activity – Aminoglutethimide/ Ketoconazole						
Replicate	Beginning Mean (nmol/mg protein/min)	End Mean (nmol/mg protein/min)	Within Replicate Mean (± sd, sem)	% CV	Overall Mean (± sd, sem)	Overall % CV
1	0.035	0.031	0.033 (0.003, 0.001)	8.83	0.045 (0.010, 0.003)	20.94
2	0.054	0.048	0.051 (0.004, 0.002)	7.09		
3	0.053	0.051	0.052 (0.002, 0.001)	4.43		
Full Enzyme Activity Control Aromatase Activity – Prochloraz/Fenarimol						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (± sd, sem)	% CV	Overall Mean (± sd, sem)	Overall % CV
1	0.067	0.055	0.061 (0.008, 0.004)	12.79	0.061 (0.010, 0.003)	17.04
2	0.059	0.044	0.052 (0.009, 0.005)	18.03		
3	0.070	0.070	0.070 (0.005, 0.002)	6.43		
Full Enzyme Activity Control Aromatase Activity - 4-Nonylphenol						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (± sd, sem)	% CV	Overall Mean (± sd, sem)	Overall % CV
1	0.054	0.049	0.051 (0.002, 0.001)	4.87	0.059 (0.007, 0.002)	11.64
2	0.059	0.057	0.058 (0.002, 0.001)	3.27		
3	0.069	0.064	0.067 (0.003, 0.001)	3.91		

Full Enzyme Activity Control Aromatase Activity – Dibenz[a,h]anthracene						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (± sd, sem)	% CV	Overall Mean (± sd, sem)	Overall % CV
1	0.048	0.050	0.049 (0.001, 0.000)	1.68	0.043 (0.006, 0.002)	14.48
2	0.035	0.040	0.038 (0.006, 0.003)	15.98		
3	0.039	0.043	0.042 (0.002, 0.001)	5.76		
Full Enzyme Activity Control Aromatase Activity – Econazole/Chrysin						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (± sd, sem)	% CV	Overall Mean (± sd, sem)	Overall % CV
1	0.073	0.072	0.072 (0.001, 0.001)	1.72	0.062 (0.012, 0.003)	19.02
2	0.070	0.062	0.066 (0.005, 0.003)	8.17		
3	0.048	0.046	0.047 (0.002, 0.001)	4.18		
Full Enzyme Activity Control Aromatase Activity – Dicofol/Atrazine						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (± sd, sem)	% CV	Overall Mean (± sd, sem)	Overall % CV
1	0.063	0.061	0.062 (0.001, 0.001)	2.28	0.057 (0.007, 0.002)	12.35
2	0.050	0.047	0.049 (0.002, 0.001)	4.64		
3	0.065	0.058	0.062 (0.005, 0.002)	8.03		

Positive Enzyme Activity Control Aromatase Activity – Aminoglutethimide/Ketoconazole						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.018	0.016	0.017 (0.001, 0.000)	4.78	0.021 (0.003, 0.001)	15.96
2	0.025	0.022	0.023 (0.002, 0.001)	7.04		
3	0.024	0.023	0.023 (0.001, 0.001)	5.19		

Positive Enzyme Activity Control Aromatase Activity – Prochloraz/Fenarimol						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.027	0.016	0.022 (0.010, 0.005)	44.31	0.022 (0.007, 0.002)	33.63
2	0.021	0.013	0.017 (0.005, 0.002)	28.47		
3	0.027	0.028	0.028 (0.002, 0.001)	8.12		
Positive Enzyme Activity Control Aromatase Activity – 4-Nonylphenol						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.023	0.021	0.022 (0.001, 0.001)	5.38	0.025 (0.003, 0.001)	11.74
2	0.026	0.024	0.025 (0.001, 0.001)	5.00		
3	0.029	0.028	0.028 (0.001, 0.000)	2.52		
Positive Enzyme Activity Control Aromatase Activity – Dibenz[a,h]anthracene						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.020	0.021	0.021 (0.001, 0.000)	4.34	0.018 (0.003, 0.001)	15.12
2	0.017	0.015	0.016 (0.002, 0.001)	9.58		
3	0.015	0.019	0.017 (0.003, 0.001)	16.50		
Positive Enzyme Activity Control Aromatase Activity – Econazole/Chrysin						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.029	0.027	0.028 (0.002, 0.001)	6.61	0.025 (0.004, 0.001)	14.99
2	0.029	0.026	0.027 (0.002, 0.001)	6.94		
3	0.022	0.020	0.021 (0.001, 0.001)	5.58		

Positive Enzyme Activity Control Aromatase Activity – Dicofol/Atrazine						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.029	0.025	0.027 (0.004, 0.002)	13.91	0.026 (0.002, 0.001)	8.74
2	0.027	0.026	0.027 (0.001, 0.000)	1.94		
3	0.025	0.024	0.024 (0.001, 0.000)	3.37		

Negative Enzyme Activity Control Aromatase Activity – Aminoglutethimide/Ketoconazole						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.033	0.030	0.032 (0.002, 0.001)	5.46	0.045 (0.010, 0.003)	21.99
2	0.054	0.049	0.052 (0.003, 0.001)	5.79		
3	0.053	0.046	0.050 (0.004, 0.002)	8.03		
Negative Enzyme Activity Control Aromatase Activity – Prochloraz/Fenarimol						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.066	0.059	0.062 (0.004, 0.002)	6.71	0.059 (0.010, 0.003)	16.27
2	0.058	0.040	0.049 (0.010, 0.005)	20.28		
3	0.068	0.064	0.066 (0.003, 0.001)	4.34		
Negative Enzyme Activity Control Aromatase Activity – 4-Nonylphenol						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.055	0.050	0.052 (0.003, 0.001)	5.22	0.058 (0.006, 0.002)	10.13
2	0.06	0.056	0.058 (0.002, 0.001)	4.17		
3	0.067	0.063	0.065 (0.003, 0.001)	4.47		

Negative Enzyme Activity Control Aromatase Activity – Dibenz[a,h]anthracene						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.049	0.039	0.044 (0.009, 0.005)	20.46	0.043 (0.005, 0.002)	12.36
2	0.041	0.039	0.040 (0.002, 0.001)	5.15		
3	0.045	0.044	0.045 (0.001, 0.000)	1.81		
Negative Enzyme Activity Control Aromatase Activity – Econazole/Chrysin						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.076	0.070	0.073 (0.004, 0.002)	5.14	0.060 (0.013, 0.004)	21.26
2	0.068	0.058	0.063 (0.006, 0.003)	9.63		
3	0.049	0.041	0.045 (0.005, 0.002)	10.33		
Negative Enzyme Activity Control Aromatase Activity – Dicofol/Atrazine						
Replicate	Beginning (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (±sd, sem)	% CV	Overall Mean (±sd, sem)	Overall % CV
1	0.062	0.057	0.060 (0.003, 0.001)	4.99	0.054 (0.009, 0.002)	16.07
2	0.052	0.046	0.049 (0.004, 0.002)	8.98		
3	0.048	0.058	0.053 (0.013, 0.006)	24.29		

Background Activity controls were conducted in duplicate at the beginning and end of each assay. Figure 2a (Background Enzyme Activity Control by Portion) presents the individual Background Activity values as the percent of control values from each replicate and a graphical representation of the data. The aromatase activity in these control samples was negligible (-0.4-0.8% of Full Enzyme Activity), indicating that there was no background activity (potentially caused by nonspecific turnover of reactant to product, or unintentional NADPH contamination). Contamination with NADPH was suspected in the first two Background Activity controls of prochloraz and fenarimol Replicate 2 and dicofol and atrazine, Replicate 3 at the end. This contamination however, did not interfere with the interpretation of the results. Overall, there were negligible differences between the beginning and end Background Activity values per replicate, indicating that the conditions were constant throughout each replicate test (see Appendix D, Individual Replicate Spreadsheets). The nominal difference in Background Activity by portion is illustrated in Figure 2b.

Positive and Negative controls were also included, divided evenly between the beginning and end of each assay. Positive controls contained the known aromatase inhibitor 4-OH ASDN. The 4-OH ASDN concentration used was expected to inhibit 50% of the enzyme activity (IC_{50}).

Negative controls contained lindane, a chemical known for its inability to inhibit aromatase activity. Figures 3a-b and 4a-b illustrate the Positive and Negative control results, respectively, across all 10 reference chemicals. The Positive and Negative controls behaved as expected during the aromatase assays, e.g. Positive controls had approximately 50% of the Full Enzyme Activity control due to the presence of 4-OH ASDN, and the Negative controls showed no effect on aromatase activity due to the inclusion of lindane in the reaction mixture.

4.5 *Reference Chemical Aromatase Activity*

Figure 5a-e – Concentration Response Curves – Aminoglutethimide
Figure 6a-e – Concentration Response Curves – Ketoconazole
Figure 7a-e – Concentration Response Curves - Prochloraz
Figure 8a-e – Concentration Response Curves – 4-Nonylphenol
Figure 9a-e – Concentration Response Curves – Dibenz[a,h]anthracene
Figure 10a-e – Concentration Response Curves – Fenarimol
Figure 11a-e – Concentration Response Curves – Econazole
Figure 12a-e – Concentration Response Curves – Chrysin
Figure 13a-e – Concentration Response Curves – Dicofol
Figure 14a-e – Concentration Response Curves – Atrazine

Ten Reference Chemicals were tested for aromatase inhibition over at least eight concentrations. Increasing the concentration of the Reference Chemical inhibited aromatase activity in a dose responsive manner for eight of the reference chemicals. Two of the chemicals (dibenz[a,h]anthracene and atrazine) exhibited no inhibitioin of aromatase activity at the concentrations evaluated. Low intra-assay variability was characterized by a coefficient of variance of less than 10% across triplicate samples (tubes) at most concentrations and replicates, except at the highest inhibitor concentrations, which resulted in percent of control activities near 0%. Small deviations in small results can result in large term errors. For all the replicates, outliers were rejected with a 90% confidence level using the Q-test described by Dean and Dixon (1951) as described in Section 3.7. Decreasing concentrations of reference chemical resulted in decreased inhibition of aromatase activity characterized by increased percent of control activity. This inhibition was characterized by a sigmoidal dose response (See Figures 5-14). Complete curves (inhibition in the full range of approximately 0-100%) were obtained for seven of the eight inhibitors. Chrysin had a slightly compressed curve (approximately 13-90% inhibition).

Reference Chemical Aromatase Activity – Percent of Control									
Reference Chemical	Rep	Log [reference chemical]	Percent of Control			Overall			
			Tube 1	Tube 2	Tube 3	Mean	sd	sem	%CV
AG	1	-10	105.35	107.00	101.42	104.59	2.87	1.66	2.74
AG	1	-9	107.23	103.81	101.86	104.30	2.72	1.57	2.61
AG	1	-8	107.04	106.45	104.35	105.95	1.41	0.82	1.33
AG	1	-7	98.79	102.91	103.05	101.58	2.42	1.40	2.38
AG	1	-6	95.40	97.49	100.18	97.69	2.40	1.38	2.45
AG	1	-5	87.57	86.40	85.60	86.52	0.99	0.57	1.15
AG	1	-4	37.89	37.82	37.55	37.75	0.18	0.10	0.48
AG	1	-3	2.91	3.52	3.61	3.35	0.38	0.22	11.38
AG	2	-9	102.04	103.38	91.40	98.94	6.56	3.79	6.63
AG	2	-7	105.37	108.38	105.42	106.39	1.72	1.00	1.62
AG	2	-6	107.50	102.02	103.03	104.18	2.92	1.68	2.80
AG	2	-5	90.85	91.88	93.16	91.96	1.16	0.67	1.26
AG	2	-4.3	61.84	58.67	61.52	60.68	1.75	1.01	2.88
AG	2	-4	43.17	42.61	41.66	42.48	0.76	0.44	1.80

*Outliers are confirmed by $(X_1-X_2)/W > Q_{critical}$, where $Q_{critical} = 0.94$ with n =3 (See Section 3.7).
**No data collected.

Reference Chemical Aromatase Activity -- Percent of Control									
Reference Chemical	Rep	Log [reference chemical]	Percent of Control			Overall			
			Tube 1	Tube 2	Tube 3	Mean	sd	sem	%CV
AG	2	-3.3	13.41	12.87	12.95	13.08	0.29	0.17	2.23
AG	2	-3	6.38	8.45	5.96	6.93	1.33	0.77	19.24
AG	3	-9	98.45	104.04	103.22	101.90	3.02	1.74	2.96
AG	3	-7	103.55	104.89	97.39	101.94	4.00	2.31	3.92
AG	3	-6	100.45	103.62	101.22	101.76	1.65	0.95	1.62
AG	3	-5	88.00	89.47	88.48	88.65	0.75	0.43	0.85
AG	3	-4.3	55.34	56.27	57.56	56.39	1.11	0.64	1.98
AG	3	-4	38.90	40.72	40.31	39.98	0.95	0.55	2.39
AG	3	-3.3	12.55	12.63	11.15	12.11	0.83	0.48	6.87
AG	3	-3	5.20	5.55	6.21	5.65	0.51	0.30	9.07
KCZ	1	-4	0.12	0.25	0.11	0.16	0.08	0.05	48.81
KCZ	1	-5	3.14	3.38	3.71	3.41	0.29	0.17	8.39
KCZ	1	-5.3	7.15	7.08	7.68	7.30	0.33	0.19	4.49
KCZ	1	-6	27.38	28.72	26.84	27.65	0.97	0.56	3.50
KCZ	1	-7	76.19	75.56	77.48	76.41	0.98	0.57	1.28
KCZ	1	-8	94.45	97.52	94.52	95.50	1.75	1.01	1.84
KCZ	1	-9	97.07	96.56	98.48	97.37	0.99	0.57	1.02
KCZ	1	-10	97.41	95.23	95.72	96.12	1.14	0.66	1.19
KCZ	2	-4	0.26	0.26	0.20	0.24	0.03	0.02	14.43
KCZ	2	-5	4.79	4.43	4.76	4.66	0.20	0.12	4.29
KCZ	2	-5.3	9.89	8.71	8.96	9.19	0.62	0.36	6.77
KCZ	2	-6	30.60	32.03	33.14	31.92	1.27	0.74	3.99
KCZ	2	-7	84.49	80.37	81.33	82.06	2.16	1.24	2.63
KCZ	2	-8	103.74	97.85	97.46	99.68	3.52	2.03	3.53
KCZ	2	-9	103.42	99.34	99.19	100.65	2.40	1.39	2.38
KCZ	2	-10	101.79	95.80	97.57	98.39	3.08	1.78	3.13
KCZ	3	-4	0.42	0.20	0.21	0.28	0.12	0.07	44.90
KCZ	3	-5	4.28	4.18	3.97	4.14	0.16	0.09	3.82
KCZ	3	-5.3	8.29	8.24	8.30	8.28	0.03	0.02	0.39
KCZ	3	-6	25.40	25.03	25.12	25.18	0.19	0.11	0.77
KCZ	3	-7	84.03	85.01	83.13	84.06	0.94	0.54	1.12
KCZ	3	-8	98.66	92.61	99.62	96.96	3.80	2.19	3.92
KCZ	3	-9	101.37	100.78	104.59	102.25	2.05	1.18	2.01
KCZ	3	-10	105.08	101.38	98.45	101.64	3.32	1.92	3.27
PCZ	1	-10	114.79	95.52	111.99	107.43	10.41	6.01	9.69
PCZ	1	-9	111.19	103.27	100.11	104.86	5.71	3.30	5.44
PCZ	1	-8	78.19	74.47	75.18	75.95	1.97	1.14	2.60
PCZ	1	-7	21.68	21.91	20.83	21.47	0.57	0.33	2.65
PCZ	1	-6	2.29	2.34	2.66	2.43	0.20	0.12	8.26
PCZ	1	-5	0.04	-0.14	-0.01	-0.04	0.09	0.05	-253.41
PCZ	1	-4	-0.16	-0.29	-0.17	-0.21	0.07	0.04	-35.00
PCZ	1	-3	0.66	-0.18	-0.29	0.06	0.52	0.30	820.50
PCZ	2	-10	99.12	106.29	98.36	101.26	4.38	2.53	4.32
PCZ	2	-9	101.66	109.49	73.41	94.85	18.98	10.96	20.01
PCZ	2	-8.52	91.70	97.13	89.84	92.89	3.79	2.19	4.08

*Outliers are confirmed by $(X_1-X_2)/W > Q_{critical}$, where $Q_{critical} = 0.94$ with n =3 (See Section 3.7).
**No data collected.

Reference Chemical Aromatase Activity – Percent of Control									
Reference Chemical	Rep	Log [reference chemical]	Percent of Control			Overall			
			Tube 1	Tube 2	Tube 3	Mean	sd	sem	%CV
PCZ	2	-8	68.32	77.95	64.45	70.24	6.95	4.01	9.90
PCZ	2	-7.52	35.41	41.73	36.11	37.75	3.46	2.00	9.18
PCZ	2	-7	5.17	6.69	3.75	5.20	1.47	0.85	28.26
PCZ	2	-6	-17.46	-17.54	-17.70	-17.57	0.12	0.07	-0.70
PCZ	2	-5	-20.26	-20.60	-21.00	-20.62	0.37	0.21	-1.80
PCZ	3	-10	105.47	100.52	97.37	101.12	4.08	2.36	4.04
PCZ	3	-9	101.36	98.85	95.22	98.48	3.09	1.78	3.13
PCZ	3	-8.52	90.39	85.48	86.28	87.38	2.63	1.52	3.01
PCZ	3	-8	71.64	75.96	70.05	72.55	3.06	1.77	4.22
PCZ	3	-7.52	45.67	48.29	46.08	46.68	1.41	0.81	3.02
PCZ	3	-7	21.08	20.78	19.62	20.49	0.77	0.45	3.76
PCZ	3	-6	2.86	2.67	2.64	2.72	0.12	0.07	4.38
PCZ	3	-5	0.40	0.22	0.18	0.27	0.12	0.07	43.95
NYP	1	-10	105.67	105.03	102.94	104.55	1.43	0.82	1.37
NYP	1	-9	106.47	104.87	103.56	104.97	1.46	0.84	1.39
NYP	1	-8	105.01	104.29	103.93	104.41	0.55	0.32	0.53
NYP	1	-7	104.41	103.46	104.13	104.00	0.49	0.28	0.47
NYP	1	-6	105.98	103.01	104.61	104.53	1.49	0.86	1.42
NYP	1	-5	87.68	88.72	89.06	88.49	0.72	0.42	0.81
NYP	1	-4	7.87	7.90	7.25	7.67	0.37	0.21	4.78
NYP	1	-3	0.56	0.32	0.26	0.38	0.16	0.09	41.78
NYP	2	-9	99.31	100.30	99.33	99.65	0.57	0.33	0.57
NYP	2	-8	102.13	101.54	100.22	101.30	0.98	0.56	0.97
NYP	2	-7	90.20	101.70	104.42	98.77	7.55	4.36	7.64
NYP	2	-6	103.91	102.25	100.24	102.13	1.84	1.06	1.80
NYP	2	-5	88.88	87.26	87.17	87.77	0.96	0.56	1.10
NYP	2	-4.3	22.97	22.28	22.02	22.42	0.49	0.28	2.19
NYP	2	-4	7.64	8.42	7.48	7.85	0.50	0.29	6.41
NYP	2	-3	0.65	0.40	0.21	0.42	0.22	0.13	52.54
NYP	3	-9	99.68	98.28	97.36	98.44	1.17	0.67	1.19
NYP	3	-8	97.34	99.27	98.69	98.43	0.99	0.57	1.01
NYP	3	-7	98.92	99.73	98.22	98.96	0.76	0.44	0.76
NYP	3	-6	98.01	98.55	98.07	98.21	0.30	0.17	0.30
NYP	3	-5	87.83	88.21	86.90	87.65	0.67	0.39	0.77
NYP	3	-4.3	23.63	23.53	20.30	22.49	1.89	1.09	8.42
NYP	3	-4	7.55	7.98	8.49	8.01	0.47	0.27	5.88
NYP	3	-3	0.05	0.22	0.23	0.17	0.10	0.06	60.70
DBA	1	-4	95.61	98.07	99.69	97.79	2.05	1.19	2.10
DBA	1	-5	93.92	100.30	99.37	97.86	3.45	1.99	3.52
DBA	1	-5.3	98.28	99.78	96.37	98.15	1.71	0.99	1.74
DBA	1	-6	100.05	99.99	99.41	99.82	0.35	0.20	0.35
DBA	1	-7	100.03	98.37	99.21	99.20	0.83	0.48	0.84
DBA	1	-8	99.68	101.24	102.91	101.28	1.61	0.93	1.59
DBA	1	-9	103.61	103.40	102.76	103.26	0.45	0.26	0.43
DBA	1	-10	103.94	100.37	96.27	100.19	3.84	2.22	3.83

*Outliers are confirmed by $(X_1-X_2)/W > Q_{critical}$, where $Q_{critical} = 0.94$ with n =3 (See Section 3.7).
**No data collected.

Reference Chemical Aromatase Activity – Percent of Control									
Reference Chemical	Rep	Log [reference chemical]	Percent of Control			Overall			
			Tube 1	Tube 2	Tube 3	Mean	sd	sem	%CV
DBA	2	-4	108.62	109.74	107.20	108.52	1.27	0.73	1.17
DBA	2	-5	120.39	105.87	110.85	112.37	7.38	4.26	6.57
DBA	2	-5.3	114.83	94.84	112.56	107.41	10.94	6.32	10.19
DBA	2	-6	112.31	108.04	108.40	109.58	2.37	1.37	2.16
DBA	2	-7	114.27	110.54	109.16	111.32	2.64	1.53	2.37
DBA	2	-8	84.76	107.12	111.86	101.25	14.47	8.36	14.30
DBA	2	-9	115.57	109.65	115.95	113.72	3.53	2.04	3.11
DBA	2	-10	109.63	108.04	111.35	109.67	1.66	0.96	1.51
DBA	3	-4	103.34	107.52	104.27	105.04	2.19	1.27	2.09
DBA	3	-5	105.48	110.95	111.01	109.15	3.18	1.83	2.91
DBA	3	-5.3	109.10	111.52	108.09	109.57	1.76	1.02	1.61
DBA	3	-6	106.03	110.73	86.90	101.22	12.62	7.29	12.47
DBA	3	-7	107.62	107.46	109.36	108.15	1.05	0.61	0.97
DBA	3	-8	108.62	106.91	110.61	108.71	1.85	1.07	1.70
DBA	3	-9	110.98	107.11	110.82	109.64	2.19	1.26	2.00
DBA	3	-10	106.59	107.69	108.91	107.73	1.16	0.67	1.08
FRM	1	-10	104.51	90.40	97.95	97.62	7.06	4.08	7.23
FRM	1	-9	106.99	102.61	97.94	102.51	4.53	2.61	4.41
FRM	1	-8	108.76	104.40	103.20	105.45	2.93	1.69	2.77
FRM	1	-7	97.55	102.12	98.89	99.52	2.35	1.36	2.36
FRM	1	-6	94.39	85.77	84.79	88.32	5.28	3.05	5.98
FRM	1	-5	41.17	38.70	38.18	39.35	1.60	0.92	4.06
FRM	1	-4	5.78	5.34	5.36	5.49	0.25	0.14	4.52
FRM	1	-3	0.95	0.70	0.87	0.84	0.13	0.07	15.20
FRM	2	-9	84.92	83.28	**	84.10	1.16	0.82	1.38
FRM	2	-7	77.36	78.82	70.78	75.65	4.28	2.47	5.66
FRM	2	-6	72.22	78.22	67.86	72.77	5.20	3.00	7.15
FRM	2	-5.52	47.93	54.48	46.25	49.55	4.35	2.51	8.78
FRM	2	-5	20.05	21.47	19.99	20.50	0.84	0.48	4.09
FRM	2	-4.52	-1.27	-0.17	3.83	0.797	2.68	1.55	336.89
FRM	2	-4	-14.17	-13.55	-16.28	-14.67	1.43	0.83	-9.76
FRM	2	-3	-20.05	-19.28	-20.16	-19.83	0.48	0.28	-2.42
FRM	3	-9	82.43	93.09	95.81	90.44	7.07	4.08	7.82
FRM	3	-7	84.70	84.70	82.36	83.92	1.35	0.78	1.61
FRM	3	-6	81.73	77.64	76.59	78.65	2.72	1.57	3.45
FRM	3	-5.52	65.88	64.33	64.47	64.89	0.86	0.49	1.32
FRM	3	-5	38.78	37.35	36.28	37.47	1.25	0.72	3.35
FRM	3	-4.52	17.57	19.21	17.49	18.09	0.97	0.56	5.37
FRM	3	-4	6.19	5.81	5.55	5.85	0.32	0.19	5.50
FRM	3	-3	1.27	1.35	1.16	1.26	0.10	0.06	7.57
ECZ	1	-10	95.05	94.48	96.10	95.21	0.82	0.47	0.86
ECZ	1	-9	62.70	63.33	61.69	62.57	0.83	0.48	1.32
ECZ	1	-8	12.07	12.48	10.94	11.83	0.80	0.46	6.74
ECZ	1	-7	1.14	1.91	1.64	1.56	0.39	0.23	24.99
ECZ	1	-6	0.08	0.22	-0.18	0.04	0.20	0.12	507.44

*Outliers are confirmed by $(X_1-X_2)/W > Q_{critical}$, where $Q_{critical} = 0.94$ with n =3 (See Section 3.7).
**No data collected.

Reference Chemical Aromatase Activity – Percent of Control									
Reference Chemical	Rep	Log [reference chemical]	Percent of Control			Overall			
			Tube 1	Tube 2	Tube 3	Mean	sd	sem	%CV
ECZ	1	-5	-0.22	-0.03	-0.24	-0.16	0.12	0.07	-70.96
ECZ	1	-4	-0.01	-0.30	-0.24	-0.18	0.15	0.09	-83.50
ECZ	1	-3	0.60	0.24	-0.04	0.27	0.32	0.19	120.31
ECZ	2	-10	99.32	98.51	98.83	98.89	0.41	0.24	0.41
ECZ	2	-9.52	93.64	92.36	91.97	92.66	0.87	0.50	0.94
ECZ	2	-9	66.44	65.69	67.03	66.39	0.67	0.39	1.01
ECZ	2	-8.52	37.16	37.00	38.04	37.40	0.56	0.32	1.50
ECZ	2	-8	15.64	15.07	15.08	15.26	0.33	0.19	2.14
ECZ	2	-7	1.81	1.60	1.51	1.64	0.15	0.09	9.39
ECZ	2	-6	0.79	-0.10	0.27	0.32	0.45	0.26	139.72
ECZ	2	-5	1.62	0.84	0.67	1.04	0.51	0.29	48.55
ECZ	3	-10	90.47	92.73	93.83	92.34	1.71	0.99	1.86
ECZ	3	-9.52	86.72	87.39	86.91	87.01	0.35	0.20	0.40
ECZ	3	-9	65.11	62.20	61.85	63.05	1.79	1.03	2.84
ECZ	3	-8.52	32.45	33.76	33.51	33.24	0.70	0.40	2.09
ECZ	3	-8	12.86	13.64	14.05	13.52	0.60	0.35	4.47
ECZ	3	-7	1.76	1.60	1.36	1.57	0.20	0.12	12.80
ECZ	3	-6	0.41	0.02	0.19	0.21	0.20	0.11	94.61
ECZ	3	-5	0.24	0.03	0.32	0.20	0.15	0.09	76.16
CYN	1	-10	96.99	98.94	100.36	98.76	1.69	0.98	1.71
CYN	1	-9	103.58	101.04	101.61	102.08	1.33	0.77	1.31
CYN	1	-8	102.88	100.58	102.63	102.03	1.26	0.73	1.24
CYN	1	-7	94.89	98.04	66.69	86.54	17.26	9.97	19.95
CYN	1	-6	75.93	75.00	76.37	75.77	0.70	0.40	0.92
CYN	1	-5.3	37.21	38.78	37.66	37.88	0.81	0.47	2.13
CYN	1	-5	23.65	23.86	22.70	23.40	0.62	0.36	2.64
CYN	1	-4	16.65	9.35	9.08	11.69	4.29	2.48	36.73
CYN	2	-10	66.49	67.96	68.29	67.58	0.96	0.55	1.42
CYN	2	-8	85.30	82.38	83.03	83.57	1.53	0.89	1.83
CYN	2	-7	95.16	98.26	96.54	96.65	1.55	0.90	1.61
CYN	2	-6	76.98	75.04	73.04	75.02	1.97	1.14	2.63
CYN	2	-5.6	54.52	57.08	56.85	56.15	1.42	0.82	2.52
CYN	2	-5.3	42.45	39.66	42.63	41.58	1.67	0.96	4.00
CYN	2	-5	27.29	28.04	27.93	27.75	0.41	0.23	1.46
CYN	2	-4	20.08	9.93	17.87	15.96	5.34	3.08	33.44
CYN	3	-10	83.33	78.69	83.66	81.89	2.78	1.60	3.39
CYN	3	-8	90.05	89.50	89.33	89.63	0.38	0.22	0.42
CYN	3	-7	97.68	95.93	94.30	95.97	1.69	0.98	1.76
CYN	3	-6	75.63	76.83	76.50	76.32	0.62	0.36	0.81
CYN	3	-5.6	58.89	57.21	55.67	57.26	1.61	0.93	2.81
CYN	3	-5.3	40.11	41.58	40.05	40.58	0.87	0.50	2.14
CYN	3	-5	26.54	27.59	27.56	27.23	0.60	0.35	2.20
CYN	3	-4	13.05	14.62	14.59	14.09	0.90	0.52	6.37
DCF	1	-10	102.19	100.97	93.47	98.88	4.72	2.73	4.78
DCF	1	-9	100.27	102.18	101.20	101.22	0.96	0.55	0.94

*Outliers are confirmed by $(X_1-X_2)/W > Q_{critical}$, where $Q_{critical} = 0.94$ with n =3 (See Section 3.7).
**No data collected.

Reference Chemical Aromatase Activity – Percent of Control									
Reference Chemical	Rep	Log [reference chemical]	Percent of Control			Overall			
			Tube 1	Tube 2	Tube 3	Mean	sd	sem	%CV
DCF	1	-8	103.85	97.90	99.03	100.26	3.16	1.82	3.15
DCF	1	-7	102.70	103.44	91.87	99.34	6.48	3.74	6.52
DCF	1	-6	92.07	96.28	93.03	93.79	2.21	1.27	2.35
DCF	1	-5	61.94	62.37	65.74	63.35	2.08	1.20	3.28
DCF	1	-4	51.93*	28.40	29.44	28.92	0.74	0.52	2.54
DCF	1	-3	5.39	2.37	3.34	3.70	1.54	0.89	41.67
DCF	2	-8	101.02	101.98	105.81	102.94	2.53	1.46	2.46
DCF	2	-6	96.15	96.97	94.59	95.90	1.21	0.70	1.26
DCF	2	-5.52	85.60	87.90	84.69	86.06	1.65	0.96	1.92
DCF	2	-5	80.56	73.92	67.57	74.02	6.50	3.75	8.78
DCF	2	-4.52	65.32	69.37	67.85	67.51	2.05	1.18	3.03
DCF	2	-4	36.27	36.87	39.54	37.56	1.74	1.01	4.63
DCF	2	-3.52	16.34	20.87	13.49	16.90	3.72	2.15	22.02
DCF	2	-3	6.49	5.71	4.42	5.54	1.05	0.60	18.87
DCF	3	-8	96.97	98.71	98.93	98.20	1.07	0.62	1.09
DCF	3	-6	90.51	87.03	91.00	89.51	2.16	1.25	2.42
DCF	3	-5.52	83.43	85.75	80.64	83.27	2.56	1.48	3.07
DCF	3	-5	66.52	56.03	62.28	61.61	5.28	3.05	8.57
DCF	3	-4.52	50.17	51.46	59.46	53.70	5.03	2.91	9.37
DCF	3	-4	31.45	25.74	29.82	29.00	2.94	1.70	10.14
DCF	3	-3.52	14.48	7.64	8.60	10.24	3.70	2.14	36.16
DCF	3	-3	-0.29	0.95	-0.15	0.17	0.68	0.39	399.48
ATZ	1	-10	100.96	98.12	97.69	98.92	1.78	1.03	1.80
ATZ	1	-9	98.66	100.88	98.97	99.50	1.20	0.69	1.21
ATZ	1	-8	103.54	107.42	99.10	103.35	4.16	2.40	4.03
ATZ	1	-7	99.33	96.81	98.72	98.29	1.31	0.76	1.34
ATZ	1	-6	101.95	99.58	104.53	102.02	2.48	1.43	2.43
ATZ	1	-5	101.16	97.38	97.80	98.78	2.07	1.20	2.10
ATZ	1	-4	94.43	97.21	95.39	95.68	1.41	0.82	1.48
ATZ	1	-3	71.53	82.58	78.27	77.46	5.57	3.22	7.19
ATZ	2	-10	97.20	98.64	97.40	97.75	0.78	0.45	0.80
ATZ	2	-9	96.63	100.61	99.89	99.04	2.12	1.22	2.14
ATZ	2	-8	102.45	98.34	98.87	99.89	2.24	1.29	2.24
ATZ	2	-7	99.01	98.63	96.16	97.93	1.55	0.89	1.58
ATZ	2	-6	99.23	96.09	98.71	98.01	1.68	0.97	1.72
ATZ	2	-5	102.03	102.58	101.73	102.11	0.43	0.25	0.42
ATZ	2	-4	95.03	95.48	96.34	95.62	0.67	0.38	0.70
ATZ	2	-3	59.49*	89.06	89.44	89.25	0.27	0.19	0.30
ATZ	3	-10	97.23	169.76*	95.30	96.27	1.36	0.97	1.42
ATZ	3	-9	96.38	94.72	94.58	95.23	1.00	0.58	1.05
ATZ	3	-8	97.38	94.40	95.75	95.84	1.49	0.86	1.56
ATZ	3	-7	104.02	94.99	95.13	98.05	5.17	2.99	5.28
ATZ	3	-6	96.69	95.11	94.00	95.27	1.35	0.78	1.42
ATZ	3	-5	96.39	96.00	97.53	96.64	0.80	0.46	0.82
ATZ	3	-4	92.01	90.14	91.52	91.22	0.97	0.56	1.06
ATZ	3	-3	79.03	82.59	80.56	80.73	1.79	1.03	2.21

*Outliers are confirmed by $(X_1-X_2)/W > Q_{critical}$, where $Q_{critical} = 0.94$ with n =3 (See Section 3.7).
**No data collected.

The following table illustrates the inter-assay variability between aromatase inhibition effected by the same chemical. Like the intra-assay variability, the coefficient of variation in percent of control between replicates was less at lower reference chemical concentrations (higher enzyme activity). In addition, high variability was found between replicates of prochloraz and fenarimol because the possible NADPH contamination increased the background activity. Because the background values are subtracted from all the raw data, the tubes with little activity were calculated as negative percent of Full Enzyme Activity.

Mean Reference Chemical Aromatase Activity – Percent of Control								
Reference Chemical	Log [reference chemical]	Mean Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	sd	sem	% CV
Aminoglutethimide	-10	104.59	*	*	104.59			
Aminoglutethimide	-9	104.30	98.94	101.90	101.71	2.68	1.55	2.64
Aminoglutethimide	-8	105.95	*	*	105.95			
Aminoglutethimide	-7	101.58	106.39	101.94	103.31	2.68	1.55	2.59
Aminoglutethimide	-6	97.69	104.18	101.76	101.21	3.28	1.89	3.24
Aminoglutethimide	-5	86.52	91.96	88.65	89.05	2.74	1.58	3.08
Aminoglutethimide	-4.3	*	60.68	56.39	58.53	3.03	2.14	5.18
Aminoglutethimide	-4	37.75	42.48	39.98	40.07	2.36	1.37	5.90
Aminoglutethimide	-3.3	*	13.08	12.11	12.59	0.68	0.48	5.43
Aminoglutethimide	-3	3.35	6.93	5.65	5.31	1.82	1.05	34.20
Ketoconazole	-10	96.12	98.39	101.64	98.71	2.77	1.60	2.81
Ketoconazole	-9	97.37	100.65	102.25	100.09	2.49	1.44	2.48
Ketoconazole	-8	95.50	99.68	96.96	97.38	2.12	1.23	2.18
Ketoconazole	-7	76.41	82.06	84.06	80.84	3.97	2.29	4.91
Ketoconazole	-6	27.65	31.92	25.18	28.25	3.41	1.97	12.07
Ketoconazole	-5.3	7.30	9.19	8.28	8.26	0.94	0.54	11.41
Ketoconazole	-5	3.41	4.66	4.14	4.07	0.63	0.36	15.43
Ketoconazole	-4	0.16	0.24	0.28	0.23	0.06	0.03	26.45
Prochloraz	-10	107.43	101.26	101.12	103.27	3.61	2.08	3.49
Prochloraz	-9	104.86	94.85	98.48	99.40	5.06	2.92	5.10
Prochloraz	-8.52	*	92.89	87.38	90.14	3.89	2.75	4.32
Prochloraz	-8	75.95	70.24	72.55	72.91	2.87	1.66	3.94
Prochloraz	-7.52	*	37.75	46.68	42.22	6.31	4.46	14.96
Prochloraz	-7	21.47	5.20	20.49	15.72	9.12	5.27	58.03
Prochloraz	-6	2.43	-17.57	2.72	-4.14	11.63	6.71	-281.1
Prochloraz	-5	-0.04	-20.62	0.27	-6.80	11.97	6.91	-176.1
Prochloraz	-4	-0.21	*	*	-0.21			
Prochloraz	-3	0.06	*	*	0.06			
4-Nonylphenol	-10	104.55	*	*	104.55			
4-Nonylphenol	-9	104.97	99.65	98.44	101.02	3.47	2.00	3.44
4-Nonylphenol	-8	104.41	101.30	98.43	101.38	2.99	1.73	2.95
4-Nonylphenol	-7	104.00	98.77	98.96	100.58	2.97	1.71	2.95
4-Nonylphenol	-6	104.53	102.13	98.21	101.63	3.19	1.84	3.14
4-Nonylphenol	-5	88.49	87.77	87.65	87.97	0.45	0.26	0.52
4-Nonylphenol	-4.3	*	22.42	22.49	22.46	0.04	0.03	0.20
4-Nonylphenol	-4	7.67	7.85	8.01	7.84	0.17	0.10	2.13
4-Nonylphenol	-3	0.38	0.42	0.17	0.32	0.14	0.08	42.27

*Empty cells result from changes in reference chemical concentration following examination of the results of the first replicate (see Section 3.2).

Mean Reference Chemical Aromatase Activity – Percent of Control								
Reference Chemical	Log [reference chemical]	Mean Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	sd	sem	% CV
Dibenz[a,h]anth-racene	-10	100.19	109.67	107.73	105.87	5.01	2.89	4.73
Dibenz[a,h]anth - racene	-9	103.26	113.72	109.64	108.87	5.27	3.05	4.85
Dibenz[a,h]anth - racene	-8	101.28	101.25	108.71	103.75	4.30	2.48	4.15
Dibenz[a,h]anth - racene	-7	99.20	111.32	108.15	106.22	6.28	3.63	5.92
Dibenz[a,h]anth - racene	-6	99.82	109.58	101.22	103.54	5.28	3.05	5.10
Dibenz[a,h]anth - racene	-5.3	98.15	107.41	109.57	105.04	6.07	3.50	5.78
Dibenz[a,h]anth - racene	-5	97.87	112.37	109.15	106.46	7.62	4.40	7.15
Dibenz[a,h]anth - racene	-4	97.79	108.52	105.04	103.78	5.47	3.16	5.27
Fenarimol	-10	97.62	*	*	97.62			
Fenarimol	-9	102.51	84.10	90.44	92.35	9.35	5.40	10.13
Fenarimol	-8	105.45	*	*	105.45			
Fenarimol	-7	99.52	75.65	83.92	86.36	12.12	7.00	14.03
Fenarimol	-6	88.32	72.77	78.65	79.91	7.85	4.53	9.82
Fenarimol	-5.52	*	49.55	64.89	57.22	10.85	7.67	18.96
Fenarimol	-5	39.35	20.50	37.47	32.44	10.38	5.99	32.00
Fenarimol	-4.52	*	0.80	18.09	9.44	12.23	8.65	129.49
Fenarimol	-4	5.49	-14.67	5.85	-1.11	11.74	6.78	-1060
Fenarimol	-3	0.84	-19.83	1.26	-5.91	12.06	6.96	-204.0
Econazole	-10	95.21	98.89	92.34	95.48	3.28	1.89	3.44
Econazole	-9.52	*	92.66	87.01	89.83	4.00	2.82	4.45
Econazole	-9	62.57	66.39	63.05	64.00	2.08	1.20	3.25
Econazole	-8.52	*	37.40	33.24	35.32	2.94	2.08	8.33
Econazole	-8	11.83	15.26	13.52	13.54	1.72	0.99	12.68
Econazole	-7	1.56	1.64	1.57	1.59	0.04	0.02	2.62
Econazole	-6	0.04	0.32	0.21	0.19	0.14	0.08	74.56
Econazole	-5	-0.16	1.04	0.20	0.36	0.62	0.36	171.69
Econazole	-4	-0.18	*	*	-0.18			
Econazole	-3	0.27	*	*	0.27			
Chrysin	-10	98.76	67.58	81.89	82.75	15.61	9.01	18.86
Chrysin	-9	102.08	*	*	102.08			
Chrysin	-8	102.03	83.57	89.63	91.74	9.41	5.43	10.26
Chrysin	-7	86.54	96.65	95.97	93.05	5.65	3.26	6.07
Chrysin	-6	75.77	75.02	76.32	75.70	0.65	0.38	0.86
Chrysin	-5.6	*	56.15	57.26	56.70	0.78	0.55	1.38
Chrysin	-5.3	37.88	41.58	40.58	40.01	1.91	1.10	4.78
Chrysin	-5	23.40	27.75	27.23	26.13	2.37	1.37	9.09
Chrysin	-4	11.69	15.96	14.09	13.91	2.14	1.23	15.37

*Empty cells result from changes in reference chemical concentration following examination of the results of the first replicate (see Section 3.2).

Mean Reference Chemical Aromatase Activity – Percent of Control								
Reference Chemical	Log [reference chemical]	Mean Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	sd	sem	% CV
Dicofol	-10	98.88	*	*	98.88			
Dicofol	-9	101.22	*	*	101.22			
Dicofol	-8	100.26	102.94	98.20	100.47	2.37	1.37	2.36
Dicofol	-7	99.34	*	*	99.34			
Dicofol	-6	93.79	95.90	89.51	93.07	3.26	1.88	3.50
Dicofol	-5.52	*	86.06	83.27	84.67	1.97	1.40	2.33
Dicofol	-5	63.35	74.02	61.61	66.33	6.72	3.88	10.13
Dicofol	-4.52	*	67.51	53.70	60.61	9.77	6.91	16.12
Dicofol	-4	28.92	37.56	29.00	31.83	4.96	2.87	15.60
Dicofol	-3.52	*	16.90	10.24	13.57	4.71	3.33	34.70
Dicofol	-3	3.70	5.54	0.17	3.14	2.73	1.58	87.00
Atrazine	-10	98.92	97.75	96.27	97.65	1.33	0.77	1.36
Atrazine	-9	99.50	99.04	95.23	97.92	2.35	1.36	2.40
Atrazine	-8	103.35	99.89	95.84	99.69	3.76	2.17	3.77
Atrazine	-7	98.29	97.93	98.05	98.09	0.18	0.10	0.18
Atrazine	-6	102.02	98.01	95.27	98.43	3.40	1.96	3.45
Atrazine	-5	98.78	102.11	96.64	99.18	2.76	1.59	2.78
Atrazine	-4	95.68	95.62	91.22	94.17	2.55	1.47	2.71
Atrazine	-3	77.46	89.25	80.73	82.48	6.09	3.51	7.38

*Empty cells result from changes in reference chemical concentration following examination of the results of the first replicate (see Section 3.2).

4.6 IC_{50} And Slope Determination

The dose-response equation presented in Section 3.7 was fitted to the percent of control data versus varying reference chemical concentration plots discussed in Section 4.5. The individual curves fit to the data from each of the three replicates resulted in values for each reference chemical of T (Top), B (Bottom), μ (log IC_{50}) and β (Hill Slope). The results for all ten reference chemicals are summarized in the following table. Arithmetic calculation of the mean IC_{50} resulted in values of 2 nM to 65 μM (econazole and aminoglutethimide, respectively). The inter-assay variability (%CV) of the arithmetic IC_{50} determination was under 20%, except for dicofol, which had an inter-assay %CV of 54%.

IC_{50} and Slope Results									
Reference Chemical	Repl	Log [IC_{50}]	se log [IC_{50}]	IC_{50} (μM)	Slope	se slope	Status*	Overall IC_{50} (±sd, sem)	Overall %CV
AMINOGLUTETHIMIDE									
1	1	-4.196	0.043	63.73	-0.8613	0.0574	Complete	64.76 (2.63, 1.52)	4.06
	2	-4.169	0.037	67.75	-1.0799	0.1049	Complete		
	3	-4.202	0.023	62.80	-0.9987	0.0532	Complete		
KETOCONAZOLE									
2	1	-6.410	0.014	0.3893	-0.9736	0.0226	Complete	0.411 (0.047, 0.027)	11.52
	2	-6.332	0.026	0.4651	-0.9924	0.0456	Complete		
	3	-6.422	0.027	0.3781	-1.1402	0.0562	Complete		

*Status = codes as described in the statistical analysis section that describe the completeness of the data for generating the dose-response curve.

IC_{50} and Slope Results									
Reference Chemical	Repl	Log [IC_{50}]	se log [IC_{50}]	IC_{50} (µM)	Slope	se slope	Status*	Overall IC_{50} (±sd, sem)	Overall %CV
PROCHLORAZ									
3	1	-7.620	0.043	0.02397	-0.9841	0.0760	Complete	0.02583 (0.0023, 0.0013)	8.95
	2	-7.546	0.053	0.02842	-1.0622	0.1284	Complete		
	3	-7.600	0.025	0.02511	-0.9521	0.0485	Complete		
4-NONYLPHENOL									
4	1	-4.602	0.012	25.03	-1.8580	0.0488	Complete	26.31 (1.23, 0.71)	4.67
	2	-4.578	0.022	26.43	-1.9735	0.1127	Complete		
	3	-4.561	0.008	27.48	-2.0307	0.0429	Complete		
DIBENZ[a,h]ANTHRACENE									
5	1	NO INHIBITION							
	2								
	3								
FENARIMOL									
6	1	-5.197	0.046	6.347	-1.0253	0.1021	Complete	7.05 (0.69, 0.40)	9.72
	2	-5.149	0.039	7.101	-1.0224	0.0813	Complete		
	3	-5.113	0.033	7.716	-1.0465	0.0720	Complete		
ECONAZOLE									
7	1	-8.794	0.007	0.001605	-1.0946	0.0166	Complete	0.0017 (0.0001, 0.0000)	4.18
	2	-8.765	0.013	0.001719	-1.0703	0.0287	Complete		
	3	-8.761	0.014	0.001734	-1.1339	0.0375	Complete		
CHRYSIN									
8	1	-5.613	0.089	2.438	-1.0139	0.1768	Complete	3.06 (0.56, 0.33)	18.44
	2	-5.451	0.081	3.537	-1.5485	0.3849	Complete		
	3	-5.493	0.040	3.212	-1.3875	0.1567	Complete		
DICOFOL									
9	1	-4.576	0.086	26.56	-0.6920	0.0723	Complete	53.13 (28.68, 16.56)	53.99
	2	-4.078	0.133	83.54	-0.6235	0.0799	Complete		
	3	-4.307	0.110	49.28	-0.6028	0.0693	Complete		
ATRAZINE									
10	1	NO INHIBITION							
	2								
	3								

*Status = codes as described in the statistical analysis section that describe the completeness of the data for generating the dose-response curve.

4.7 Statistical Analysis

Figure 15a. Aminoglutethimide Statistical Average Concentration Response Curve
Figure 15b. Ketoconazole Statistical Average Concentration Response Curve
Figure 15c. Prochloraz Statistical Average Concentration Response Curve
Figure 15d. 4-Nonylphenol Statistical Average Concentration Response Curve
Figure 15e. Fenarimol Statistical Average Concentration Response Curve
Figure 15f. Econazole Statistical Average Concentration Response Curve
Figure 15g. Chrysin Statistical Average Concentration Response Curve
Figure 15h. Dicofol Statistical Average Concentration Response Curve
Figure 16. Validation of the Placental Aromatase Assay (WIL-431009) Reference Chemical 1- Aminoglutethimide Response Curve Summary Table
Figure 17. Validation of the Placental Aromatase Assay (WIL-431009) Reference Chemical 2-Ketoconazole Response Curve Summary Table
Figure 18. Validation of the Placental Aromatase Assay (WIL-431009) Reference Chemical 3-Prochloraz Response Curve Summary Table
Figure 19. Validation of the Placental Aromatase Assay (WIL-431009) Reference Chemical 4-4-Nonylphenol Response Curve Summary Table
Figure 20. Validation of the Placental Aromatase Assay (WIL-431009) Reference Chemical 6-Fenarimol Response Curve Summary Table
Figure 21. Validation of the Placental Aromatase Assay (WIL-431009) Reference Chemical 7-Econazole Response Curve Summary Table
Figure 22. Validation of the Placental Aromatase Assay (WIL-431009) Reference Chemical 8-Chrysin Response Curve Summary Table
Figure 23. Validation of the Placental Aromatase Assay (WIL-431009) Reference Chemical 9-Dicofol Response Curve Summary Table

The Log IC_{50} (μ) and slope (β) were subjected to random effects analysis of variance to determine the within replicate standard error (see table in Section 4.6, IC_{50} and Slope Results). Log IC_{50} and slope were also compared across replicates based on random effects analysis of variance to determine Top_{avg}, $Bottom_{avg}$, μ_{AVG} and β_{AVG}. 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 are summarized in a results table. From the statistical analysis described in Section 3.7, the overall IC_{50} based on μ_{AVG} and β_{AVG} was between 2 nM and 64 μM for all reference chemicals (see table below, and Figures 15 a-h, Statistical Average Concentration Response curves). The Top, Bottom, Log IC_{50} (μ) and β (slope) parameters and their associated 95% confidence intervals are plotted per replicate and across replicates (e.g. per chemical) for each reference chemical in Figures 16 through 23.

Reference Chemical	Top_{avg}	$Bottom_{avg}$	μ_{avg}	IC_{50} (μM)	β_{avg}
Aminoglutethimide	103.143	-1.108	-4.193	64.12	-0.963
Ketoconazole	99.435	0.187	-6.390	0.4074	-1.026
Prochloraz	103.824	-6.695	-7.597	0.02529	-0.971
4-Nonylphenol	101.226	0.444	-4.579	26.36	-1.952
Fenarimol	90.229	-6.474	-5.145	7.161	-1.034
Econazole	100.533	0.315	-8.776	0.001675	-1.094
Chrysin	90.296	13.302	-5.503	3.141	-1.258
Dicofol	100.860	-10.380	-4.334	46.34	-0.639

The Full Enzyme Activity, Background Activity, Positive control and Negative control values among replicates were analyzed by 3 factor mixed effects analysis of variance with the percent of control aromatase activity as the response variable. P-values for replicate, portion, and replicate by portion are presented in Appendix E (Statistical Analysis Report). In addition, estimates for the LSMeans, standard errors and 95% Confidence Intervals are presented for percent of control aromatase activity across replicates and across chemicals.

Statistical evaluation of all control activity was based on values normalized to a percent of the Full Enzyme Activity control. These data, summarized in the Statistician's Report in Appendix E, shows there is a statistically significant portion effect in the Full Enzyme Activity, Negative and Positive controls, in all cases showing higher activity in the beginning replicates than in the end. There was also a statistically significant portion by chemical effect on the Full Enzyme Activity controls. In all other instances (Chemical, Chemical by Portion, Replicate within Chemical and Portion by Replicate within Chemical) the differences in control values within control type were statistically insignificant or not calculable within the restraints set by the model in the Statistical Analysis Plan.

5.0 Discussion

Ten chemicals were tested for their ability to inhibit the enzyme aromatase at concentrations ranging from 10^{-3}M to 10^{-10}M (10^{-4}M if limited by solubility). Of the 10, eight were found to inhibit aromatase with IC_{50} values ranging from 2nM to 65μM. The most effective inhibitor (IC_{50}=2nM) was econazole, a potent imidazole anti-fungal. The least effective inhibitor was aminoglutethimide (IC_{50}=65μM). Atrazine and dibenz[a,h]anthracene did not inhibit aromatase in the concentration range tested, as expected. Contrary to the expectation of the EDSP, the supposed non-inhibitor 4-nonylphenol was found to inhibit aromatase with an IC_{50} of approximately 26μM.

The Full Enzyme Activity controls demonstrated that the enzyme was active on each assay day. The Full Enzyme Activity controls also suggest a slight portion effect, indicating that the enzyme loses activity over time when diluted for use in the assay. However, this decrease in activity was not enough to affect the utility of the assay in identifying aromatase inhibitors. The Background Activity controls confirmed that there was no nonspecific turnover of androstenedione to estrone, although in more than one instance (prochloraz and fenarimol Replicate 2, dicofol and atrazine Replicate 3) there was evidence of NADPH contamination of the Background Activity controls. However, this did not adversely affect the calculation of the IC_{50}. The Positive and Negative controls responded as expected to the presence of 4-OH ASDN (50% inhibition at the concentration used) or lindane (no inhibition), respectively.

6.0 Conclusion

The human placental aromatase assay responds to known aromatase inhibitors and shows no response to non-inhibitors across a 10^{7}-fold concentration range. The assay identifies IC_{50} values that correlate well to the inhibition of human recombinant aromatase (WIL-431011) by the same chemicals. The data generated for the validation of the human placental aromatase assay and discussed in this report demonstrates that the assay can generate reliable and reproducible data, but is susceptible to some factors that affect the determined IC_{50}, which is the critical value for comparing compounds suspected of endocrine disruption via aromatase inhibition. Identifying these factors, which include determining the criteria for nullifying the results of potentially compromised assays as well as determining criteria for discarding outliers within replicates will increase the utility of the aromatase assay.

7.0 References

Bowman, C.J. WA 4-16, Task 4. Validation Of The Placental Aromatase Assay: Positive Control Study. (Study No. WIL 431006). WIL Research Laboratories, LLC, Ashland, OH, Draft

Thomas-Wohlever, J.A. WA 4-16, Task 5. Validation Of The Placental Aromatase Assay For Endocrine Disruptor Screening. (Study No. WIL 431007). WIL Research Laboratories, LLC, Ashland, OH, Draft

Thomas-Wohlever, J.A. WA 4-16, Task 6. Validation Of The Placental Aromatase Assay Using Prepared Microsomes . (Study No. WIL 431008). WIL Research Laboratories, LLC, Ashland, OH, Draft

Thomas-Wohlever, J.A. WA 4-16, Task 7: Validation Of The Placental Aromatase Assay For Endocrine Disruptor Screening Using Prepared Microsomes. (Study No. WIL-431009). WIL Research Laboratories, LLC, Ashland, OH, Draft

Thomas-Wohlever, J.A. WA 4-17, Task 4: Validation Of The Aromatase Assay For Endocrine Disruptor Screening Using Recombinant Microsomes. (Study No. WIL-431011). WIL Research Laboratories, LLC, Ashland, OH, Draft

Dean, R.B., Dixon, W.J. Simplified Statistics for Small Numbers of Observations. University of Oregon, Eugene, OR, 1951

Microsomal Aromatase Prevalidation Study Reports (WA 4-10), Battelle Memorial Institute, Columbus, OH, 2004.

Pre-Optimization Experiments for Substrate Characterization for Human Recombinant and Human Placental Microsomes (WA 2-24). Battelle Memorial Institute, Columbus,OH, 2003

Battelle. Final Detailed Review Paper on Aromatase, (WA 2-7 and 5-5, Task 2), Battelle Memorial Institute, Columbus, OH, 2005

8.0 Protocol Deviations

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 5.2** states that the microsomes will be diluted in buffer to an approximate protein concentration of 0.025 mg/mL. During the aromatase assay on study day 2 for reference chemical 5, the aliquot of microsomes (MET-0297A #29) used for that study day did not contain sufficient volume of microsomes to prepare the 0.025 mg/mL microsomal dilution as called for in the protocol. As a result, a microsomal dilution was prepared at a concentration of 0.02 mg/mL.

- **Protocol Section 7.** states that the volume of duplicate aliquot vials should be 0.5 µL. During the aromatase assay on study day 1 for reference chemical 5, for WTDMS count file 111 (LSC vial numbers: 1825-1828), a pipetting error occurred resulting in insufficient volume of aqueous layer for duplicate 500 µL aliquots of both the full enzyme activity and background activity control. As a result, duplicate 200 µL aliquots of the controls were aliquoted into LSC vial numbers 1825-1828.

- **Protocol Section 7.** states that the volume of duplicate aliquot vials should be 0.5 µL. During the aromatase assay on study day 2 for reference chemicals 3 and 6, for WTDMS count file 31 (LSC vial numbers: 0449-0480), the LSC vial order was inadvertently lost during the pipetting of duplicate 500 µL samples of the aqueous layer and not discovered until the final sample was aliquoted, resulting in LSC vials with unknown sample. As a result, the vial numbers (0449-0480) were removed from the caps of the unknown vials and placed onto the caps of new scintillation vials and duplicate 200 µL aliquots were pipetted into the appropriately numbered LSC vials. For vial numbers 0479 and 0480 (Reference Chemical 6, dilution 8, replicate 3) there was insufficient sample volume for LSC analysis and as a result, only scintillation cocktail was placed into the vials and analyzed by LSC.

These deviations did not negatively impact the quality or integrity of the data nor the outcome of the study.

9.0 Key Personnel and Report Submission

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Manager, Reporting and
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Date

FIGURES 1-23

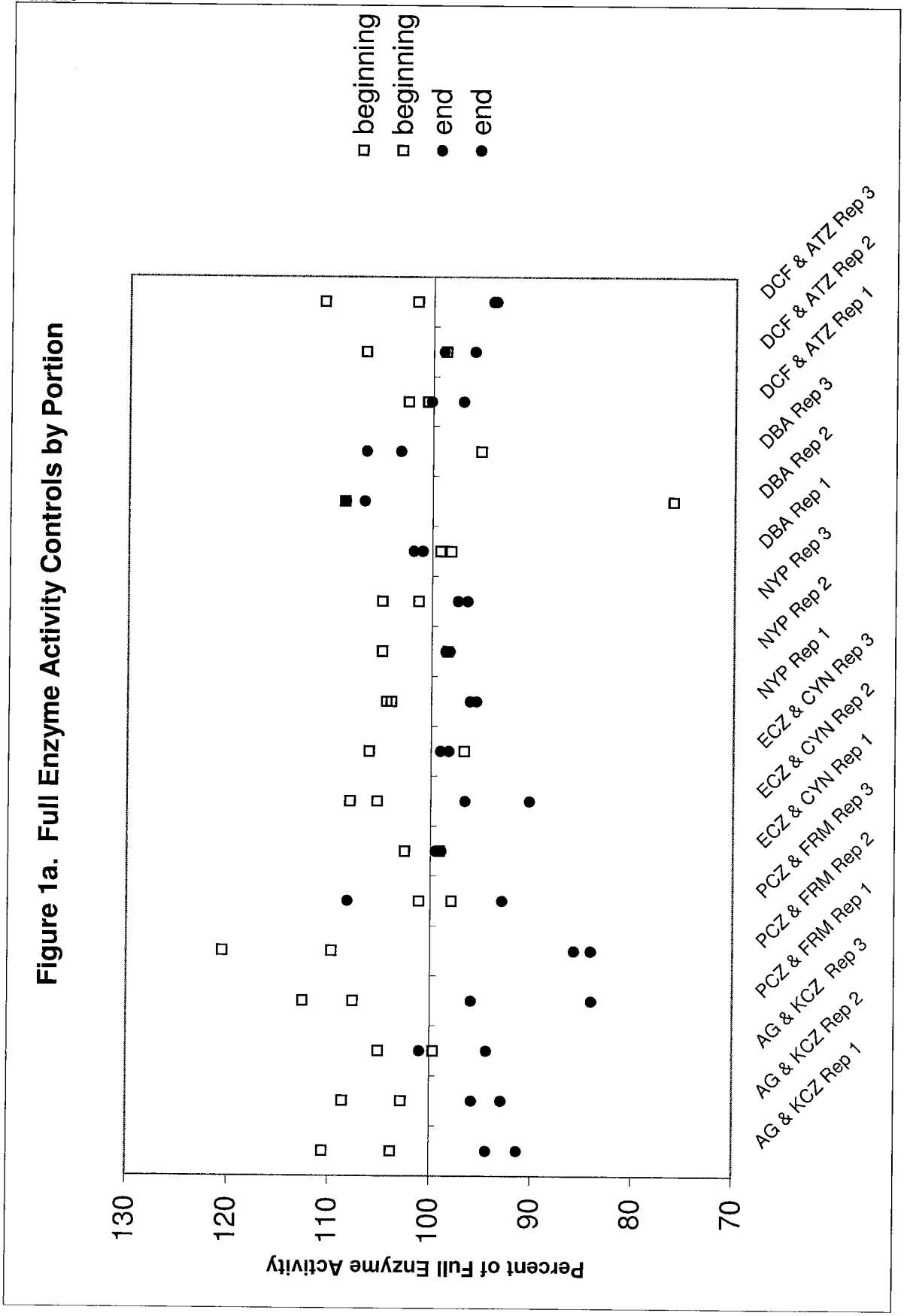


Figure 1a. Full Enzyme Activity Controls by Portion
beginning
beginning
end
end
Percent of Full Enzyme Activity
130
120
110
100
90
80
70
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

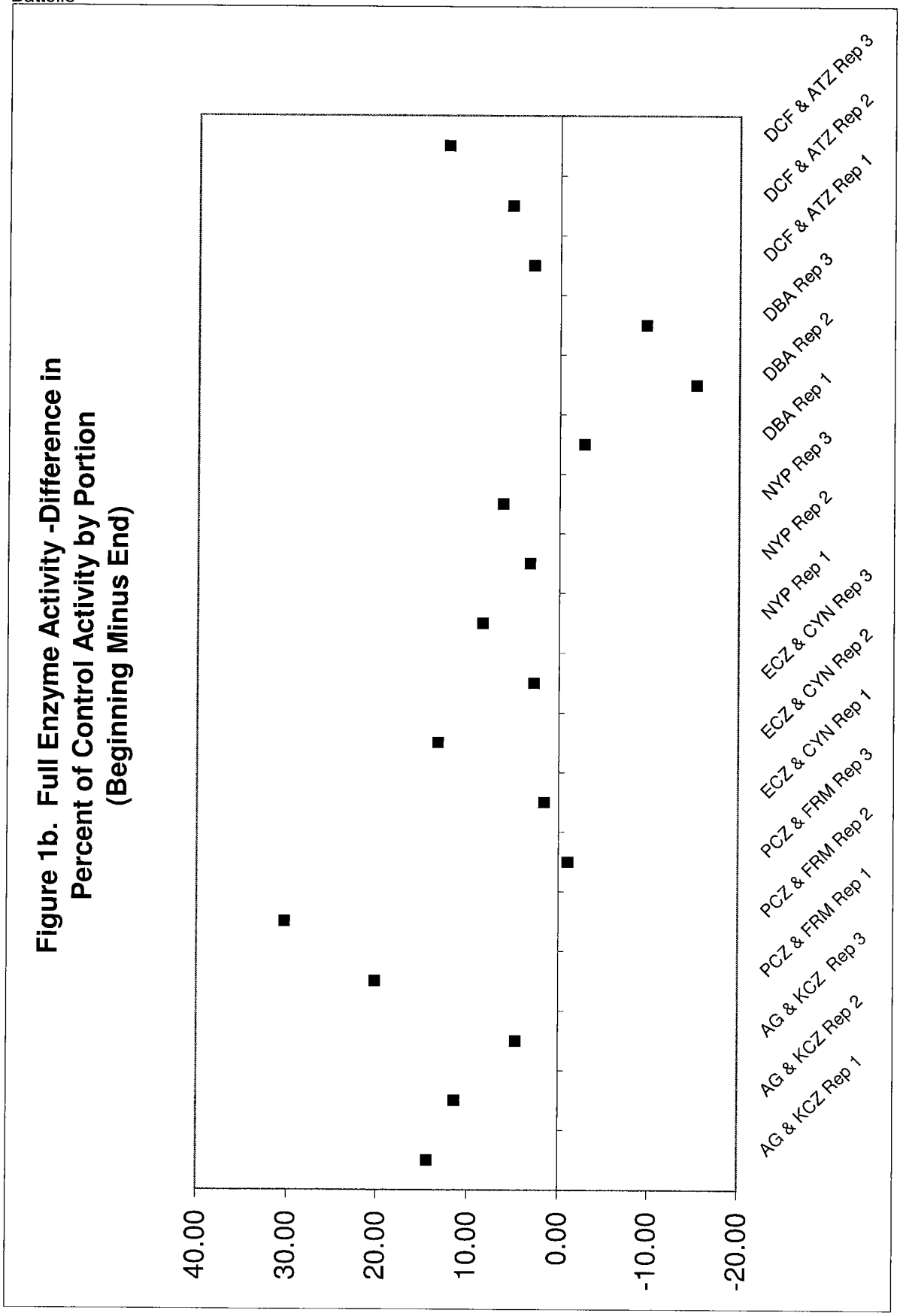


Figure 1b. Full Enzyme Activity -Difference in Percent of Control Activity by Portion (Beginning Minus End)
40.00
30.00
20.00
10.00
0.00
-10.00
-20.00
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

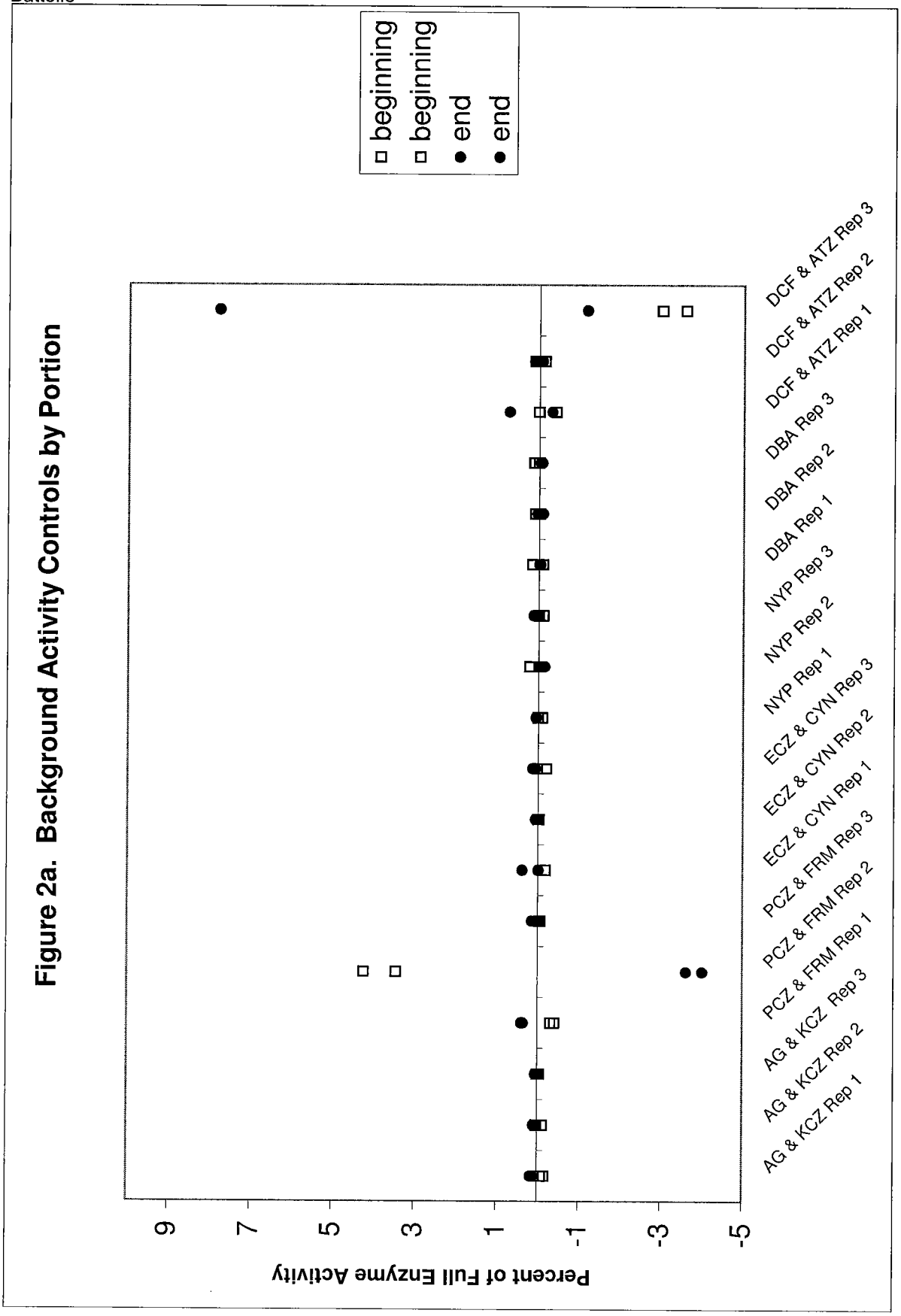


Figure 2a. Background Activity Controls by Portion
beginning
beginning
end
end
Percent of Full Enzyme Activity
9
7
5
3
1
-1
-3
-5
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

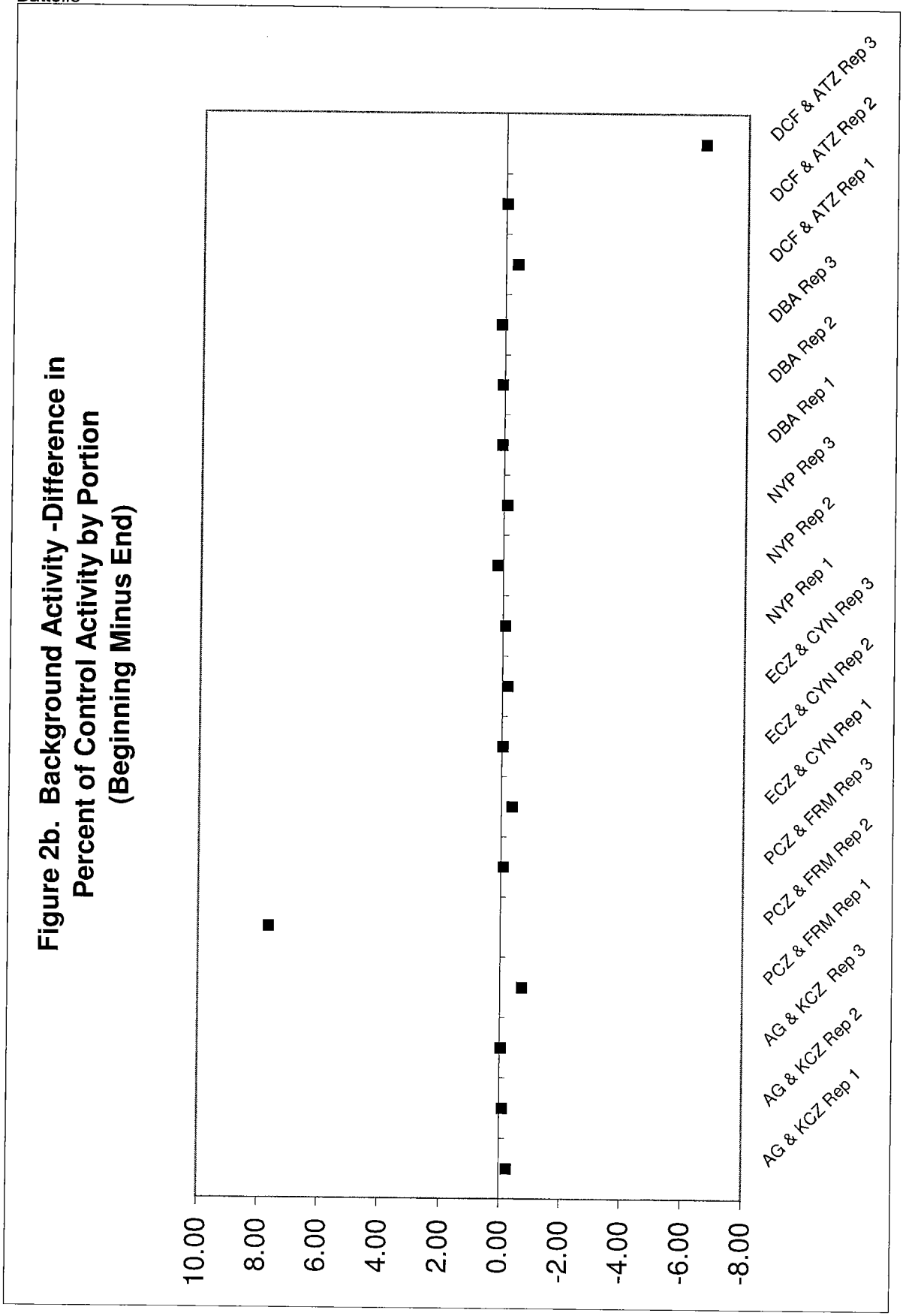


Figure 2b. Background Activity -Difference in Percent of Control Activity by Portion (Beginning Minus End)
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

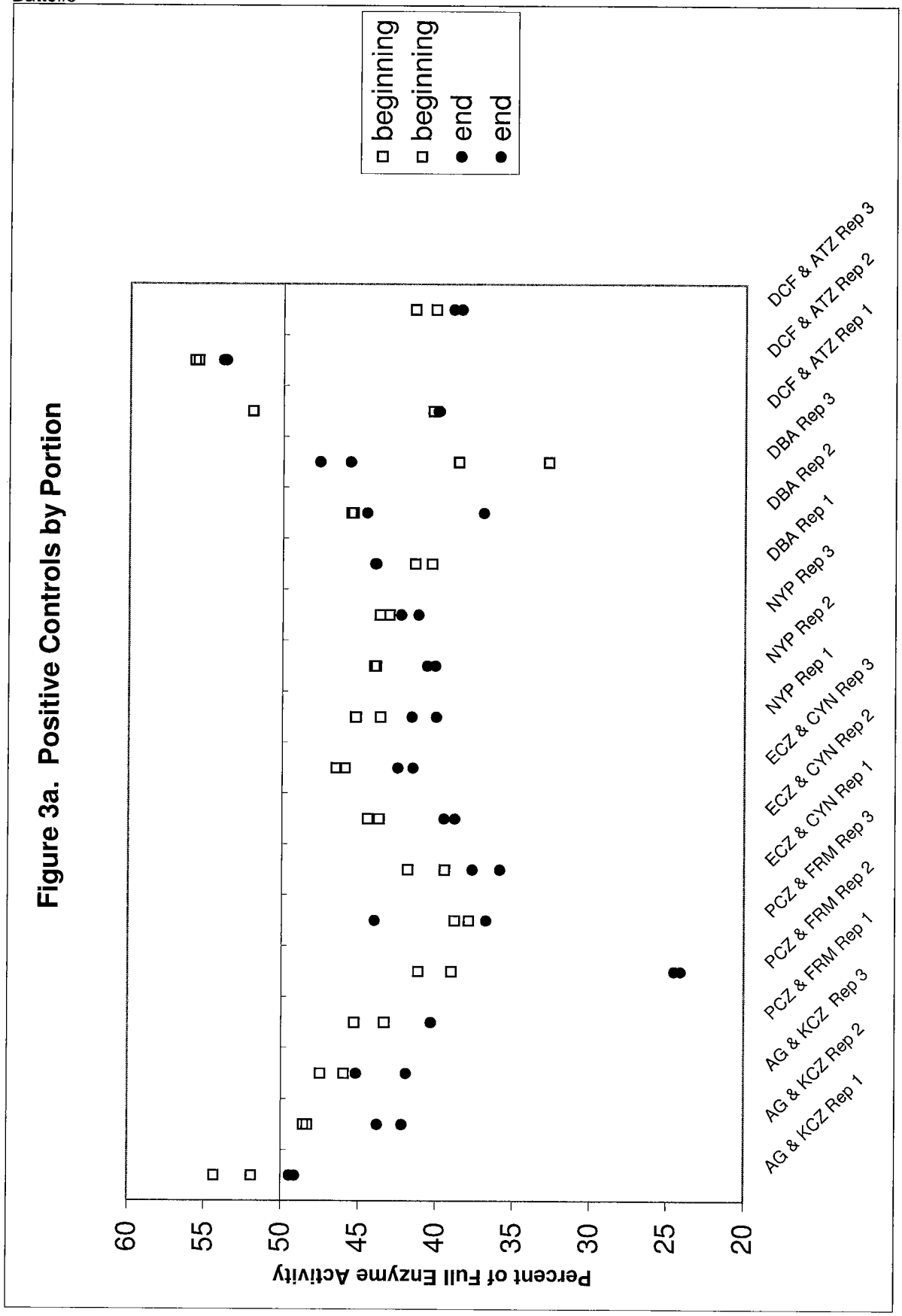


Figure 3a. Positive Controls by Portion
beginning
beginning
end
end
Percent of Full Enzyme Activity
60
55
50
45
40
35
30
25
20
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

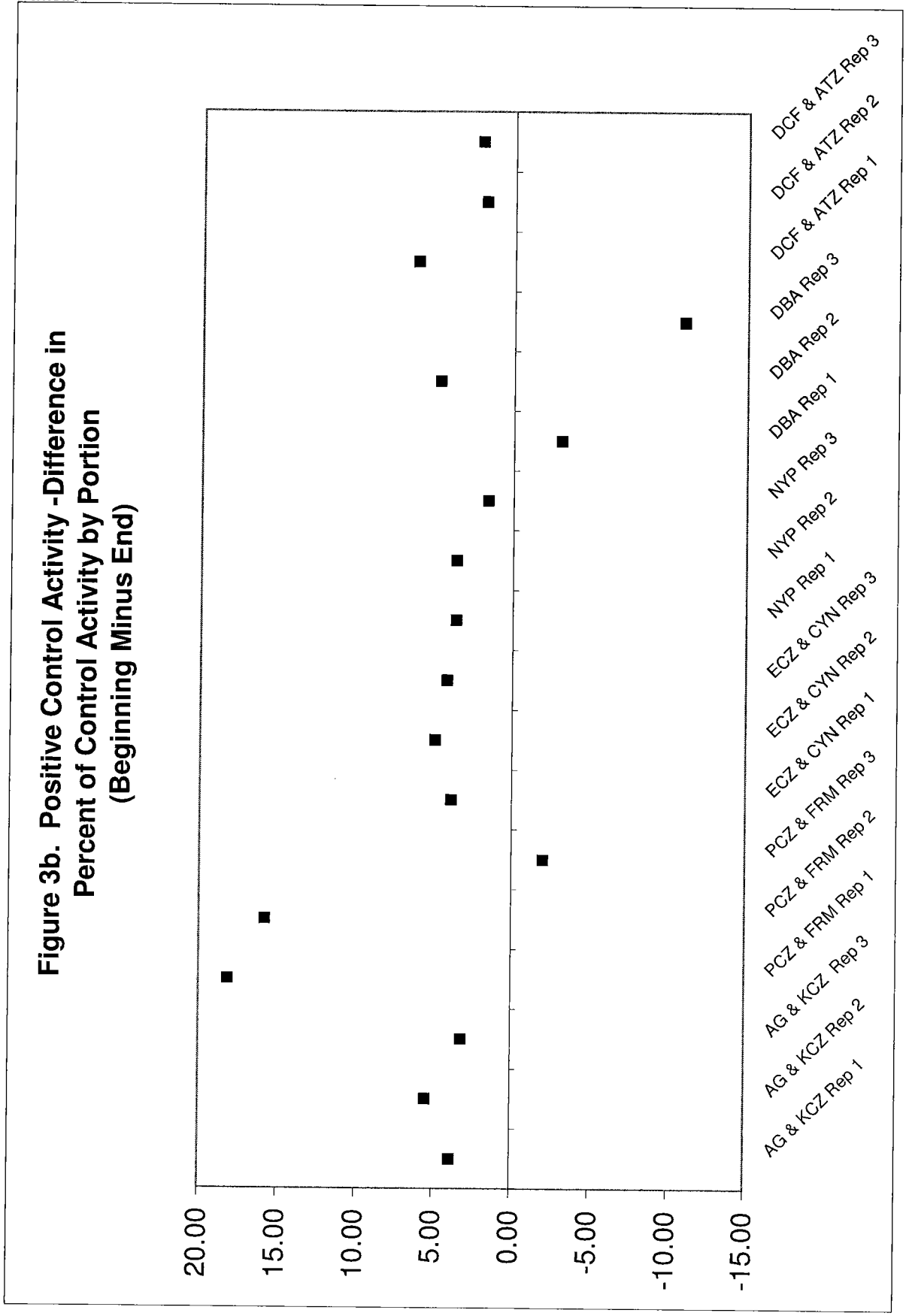


Figure 3b. Positive Control Activity -Difference in Percent of Control Activity by Portion (Beginning Minus End)
20.00
15.00
10.00
5.00
0.00
-5.00
-10.00
-15.00
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

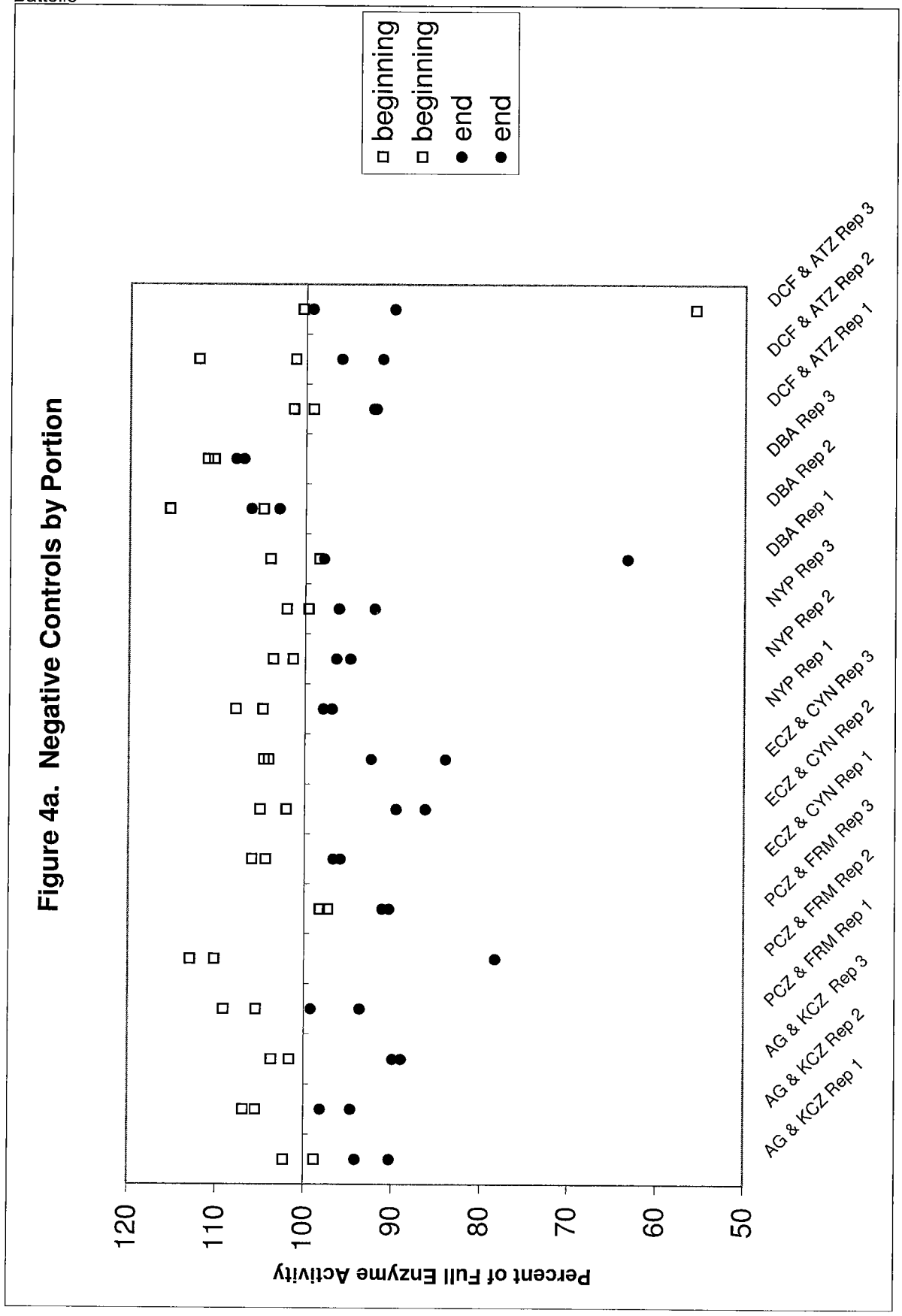


Figure 4a. Negative Controls by Portion
beginning
beginning
end
end
Percent of Full Enzyme Activity
120
110
100
90
80
70
60
50
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

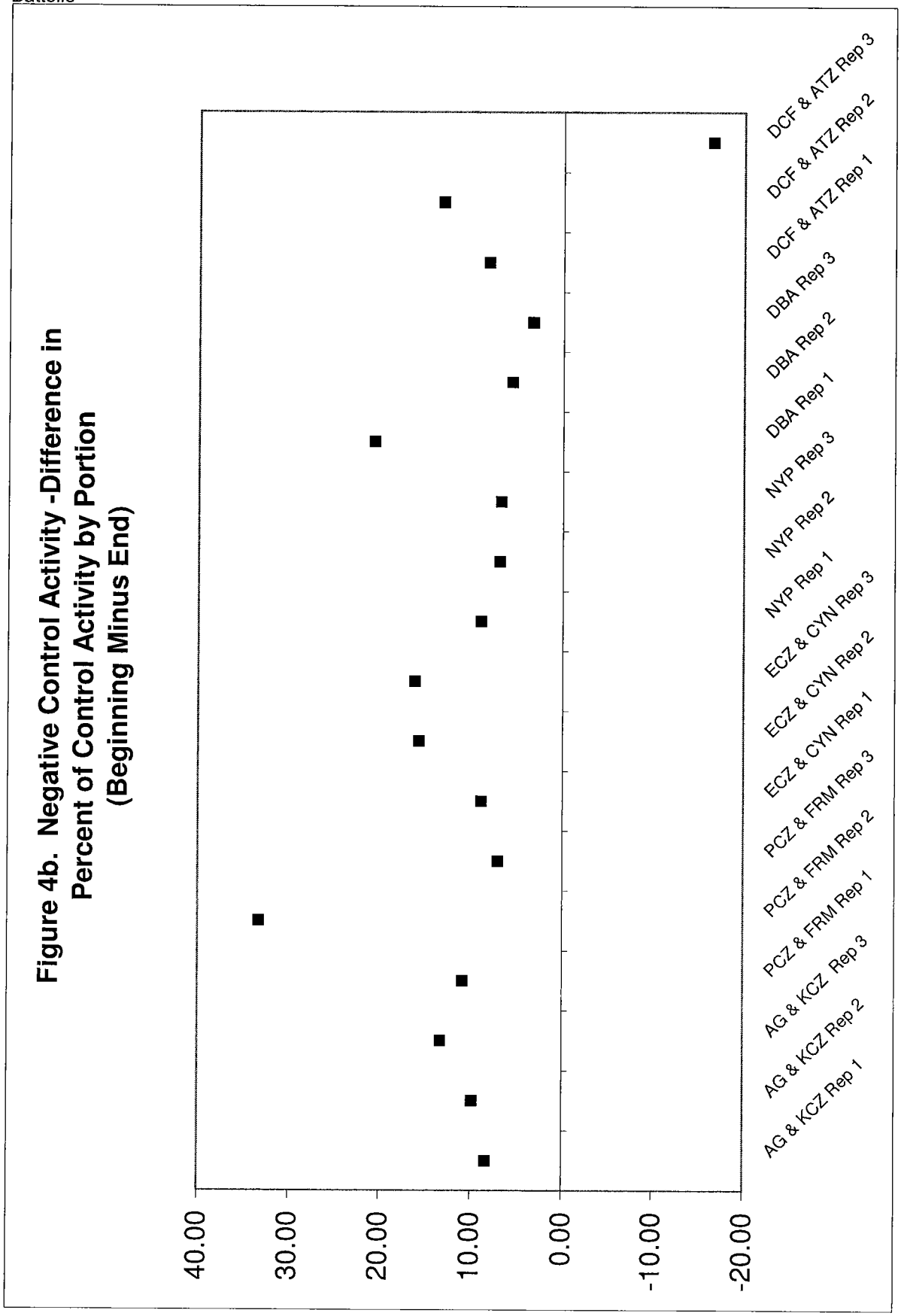
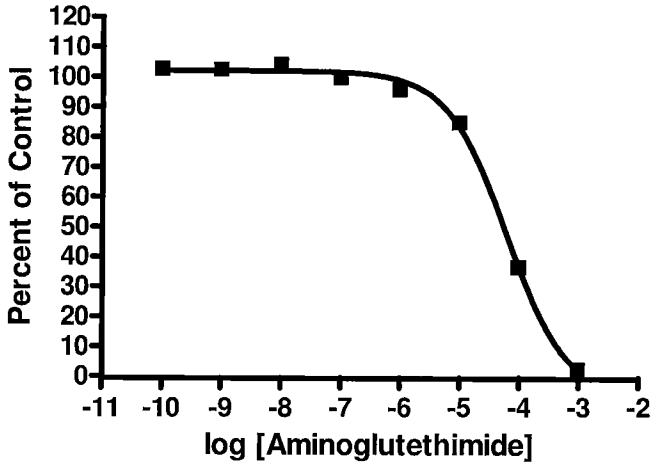


Figure 4b. Negative Control Activity -Difference in Percent of Control Activity by Portion (Beginning Minus End)
40.00
30.00
20.00
10.00
0.00
-10.00
-20.00
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

WIL WA416 TK7

Figure 5a.
Aminoglutethimide Response Curve
Replicate 1

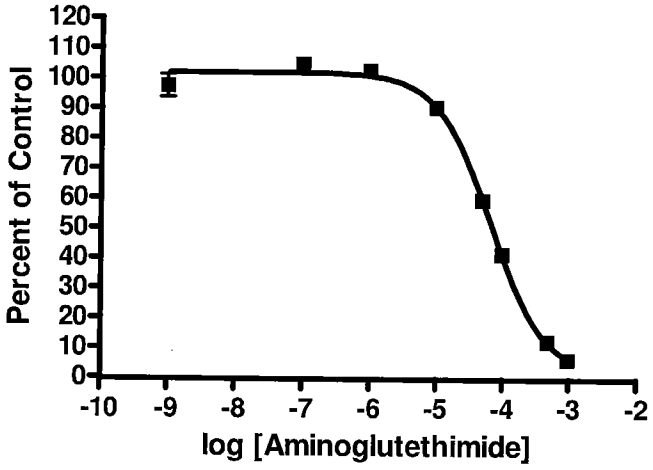


log [RC1]	Replicate 1		
	Y1	Y2	Y3
-10.00	105.35	107.00	101.42
-9.00	107.23	103.81	101.86
-8.00	107.04	106.45	104.35
-7.00	98.79	102.91	103.05
-6.00	95.40	97.49	100.18
-5.00	87.57	86.40	85.60
-4.30			
-4.00	37.89	37.82	37.55
-3.30			
-3.00	2.91	3.52	3.61

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-6.1977
TOP	103.72
LOGEC50	-4.1957
HILLSLOPE	-0.86132
EC50	6.3727e-005
Std. Error	
BOTTOM	2.9913
TOP	0.69837
LOGEC50	0.043444
HILLSLOPE	0.057351
95% Confidence Intervals	
BOTTOM	-12.437 to 0.042008
TOP	102.26 to 105.18
LOGEC50	-4.2863 to -4.1051
HILLSLOPE	-0.98095 to -0.74169
EC50	5.1724e-005 to 7.8514e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99606
Absolute Sum of Squares	122.50
Sy.x	2.4748
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA416 TK7

Figure 5b.
Aminoglutethimide Response Curve
Replicate 2

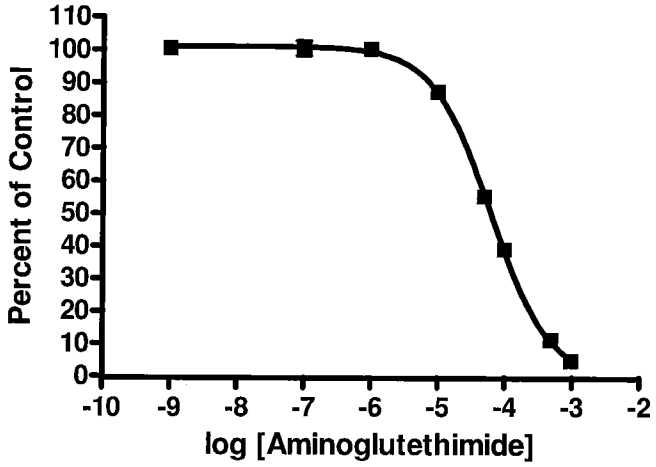


log [RC1]	Replicate 2		
	Y1	Y2	Y3
-10.00			
-9.00	102.04	103.38	91.40
-8.00			
-7.00	105.37	108.38	105.42
-6.00	107.50	102.02	103.03
-5.00	90.85	91.88	93.16
-4.30	61.84	58.67	61.52
-4.00	43.17	42.61	41.66
-3.30	13.41	12.87	12.95
-3.00	6.38	8.45	5.96

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	2.1212
TOP	103.48
LOGEC50	-4.1691
HILLSLOPE	-1.0799
EC50	6.7752e-005
Std. Error	
BOTTOM	2.9717
TOP	1.1742
LOGEC50	0.036583
HILLSLOPE	0.10493
95% Confidence Intervals	
BOTTOM	-4.0778 to 8.3202
TOP	101.03 to 105.93
LOGEC50	-4.2454 to -4.0928
HILLSLOPE	-1.2988 to -0.86097
EC50	5.6834e-005 to 8.0767e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99369
Absolute Sum of Squares	222.58
Sy.x	3.3360
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA416 TK7

Figure 5c.
Aminoglutethimide Response Curve
Replicate 3

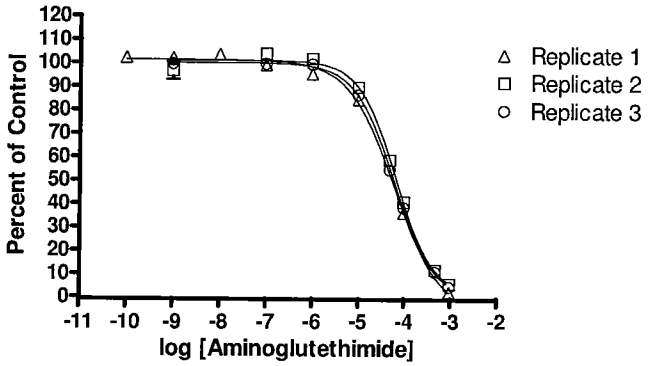


log [RC1]	Replicate 3		
	Y1	Y2	Y3
-10.00			
-9.00	98.45	104.04	103.22
-8.00			
-7.00	103.55	104.89	97.39
-6.00	100.45	103.62	101.22
-5.00	88.00	89.47	88.48
-4.30	55.34	56.27	57.56
-4.00	38.90	40.72	40.31
-3.30	12.55	12.63	11.15
-3.00	5.20	5.55	6.21

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.11287
TOP	102.47
LOGEC50	-4.2021
HILLSLOPE	-0.99869
EC50	6.2798e-005
Std. Error	
BOTTOM	1.7640
TOP	0.68013
LOGEC50	0.022509
HILLSLOPE	0.053243
95% Confidence Intervals	
BOTTOM	-3.5668 to 3.7925
TOP	101.05 to 103.89
LOGEC50	-4.2490 to -4.1551
HILLSLOPE	-1.1098 to -0.88763
EC50	5.6363e-005 to 6.9968e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99798
Absolute Sum of Squares	70.520
Sy.x	1.8778
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA416 TK7

Figure 5d.
Aminoglutethimide Response Curve
Replicates 1, 2, 3

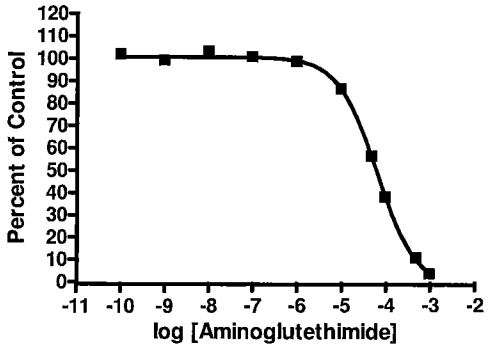


log [RC1]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	105.35	107.00	101.42						
-9.00	107.23	103.81	101.86	102.04	103.38	91.40	98.45	104.04	103.22
-8.00	107.04	106.45	104.35						
-7.00	98.79	102.91	103.05	105.37	108.38	105.42	103.55	104.89	97.39
-6.00	95.40	97.49	100.18	107.50	102.02	103.03	100.45	103.62	101.22
-5.00	87.57	86.40	85.60	90.85	91.88	93.16	88.00	89.47	88.48
-4.30				61.84	58.67	61.52	55.34	56.27	57.56
-4.00	37.89	37.82	37.55	43.17	42.61	41.66	38.90	40.72	40.31
-3.30				13.41	12.87	12.95	12.55	12.63	11.15
-3.00	2.91	3.52	3.61	6.38	8.45	5.96	5.20	5.55	6.21

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-6.1977	2.1212	0.11287
TOP	103.72	103.48	102.47
LOGEC50	-4.1957	-4.1691	-4.2021
HILLSLOPE	-0.86132	-1.0799	-0.99869
EC50	6.3727e-005	6.7752e-005	6.2798e-005
Std. Error			
BOTTOM	2.9913	2.9717	1.7640
TOP	0.69837	1.1742	0.68013
LOGEC50	0.043444	0.036583	0.022509
HILLSLOPE	0.057351	0.10493	0.053243
95% Confidence Intervals			
BOTTOM	-12.437 to 0.042008	-4.0778 to 8.3202	-3.5668 to 3.7925
TOP	102.26 to 105.18	101.03 to 105.93	101.05 to 103.89
LOGEC50	-4.2863 to -4.1051	-4.2454 to -4.0928	-4.2490 to -4.1551
HILLSLOPE	-0.98095 to -0.74169	-1.2988 to -0.86097	-1.1098 to -0.88763
EC50	5.1724e-005 to 7.8514e-005	5.6834e-005 to 8.0767e-005	5.6363e-005 to 6.9968e-005
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.99606	0.99369	0.99798
Absolute Sum of Squares	122.50	222.58	70.520
Sy.x	2.4748	3.3360	1.8778
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

WIL WA416 TK7

Figure 5e.
Aminoglutethimide Response Curve
Average of All Replicates



log [RC1]	All Aminoglutethimide Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	105.35	107.00	101.42						
-9.00	107.23	103.81	101.86	102.04	103.38	91.40	98.45	104.04	103.22
-8.00	107.04	106.45	104.35						
-7.00	98.79	102.91	103.05	105.37	108.38	105.42	103.55	104.89	97.39
-6.00	95.40	97.49	100.18	107.50	102.02	103.03	100.45	103.62	101.22
-5.00	87.57	86.40	85.60	90.85	91.88	93.16	88.00	89.47	88.48
-4.30				61.84	58.67	61.52	55.34	56.27	57.56
-4.00	37.89	37.82	37.55	43.17	42.61	41.66	38.90	40.72	40.31
-3.30				13.41	12.87	12.95	12.55	12.63	11.15
-3.00	2.91	3.52	3.61	6.38	8.45	5.96	5.20	5.55	6.21

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-1.020
TOP	103.2
LOGEC50	-4.181
HILLSLOPE	-0.9793
EC50	6.593e-005
Std. Error	
BOTTOM	1.701
TOP	0.5506
LOGEC50	0.02212
HILLSLOPE	0.04670
95% Confidence Intervals	
BOTTOM	-4.417 to 2.376
TOP	102.1 to 104.3
LOGEC50	-4.225 to -4.137
HILLSLOPE	-1.073 to -0.8860
EC50	5.956e-005 to 7.299e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9944
Absolute Sum of Squares	587.3
Sy.x	2.939
Data	
Number of X values	10
Number of Y replicates	9
Total number of values	72
Number of missing values	18

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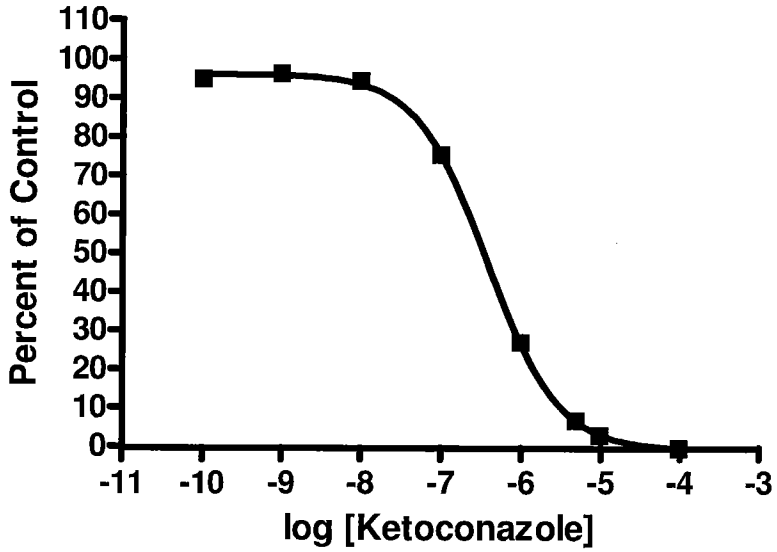
Figure 6a.
Ketoconazole Response Curve
Replicate 1

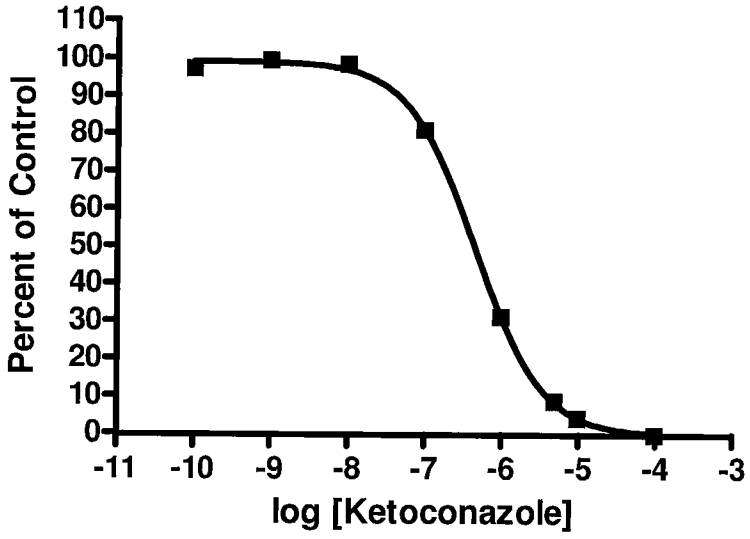


log [RC2]	Replicate 1		
	Y1	Y2	Y3
-4.00	0.12	0.25	0.11
-5.00	3.14	3.38	3.71
-5.30	7.15	7.08	7.68
-6.00	27.38	28.72	26.84
-7.00	76.19	75.56	77.48
-8.00	94.45	97.52	94.52
-9.00	97.07	96.56	98.48
-10.00	97.41	95.23	95.72

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.32242
TOP	97.250
LOGEC50	-6.4097
HILLSLOPE	-0.97363
EC50	3.8933e-007
Std. Error	
BOTTOM	0.51388
TOP	0.39173
LOGEC50	0.013588
HILLSLOPE	0.022587
95% Confidence Intervals	
BOTTOM	-1.3944 to 0.74953
TOP	96.433 to 98.067
LOGEC50	-6.4380 to -6.3813
HILLSLOPE	-1.0207 to -0.92651
EC50	3.6473e-007 to 4.1558e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99947
Absolute Sum of Squares	22.257
Sy.x	1.0549
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA416 TK7

Figure 6b.
Ketoconazole Response Curve
Replicate 2



log [RC2]	Replicate 2		
	Y1	Y2	Y3
-4.00	0.26	0.26	0.20
-5.00	4.79	4.43	4.76
-5.30	9.89	8.71	8.96
-6.00	30.60	32.03	33.14
-7.00	84.49	80.37	81.33
-8.00	103.74	97.85	97.46
-9.00	103.42	99.34	99.19
-10.00	101.79	95.80	97.57

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.091124
TOP	100.28
LOGEC50	-6.3325
HILLSLOPE	-0.99241
EC50	4.6510e-007
Std. Error	
BOTTOM	1.0465
TOP	0.77080
LOGEC50	0.025835
HILLSLOPE	0.045626
95% Confidence Intervals	
BOTTOM	-2.0919 to 2.2741
TOP	98.675 to 101.89
LOGEC50	-6.3863 to -6.2786
HILLSLOPE	-1.0876 to -0.89724
EC50	4.1082e-007 to 5.2655e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99801
Absolute Sum of Squares	88.858
Sy.x	2.1078
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

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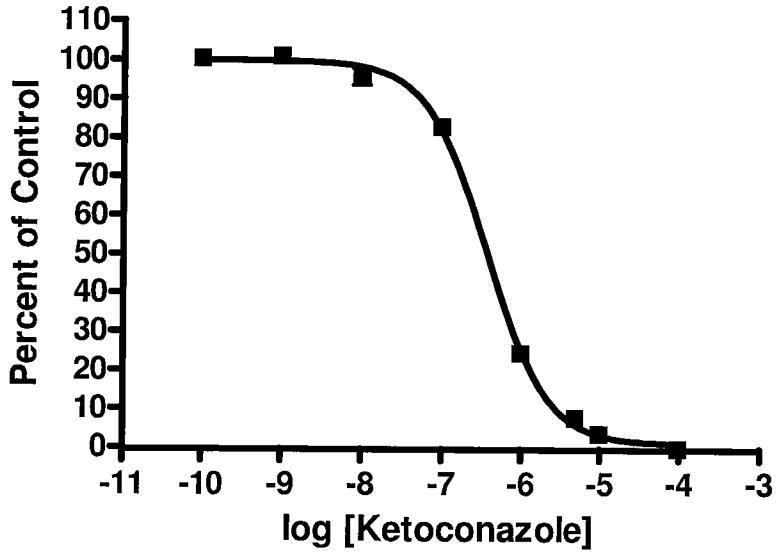
Figure 6c.
Ketoconazole Response Curve
Replicate 3



log [RC2]	Replicate 3		
	Y1	Y2	Y3
-4.00	0.42	0.20	0.21
-5.00	4.28	4.18	3.97
-5.30	8.29	8.24	8.30
-6.00	25.40	25.03	25.12
-7.00	84.03	85.01	83.13
-8.00	98.66	92.61	99.62
-9.00	101.37	100.78	104.59
-10.00	105.08	101.38	98.45

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	1.5025
TOP	101.05
LOGEC50	-6.4224
HILLSLOPE	-1.1402
EC50	3.7808e-007
Std. Error	
BOTTOM	1.0007
TOP	0.82905
LOGEC50	0.026862
HILLSLOPE	0.056233
95% Confidence Intervals	
BOTTOM	-0.58489 to 3.5900
TOP	99.325 to 102.78
LOGEC50	-6.4785 to -6.3664
HILLSLOPE	-1.2575 to -1.0229
EC50	3.3231e-007 to 4.3015e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99769
Absolute Sum of Squares	108.32
Sy.x	2.3272
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

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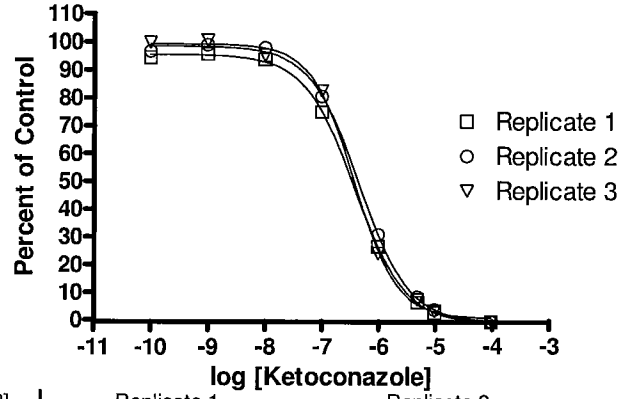
Figure 6d.
Ketoconazole Response Curve
Replicates 1, 2, 3



log [RC2]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	0.12	0.25	0.11	0.26	0.26	0.20	0.42	0.20	0.21
-5.00	3.14	3.38	3.71	4.79	4.43	4.76	4.28	4.18	3.97
-5.30	7.15	7.08	7.68	9.89	8.71	8.96	8.29	8.24	8.30
-6.00	27.38	28.72	26.84	30.60	32.03	33.14	25.40	25.03	25.12
-7.00	76.19	75.56	77.48	84.49	80.37	81.33	84.03	85.01	83.13
-8.00	94.45	97.52	94.52	103.74	97.85	97.46	98.66	92.61	99.62
-9.00	97.07	96.56	98.48	103.42	99.34	99.19	101.37	100.78	104.59
-10.00	97.41	95.23	95.72	101.79	95.80	97.57	105.08	101.38	98.45

Sigmoidal dose-response (variable slope)	Replicate 1	Replicate 2	Replicate 3
Best-fit values			
BOTTOM	-0.32242	0.091124	1.5025
TOP	97.250	100.28	101.05
LOGEC50	-6.4097	-6.3325	-6.4224
HILLSLOPE	-0.97363	-0.99241	-1.1402
EC50	3.8933e-007	4.6510e-007	3.7808e-007
Std. Error			
BOTTOM	0.51388	1.0465	1.0007
TOP	0.39173	0.77080	0.82905
LOGEC50	0.013588	0.025835	0.026862
HILLSLOPE	0.022587	0.045626	0.056233
95% Confidence Intervals			
BOTTOM	-1.3944 to 0.74953	-2.0919 to 2.2741	-0.58489 to 3.5900
TOP	96.433 to 98.067	98.675 to 101.89	99.325 to 102.78
LOGEC50	-6.4380 to -6.3813	-6.3863 to -6.2786	-6.4785 to -6.3664
HILLSLOPE	-1.0207 to -0.92651	-1.0876 to -0.89724	-1.2575 to -1.0229
EC50	3.6473e-007 to 4.1558e-007	4.1082e-007 to 5.2655e-007	3.3231e-007 to 4.3015e-007
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.99947	0.99801	0.99769
Absolute Sum of Squares	22.257	88.858	108.32
Sy.x	1.0549	2.1078	2.3272
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

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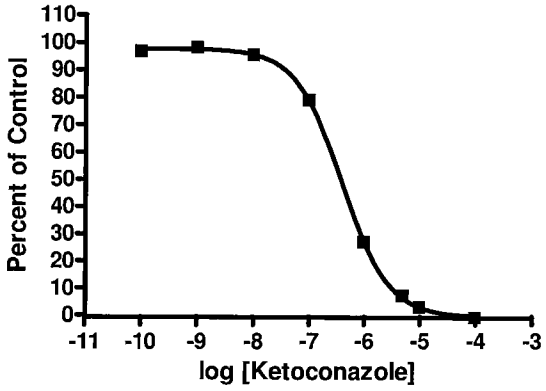
Figure 6e.
Ketoconazole Response Curve
Average of All Replicates

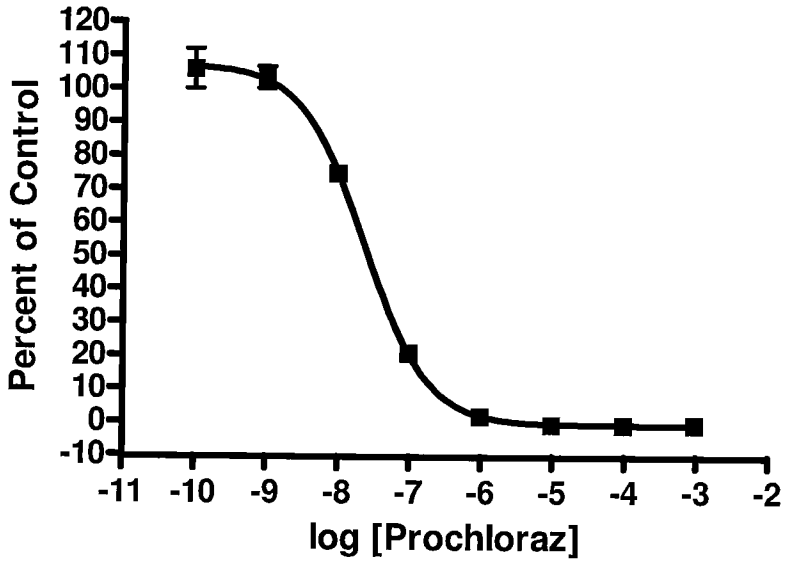


log [RC2]	All Ketoconazole Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	0.12	0.25	0.11	0.26	0.26	0.20	0.42	0.20	0.21
-5.00	3.14	3.38	3.71	4.79	4.43	4.76	4.28	4.18	3.97
-5.30	7.15	7.08	7.68	9.89	8.71	8.96	8.29	8.24	8.30
-6.00	27.38	28.72	26.84	30.60	32.03	33.14	25.40	25.03	25.12
-7.00	76.19	75.56	77.48	84.49	80.37	81.33	84.03	85.01	83.13
-8.00	94.45	97.52	94.52	103.74	97.85	97.46	98.66	92.61	99.62
-9.00	97.07	96.56	98.48	103.42	99.34	99.19	101.37	100.78	104.59
-10.00	97.41	95.23	95.72	101.79	95.80	97.57	105.08	101.38	98.45

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.4277
TOP	99.56
LOGEC50	-6.389
HILLSLOPE	-1.027
EC50	4.082e-007
Std. Error	
BOTTOM	0.6980
TOP	0.5407
LOGEC50	0.01811
HILLSLOPE	0.03294
95% Confidence Intervals	
BOTTOM	-0.9664 to 1.822
TOP	98.48 to 100.6
LOGEC50	-6.425 to -6.353
HILLSLOPE	-1.092 to -0.9609
EC50	3.756e-007 to 4.437e-007
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9967
Absolute Sum of Squares	448.8
Sy.x	2.569
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

WIL WA416 TK7

Figure 7a.
Prochloraz Response Curve
Replicate 1

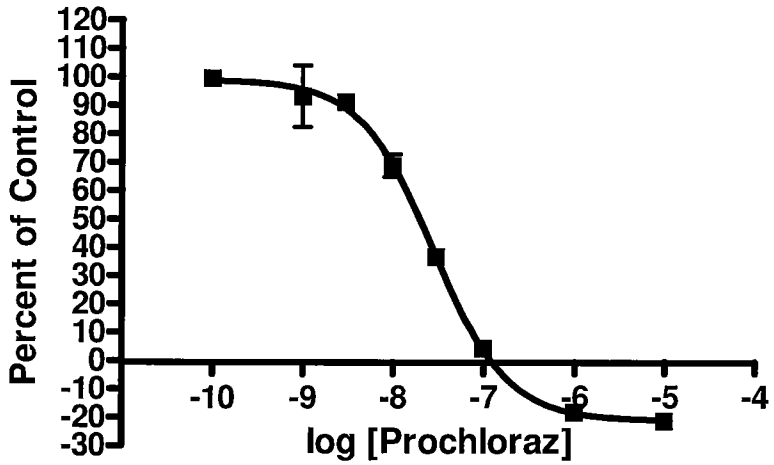


log [RC3]	Replicate 1		
	Y1	Y2	Y3
-10.00	114.79	95.52	111.99
-9.00	111.19	103.27	100.11
-8.52			
-8.00	78.19	74.47	75.18
-7.52			
-7.00	21.68	21.91	20.83
-6.00	2.29	2.34	2.66
-5.00	0.04	-0.14	-0.01
-4.00	-0.16	-0.29	-0.17
-3.00	0.66	-0.18	-0.29

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.16066
TOP	108.57
LOGEC50	-7.6203
HILLSLOPE	-0.98409
EC50	2.3971e-008
Std. Error	
BOTTOM	1.1985
TOP	1.9280
LOGEC50	0.043119
HILLSLOPE	0.076004
95% Confidence Intervals	
BOTTOM	-2.6608 to 2.3394
TOP	104.55 to 112.60
LOGEC50	-7.7103 to -7.5304
HILLSLOPE	-1.1426 to -0.82555
EC50	1.9486e-008 to 2.9487e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99411
Absolute Sum of Squares	295.21
Sy.x	3.8420
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA416 TK7

Figure 7b.
Prochloraz Response Curve
Replicate 2

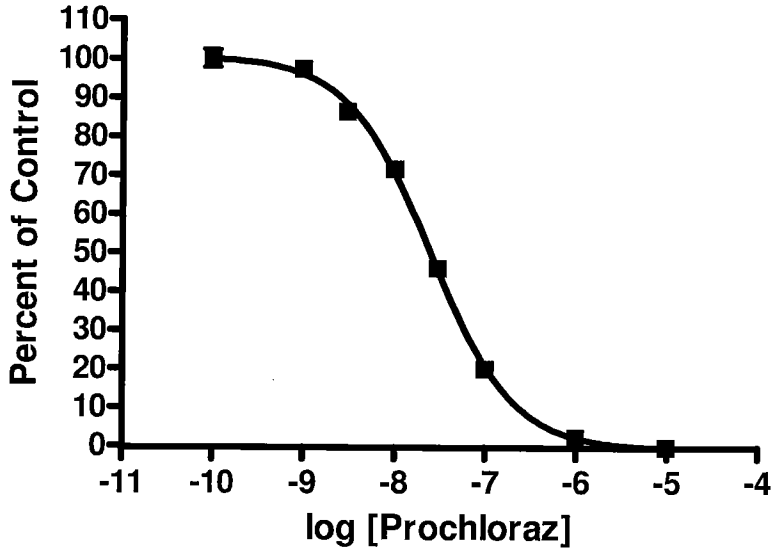


log [RC3]	Replicate 2		
	Y1	Y2	Y3
-10.00	99.12	106.29	98.36
-9.00	101.66	109.49	73.41
-8.52	91.70	97.13	89.84
-8.00	68.32	77.95	64.45
-7.52	35.41	41.73	36.11
-7.00	5.17	6.69	3.75
-6.00	-17.46	-17.54	-17.70
-5.00	-20.26	-20.60	-21.00

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-20.489
TOP	100.78
LOGEC50	-7.5464
HILLSLOPE	-1.0622
EC50	2.8417e-008
Std. Error	
BOTTOM	3.1592
TOP	3.1791
LOGEC50	0.052897
HILLSLOPE	0.12842
95% Confidence Intervals	
BOTTOM	-27.079 to -13.899
TOP	94.147 to 107.41
LOGEC50	-7.6568 to -7.4361
HILLSLOPE	-1.3301 to -0.79431
EC50	2.2042e-008 to 3.6638e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.98304
Absolute Sum of Squares	953.37
Sy.x	6.9042
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA416 TK7

Figure 7c.
Prochloraz Response Curve
Replicate 3

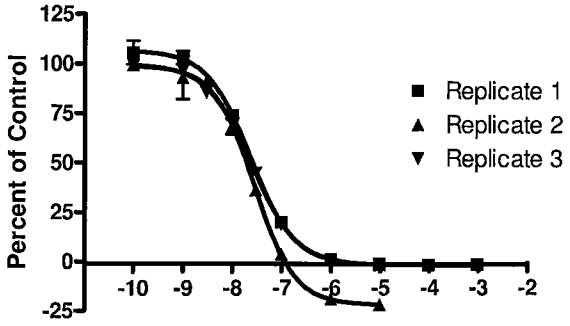


log [RC3]	Replicate 3		
	Y1	Y2	Y3
-10.00	105.47	100.52	97.37
-9.00	101.36	98.85	95.22
-8.52	90.39	85.48	86.28
-8.00	71.64	75.96	70.05
-7.52	45.67	48.29	46.08
-7.00	21.08	20.78	19.62
-6.00	2.86	2.67	2.64
-5.00	0.40	0.22	0.18

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.30247
TOP	101.63
LOGEC50	-7.6002
HILLSLOPE	-0.95206
EC50	2.5106e-008
Std. Error	
BOTTOM	1.1467
TOP	1.2295
LOGEC50	0.024521
HILLSLOPE	0.048475
95% Confidence Intervals	
BOTTOM	-2.6946 to 2.0896
TOP	99.069 to 104.20
LOGEC50	-7.6514 to -7.5491
HILLSLOPE	-1.0532 to -0.85094
EC50	2.2317e-008 to 2.8244e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99687
Absolute Sum of Squares	116.29
Sy.x	2.4113
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA416 TK7

Figure 7d.
Prochloraz Response Curve
Replicates 1, 2, 3



log [RC3]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	114.79	95.52	111.99	99.12	106.29	98.36	105.47	100.52	97.37
-9.00	111.19	103.27	100.11	101.66	109.49	73.41	101.36	98.85	95.22
-8.52				91.70	97.13	89.84	90.39	85.48	86.28
-8.00	78.19	74.47	75.18	68.32	77.95	64.45	71.64	75.96	70.05
-7.52				35.41	41.73	36.11	45.67	48.29	46.08
-7.00	21.68	21.91	20.83	5.17	6.69	3.75	21.08	20.78	19.62
-6.00	2.29	2.34	2.66	-17.46	-17.54	-17.70	2.86	2.67	2.64
-5.00	0.04	-0.14	-0.01	-20.26	-20.60	-21.00	0.40	0.22	0.18
-4.00	-0.16	-0.29	-0.17						
-3.00	0.66	-0.18	-0.29						

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-0.16066	-20.489	-0.30247
TOP	108.57	100.78	101.63
LOGEC50	-7.6203	-7.5464	-7.6002
HILLSLOPE	-0.98409	-1.0622	-0.95206
EC50	2.3971e-008	2.8417e-008	2.5106e-008
Std. Error			
BOTTOM	1.1985	3.1592	1.1467
TOP	1.9280	3.1791	1.2295
LOGEC50	0.043119	0.052897	0.024521
HILLSLOPE	0.076004	0.12842	0.048475
95% Confidence Intervals			
BOTTOM	-2.6608 to 2.3394	-27.079 to -13.899	-2.6946 to 2.0896
TOP	104.55 to 112.60	94.147 to 107.41	99.069 to 104.20
LOGEC50	-7.7103 to -7.5304	-7.6568 to -7.4361	-7.6514 to -7.5491
HILLSLOPE	-1.1426 to -0.82555	-1.3301 to -0.79431	-1.0532 to -0.85094
EC50	1.9486e-008 to 2.9487e-008	2.2042e-008 to 3.6638e-008	2.2317e-008 to 2.8244e-008
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.99411	0.98304	0.99687
Absolute Sum of Squares	295.21	953.37	116.29
Sy.x	3.8420	6.9042	2.4113
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

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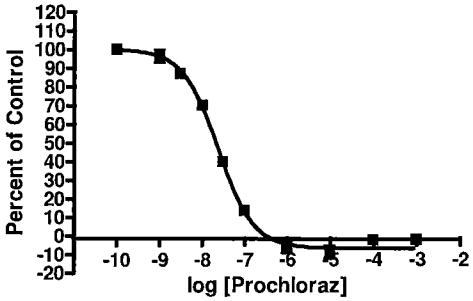
Figure 7e.
Prochloraz Response Curve
Average of All Replicates



log [RC3]	All Prochloraz Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	114.79	95.52	111.99	99.12	106.29	98.36	105.47	100.52	97.37
-9.00	111.19	103.27	100.11	101.66	109.49	73.41	101.36	98.85	95.22
-8.52				91.70	97.13	89.84	90.39	85.48	86.28
-8.00	78.19	74.47	75.18	68.32	77.95	64.45	71.64	75.96	70.05
-7.52				35.41	41.73	36.11	45.67	48.29	46.08
-7.00	21.68	21.91	20.83	5.17	6.69	3.75	21.08	20.78	19.62
-6.00	2.29	2.34	2.66	-17.46	-17.54	-17.70	2.86	2.67	2.64
-5.00	0.04	-0.14	-0.01	-20.26	-20.60	-21.00	0.40	0.22	0.18
-4.00	-0.16	-0.29	-0.17						
-3.00	0.66	-0.18	-0.29						

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-4.933
TOP	103.2
LOGEC50	-7.618
HILLSLOPE	-1.033
EC50	2.408e-008
Std. Error	
BOTTOM	1.733
TOP	2.177
LOGEC50	0.04137
HILLSLOPE	0.09024
95% Confidence Intervals	
BOTTOM	-8.395 to -1.471
TOP	98.83 to 107.5
LOGEC50	-7.701 to -7.536
HILLSLOPE	-1.213 to -0.8529
EC50	1.991e-008 to 2.913e-008
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9717
Absolute Sum of Squares	4140
Sy.x	7.803
Data	
Number of X values	10
Number of Y replicates	9
Total number of values	72
Number of missing values	18

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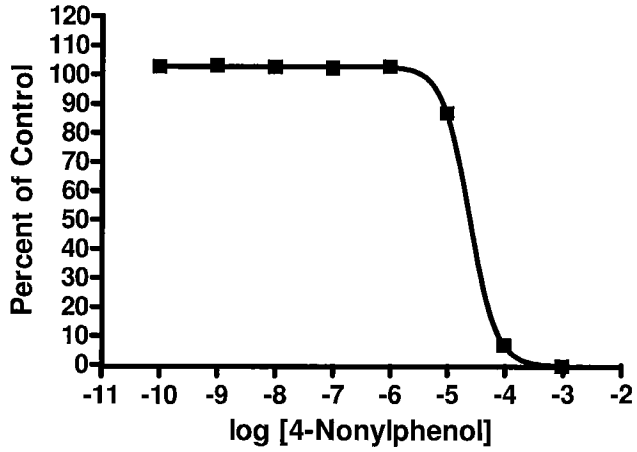
Figure 8a.
4-Nonylphenol Response Curve
Replicate 1



log [RC4]	Replicate 1		
	Y1	Y2	Y3
-10.00	105.67	105.03	102.94
-9.00	106.47	104.87	103.56
-8.00	105.01	104.29	103.93
-7.00	104.41	103.46	104.13
-6.00	105.98	103.01	104.61
-5.00	87.68	88.72	89.06
-4.30			
-4.00	7.87	7.90	7.25
-3.00	0.56	0.32	0.26

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.27770
TOP	104.54
LOGEC50	-4.6016
HILLSLOPE	-1.8580
EC50	2.5025e-005
Std. Error	
BOTTOM	0.54593
TOP	0.23856
LOGEC50	0.012219
HILLSLOPE	0.048837
95% Confidence Intervals	
BOTTOM	-0.86111 to 1.4165
TOP	104.05 to 105.04
LOGEC50	-4.6271 to -4.5761
HILLSLOPE	-1.9598 to -1.7561
EC50	2.3599e-005 to 2.6538e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99962
Absolute Sum of Squares	16.836
Sy.x	0.91749
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

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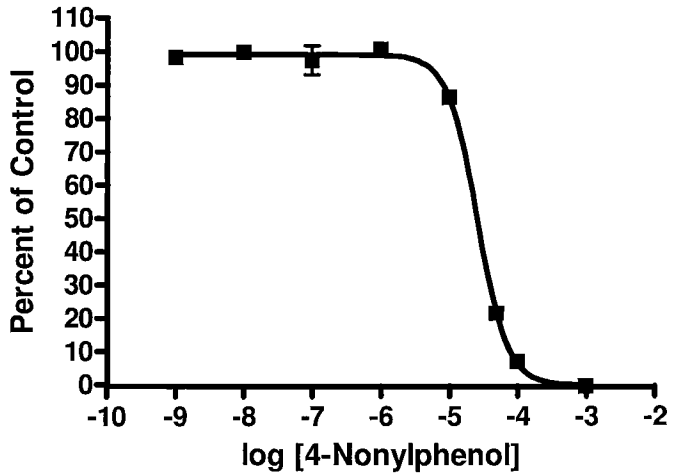
Figure 8b.
4-Nonylphenol Response Curve
Replicate 2



log [RC4]	Replicate 2		
	Y1	Y2	Y3
-10.00			
-9.00	99.31	100.30	99.33
-8.00	102.13	101.54	100.22
-7.00	90.20	101.70	104.42
-6.00	103.91	102.25	100.24
-5.00	88.88	87.26	87.17
-4.30	22.97	22.28	22.02
-4.00	7.64	8.42	7.48
-3.00	0.65	0.40	0.21

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.62588
TOP	100.51
LOGEC50	-4.5779
HILLSLOPE	-1.9735
EC50	2.6431e-005
Std. Error	
BOTTOM	1.4407
TOP	0.79439
LOGEC50	0.021633
HILLSLOPE	0.11266
95% Confidence Intervals	
BOTTOM	-2.3795 to 3.6312
TOP	98.851 to 102.17
LOGEC50	-4.6230 to -4.5328
HILLSLOPE	-2.2086 to -1.7385
EC50	2.3822e-005 to 2.9325e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99663
Absolute Sum of Squares	149.91
Sy.x	2.7378
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

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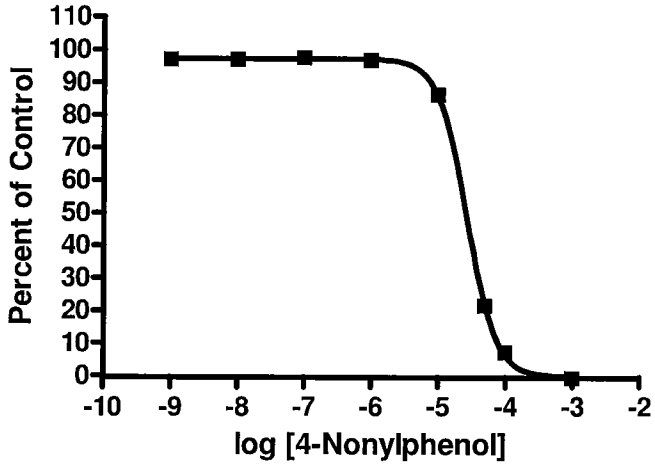
Figure 8c.
4-Nonylphenol Response Curve
Replicate 3



log [RC4]	Replicate 3		
	Y1	Y2	Y3
-10.00			
-9.00	99.68	98.28	97.36
-8.00	97.34	99.27	98.69
-7.00	98.92	99.73	98.22
-6.00	98.01	98.55	98.07
-5.00	87.83	88.21	86.90
-4.30	23.63	23.53	20.30
-4.00	7.55	7.98	8.49
-3.00	0.05	0.22	0.23

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.56287
TOP	98.578
LOGEC50	-4.5610
HILLSLOPE	-2.0307
EC50	2.7481e-005
Std. Error	
BOTTOM	0.50455
TOP	0.27912
LOGEC50	0.0076795
HILLSLOPE	0.042936
95% Confidence Intervals	
BOTTOM	-0.48961 to 1.6154
TOP	97.996 to 99.160
LOGEC50	-4.5770 to -4.5450
HILLSLOPE	-2.1203 to -1.9412
EC50	2.6486e-005 to 2.8514e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99957
Absolute Sum of Squares	18.569
Sy.x	0.96357
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

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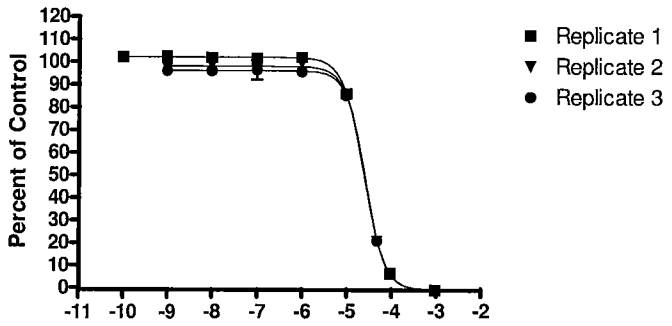
Figure 8d.
4-Nonylphenol Response Curve
Replicates 1, 2, 3



log [RC4]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	105.67	105.03	102.94						
-9.00	106.47	104.87	103.56	99.31	100.30	99.33	99.68	98.28	97.36
-8.00	105.01	104.29	103.93	102.13	101.54	100.22	97.34	99.27	98.69
-7.00	104.41	103.46	104.13	90.20	101.70	104.42	98.92	99.73	98.22
-6.00	105.98	103.01	104.61	103.91	102.25	100.24	98.01	98.55	98.07
-5.00	87.68	88.72	89.06	88.88	87.26	87.17	87.83	88.21	86.90
-4.30				22.97	22.28	22.02	23.63	23.53	20.30
-4.00	7.87	7.90	7.25	7.64	8.42	7.48	7.55	7.98	8.49
-3.00	0.56	0.32	0.26	0.65	0.40	0.21	0.05	0.22	0.23

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	0.27770	0.62588	0.56287
TOP	104.54	100.51	98.578
LOGEC50	-4.6016	-4.5779	-4.5610
HILLSLOPE	-1.8580	-1.9735	-2.0307
EC50	2.5025e-005	2.6431e-005	2.7481e-005
Std. Error			
BOTTOM	0.54593	1.4407	0.50455
TOP	0.23856	0.79439	0.27912
LOGEC50	0.012219	0.021633	0.0076795
HILLSLOPE	0.048837	0.11266	0.042936
95% Confidence Intervals			
BOTTOM	-0.86111 to 1.4165	-2.3795 to 3.6312	-0.48961 to 1.6154
TOP	104.05 to 105.04	98.851 to 102.17	97.996 to 99.160
LOGEC50	-4.6271 to -4.5761	-4.6230 to -4.5328	-4.5770 to -4.5450
HILLSLOPE	-1.9598 to -1.7561	-2.2086 to -1.7385	-2.1203 to -1.9412
EC50	2.3599e-005 to 2.6538e-005	2.3822e-005 to 2.9325e-005	2.6486e-005 to 2.8514e-005
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.99962	0.99663	0.99957
Absolute Sum of Squares	16.836	149.91	18.569
Sy.x	0.91749	2.7378	0.96357
Data			
Number of X values	9	9	9
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	3	3	3

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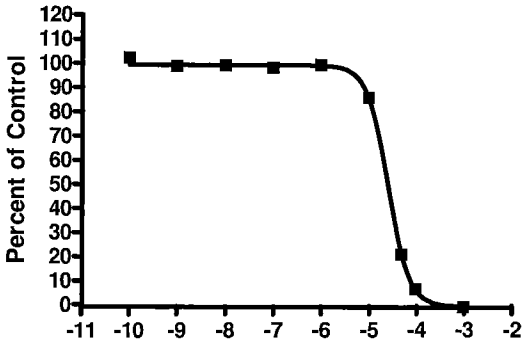
Figure 8e.
4-Nonylphenol Response Curve
Average of All Replicates



log [RC4]	All 4-Nonylphenol Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	105.67	105.03	102.94						
-9.00	106.47	104.87	103.56	99.31	100.30	99.33	99.68	98.28	97.36
-8.00	105.01	104.29	103.93	102.13	101.54	100.22	97.34	99.27	98.69
-7.00	104.41	103.46	104.13	90.20	101.70	104.42	98.92	99.73	98.22
-6.00	105.98	103.01	104.61	103.91	102.25	100.24	98.01	98.55	98.07
-5.00	87.68	88.72	89.06	88.88	87.26	87.17	87.83	88.21	86.90
-4.30				22.97	22.28	22.02	23.63	23.53	20.30
-4.00	7.87	7.90	7.25	7.64	8.42	7.48	7.55	7.98	8.49
-3.00	0.56	0.32	0.26	0.65	0.40	0.21	0.05	0.22	0.23

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.4578
TOP	101.5
LOGEC50	-4.583
HILLSLOPE	-1.938
EC50	2.615e-005
Std. Error	
BOTTOM	0.7829
TOP	0.4088
LOGEC50	0.01242
HILLSLOPE	0.06017
95% Confidence Intervals	
BOTTOM	-1.106 to 2.021
TOP	100.6 to 102.3
LOGEC50	-4.607 to -4.558
HILLSLOPE	-2.058 to -1.818
EC50	2.469e-005 to 2.768e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9967
Absolute Sum of Squares	438.3
Sy.x	2.539
Data	
Number of X values	9
Number of Y replicates	9
Total number of values	72
Number of missing values	9

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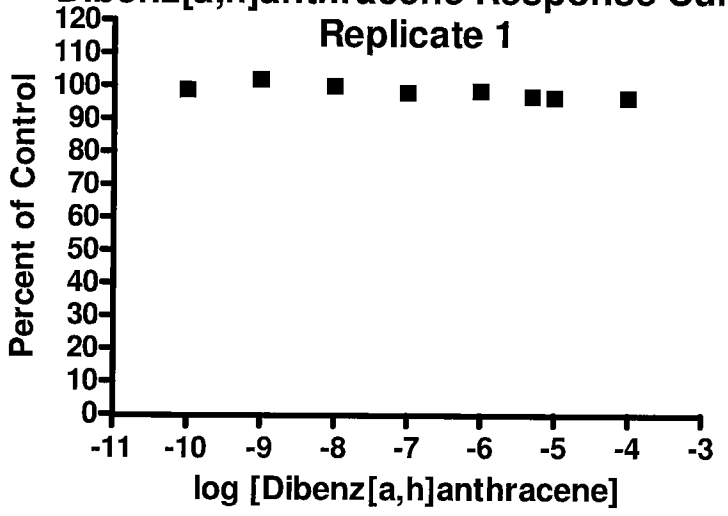
Figure 9a.
Dibenz[a,h]anthracene Response Curve
Replicate 1



log [RC5]	Replicate 1		
	Y1	Y2	Y3
-4.00	95.61	98.07	99.69
-5.00	93.92	100.30	99.37
-5.30	98.28	99.78	96.37
-6.00	100.05	99.99	99.41
-7.00	100.03	98.37	99.21
-8.00	99.68	101.24	102.91
-9.00	103.61	103.40	102.76
-10.00	103.94	100.37	96.27

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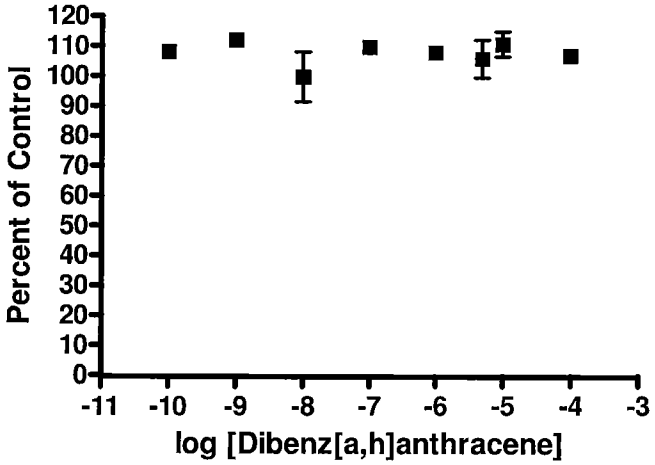
Figure 9b.
Dibenz[a,h]anthracene Response Curve
Replicate 2



log [RC5]	Replicate 2		
	Y1	Y2	Y3
-4.00	108.62	109.74	107.20
-5.00	120.39	105.87	110.85
-5.30	114.83	94.84	112.56
-6.00	112.31	108.04	108.40
-7.00	114.27	110.54	109.16
-8.00	84.76	107.12	111.86
-9.00	115.57	109.65	115.95
-10.00	109.63	108.04	111.35

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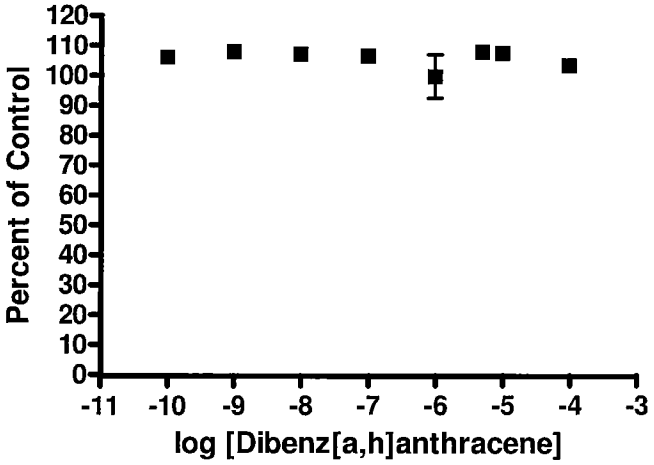
Figure 9c.
Dibenz[a,h]anthracene Response Curve
Replicate 3



log [RC5]	Replicate 3		
	Y1	Y2	Y3
-4.00	103.34	107.52	104.27
-5.00	105.48	110.95	111.01
-5.30	109.10	111.52	108.09
-6.00	106.03	110.73	86.90
-7.00	107.62	107.46	109.36
-8.00	108.62	106.91	110.61
-9.00	110.98	107.11	110.82
-10.00	106.59	107.69	108.91

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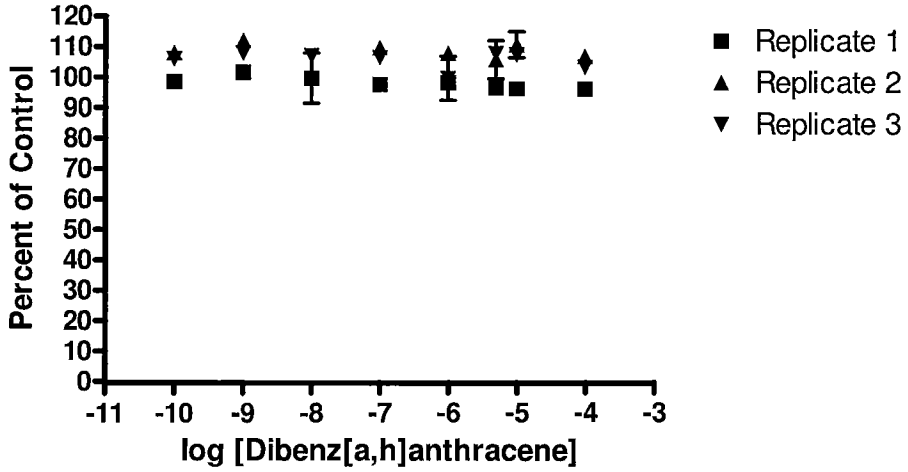
Figure 9d.
Dibenz[a,h]anthracene Response Curve
Replicates 1, 2, 3



log [RC5]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	95.61	98.07	99.69	108.62	109.74	107.20	103.34	107.52	104.27
-5.00	93.92	100.30	99.37	120.39	105.87	110.85	105.48	110.95	111.01
-5.30	98.28	99.78	96.37	114.83	94.84	112.56	109.10	111.52	108.09
-6.00	100.05	99.99	99.41	112.31	108.04	108.40	106.03	110.73	86.90
-7.00	100.03	98.37	99.21	114.27	110.54	109.16	107.62	107.46	109.36
-8.00	99.68	101.24	102.91	84.76	107.12	111.86	108.62	106.91	110.61
-9.00	103.61	103.40	102.76	115.57	109.65	115.95	110.98	107.11	110.82
-10.00	103.94	100.37	96.27	109.63	108.04	111.35	106.59	107.69	108.91

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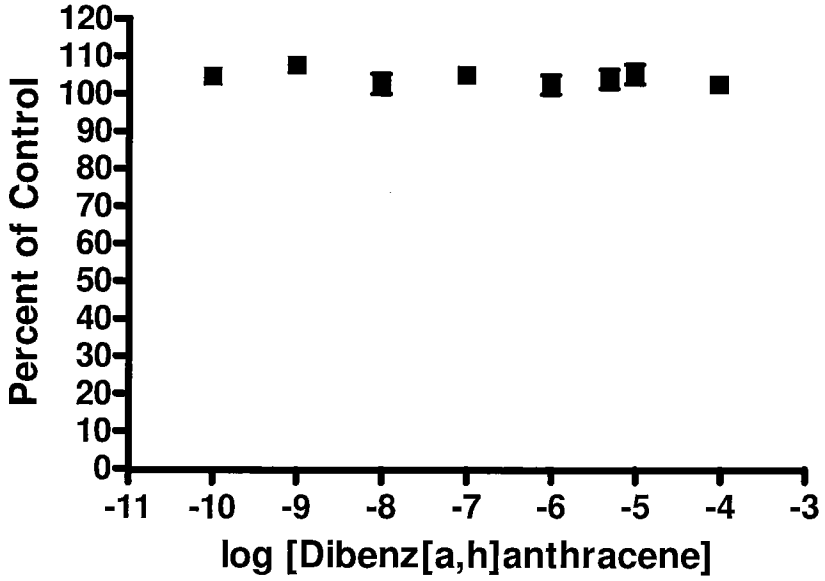
Figure 9e.
Dibenz[a,h]anthracene Response Curve
Average of All Replicates



log [RC5]	All Dibenz[a,h]anthracene Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	95.61	98.07	99.69	108.62	109.74	107.20	103.34	107.52	104.27
-5.00	93.92	100.30	99.37	120.39	105.87	110.85	105.48	110.95	111.01
-5.30	98.28	99.78	96.37	114.83	94.84	112.56	109.10	111.52	108.09
-6.00	100.05	99.99	99.41	112.31	108.04	108.40	106.03	110.73	86.90
-7.00	100.03	98.37	99.21	114.27	110.54	109.16	107.62	107.46	109.36
-8.00	99.68	101.24	102.91	84.76	107.12	111.86	108.62	106.91	110.61
-9.00	103.61	103.40	102.76	115.57	109.65	115.95	110.98	107.11	110.82
-10.00	103.94	100.37	96.27	109.63	108.04	111.35	106.59	107.69	108.91

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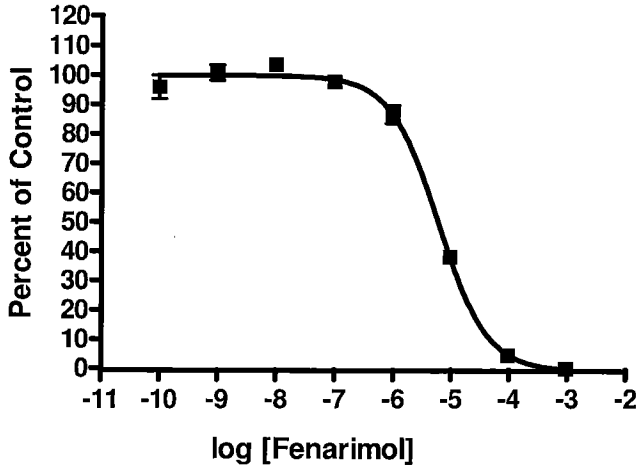
Figure 10a.
Fenarimol Response Curve
Replicate 1



log [RC6]	Replicate 1		
	Y1	Y2	Y3
-10.00	104.51	90.40	97.95
-9.00	106.99	102.61	97.94
-8.00	108.76	104.40	103.20
-7.00	97.55	102.12	98.89
-6.00	94.39	85.77	84.79
-5.52			
-5.00	41.17	38.70	38.18
-4.52			
-4.00	5.78	5.34	5.36
-3.00	0.95	0.70	0.87

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.0781259
TOP	101.663
LOGEC50	-5.19742
HILLSLOPE	-1.02526
EC50	6.34715e-006
Std. Error	
BOTTOM	2.12828
TOP	1.23892
LOGEC50	0.0457651
HILLSLOPE	0.102066
95% Confidence Intervals	
BOTTOM	-4.36147 to 4.51772
TOP	99.0783 to 104.247
LOGEC50	-5.29289 to -5.10196
HILLSLOPE	-1.23816 to -0.812346
EC50	5.09463e-006 to 7.90761e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.992264
Absolute Sum of Squares	329.324
Sy.x	4.05786
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

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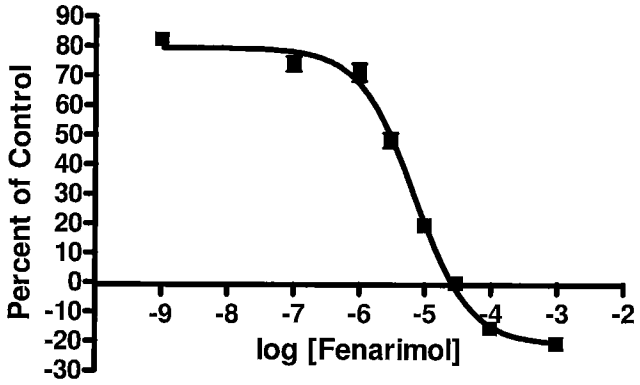
Figure 10b.
Fenarimol Response Curve
Replicate 2



log [RC6]	Replicate 2		
	Y1	Y2	Y3
-10.00			
-9.00	84.92	83.28	
-8.00			
-7.00	77.36	78.82	70.78
-6.00	72.22	78.22	67.86
-5.52	47.93	54.48	46.25
-5.00	20.05	21.47	19.99
-4.52	-1.27	-0.17	3.83
-4.00	-14.17	-13.55	-16.28
-3.00	-20.05	-19.28	-20.16

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-20.1991
TOP	81.0384
LOGEC50	-5.14867
HILLSLOPE	-1.02236
EC50	7.10118e-006
Std. Error	
BOTTOM	2.11630
TOP	1.84701
LOGEC50	0.0392115
HILLSLOPE	0.0813387
95% Confidence Intervals	
BOTTOM	-24.6285 to -15.7697
TOP	77.1726 to 84.9042
LOGEC50	-5.23074 to -5.06660
HILLSLOPE	-1.19261 to -0.852122
EC50	5.87842e-006 to 8.57828e-006
Goodness of Fit	
Degrees of Freedom	19
R^2	0.991697
Absolute Sum of Squares	291.517
Sy.x	3.91701
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	23
Number of missing values	7

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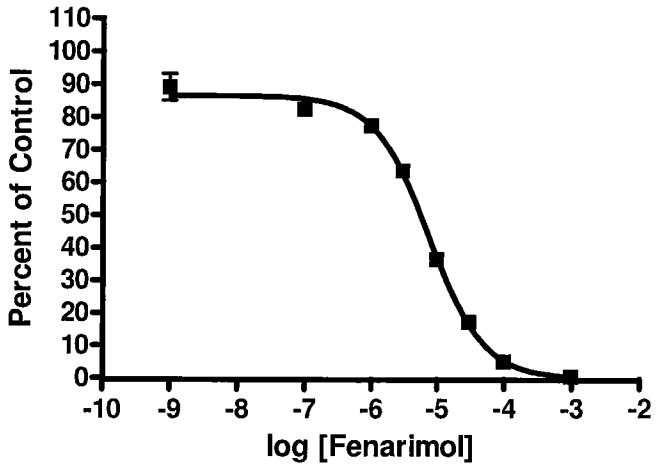
Figure 10c.
Fenarimol Response Curve
Replicate 3



log [RC6]	Replicate 3		
	Y1	Y2	Y3
-10.00			
-9.00	82.43	93.09	95.81
-8.00			
-7.00	84.70	84.70	82.36
-6.00	81.73	77.64	76.59
-5.52	65.88	64.33	64.47
-5.00	38.78	37.35	36.28
-4.52	17.57	19.21	17.49
-4.00	6.19	5.81	5.55
-3.00	1.27	1.35	1.16

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.604631
TOP	87.8319
LOGEC50	-5.11262
HILLSLOPE	-1.04648
EC50	7.71572e-006
Std. Error	
BOTTOM	1.61030
TOP	1.25539
LOGEC50	0.0334714
HILLSLOPE	0.0720153
95% Confidence Intervals	
BOTTOM	-2.75444 to 3.96371
TOP	85.2132 to 90.4507
LOGEC50	-5.18245 to -5.04280
HILLSLOPE	-1.19671 to -0.896258
EC50	6.56984e-006 to 9.06145e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.993616
Absolute Sum of Squares	178.576
Sy.x	2.98811
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

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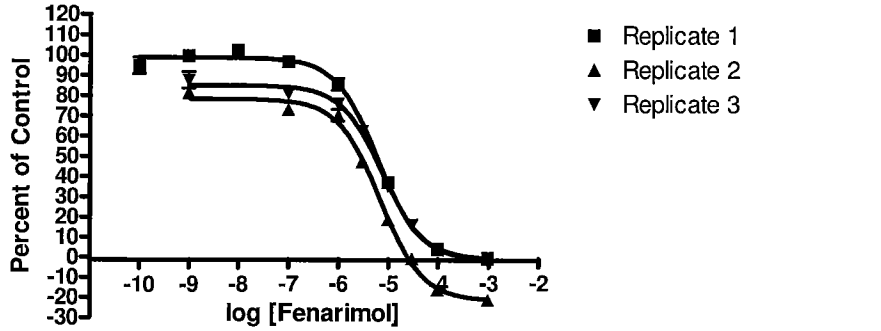
Figure 10d.
Fenarimol Response Curve
Replicates 1, 2, 3



log [RC6]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	104.51	90.40	97.95						
-9.00	106.99	102.61	97.94	84.92	83.28		82.43	93.09	95.81
-8.00	108.76	104.40	103.20						
-7.00	97.55	102.12	98.89	77.36	78.82	70.78	84.70	84.70	82.36
-6.00	94.39	85.77	84.79	72.22	78.22	67.86	81.73	77.64	76.59
-5.52				47.93	54.48	46.25	65.88	64.33	64.47
-5.00	41.17	38.70	38.18	20.05	21.47	19.99	38.78	37.35	36.28
-4.52				-1.27	-0.17	3.83	17.57	19.21	17.49
-4.00	5.78	5.34	5.36	-14.17	-13.55	-16.28	6.19	5.81	5.55
-3.00	0.95	0.70	0.87	-20.05	-19.28	-20.16	1.27	1.35	1.16

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	0.0781259	-20.1991	0.604631
TOP	101.663	81.0384	87.8319
LOGEC50	-5.19742	-5.14867	-5.11262
HILLSLOPE	-1.02526	-1.02236	-1.04648
EC50	6.34715e-006	7.10118e-006	7.71572e-006
Std. Error			
BOTTOM	2.12828	2.11630	1.61030
TOP	1.23892	1.84701	1.25539
LOGEC50	0.0457651	0.0392115	0.0334714
HILLSLOPE	0.102066	0.0813387	0.0720153
95% Confidence Intervals			
BOTTOM	-4.36147 to 4.51772	-24.6285 to -15.7697	-2.75444 to 3.96371
TOP	99.0783 to 104.247	77.1726 to 84.9042	85.2132 to 90.4507
LOGEC50	-5.29289 to -5.10196	-5.23074 to -5.06660	-5.18245 to -5.04280
HILLSLOPE	-1.23816 to -0.812346	-1.19261 to -0.852122	-1.19671 to -0.896258
EC50	5.09463e-006 to 7.90761e-006	5.87842e-006 to 8.57828e-006	6.56984e-006 to 9.06145e-006
Goodness of Fit			
Degrees of Freedom	20	19	20
R^2	0.992264	0.991697	0.993616
Absolute Sum of Squares	329.324	291.517	178.576
Sy.x	4.05786	3.91701	2.98811
Data			
Number of X values	10	10	10
Number of Y replicates	3	3	3
Total number of values	24	23	24
Number of missing values	6	7	6

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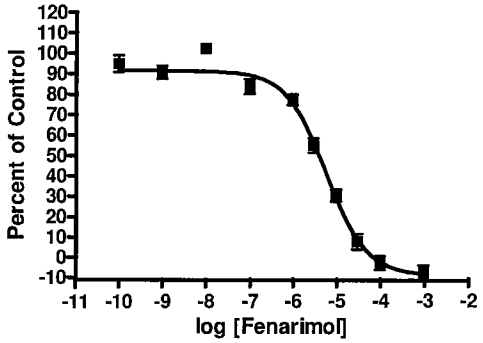
Figure 10e.
Fenarimol Response Curve
Average of All Replicates

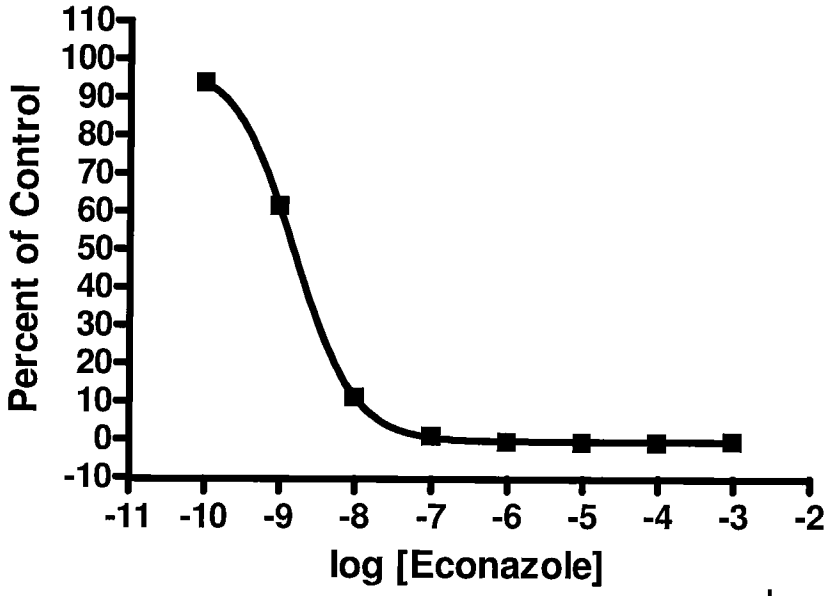


log [RC6]	All Fenarimol Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	104.51	90.40	97.95						
-9.00	106.99	102.61	97.94	84.92	83.28		82.43	93.09	95.81
-8.00	108.76	104.40	103.20						
-7.00	97.55	102.12	98.89	77.36	78.82	70.78	84.70	84.70	82.36
-6.00	94.39	85.77	84.79	72.22	78.22	67.86	81.73	77.64	76.59
-5.52				47.93	54.48	46.25	65.88	64.33	64.47
-5.00	41.17	38.70	38.18	20.05	21.47	19.99	38.78	37.35	36.28
-4.52				-1.27	-0.17	3.83	17.57	19.21	17.49
-4.00	5.78	5.34	5.36	-14.17	-13.55	-16.28	6.19	5.81	5.55
-3.00	0.95	0.70	0.87	-20.05	-19.28	-20.16	1.27	1.35	1.16

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-7.452
TOP	94.15
LOGEC50	-5.225
HILLSLOPE	-0.9335
EC50	5.956e-006
Std. Error	
BOTTOM	3.068
TOP	2.174
LOGEC50	0.06039
HILLSLOPE	0.1070
95% Confidence Intervals	
BOTTOM	-13.58 to -1.323
TOP	89.80 to 98.49
LOGEC50	-5.346 to -5.104
HILLSLOPE	-1.147 to -0.7198
EC50	4.511e-006 to 7.862e-006
Goodness of Fit	
Degrees of Freedom	67
R^2	0.9491
Absolute Sum of Squares	6157
Sy.x	9.586
Data	
Number of X values	10
Number of Y replicates	9
Total number of values	71
Number of missing values	19

WIL WA416 TK7

Figure 11a.
Econazole Response Curve
Replicate 1

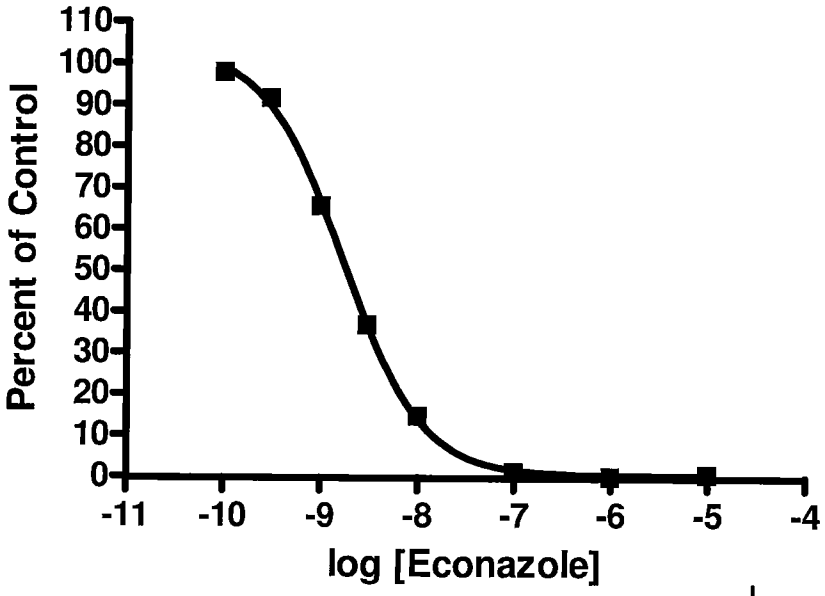


log [RC7]	Replicate 1		
	Y1	Y2	Y3
-10.00	95.05	94.48	96.10
-9.52			
-9.00	62.70	63.33	61.69
-8.52			
-8.00	12.07	12.48	10.94
-7.00	1.14	1.91	1.64
-6.00	0.08	0.22	-0.18
-5.00	-0.22	-0.03	-0.24
-4.00	-0.01	-0.30	-0.24
-3.00	0.60	0.24	-0.04

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.0565812
TOP	99.7765
LOGEC50	-8.79444
HILLSLOPE	-1.09464
EC50	1.60530e-009
Std. Error	
BOTTOM	0.144483
TOP	0.463444
LOGEC50	0.00689144
HILLSLOPE	0.0165721
95% Confidence Intervals	
BOTTOM	-0.244811 to 0.357973
TOP	98.8097 to 100.743
LOGEC50	-8.80882 to -8.78007
HILLSLOPE	-1.12921 to -1.06007
EC50	1.55304e-009 to 1.65933e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.999798
Absolute Sum of Squares	5.74593
Sy.x	0.536001
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA416 TK7

Figure 11b.
Econazole Response Curve
Replicate 2

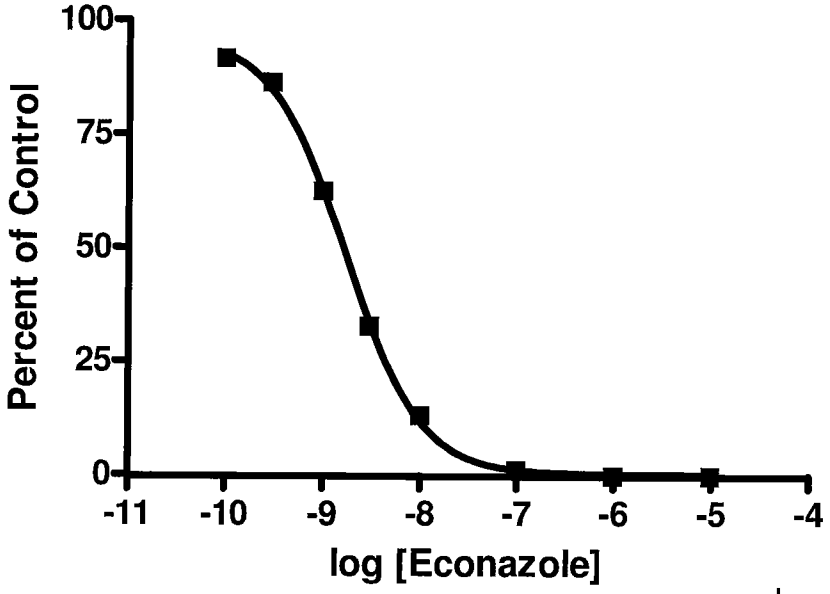


log [RC7]	Replicate 2		
	Y1	Y2	Y3
-10.00	99.32	98.51	98.83
-9.52	93.64	92.36	91.97
-9.00	66.44	65.69	67.03
-8.52	37.16	37.00	38.04
-8.00	15.64	15.07	15.08
-7.00	1.81	1.60	1.51
-6.00	0.79	0.50	0.27
-5.00	1.62	0.84	0.67

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.741611
TOP	104.729
LOGEC50	-8.76465
HILLSLOPE	-1.07030
EC50	1.71930e-009
Std. Error	
BOTTOM	0.394959
TOP	0.966606
LOGEC50	0.0125901
HILLSLOPE	0.0287428
95% Confidence Intervals	
BOTTOM	-0.0822728 to 1.56549
TOP	102.713 to 106.745
LOGEC50	-8.79091 to -8.73839
HILLSLOPE	-1.13026 to -1.01034
EC50	1.61841e-009 to 1.82648e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.999303
Absolute Sum of Squares	25.3044
Sy.x	1.12482
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA416 TK7

Figure 11c.
Econazole Response Curve
Replicate 3



log [RC7]	Replicate 3		
	Y1	Y2	Y3
-10.00	90.47	92.73	93.83
-9.52	86.72	87.39	86.91
-9.00	65.11	62.20	61.85
-8.52	32.45	33.76	33.51
-8.00	12.86	13.64	14.05
-7.00	1.76	1.60	1.36
-6.00	0.41	0.02	0.19
-5.00	0.24	0.03	0.32

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.529437
TOP	97.0943
LOGEC50	-8.76104
HILLSLOPE	-1.13389
EC50	1.73363e-009
Std. Error	
BOTTOM	0.454808
TOP	1.04556
LOGEC50	0.0145225
HILLSLOPE	0.037452
95% Confidence Intervals	
BOTTOM	-0.419292 to 1.47817
TOP	94.9133 to 99.2753
LOGEC50	-8.79134 to -8.73075
HILLSLOPE	-1.21201 to -1.05576
EC50	1.61682e-009 to 1.85887e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.998929
Absolute Sum of Squares	34.6309
Sy.x	1.31588
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

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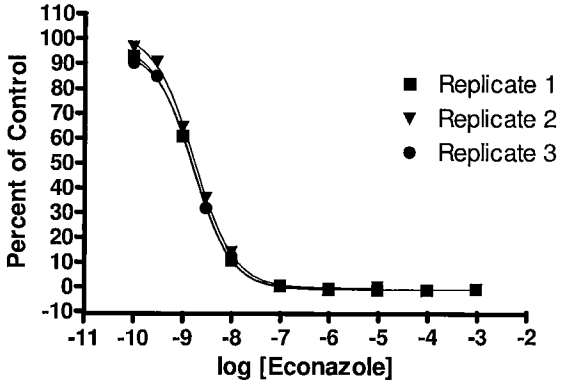
Figure 11d.
Econazole Response Curve
Replicates 1, 2, 3

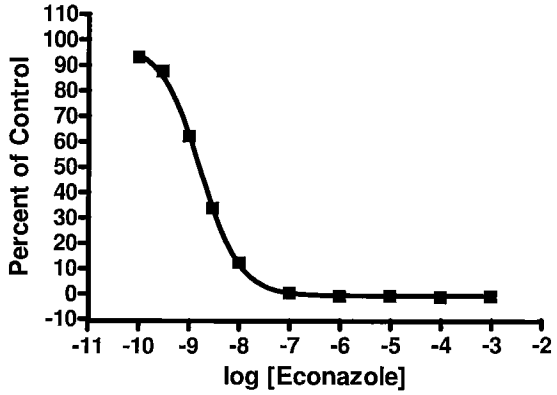


log [RC7]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	95.05	94.48	96.10	99.32	98.51	98.83	90.47	92.73	93.83
-9.52				93.64	92.36	91.97	86.72	87.39	86.91
-9.00	62.70	63.33	61.69	66.44	65.69	67.03	65.11	62.20	61.85
-8.52				37.16	37.00	38.04	32.45	33.76	33.51
-8.00	12.07	12.48	10.94	15.64	15.07	15.08	12.86	13.64	14.05
-7.00	1.14	1.91	1.64	1.81	1.60	1.51	1.76	1.60	1.36
-6.00	0.08	0.22	-0.18	0.79	0.50	0.27	0.41	0.02	0.19
-5.00	-0.22	-0.03	-0.24	1.62	0.84	0.67	0.24	0.03	0.32
-4.00	-0.01	-0.30	-0.24						
-3.00	0.60	0.24	-0.04						

Sigmoidal dose-response (variable slope)	Replicate 1	Replicate 2	Replicate 3
Best-fit values			
BOTTOM	0.0565812	0.741611	0.529437
TOP	99.7765	104.729	97.0943
LOGEC50	-8.79444	-8.76465	-8.76104
HILLSLOPE	-1.09464	-1.07030	-1.13389
EC50	1.60530e-009	1.71930e-009	1.73363e-009
Std. Error			
BOTTOM	0.144483	0.394959	0.454808
TOP	0.463444	0.966606	1.04556
LOGEC50	0.00689144	0.0125901	0.0145225
HILLSLOPE	0.0165721	0.0287428	0.037452
95% Confidence Intervals			
BOTTOM	-0.244811 to 0.357973	-0.0822728 to 1.56549	-0.419292 to 1.47817
TOP	98.8097 to 100.743	102.713 to 106.745	94.9133 to 99.2753
LOGEC50	-8.80882 to -8.78007	-8.79091 to -8.73839	-8.79134 to -8.73075
HILLSLOPE	-1.12921 to -1.06007	-1.13026 to -1.01034	-1.21201 to -1.05576
EC50	1.55304e-009 to 1.65933e-009	1.61841e-009 to 1.82648e-009	1.61682e-009 to 1.85887e-009
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.999798	0.999303	0.998929
Absolute Sum of Squares	5.74593	25.3044	34.6309
Sy.x	0.536001	1.12482	1.31588
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

WIL WA416 TK7

Figure 11e.
Econazole Response Curve
Average of All Replicates

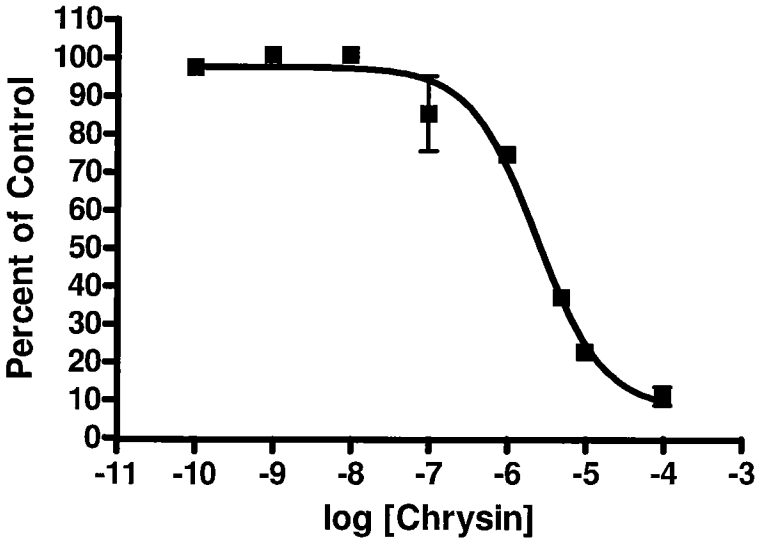


log [RC7]	All Econazole Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	95.05	94.48	96.10	99.32	98.51	98.83	90.47	92.73	93.83
-9.52				93.64	92.36	91.97	86.72	87.39	86.91
-9.00	62.70	63.33	61.69	66.44	65.69	67.03	65.11	62.20	61.85
-8.52				37.16	37.00	38.04	32.45	33.76	33.51
-8.00	12.07	12.48	10.94	15.64	15.07	15.08	12.86	13.64	14.05
-7.00	1.14	1.91	1.64	1.81	1.60	1.51	1.76	1.60	1.36
-6.00	0.08	0.22	-0.18	0.79	0.50	0.27	0.41	0.02	0.19
-5.00	-0.22	-0.03	-0.24	1.62	0.84	0.67	0.24	0.03	0.32
-4.00	-0.01	-0.30	-0.24						
-3.00	0.60	0.24	-0.04						

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.3431
TOP	100.7
LOGEC50	-8.767
HILLSLOPE	-1.093
EC50	1.708e-009
Std. Error	
BOTTOM	0.3513
TOP	0.9362
LOGEC50	0.01291
HILLSLOPE	0.03040
95% Confidence Intervals	
BOTTOM	-0.3586 to 1.045
TOP	98.81 to 102.6
LOGEC50	-8.793 to -8.742
HILLSLOPE	-1.154 to -1.033
EC50	1.610e-009 to 1.813e-009
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9975
Absolute Sum of Squares	255.0
Sy.x	1.937
Data	
Number of X values	10
Number of Y replicates	9
Total number of values	72
Number of missing values	18

WIL WA416 TK7

Figure 12a.
Chrysin Response Curve
Replicate 1

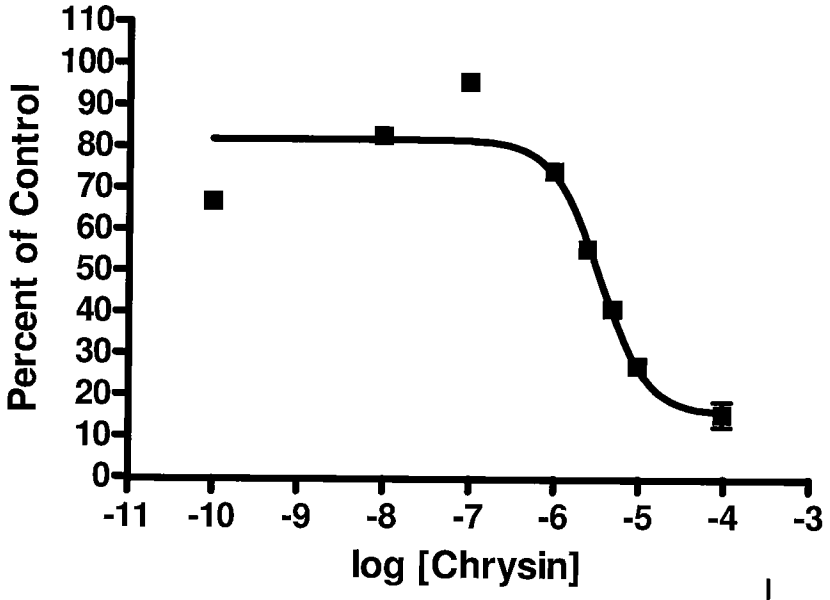


log [RC8]	Replicate 1		
	Y1	Y2	Y3
-10.00	96.99	98.94	100.36
-9.00	103.58	101.04	101.61
-8.00	102.88	100.58	102.63
-7.00	94.89	98.04	66.69
-6.00	75.93	75.00	76.37
-5.60			
-5.30	37.21	38.78	37.66
-5.00	23.65	23.86	22.70
-4.00	16.65	9.35	9.08

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	8.33314
TOP	98.8245
LOGEC50	-5.61297
HILLSLOPE	-1.01386
EC50	2.43796e-006
Std. Error	
BOTTOM	5.09269
TOP	2.24963
LOGEC50	0.0885268
HILLSLOPE	0.176793
95% Confidence Intervals	
BOTTOM	-2.29021 to 18.9565
TOP	94.1317 to 103.517
LOGEC50	-5.79764 to -5.42831
HILLSLOPE	-1.38265 to -0.645071
EC50	1.59352e-006 to 3.72986e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.966344
Absolute Sum of Squares	1004.42
Sy.x	7.08666
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

WIL WA416 TK7

Figure 12b.
Chrysin Response Curve
Replicate 2



log [RC8]	Replicate 2		
	Y1	Y2	Y3
-10.00	66.49	67.96	68.29
-9.00			
-8.00	85.30	82.38	83.03
-7.00	95.16	98.26	96.54
-6.00	76.98	75.04	73.04
-5.60	54.52	57.08	56.85
-5.30	42.45	39.66	42.63
-5.00	27.29	28.04	27.93
-4.00	20.08	9.93	17.87

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	16.1193
TOP	82.6233
LOGEC50	-5.45139
HILLSLOPE	-1.54852
EC50	3.53678e-006
Std. Error	
BOTTOM	4.97480
TOP	2.78731
LOGEC50	0.0808487
HILLSLOPE	0.384855
95% Confidence Intervals	
BOTTOM	5.74182 to 26.4967
TOP	76.8090 to 88.4376
LOGEC50	-5.62004 to -5.28274
HILLSLOPE	-2.35132 to -0.745708
EC50	2.39860e-006 to 5.21505e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.915566
Absolute Sum of Squares	1396.52
Sy.x	8.35620
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

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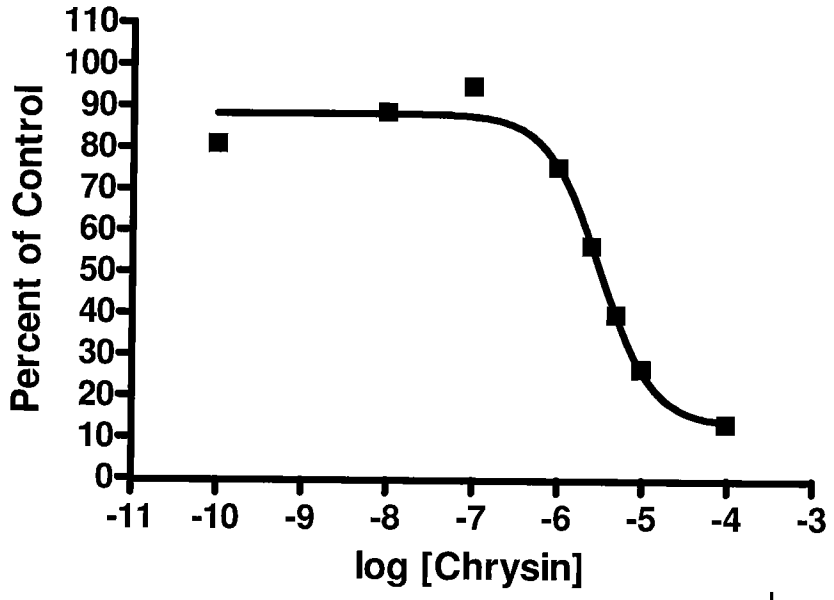
Figure 12c.
Chrysin Response Curve
Replicate 3



log [RC8]	Replicate 3		
	Y1	Y2	Y3
-10.00	83.33	78.69	83.66
-9.00			
-8.00	90.05	89.50	89.33
-7.00	97.68	95.93	94.30
-6.00	75.63	76.83	76.50
-5.60	58.89	57.21	55.67
-5.30	40.11	41.58	40.05
-5.00	26.54	27.59	27.56
-4.00	13.05	14.62	14.59

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	13.8263
TOP	89.1890
LOGEC50	-5.49328
HILLSLOPE	-1.38750
EC50	3.21162e-006
Std. Error	
BOTTOM	2.59549
TOP	1.44391
LOGEC50	0.0398825
HILLSLOPE	0.156717
95% Confidence Intervals	
BOTTOM	8.41209 to 19.2405
TOP	86.1770 to 92.2010
LOGEC50	-5.57647 to -5.41008
HILLSLOPE	-1.71441 to -1.06059
EC50	2.65173e-006 to 3.88972e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.981600
Absolute Sum of Squares	358.418
Sy.x	4.23331
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

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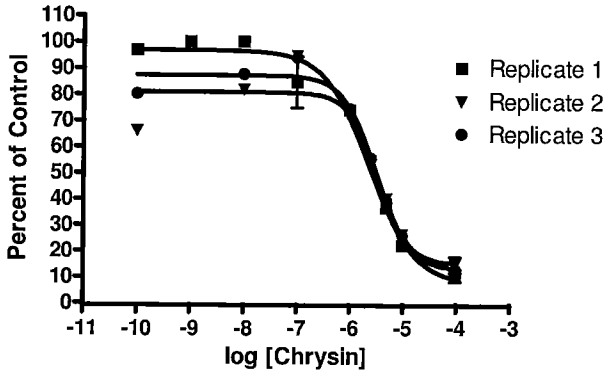
Figure 12d.
Chrysin Response Curve
Replicates 1, 2, 3

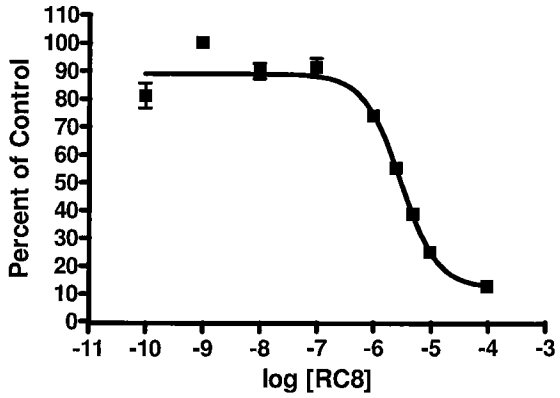


log [RC8]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	96.99	98.94	100.36	66.49	67.96	68.29	83.33	78.69	83.66
-9.00	103.58	101.04	101.61						
-8.00	102.88	100.58	102.63	85.30	82.38	83.03	90.05	89.50	89.33
-7.00	94.89	98.04	66.69	95.16	98.26	96.54	97.68	95.93	94.30
-6.00	75.93	75.00	76.37	76.98	75.04	73.04	75.63	76.83	76.50
-5.60				54.52	57.08	56.85	58.89	57.21	55.67
-5.30	37.21	38.78	37.66	42.45	39.66	42.63	40.11	41.58	40.05
-5.00	23.65	23.86	22.70	27.29	28.04	27.93	26.54	27.59	27.56
-4.00	16.65	9.35	9.08	20.08	9.93	17.87	13.05	14.62	14.59

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	8.33314	16.1193	13.8263
TOP	98.8245	82.6233	89.1890
LOGEC50	-5.61297	-5.45139	-5.49328
HILLSLOPE	-1.01386	-1.54852	-1.38750
EC50	2.43796e-006	3.53678e-006	3.21162e-006
Std. Error			
BOTTOM	5.09269	4.97480	2.59549
TOP	2.24963	2.78731	1.44391
LOGEC50	0.0885268	0.0808487	0.0398825
HILLSLOPE	0.176793	0.384855	0.156717
95% Confidence Intervals			
BOTTOM	-2.29021 to 18.9565	5.74182 to 26.4967	8.41209 to 19.2405
TOP	94.1317 to 103.517	76.8090 to 88.4376	86.1770 to 92.2010
LOGEC50	-5.79764 to -5.42831	-5.62004 to -5.28274	-5.57647 to -5.41008
HILLSLOPE	-1.38265 to -0.645071	-2.35132 to -0.745708	-1.71441 to -1.06059
EC50	1.59352e-006 to 3.72986e-006	2.39860e-006 to 5.21505e-006	2.65173e-006 to 3.88972e-006
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.966344	0.915566	0.981600
Absolute Sum of Squares	1004.42	1396.52	358.418
Sy.x	7.08666	8.35620	4.23331
Data			
Number of X values	9	9	9
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	3	3	3

WIL WA416 TK7

Figure 12e.
Chrysin Response Curve
Average of All Replicates



log [RC8]	All Chrysin Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	96.99	98.94	100.36	66.49	67.96	68.29	83.33	78.69	83.66
-9.00	103.58	101.04	101.61						
-8.00	102.88	100.58	102.63	85.30	82.38	83.03	90.05	89.50	89.33
-7.00	94.89	98.04	66.69	95.16	98.26	96.54	97.68	95.93	94.30
-6.00	75.93	75.00	76.37	76.98	75.04	73.04	75.63	76.83	76.50
-5.60				54.52	57.08	56.85	58.89	57.21	55.67
-5.30	37.21	38.78	37.66	42.45	39.66	42.63	40.11	41.58	40.05
-5.00	23.65	23.86	22.70	27.29	28.04	27.93	26.54	27.59	27.56
-4.00	16.65	9.35	9.08	20.08	9.93	17.87	13.05	14.62	14.59

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	13.20
TOP	90.59
LOGEC50	-5.516
HILLSLOPE	-1.333
EC50	3.047e-006
Std. Error	
BOTTOM	2.782
TOP	1.463
LOGEC50	0.04464
HILLSLOPE	0.1569
95% Confidence Intervals	
BOTTOM	7.640 to 18.75
TOP	87.67 to 93.51
LOGEC50	-5.605 to -5.427
HILLSLOPE	-1.646 to -1.019
EC50	2.481e-006 to 3.741e-006
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9387
Absolute Sum of Squares	4103
Sy.x	7.768
Data	
Number of X values	9
Number of Y replicates	9
Total number of values	72
Number of missing values	9

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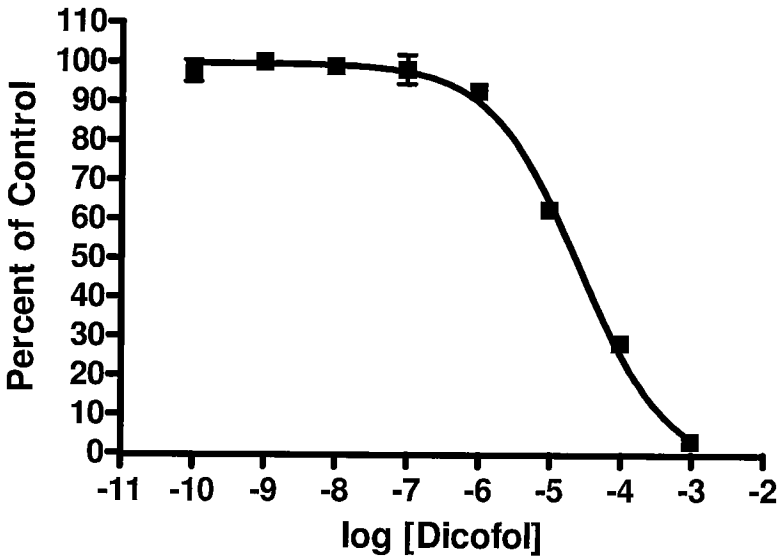
Figure 13a.
Dicofol Response Curve
Replicate 1



log [RC9]	Replicate 1		
	Y1	Y2	Y3
-10.00	102.19	100.97	93.47
-9.00	100.27	102.18	101.20
-8.00	103.85	97.90	99.03
-7.00	102.70	103.44	91.87
-6.00	92.07	96.28	93.03
-5.52			
-5.00	61.94	62.37	65.74
-4.52			
-4.00	~~51.93~~	28.40	29.44
-3.52			
-3.00	5.39	2.37	3.34

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-3.408898
TOP	100.9096
LOGEC50	-4.575840
HILLSLOPE	-0.6919932
EC50	2.655587e-005
Std. Error	
BOTTOM	4.305249
TOP	1.127295
LOGEC50	0.08559638
HILLSLOPE	0.07234772
95% Confidence Intervals	
BOTTOM	-12.41978 to 5.601989
TOP	98.55020 to 103.2691
LOGEC50	-4.754993 to -4.396687
HILLSLOPE	-0.8434170 to -0.5405694
EC50	1.757952e-005 to 4.011561e-005
Goodness of Fit	
Degrees of Freedom	19
R^2	0.9915855
Absolute Sum of Squares	240.5932
Sy.x	3.558483
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	23
Number of missing values	10

Data noted by a strike through have been eliminated as outliers by the Q-test as described in the text.

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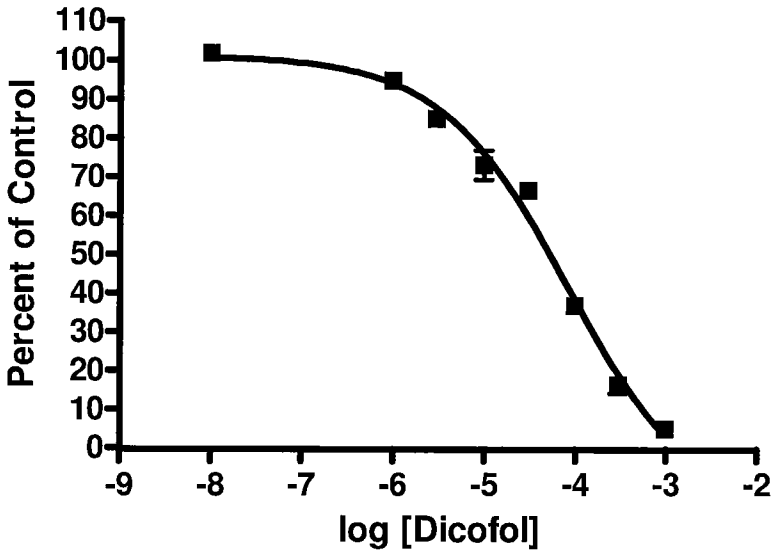
Figure 13b.
Dicofol Response Curve
Replicate 2

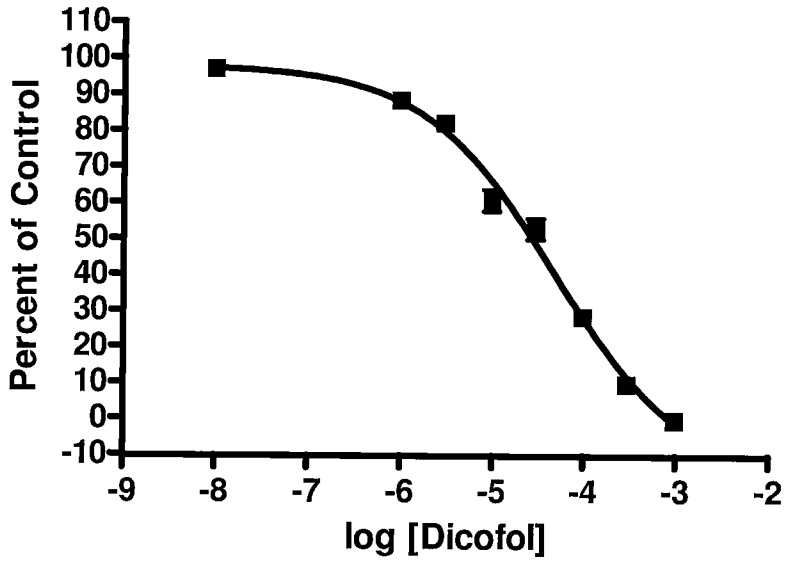


log [RC9]	Replicate 2		
	Y1	Y2	Y3
-10.00			
-9.00			
-8.00	101.02	101.98	105.81
-7.00			
-6.00	96.15	96.97	94.59
-5.52	85.60	87.90	84.69
-5.00	80.56	73.92	67.57
-4.52	65.32	69.37	67.85
-4.00	36.27	36.87	39.54
-3.52	16.34	20.87	13.49
-3.00	6.49	5.71	4.42

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-17.16866
TOP	102.2098
LOGEC50	-4.078107
HILLSLOPE	-0.6235480
EC50	8.353965e-005
Std. Error	
BOTTOM	9.803435
TOP	2.488649
LOGEC50	0.1334029
HILLSLOPE	0.07987523
95% Confidence Intervals	
BOTTOM	-37.61862 to 3.281312
TOP	97.01844 to 107.4011
LOGEC50	-4.356386 to -3.799829
HILLSLOPE	-0.7901677 to -0.4569283
EC50	4.401633e-005 to 0.0001585517
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9864443
Absolute Sum of Squares	383.7625
Sy.x	4.380425
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

WIL WA416 TK7

Figure 13c.
Dicofol Response Curve
Replicate 3



log [RC9]	Replicate 3		
	Y1	Y2	Y3
-10.00			
-9.00			
-8.00	96.97	98.71	98.93
-7.00			
-6.00	90.51	87.03	91.00
-5.52	83.43	85.75	80.64
-5.00	66.52	56.03	62.28
-4.52	50.17	51.46	59.46
-4.00	31.45	25.74	29.82
-3.52	14.48	7.64	8.60
-3.00	-0.29	0.95	-0.15

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-17.01199
TOP	99.22736
LOGEC50	-4.307286
HILLSLOPE	-0.6028344
EC50	4.928488e-005
Std. Error	
BOTTOM	7.392294
TOP	2.518384
LOGEC50	0.1104067
HILLSLOPE	0.06925347
95% Confidence Intervals	
BOTTOM	-32.43232 to -1.591665
TOP	93.97401 to 104.4807
LOGEC50	-4.537594 to -4.076978
HILLSLOPE	-0.7472971 to -0.4583717
EC50	2.900051e-005 to 8.375722e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.9880130
Absolute Sum of Squares	345.9980
Sy.x	4.159315
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

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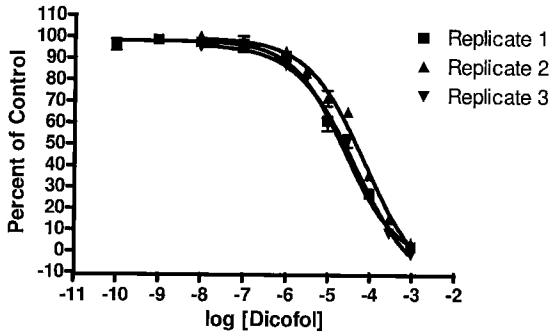
Figure 13d.
Dicofol Response Curve
Replicates 1, 2, 3



log [RC9]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	102.19	100.97	93.47						
-9.00	100.27	102.18	101.20						
-8.00	103.85	97.90	99.03	101.02	101.98	105.81	96.97	98.71	98.93
-7.00	102.70	103.44	91.87						
-6.00	92.07	96.28	93.03	96.15	96.97	94.59	90.51	87.03	91.00
-5.52				85.60	87.90	84.69	83.43	85.75	80.64
-5.00	61.94	62.37	65.74	80.56	73.92	67.57	66.52	56.03	62.28
-4.52				65.32	69.37	67.85	50.17	51.46	59.46
-4.00	~~51.93~~	28.40	29.44	36.27	36.87	39.54	31.45	25.74	29.82
-3.52				16.34	20.87	13.49	14.48	7.64	8.60
-3.00	5.39	2.37	3.34	6.49	5.71	4.42	-0.29	0.95	-0.15

Sigmoidal dose-response (variable slope)	Replicate 1	Replicate 2	Replicate 3
Best-fit values			
BOTTOM	-3.408898	-17.16866	-17.01199
TOP	100.9096	102.2098	99.22736
LOGEC50	-4.575840	-4.078107	-4.307286
HILLSLOPE	-0.6919932	-0.6235480	-0.6028344
EC50	2.655587e-005	8.353965e-005	4.928488e-005
Std. Error			
BOTTOM	4.305249	9.803435	7.392294
TOP	1.127295	2.488649	2.518384
LOGEC50	0.08559638	0.1334029	0.1104067
HILLSLOPE	0.07234772	0.07987523	0.06925347
95% Confidence Intervals			
BOTTOM	-12.41978 to 5.601989	-37.61862 to 3.281312	-32.43232 to -1.591665
TOP	98.55020 to 103.2691	97.01844 to 107.4011	93.97401 to 104.4807
LOGEC50	-4.754993 to -4.396687	-4.356386 to -3.799829	-4.537594 to -4.076978
HILLSLOPE	-0.8434170 to -0.5405694	-0.7901677 to -0.4569283	-0.7472971 to -0.4583717
EC50	1.757952e-005 to 4.011561e-005	4.401633e-005 to 0.0001585517	2.900051e-005 to 8.375722e-005
Goodness of Fit			
Degrees of Freedom	19	20	20
R^2	0.9915855	0.9864443	0.9880130
Absolute Sum of Squares	240.5932	383.7625	345.9980
Sy.x	3.558483	4.380425	4.159315
Data			
Number of X values	11	11	11
Number of Y replicates	3	3	3
Total number of values	23	24	24
Number of missing values	10	9	9

Data noted by a strike through have been eliminated as outliers by the Q-test as described in the text.

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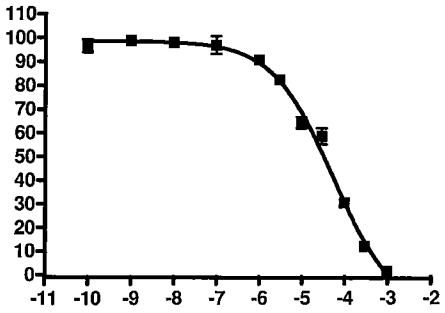
Figure 13e.
Dicofol Response Curve
Average of All Replicates



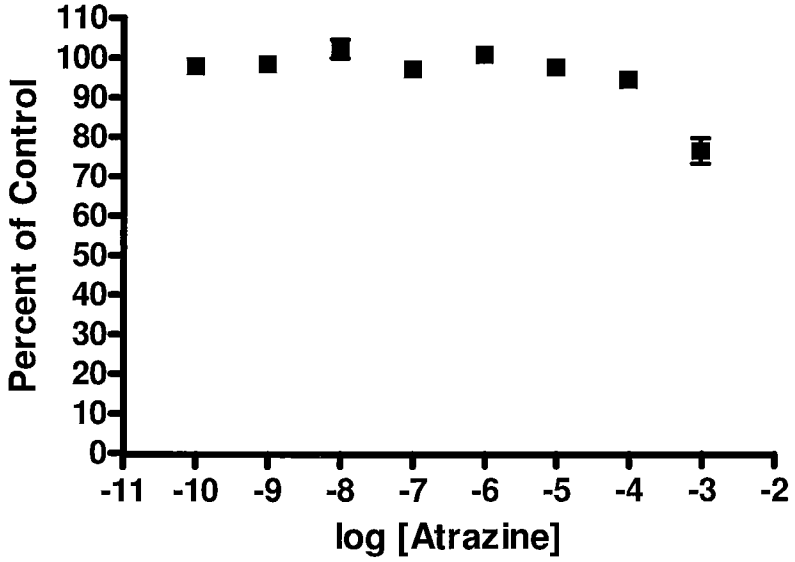
log [RC9]	All Dicofol Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	102.19	100.97	93.47						
-9.00	100.27	102.18	101.20						
-8.00	103.85	97.90	99.03	101.02	101.98	105.81	96.97	98.71	98.93
-7.00	102.70	103.44	91.87						
-6.00	92.07	96.28	93.03	96.15	96.97	94.59	90.51	87.03	91.00
-5.52				85.60	87.90	84.69	83.43	85.75	80.64
-5.00	61.94	62.37	65.74	80.56	73.92	67.57	66.52	56.03	62.28
-4.52				65.32	69.37	67.85	50.17	51.46	59.46
-4.00	~~51.93~~	28.40	29.44	36.27	36.87	39.54	31.45	25.74	29.82
-3.52				16.34	20.87	13.49	14.48	7.64	8.60
-3.00	5.39	2.37	3.34	6.49	5.71	4.42	-0.29	0.95	-0.15

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-14.82
TOP	101.0
LOGEC50	-4.252
HILLSLOPE	-0.6072
EC50	5.598e-005
Std. Error	
BOTTOM	5.838
TOP	1.378
LOGEC50	0.09128
HILLSLOPE	0.05169
95% Confidence Intervals	
BOTTOM	-26.48 to -3.154
TOP	98.24 to 103.7
LOGEC50	-4.434 to -4.070
HILLSLOPE	-0.7105 to -0.5040
EC50	3.678e-005 to 8.518e-005
Goodness of Fit	
Degrees of Freedom	67
R^2	0.9787
Absolute Sum of Squares	1953
Sy.x	5.400
Data	
Number of X values	11
Number of Y replicates	9
Total number of values	71
Number of missing values	28

Data noted by a strike through have been eliminated as outliers by the Q-test as described in the text.

WIL WA416 TK7

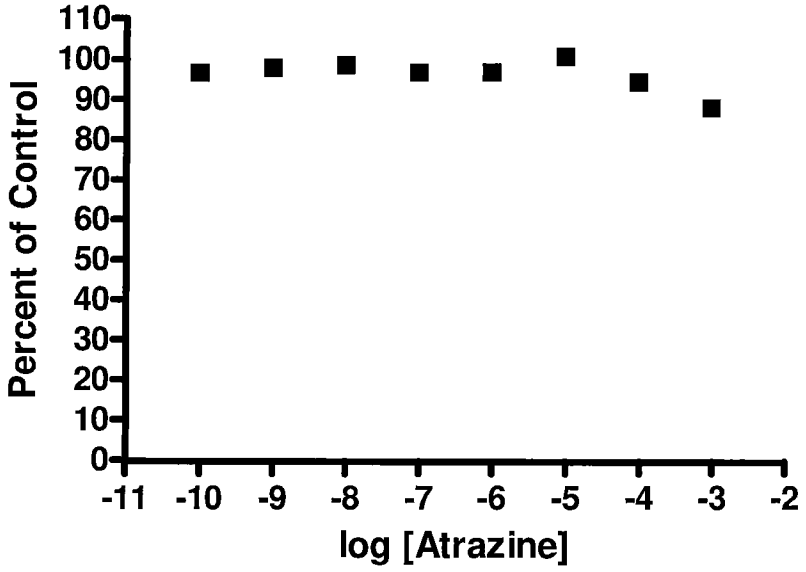
Figure 14a.
Atrazine Response Curve
Replicate 1



log [RC10]	Replicate 1		
	Y1	Y2	Y3
-10.00	100.96	98.12	97.69
-9.00	98.66	100.88	98.97
-8.00	103.54	107.42	99.10
-7.00	99.33	96.81	98.72
-6.00	101.95	99.58	104.53
-5.00	101.16	97.38	97.80
-4.00	94.43	97.21	95.39
-3.00	71.53	82.58	78.27

WIL WA416 TK7

Figure 14b.
Atrazine Response Curve
Replicate 2

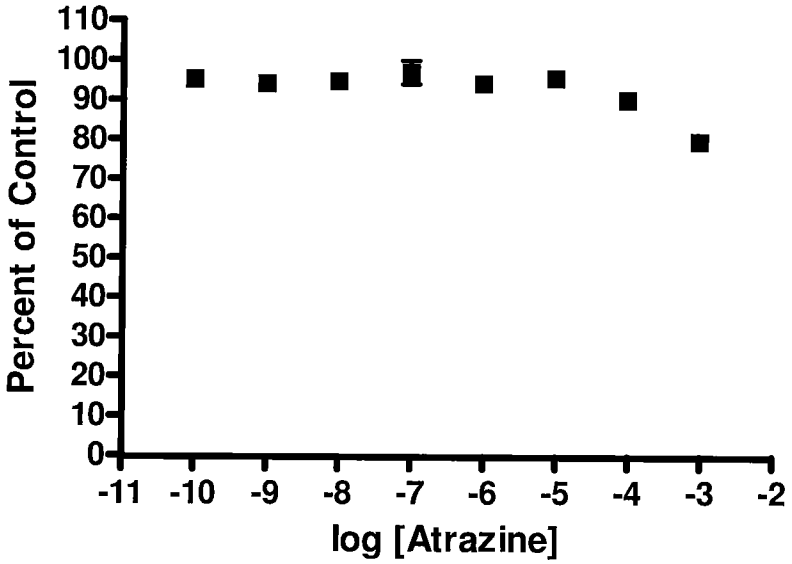


log [RC10]	Replicate 2		
	Y1	Y2	Y3
-10.00	97.20	98.64	97.40
-9.00	96.63	100.61	99.89
-8.00	102.45	98.34	98.87
-7.00	99.01	98.63	96.16
-6.00	99.23	96.09	98.71
-5.00	102.03	102.58	101.73
-4.00	95.03	95.48	96.34
-3.00	~~59.49~~	89.06	89.44

Data noted by a strike through have been eliminated as outliers by the Q-test as described in the text.

WIL WA416 TK7

Figure 14c.
Atrazine Response Curve
Replicate 3

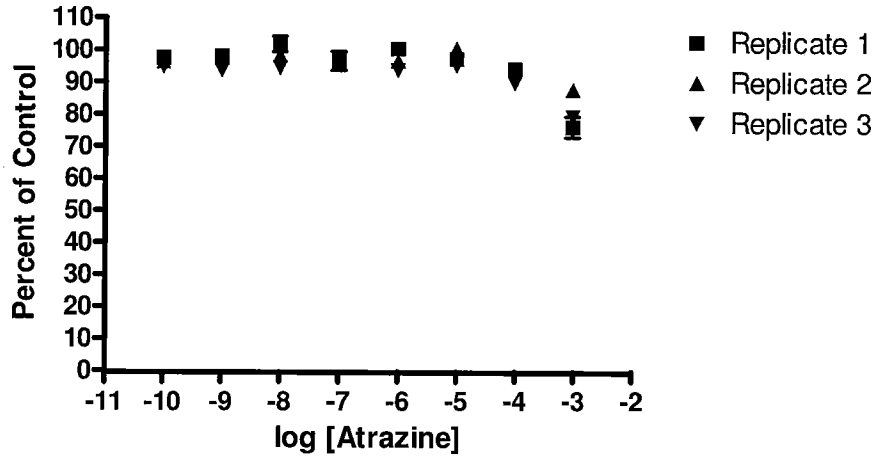


log [RC10]	Replicate 3		
	Y1	Y2	Y3
-10.00	97.23	~~169.76~~	95.30
-9.00	96.38	94.72	94.58
-8.00	97.38	94.40	95.75
-7.00	104.02	94.99	95.13
-6.00	96.69	95.11	94.00
-5.00	96.39	96.00	97.53
-4.00	92.01	90.14	91.52
-3.00	79.03	82.59	80.56

Data noted by a strike through have been eliminated as outliers by the Q-test as described in the text.

WIL WA416 TK7

Figure 14d.
Atrazine Response Curve
Replicates 1, 2, 3

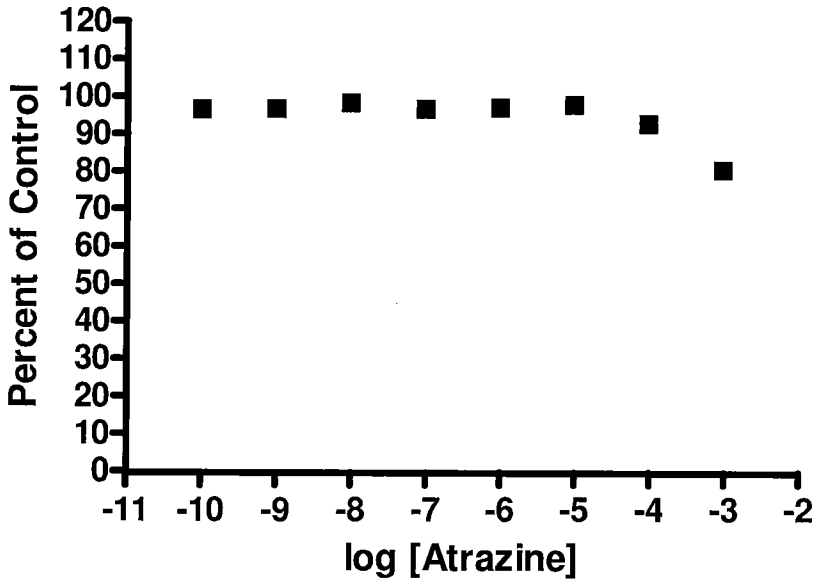


log [RC10]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	100.96	98.12	97.69	97.20	98.64	97.40	97.23	~~169.76~~	95.30
-9.00	98.66	100.88	98.97	96.63	100.61	99.89	96.38	94.72	94.58
-8.00	103.54	107.42	99.10	102.45	98.34	98.87	97.38	94.40	95.75
-7.00	99.33	96.81	98.72	99.01	98.63	96.16	104.02	94.99	95.13
-6.00	101.95	99.58	104.53	99.23	96.09	98.71	96.69	95.11	94.00
-5.00	101.16	97.38	97.80	102.03	102.58	101.73	96.39	96.00	97.53
-4.00	94.43	97.21	95.39	95.03	95.48	96.34	92.01	90.14	91.52
-3.00	71.53	82.58	78.27	~~59.49~~	89.06	89.44	79.03	82.59	80.56

Data noted by a strike through have been eliminated as outliers by the Q-test as described in the text.

WIL WA416 TK7

Figure 14e.
Atrazine Response Curve
Average of All Replicates



log [RC10]	All Atrazine Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	100.96	98.12	97.69	97.20	98.64	97.40	97.23	~~169.76~~	95.30
-9.00	98.66	100.88	98.97	96.63	100.61	99.89	96.38	94.72	94.58
-8.00	103.54	107.42	99.10	102.45	98.34	98.87	97.38	94.40	95.75
-7.00	99.33	96.81	98.72	99.01	98.63	96.16	104.02	94.99	95.13
-6.00	101.95	99.58	104.53	99.23	96.09	98.71	96.69	95.11	94.00
-5.00	101.16	97.38	97.80	102.03	102.58	101.73	96.39	96.00	97.53
-4.00	94.43	97.21	95.39	95.03	95.48	96.34	92.01	90.14	91.52
-3.00	71.53	82.58	78.27	~~59.49~~	89.06	89.44	79.03	82.59	80.56

Data noted by a strike through have been eliminated as outliers by the Q-test as described in the text.

Figure 15a. Aminoglutethimide Statistical Average Concentration Response Curve

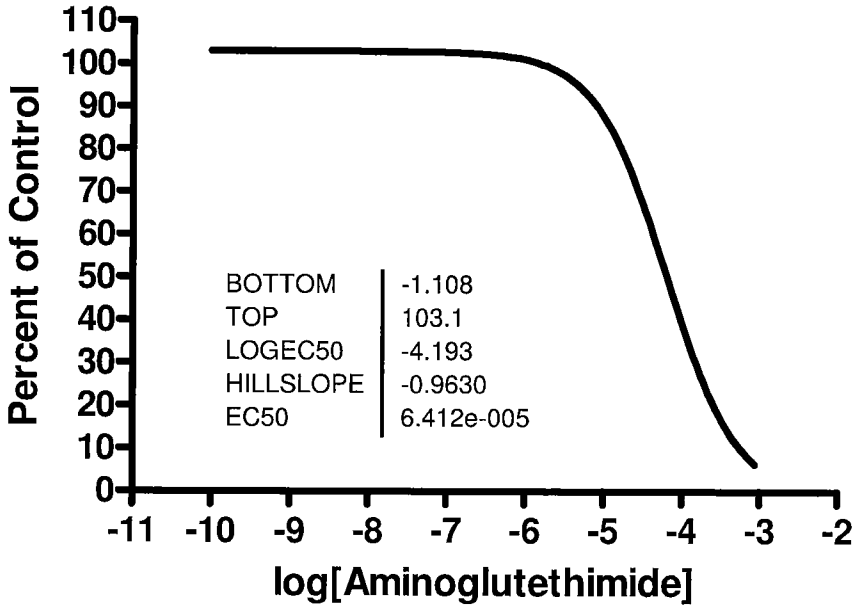


Figure 15b. Ketoconazole Statistical Average Concentration Response Curve

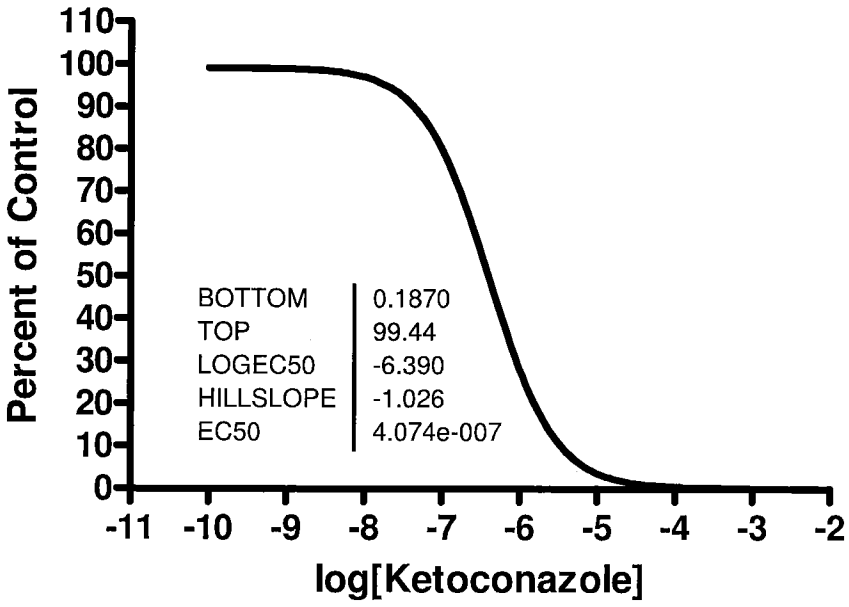


Figure 15c. Prochloraz Statistical Average Concentration Response Curve

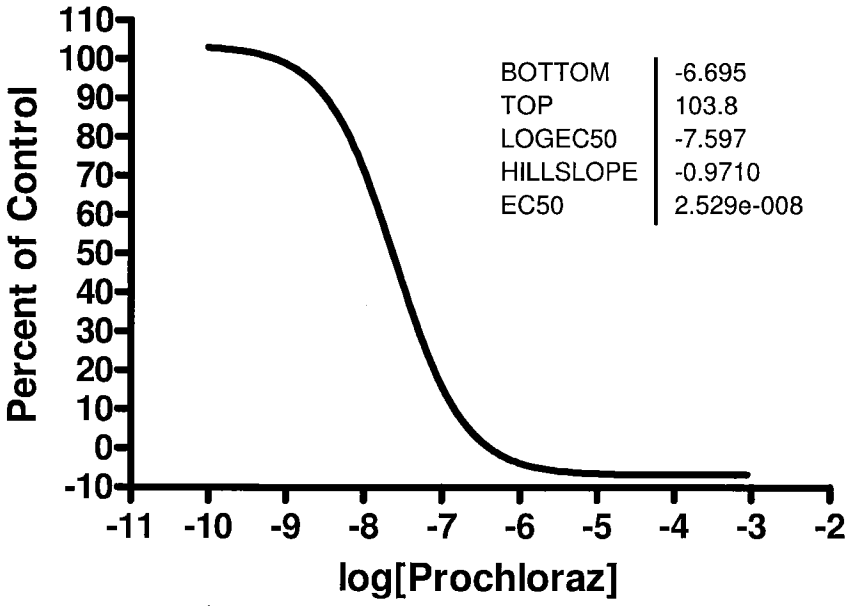


Figure 15d. 4-Nonylphenol Statistical Average Concentration Response Curve

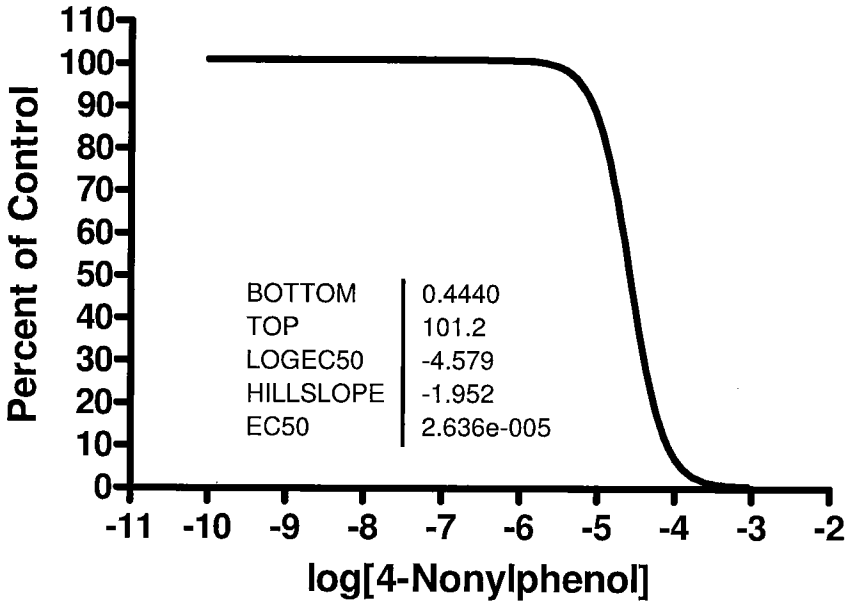


Figure 15e. Fenarimol Statistical Average Concentration Response Curve

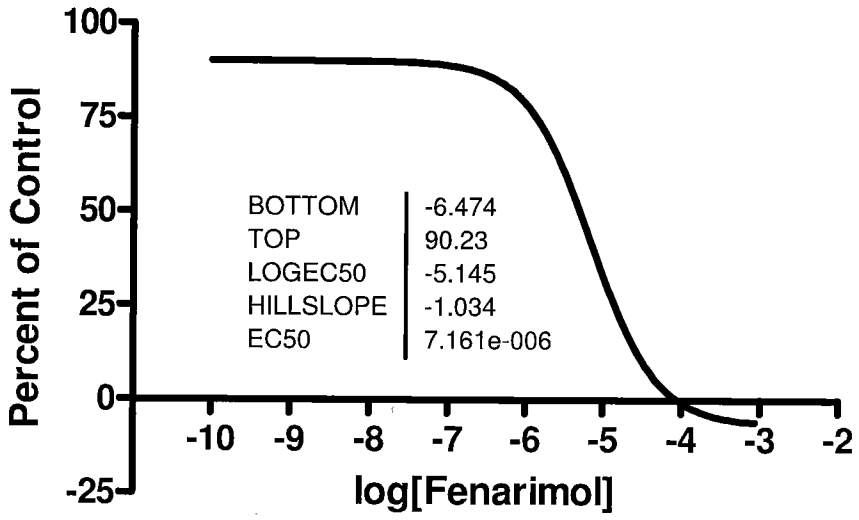


Figure 15f. Econazole Statistical Average Concentration Response Curve

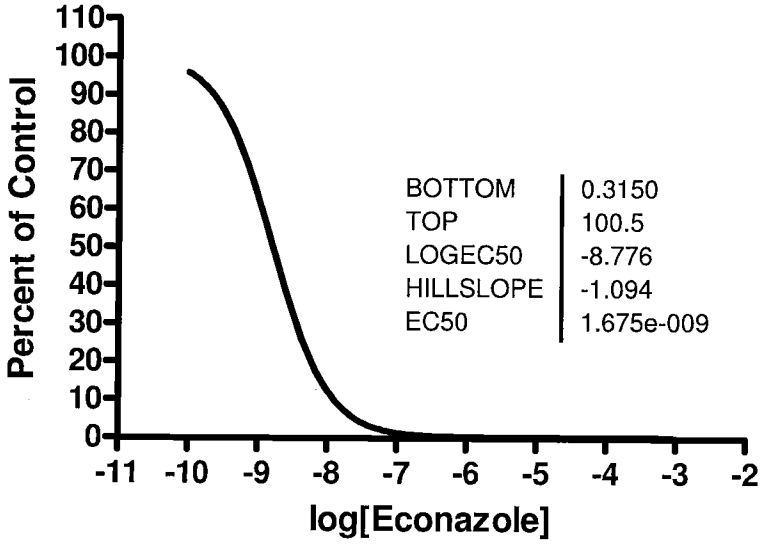


Figure 15g. Chrysin Statistical Average Concentration Response Curve

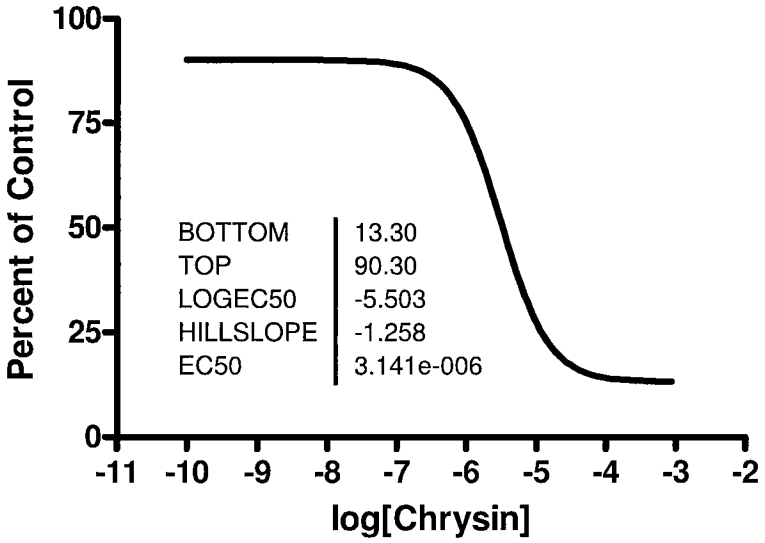


**Figure 15h. Dicofol
Statistical Average
Concentration Response Curve**

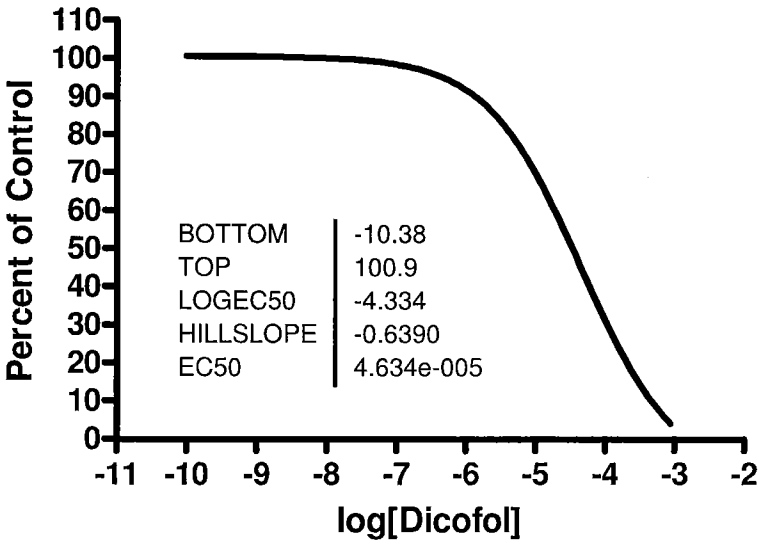


Figure 16. Validation of the Placental Aromatase Assay (WIL-431009)
Reference Chemical 1- Aminoglutethimide
Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	-6.198	2.991	-12.440	0.042
T	Top	1	103.7	0.7	102.3	105.2
μ	LOGEC50	1	-4.196	0.043	-4.286	-4.105
β	HILLSLOPE	1	-0.861	0.057	-0.981	-0.742
EC50	EC50 (μM)	1	63.73		51.72	78.51
B	Bottom	2	2.121	2.972	-4.078	8.320
T	Top	2	103.5	1.2	101.0	105.9
μ	LOGEC50	2	-4.169	0.037	-4.245	-4.093
β	HILLSLOPE	2	-1.080	0.105	-1.299	-0.861
EC50	EC50 (μM)	2	67.75		56.83	80.77
B	Bottom	3	0.113	1.764	-3.567	3.793
T	Top	3	102.5	0.7	101.1	103.9
μ	LOGEC50	3	-4.202	0.023	-4.249	-4.155
β	HILLSLOPE	3	-0.999	0.053	-1.110	-0.888
EC50	EC50 (μM)	3	62.80		56.36	69.97

Mathematical Averages

AVG LOGEC50	-4.189			
AVG HILLSLOPE	-0.980			
AVG T	103.2			
AVG B	-1.321			
		sd	sem	%CV
Avg EC50	64.76	2.63	1.52	4.06

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	-1.108	2.224	-10.679	8.462
Top	Overall	103.143	0.479	101.08	105.206
HILLSLOPE	Overall	-0.963	0.06	-1.223	-0.703
LOGEC50	Overall	-4.193	0.018	-4.27	-4.117

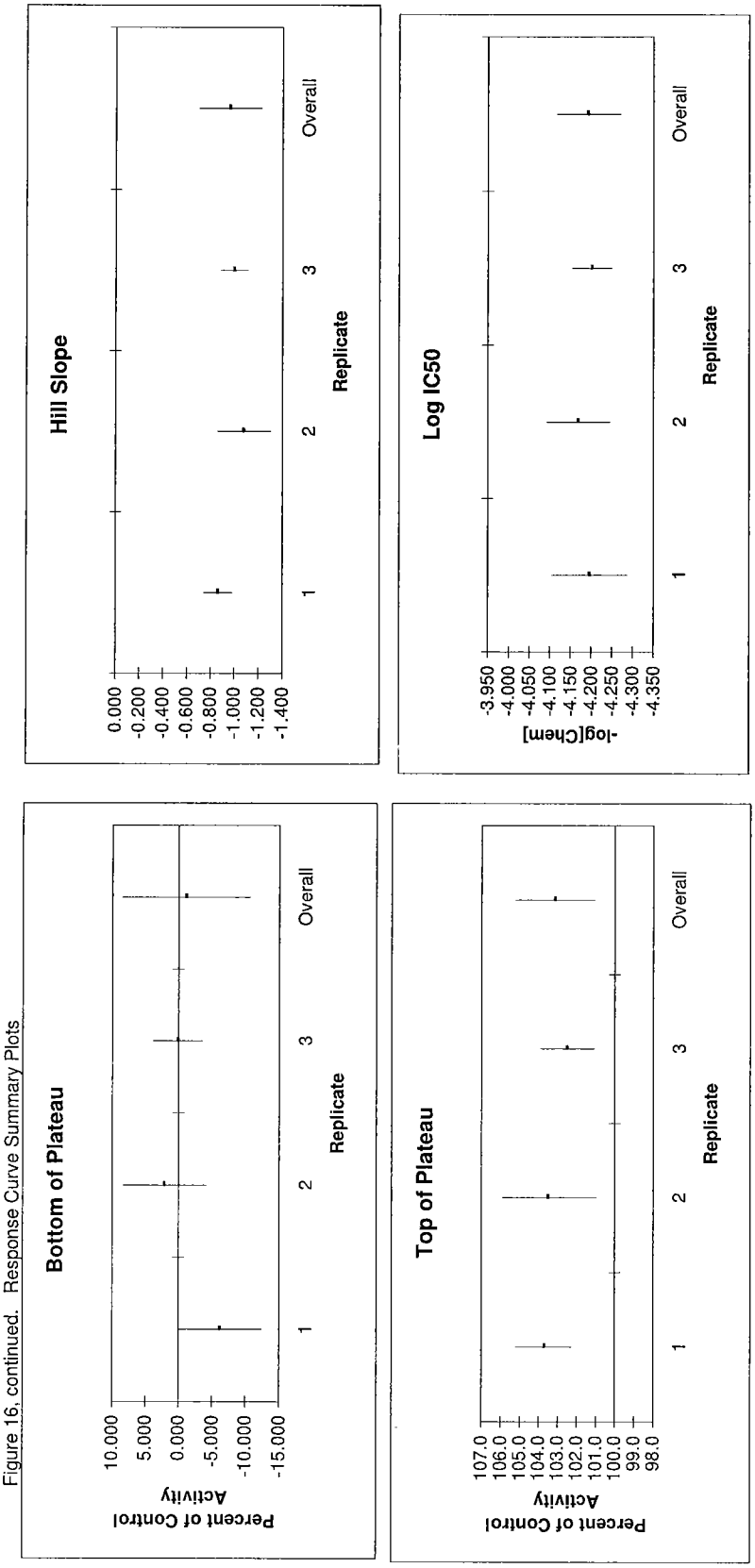


Figure 16, continued. Response Curve Summary Plots

Figure 17. Validation of the Placental Aromatase Assay (WIL-431009)

Reference Chemical 2-Ketoconazole

Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	-0.322	0.514	-1.394	0.750
T	Top	1	97.3	0.4	96.4	98.1
μ	LOGEC50	1	-6.410	0.014	-6.438	-6.381
β	HILLSLOPE	1	-0.974	0.023	-1.021	-0.927
EC50	EC50 (μM)	1	0.389		0.365	0.416
B	Bottom	2	0.091	1.047	-2.092	2.274
T	Top	2	100.3	0.8	98.7	101.9
μ	LOGEC50	2	-6.332	0.026	-6.386	-6.279
β	HILLSLOPE	2	-0.992	0.046	-1.088	-0.897
EC50	EC50 (μM)	2	0.465		0.411	0.527
B	Bottom	3	1.503	1.001	-0.585	3.590
T	Top	3	101.1	0.8	99.3	102.8
μ	LOGEC50	3	-6.422	0.027	-6.478	-6.366
β	HILLSLOPE	3	-1.140	0.056	-1.258	-1.023
EC50	EC50 (μM)	3	0.378		0.332	0.430

Mathematical Averages

AVG LOGEC50	-6.388			
AVG HILLSLOPE	-1.035			
AVG T	99.6			
AVG B	0.424			
		sd	sem	%CV
Avg EC50	0.411	0.047	0.027	11.52

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	0.187	0.539	-2.132	2.506
Top	Overall	99.435	1.197	94.285	104.585
HILLSLOPE	Overall	-1.026	0.049	-1.236	-0.816
LOGEC50	Overall	-6.39	0.027	-6.505	-6.274

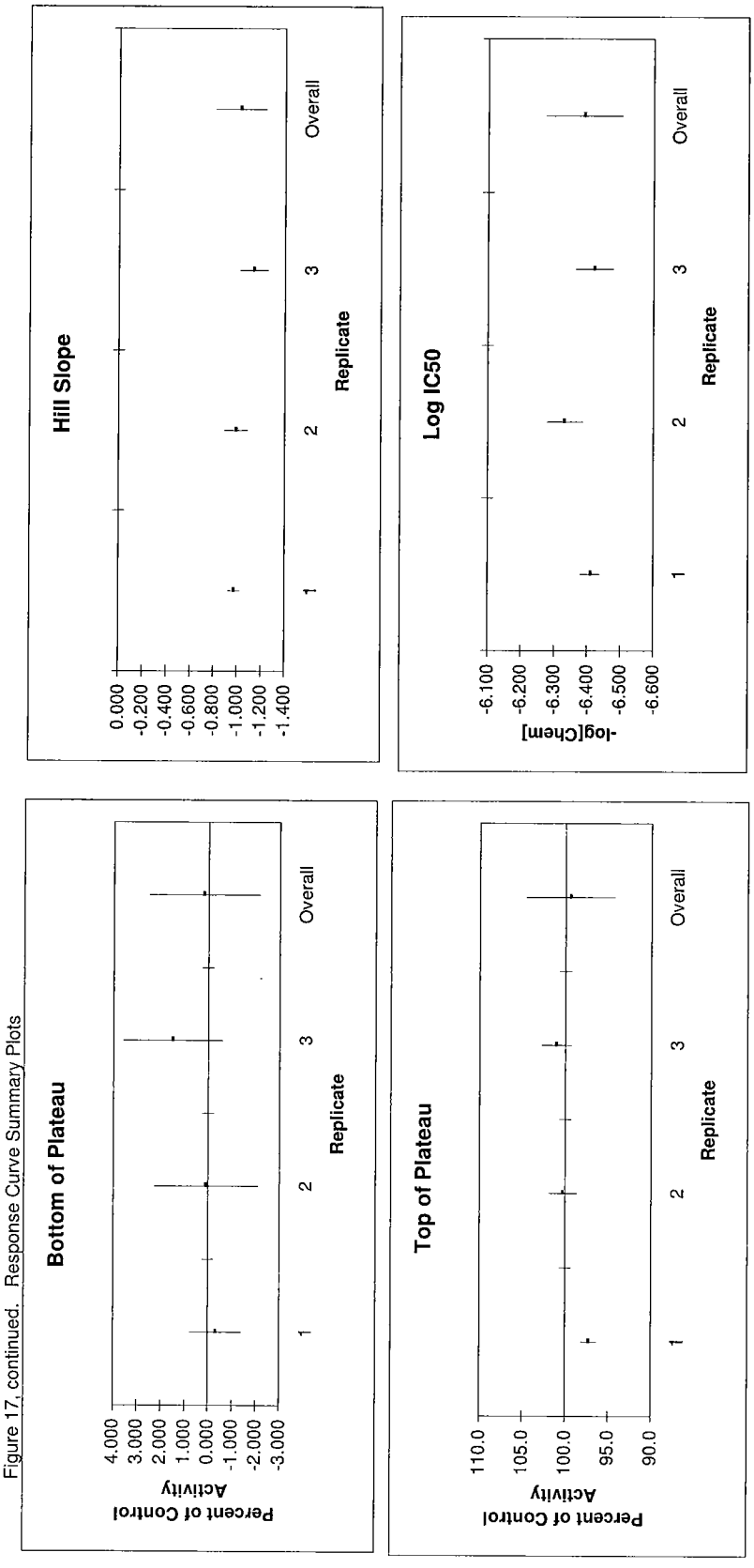


Figure 17, continued. Response Curve Summary Plots

Figure 18. Validation of the Placental Aromatase Assay (WIL-431009)

Reference Chemical 3-Prochloraz

Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	-0.161	1.199	-2.661	2.339
T	Top	1	108.6	1.9	104.6	112.6
μ	LOGEC50	1	-7.620	0.043	-7.710	-7.530
β	HILLSLOPE	1	-0.984	0.076	-1.143	-0.826
EC50	EC50 (μM)	1	0.024		0.019	0.029
B	Bottom	2	-20.490	3.159	-27.080	-13.900
T	Top	2	100.8	3.2	94.2	107.4
μ	LOGEC50	2	-7.546	0.053	-7.657	-7.436
β	HILLSLOPE	2	-1.062	0.128	-1.330	-0.794
EC50	EC50 (μM)	2	0.028		0.022	0.037
B	Bottom	3	-0.303	1.147	-2.695	2.090
T	Top	3	101.6	1.2	99.1	104.2
μ	LOGEC50	3	-7.600	0.025	-7.651	-7.549
β	HILLSLOPE	3	-0.952	0.048	-1.053	-0.851
EC50	EC50 (μM)	3	0.025		0.022	0.028

Mathematical Averages

AVG LOGEC50	-7.589				
AVG HILLSLOPE	-0.999				
AVG T	103.7				
AVG B	-6.984				
		sd	sem	%CV	
Avg EC50	0.0245	0.0008	0.0005	3.28	

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	-6.695	6.603	-35.107	21.717
Top	Overall	103.824	2.494	93.093	114.554
HILLSLOPE	Overall	-0.971	0.039	-1.138	-0.803
LOGEC50	Overall	-7.597	0.02	-7.683	-7.511

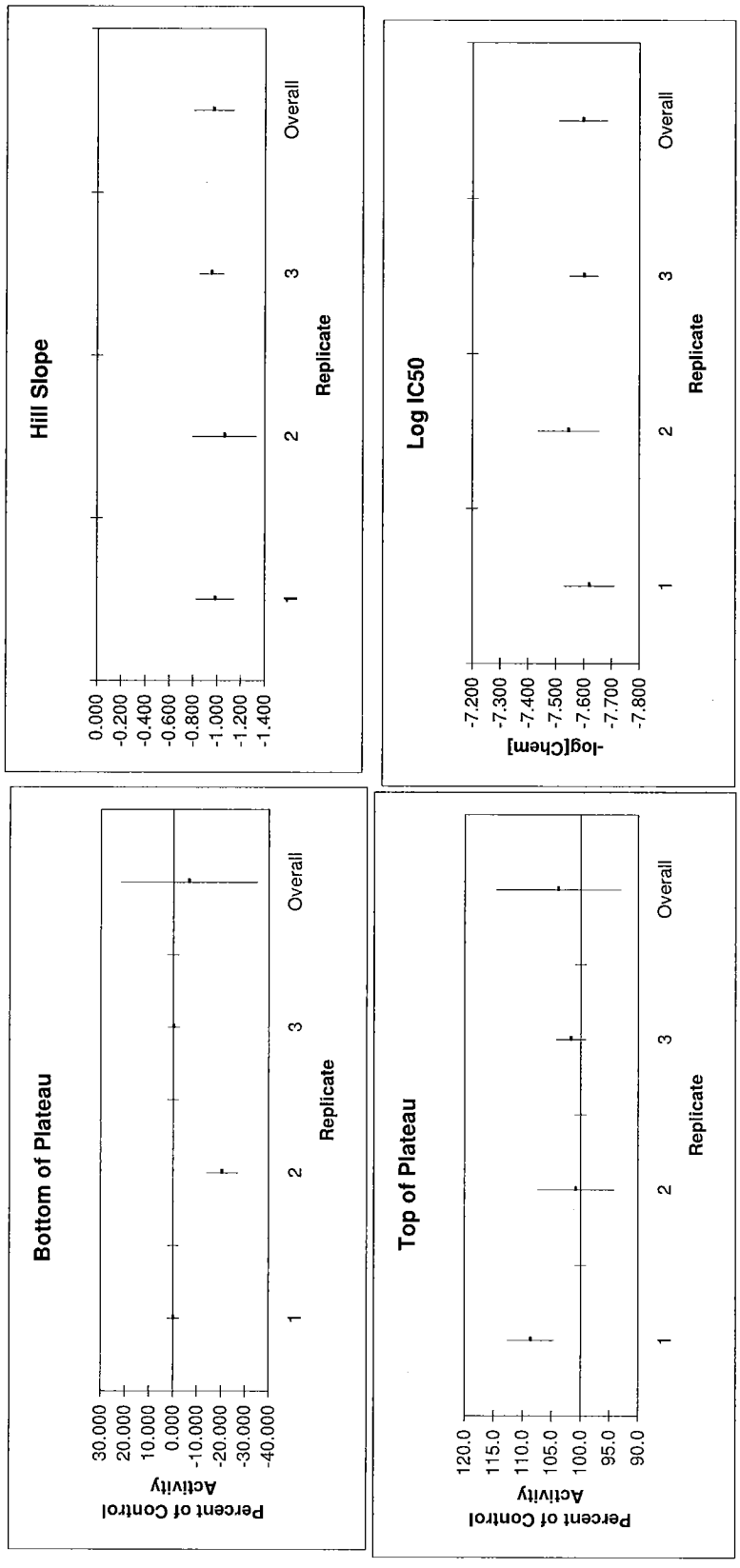


Figure 18, continued. Response Curve Summary Plots

Figure 19. Validation of the Placental Aromatase Assay (WIL-431009)

Reference Chemical 4-4-Nonylphenol

Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	0.278	0.546	-0.861	1.417
T	Top	1	104.5	0.2	104.0	105.0
μ	LOGEC50	1	-4.602	0.012	-4.627	-4.576
β	HILLSLOPE	1	-1.858	0.049	-1.960	-1.756
EC50	EC50 (μM)	1	25.03		23.60	26.54
B	Bottom	2	0.626	1.441	-2.379	3.631
T	Top	2	100.5	0.8	98.9	102.2
μ	LOGEC50	2	-4.578	0.022	-4.623	-4.533
β	HILLSLOPE	2	-1.974	0.113	-2.209	-1.739
EC50	EC50 (μM)	2	26.43		23.82	29.32
B	Bottom	3	0.563	0.505	-0.490	1.615
T	Top	3	98.6	0.3	98.0	99.2
μ	LOGEC50	3	-4.561	0.008	-4.577	-4.545
β	HILLSLOPE	3	-2.031	0.043	-2.120	-1.941
EC50	EC50 (μM)	3	27.48		26.49	28.51

Mathematical Averages

AVG LOGEC50	-4.580			
AVG HILLSLOPE	-1.954			
AVG T	101.193			
AVG B	0.489			
		sd	sem	%CV
Avg EC50	26.31	1.23	0.71	4.67

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	0.444	0.359	-1.101	1.989
Top	Overall	101.226	1.787	93.536	108.915
HILLSLOPE	Overall	-1.952	0.062	-2.219	-1.685
LOGEC50	Overall	-4.579	0.014	-4.64	-4.519

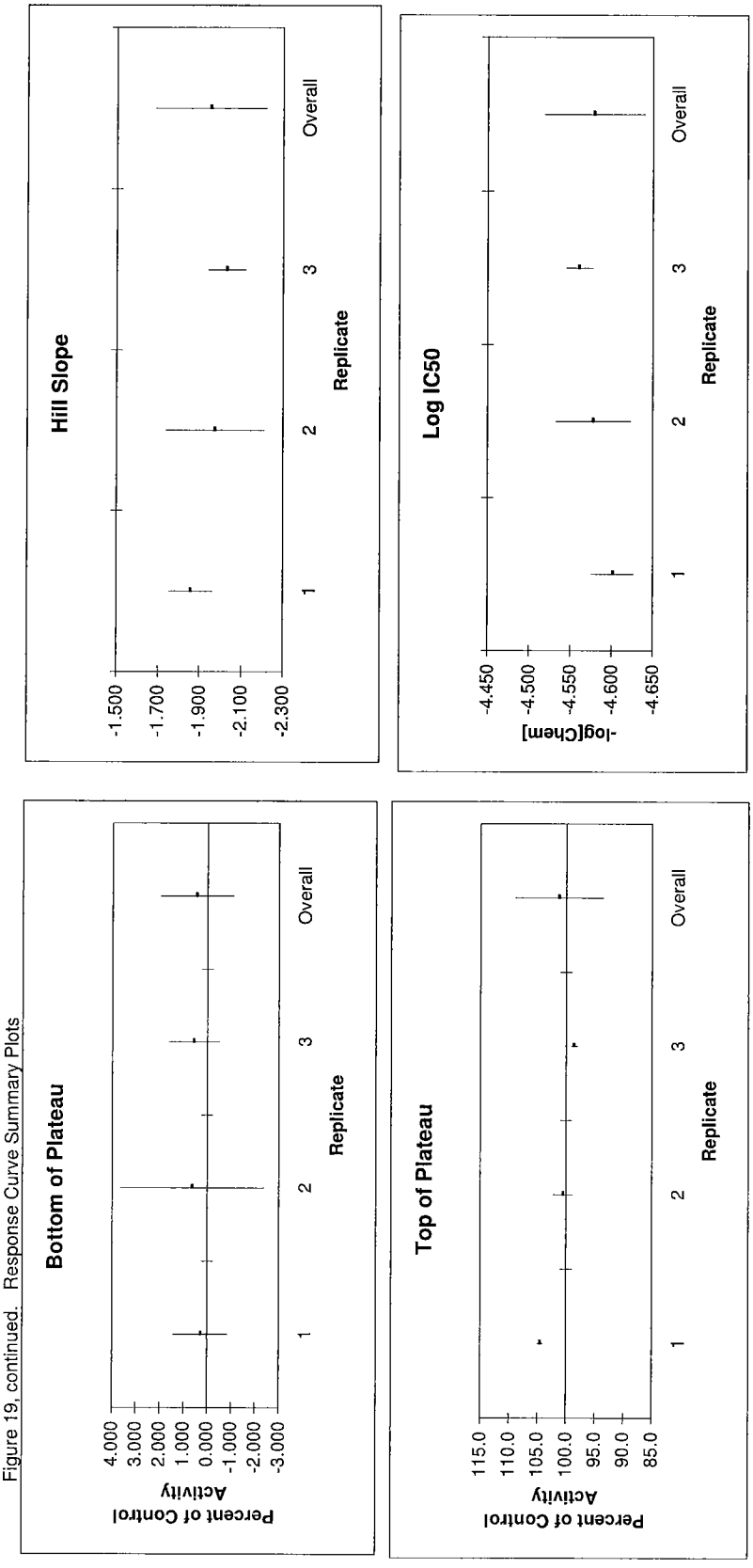


Figure 19, continued. Response Curve Summary Plots

Figure 20. Validation of the Placental Aromatase Assay (WIL-431009)

Reference Chemical 6-Fenarimol

Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	0.078	2.128	-4.361	4.518
T	Top	1	101.7	1.2	99.1	104.2
μ	LOGEC50	1	-5.197	0.046	-5.293	-5.102
β	HILLSLOPE	1	-1.025	0.102	-1.238	-0.812
EC50	EC50 (μM)	1	6.347		5.095	7.908
B	Bottom	2	-20.199	2.116	-24.629	-15.770
T	Top	2	81.0	1.8	77.2	84.9
μ	LOGEC50	2	-5.149	0.039	-5.231	-5.067
β	HILLSLOPE	2	-1.022	0.081	-1.193	-0.852
EC50	EC50 (μM)	2	7.101		5.878	8.578
B	Bottom	3	0.605	1.610	-2.754	3.964
T	Top	3	87.8	1.3	85.2	90.5
μ	LOGEC50	3	-5.113	0.033	-5.182	-5.043
β	HILLSLOPE	3	-1.046	0.072	-1.197	-0.896
EC50	EC50 (μM)	3	7.716		6.570	9.061

Mathematical Averages

AVG LOGEC50	-5.153			
AVG HILLSLOPE	-1.031			
AVG T	90.178			
AVG B	-6.505			
		sd	sem	%CV
Avg EC50	7.055	0.686	0.396	9.72

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	-6.474	6.835	-35.881	22.933
Top	Overall	90.229	6.063	64.141	116.317
HILLSLOPE	Overall	-1.034	0.048	-1.239	-0.829
LOGEC50	Overall	-5.145	0.023	-5.245	-5.045

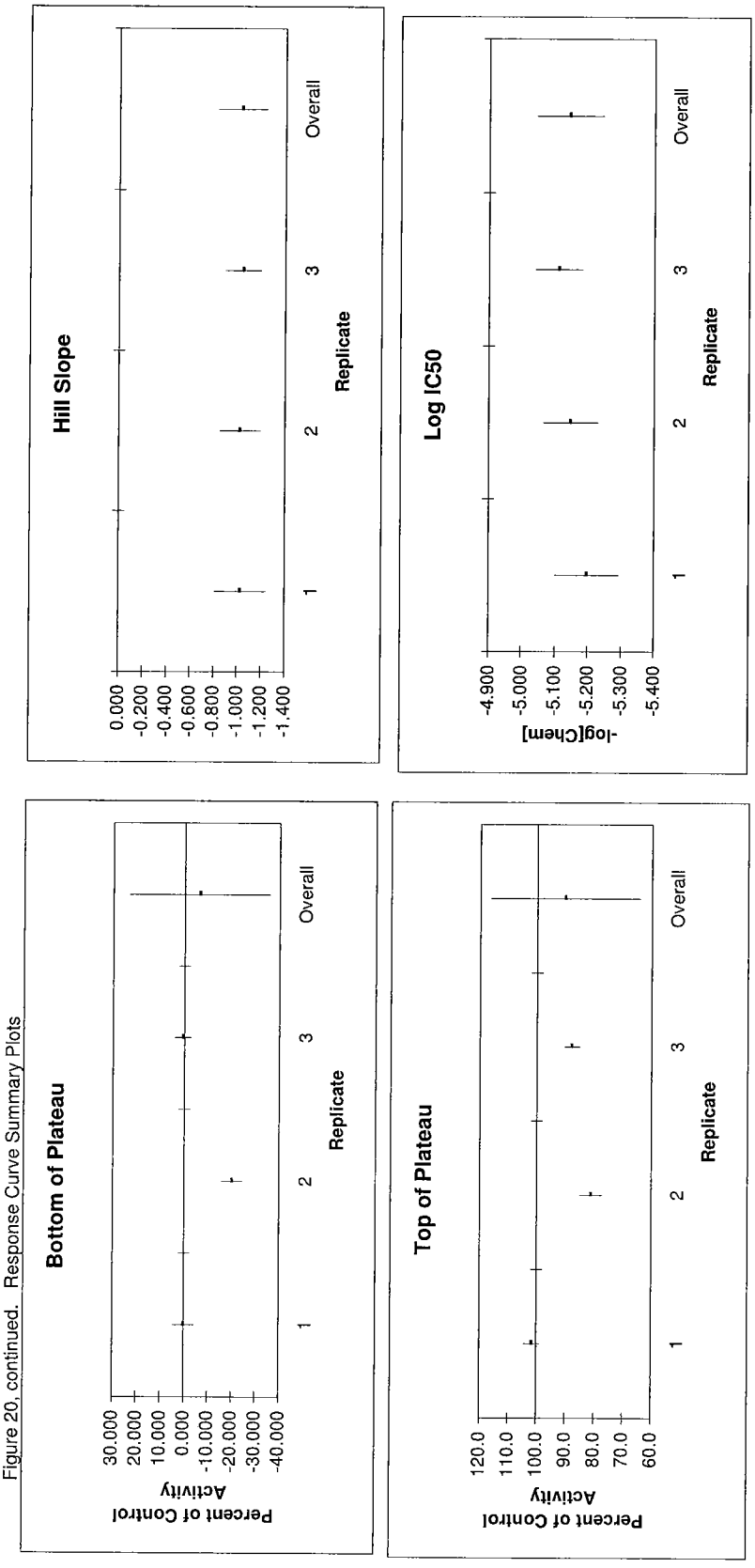


Figure 20, continued. Response Curve Summary Plots

Figure 21. Validation of the Placental Aromatase Assay (WIL-431009)

Reference Chemical 7-Econazole

Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	0.057	0.144	-0.245	0.358
T	Top	1	99.8	0.5	98.8	100.7
μ	LOGEC50	1	-8.794	0.007	-8.809	-8.780
β	HILLSLOPE	1	-1.095	0.017	-1.129	-1.060
EC50	EC50 (μM)	1	0.0016		0.0016	0.0017
B	Bottom	2	0.742	0.395	-0.082	1.565
T	Top	2	104.7	1.0	102.7	106.7
μ	LOGEC50	2	-8.765	0.013	-8.791	-8.738
β	HILLSLOPE	2	-1.070	0.029	-1.130	-1.010
EC50	EC50 (μM)	2	0.0017		0.0016	0.0018
B	Bottom	3	0.529	0.455	-0.419	1.478
T	Top	3	97.1	1.0	94.9	99.3
μ	LOGEC50	3	-8.761	0.015	-8.791	-8.731
β	HILLSLOPE	3	-1.134	0.037	-1.212	-1.056
EC50	EC50 (μM)	3	0.0017		0.0016	0.0019

Mathematical Averages

AVG LOGEC50	-8.773			
AVG HILLSLOPE	-1.100			
AVG T	100.533			
AVG B	0.443			
		sd	sem	%CV
Avg EC50	0.0017	0.0001	0.0000	4.18

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	0.315	0.238	-0.71	1.34
Top	Overall	100.533	2.2	91.066	110
HILLSLOPE	Overall	-1.094	0.013	-1.152	-1.037
LOGEC50	Overall	-8.776	0.011	-8.825	-8.727

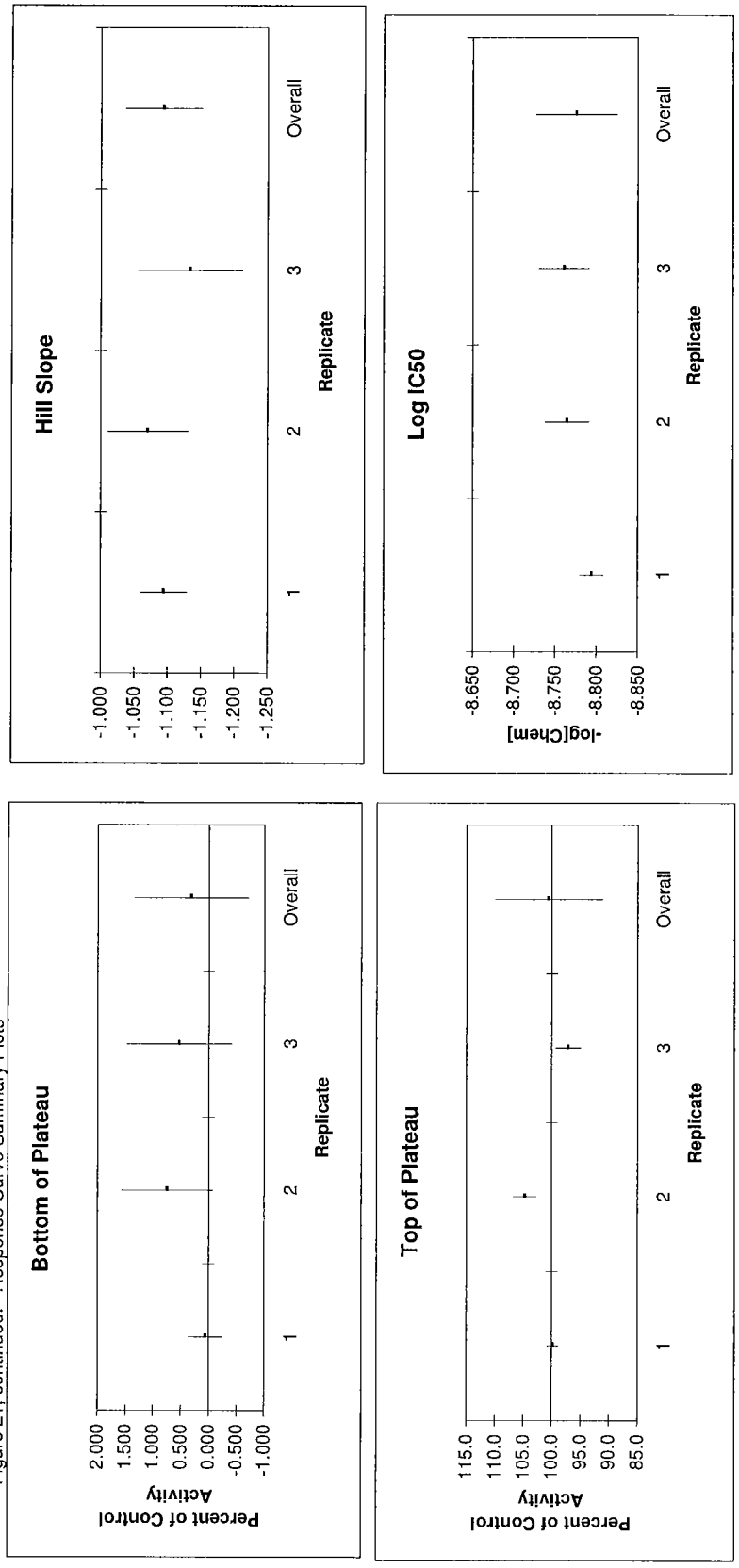


Figure 21, continued. Response Curve Summary Plots

Figure 22. Validation of the Placental Aromatase Assay (WIL-431009)

Reference Chemical 8-Chrysin

Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	8.333	5.093	-2.290	18.957
T	Top	1	98.8	2.2	94.1	103.5
μ	LOGEC50	1	-5.613	0.089	-5.798	-5.428
β	HILLSLOPE	1	-1.014	0.177	-1.383	-0.645
EC50	EC50 (μM)	1	2.438		1.594	3.730
B	Bottom	2	16.119	4.975	5.742	26.497
T	Top	2	82.6	2.8	76.8	88.4
μ	LOGEC50	2	-5.451	0.081	-5.620	-5.283
β	HILLSLOPE	2	-1.549	0.385	-2.351	-0.746
EC50	EC50 (μM)	2	3.537		2.399	5.215
B	Bottom	3	13.826	2.595	8.412	19.241
T	Top	3	89.2	1.4	86.2	92.2
μ	LOGEC50	3	-5.493	0.040	-5.576	-5.410
β	HILLSLOPE	3	-1.388	0.157	-1.714	-1.061
EC50	EC50 (μM)	3	3.212		2.652	3.890

Mathematical Averages

AVG LOGEC50	-5.519			
AVG HILLSLOPE	-1.317			
AVG T	90.2			
AVG B	12.760			
		sd	sem	%CV
Avg EC50	3.062	0.565	0.326	18.44

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	13.302	2.097	4.281	22.324
Top	Overall	90.296	4.612	70.454	110.139
HILLSLOPE	Overall	-1.258	0.157	-1.932	-0.585
LOGEC50	Overall	-5.503	0.033	-5.646	-5.36

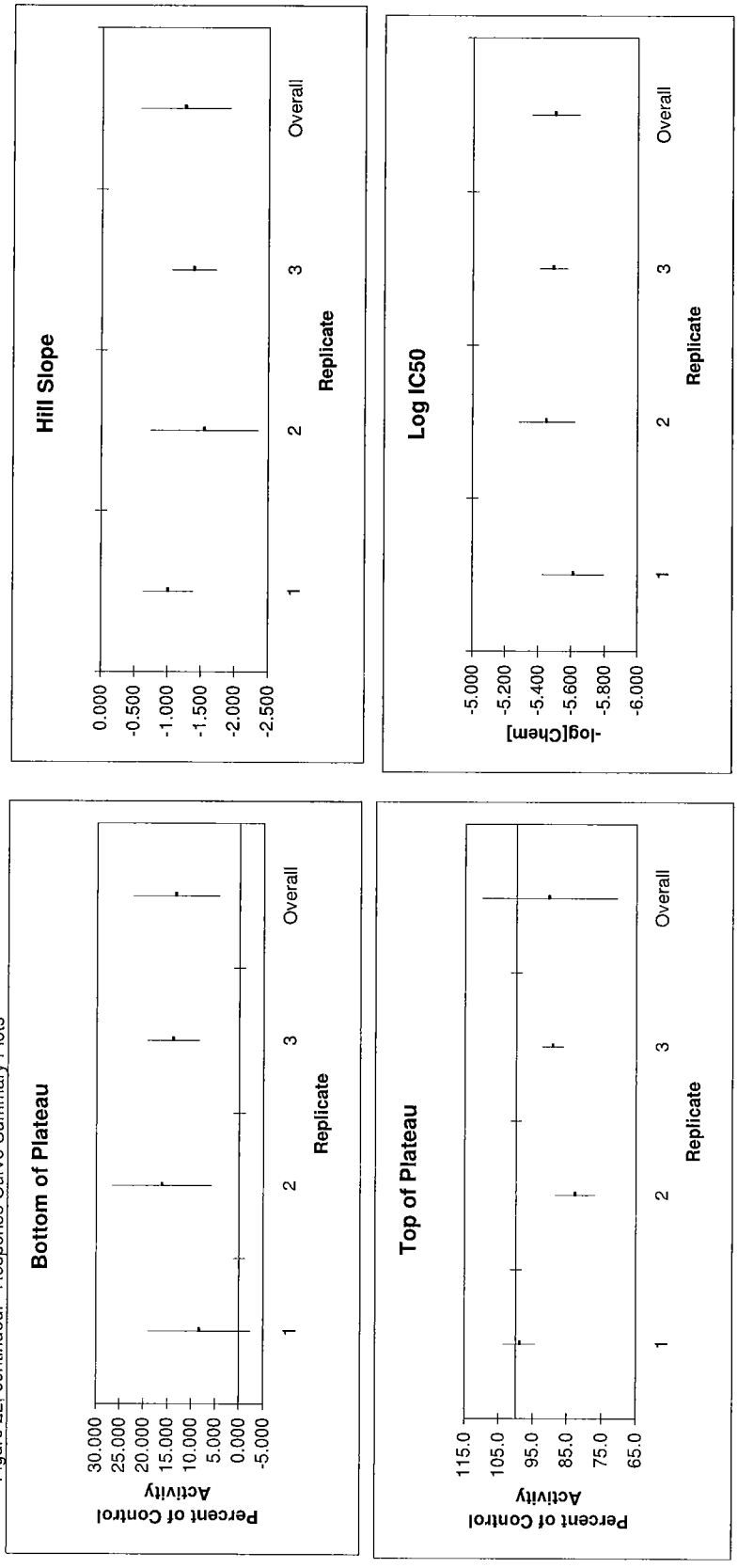


Figure 22, continued. Response Curve Summary Plots

Figure 23. Validation of the Placental Aromatase Assay (WIL-431009)

Reference Chemical 9-Dicofol

Response Curve Summary Table

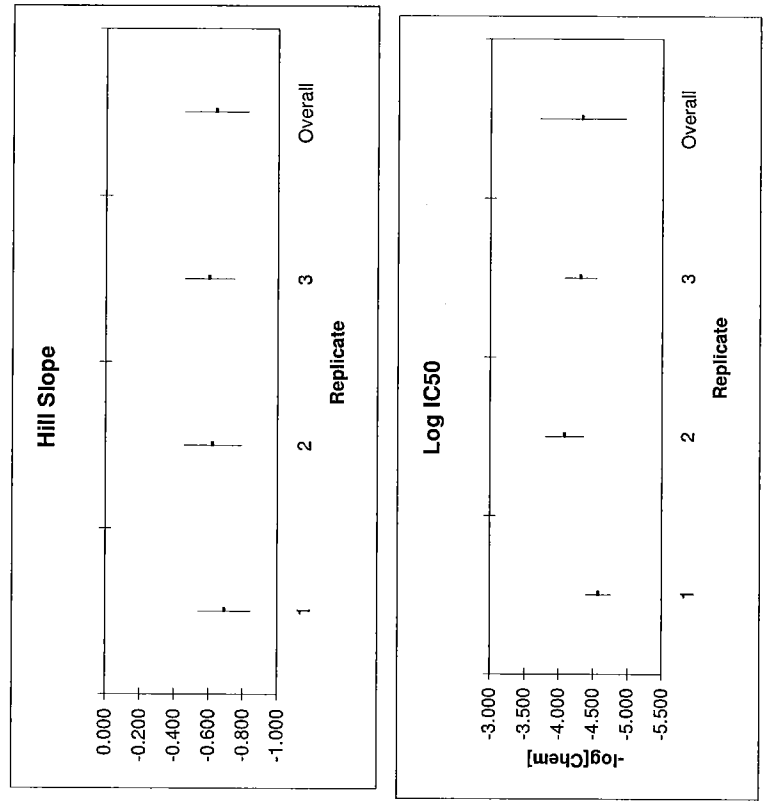
		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	-3.409	4.305	-12.420	5.602
T	Top	1	100.9	1.1	98.6	103.3
μ	LOGEC50	1	-4.576	0.086	-4.755	-4.397
β	HILLSLOPE	1	-0.692	0.072	-0.843	-0.541
EC50	EC50 (μM)	1	26.56		17.58	40.12
B	Bottom	2	-17.170	9.803	-37.620	3.281
T	Top	2	102.2	2.5	97.0	107.4
μ	LOGEC50	2	-4.078	0.133	-4.356	-3.800
β	HILLSLOPE	2	-0.624	0.080	-0.790	-0.457
EC50	EC50 (μM)	2	83.54		44.02	158.60
B	Bottom	3	-17.010	7.392	-32.430	-1.592
T	Top	3	99.2	2.5	94.0	104.5
μ	LOGEC50	3	-4.307	0.110	-4.538	-4.077
β	HILLSLOPE	3	-0.603	0.069	-0.747	-0.458
EC50	EC50 (μM)	3	49.28		29.00	83.76

Mathematical Averages

AVG LOGEC50	-4.320			
AVG HILLSLOPE	-0.639			
AVG T	100.777			
AVG B	-12.530			
		sd	sem	%CV
Avg EC50	53.13	28.68	16.56	53.99

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	-10.38	5.313	-33.241	12.482
Top	Overall	100.86	0.953	96.761	104.959
HILLSLOPE	Overall	-0.639	0.042	-0.822	-0.457
LOGEC50	Overall	-4.334	0.144	-4.954	-3.713



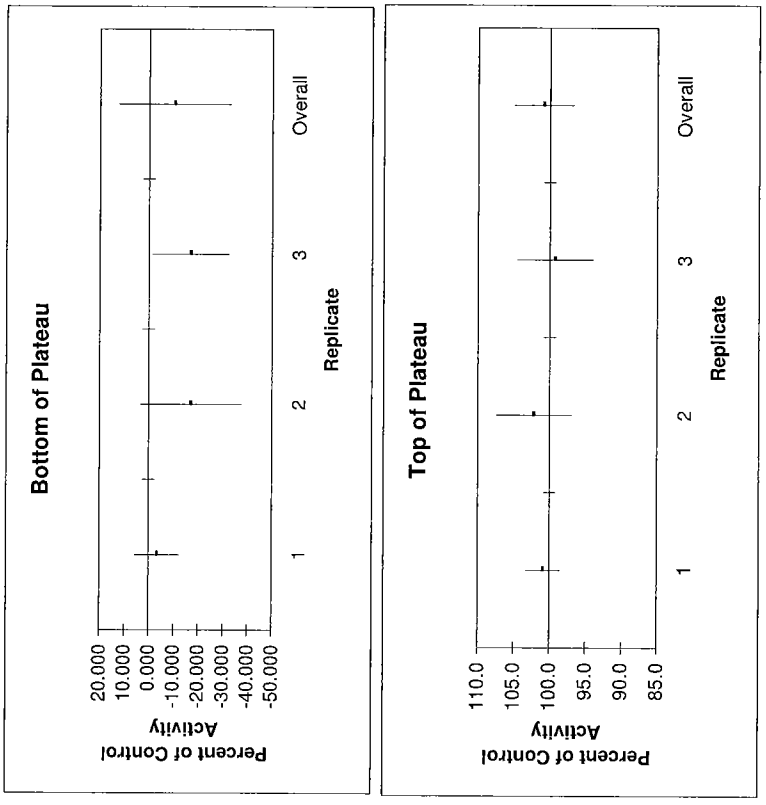


Figure 23, continued. Response Curve Summary Plots

Appendix A

Study Protocol (with Amendment)

Study Number: WIL-431009

PROTOCOL AMENDMENT I

Sponsor: Battelle Memorial Institute

EPA Contract No.: 68-W-01-023

A. Title of Study:

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

B. Protocol Additions/Modifications

1) **4.3** **Analysis of Reference & Control Chemicals:**

Section 4.3 is to be added and reads as follows:

Analysis of the reference and control 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.

2) **9.2.1** **Concentration Response Fits for the Reference Chemicals**
"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)."

Will be replaced with:

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

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

C. Reasons for Protocol Additions/Modifications:

1) For this study to meet GLP requirements Section 4.3 must be added in response to the Endocrine Disruptor Screening Program Quality Assurance Report which finds that neither the methods of analysis nor the frequency of the analyses is documented in the study protocol.

2) Battelle notified the EPA that laboratories performing the aromatase assay (including WIL) were obtaining negative values and experiencing error messages when using Prism to model the data according to the protocol. The EPA recommended solution requires that WIL amend the protocol and re-analyze the data using an unconstrained 4 parameter model and non-weighted values in the Prism software.

Approved By:
Battelle Memorial Institute

Jerry D. Johnson, PhD, DABT
Sponsor Representative — 11/22/05 Date

Prepared By:
WIL Research Laboratories, LLC

Jennifer Thomas-Wohlever, PhD
Study Director — 11/18/05 Date

Daniel W. Sved, PhD
Director, Metabolism and
Analytical Chemistry — 11/18/05 Date

Study Number: WIL-431009

PROTOCOL AMENDMENT II

Sponsor: Battelle Memorial Institute

EPA Contract No.: 68-W-01-023

A. Title of Study:

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

B. Protocol Additions/Modifications:

1) **9.2.1 Concentration Response Fits for the Reference Chemicals:**

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

$$Y = B + \frac{-}{1+10^{(X-\mu)*H}}$$

will be replaced with:

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

$$Y = B + \frac{-}{1+10^{(\mu-X)*H}}$$

C. Reasons for Protocol Additions/Modifications:

1) An error in the equation for the response curve in Amendment I necessitates Amendment II. In the revised equation the "X" and "μ" are in a different order.

Approved By:

Battelle Memorial Institute

Jerry D. Johnson, PhD, DABT
Sponsor Representative

1-6-06
Date

Prepared By:

WIL Research Laboratories, LLC

Jennifer Thomas-Wohlever, PhD
Study Director

1/4/06
Date

Daniel W. Sved, PhD
Director, Metabolism and
Analytical Chemistry

1/4/06
Date

PROTOCOL

WA 4-16 TASK 7: CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES

EPA Contract No.: 68-W-01-023

Submitted To:

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

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-9281

1 OBJECTIVE:

Task 7: The objectives of this protocol are to test 10 reference chemicals using microsomes prepared by two participating laboratories (Battelle and In Vitro) in Task 6 (WIL-431008), and to determine the intra- and interlaboratory variability in the test results. The microsomes prepared in Task 6 (WIL-431008) by Battelle and In Vitro will be distributed to each laboratory and tested as outlined in Section 8 (Table 4).

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

There is no applicable route of administration in the sense of a dose administration route for this *in vitro* test. This *in vitro* test method involves combining microsomes, substrate, appropriate co-factors and test substances in a common reaction vessel. The effect of the control chemicals on microsomal enzyme activity is evaluated by measuring the amount of the product of the enzyme-catalyzed substrate oxidation that is formed.

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Sponsor Representatives:

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David P. Houchens, PhD
Program Manager
Endocrine Disruptor Screening Program
Battelle Memorial Institute

2.2 U.S. EPA Representatives:

Gary E. Timm, MS, MA
Work Assignment Manager
Endocrine Disruptor Screening Program
U.S. EPA

Linda Phillips, PhD
Project Officer
U.S. EPA

2.3 WIL Study Director:

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2.4 WIL Deputy Director:

Justin Godsey, BS, LATG
Biologist, Metabolism

2.5 WIL Staff Involved with Study:

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President, Director

Daniel W. Sved, PhD
Director, Metabolism and Analytical Chemistry

Terry L. Johnson, PhD
Associate Director, Metabolism

Christopher J. Bowman, PhD
Staff Toxicologist, Developmental
and Reproductive Toxicology

Heather L. Osborn, BS, RQAP-GLP
Manager, Quality Assurance

Robert A. Wally, BS, RAC
Acting Manager, Reporting and
Regulatory Technical Services

Pete Resnis, BS
Senior Research Chemist, Metabolism

Aimee Mahoney, BS
Group Supervisor, Metabolism

2.6 Statistical Analysis:

Les Freshwater, MS
BioSTAT Consultants, Inc.

3 STUDY SCHEDULE:

Proposed Experimental Start Date:	July 8, 2005
Proposed Experimental Termination Date:	August 30, 2005
Proposed Unaudited Data Submission Date:	September 2, 2005
Proposed Audited Report Date:	September 23, 2005

4 REFERENCE & CONTROL CHEMICAL DATA:

Reserve samples of the reference and control chemicals used in this study will be collected by the Sponsor and will be stored at the Sponsor's facility. Therefore, no reserve samples for this study will be collected by WIL Research Laboratories, LLC.

4.1 Control Chemicals For Determination of Enzyme Activity:

The known aromatase inhibitor, 4-hydroxyandrostenedione (4-OH ASDN), is used as the positive control substance for this task. A known aromatase non-inhibitor, lindane, will be used as the negative control substance. Table 1 contains the identity and property information for these substances.

Table 1. Control Substances

Control Chemical	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

Control substance stock solutions will be prepared and analyzed by Battelle's Chemical Repository (CR) and distributed to the laboratories. Lindane will be formulated in DMSO and 4-OH ASDN will be formulated in ethanol. 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 1) 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.

4.2 Reference Chemicals:

The reference chemicals for Task 7, their properties and rationale for selection are listed in Table 2. 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 8.

Table 2. 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
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

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.

5 ASSAY MATERIALS RECEIPT AND/OR PREPARATION:

A sufficient supply of chemical reagents, radiolabeled and non-radiolabeled androstenedione, and human placental microsomal preparations will be obtained prior to initiation of the first set of experiments to ensure that sufficient quantities are available to conduct the studies. The detailed procedures for preparation of the assay substrate, assay buffer, microsomes and NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt) solution will be documented in the study records.

The procedure for identification of the test system will be that each test tube used in the conduct of the aromatase assay will be uniquely identified by applying a label or writing directly on the test tube.

5.1 Assay Substrate, [^{3}H]ASDN + ASDN:

5.1.1 Substrate Name/Supplier:

The substrate for the aromatase assay is androstenedione (ASDN). Non-radiolabeled and radiolabeled ASDN will be used. The non-radiolabeled ASDN will be provided to the laboratories by the CR. The radiolabeled androstenedione ([1β-^{3}H]-ASDN, [^{3}H]ASDN) will be provided to the laboratories by Perkin Elmer through Battelle's 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 will be assessed by RTI.

5.1.2 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 is 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 (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.

5.2 Microsomes:

Human placental microsomes (prepared in Task 6, described in WIL-431008) will be supplied to each laboratory by Battelle and In Vitro. These samples should be treated as potentially infectious and appropriate precautions must be employed. The microsomes must be stored at approximately -70 to -80 °C. The approximate protein content of the microsomes will be provided by the supplier.

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

If the human placental microsomes are supplied in aliquots in excess of what is required to conduct a single experiment, they will be thawed, pooled, homogenized, divided into appropriate aliquots for conduct of a single experiment and refrozen as described below in order to minimize and standardize the number of freeze/thaw cycles each preparation undergoes. Microsomes will be thawed quickly in a 37 ± 1 °C water bath and then will be

immediately transferred to an ice bath. The microsomes will be pooled and rehomogenized using a Potter-Elvejhem homogenizer (about 5–10 passes). The pooled sample will be aliquoted into portions appropriate for use in a single experiment (ca. 160 µL, dependent on the protein concentration of the preparation) and the samples will be flash frozen and stored at approximately -70 to -80°C for future use. Each tube will provide enough protein for a single experiment and any excess thawed microsomal preparation will be discarded.

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

5.3 Other Assay Components:

5.3.1 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).

5.3.2 Propylene Glycol:

Propylene glycol (JT Baker, Cat #9402-01, 76.1 g/mol) is added directly to the assay as described in Section 7.

5.3.3 NADPH:

NADPH (Sigma cat #1630, 833.4 g/mol) is the required co-factor for CYP19 (aromatase enzyme). The Sponsor will provide the NADPH to

be used in the assay. The final concentration in the assay will be 0.3 mM. Typically, a 6 mM stock solution is prepared in assay buffer and then 100 µL of the stock is added to the 2 mL assay volume. NADPH must be prepared fresh each day and is kept on ice.

6 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.

7 AROMATASE ASSAY (SEE APPENDIX A):

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 + ASDN substrate, NADPH, inhibitor solution 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 below.

Table 3. Human Placental Microsomal Aromatase Assay-Optimized Conditions

Microsomal Protein	0.0125 mg/mL[a]
NADPH	0.3 mM[a]
[^{3}H]ASDN + ASDN	100 nM[a]
Incubation Time	15 minutes

[a] Final concentrations

The tubes and the microsomal suspension will be placed in a 37 ± 1°C water bath for at least five minutes prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension to the reaction mixture in the labeled test tube. The total assay volume will be 2.0 mL, and the tubes will be incubated for 15 minutes.

The incubations will be stopped by the addition of methylene chloride (2.0 mL); the tubes will be vortex-mixed for approximately 5 seconds and placed on ice. The tubes are then vortex-mixed an additional 20-25 seconds. The tubes will then be centrifuged for 10 minutes at approximately 162 x g. The methylene chloride layer will be removed and discarded; the aqueous layers are 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, approximately 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.

Analysis of the samples will be performed using a liquid scintillation counter (LSC). Radiolabel found in the aqueous fractions represents ^{3}HOH formed. One ^{3}HOH molecule is released per molecule of ASDN converted to estrogen in a stereospecific reaction. Thus, the amount of estrogen product formed is determined by dividing the total amount of ^{3}HOH formed by the specific activity of the [^{3}H]ASDN substrate (expressed in DPM/nmol). Results will be presented as the activity (velocity) of the enzyme reaction. The activity of the enzyme reaction is expressed in nmol (mg protein)$^{-1}$min^{-1} and is calculated by dividing the amount of estrogen formed by the product of mg microsomal protein used times the incubation time, e.g. 15 minutes.

8 DETERMINATION OF THE RESPONSE OF AROMATASE ACTIVITY TO REFERENCE CHEMICALS:

This task is similar in structure to Task 5 (WIL-431007) except that the test laboratories will use the microsomes prepared in Laboratory 2 or in Laboratory 4 (neither are WIL Laboratories), according to the matrix shown below (Table 4). Ten inhibitor compounds will be tested, including the four inhibitor compounds in Task 5 (WIL-431007). The test is organized so that across the ten chemicals the allocation of microsome sources to reference chemicals is approximately balanced.

For each chemical (1 to 10) Laboratory 2 (Battelle) will supply two of laboratories RTI, Battelle, WIL and In Vitro (sometimes itself and sometimes not) and laboratory 4 (In Vitro) will supply the other two. The distribution of microsomal preparations will differ for different chemicals. Let M2 denote the microsomal preparation from Laboratory 2 and let M_4 denote the microsomal preparation from Laboratory 4. The four laboratories will use microsomal preparations from the laboratories as shown in Table 4.

Table 4. Microsome Distribution and Testing Matrix

	Analysis Laboratory			
Chemical	Laboratory 1 RTI	Laboratory 2 Battelle	Laboratory 3 WIL	Laboratory 4 In Vitro
Chemical 1	M_2	M_2	M_4	M_4
Chemical 2	M_2	M_2	M_4	M_4
Chemical 3	M_4	M_4	M_2	M_2
Chemical 4	M_2	M_4	M_2	M_4
Chemical 5	M_4	M_2	M_4	M_2
Chemical 6	M_4	M_4	M_2	M_2
Chemical 7	M_2	M_4	M_4	M_2
Chemical 8	M_2	M_4	M_4	M_2
Chemical 9	M_4	M_2	M_2	M_4
Chemical 10	M_4	M_2	M_2	M_4

Each test laboratory will analyze five chemicals with microsomal preparation made in Laboratory 2 and five chemicals with microsomal preparation made in Laboratory 4. Across the 10 chemicals the laboratories are approximately balanced for comparison of M_2 and M_4. Thus if there is no interaction between laboratory or microsome source and chemical, the laboratory effects are excluded from the comparison between microsomal preparations.

Each replicate will test the response of aromatase activity to the presence of eight concentrations of a reference chemical. The reference chemicals must be coded prior to distribution to the assaying technicians in order that the replicates are conducted blind for reference chemical identity. This task will be conducted in three independent replicates. All three replicates for a given reference chemical must be conducted by the same technician. However, the same technician is not required to perform the three replicates for all ten reference chemicals. Multiple reference chemicals may be conducted 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 assays are conducted on a given day by a single technician, those assays should use different reference chemicals. Each reference chemical will be tested at eight concentrations and there will be three (triplicate) repetitions for each concentration of a given replicate. A single replicate study with two reference chemicals is described in Table 5.

Table 5. Sample Reference Chemical Study Design

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

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

Four types of control samples will be included for each replicate day. 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 during each replicate day 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 day's set.

The assay will be conducted as described in Section 7.0 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.

9 DATA ANALYSIS:

The data analysis described in the following subsections addresses all of the experiments of this task. The laboratories will only be responsible for performing the data analysis that corresponds to the experiments that they are assigned to conduct.

9.1 Aromatase Activity and Percent of Control Calculations:

Relevant data will be 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 issued in a previous task on this work assignment (WIL Study Number WIL-431007).

9.2 Statistical Analysis:

9.2.1 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 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}.

9.2.2 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.

9.2.3 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)
- 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, the portion by reference chemical interaction, and the portion by replicate within reference chemical interaction should be non-significant. 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. Simultaneity of inference will be adjusted for by Bonferroni's method.

9.2.4 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), and Microsoft Excel, as convenient.

9.2.5 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.

10 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit with in-phase inspections to assure compliance with the study protocol and protocol amendments, WIL Standard Operating Procedures (SOPs) and the appropriate provisions of the EPA TSCA and FIFRA Good Laboratory Practice Standards (GLPs) published in the Federal Register (40 CFR Part 792 and 40 CFR Part 160). The raw data and draft report will be audited by the WIL Quality Assurance Unit prior to submission to the Sponsor to assure that the Final Report accurately describes the conduct and the findings of the study. Quality control (QC) and quality assurance (QA) procedures will follow those outlined in the Quality Assurance Project Plan (QAPP) that will be prepared for this study.

Data requiring statistical analysis will be analyzed by BioSTAT Consultants, Inc. following the current procedural guidelines of BioSTAT Consultants Inc. BioSTAT Consultants Inc. will provide a statistical analysis report, which will be included as an appendix to the final report. Quality Assurance auditing of the statistical report (for internal consistency with the study report) will be conducted under the direction of the Quality Assurance Unit of WIL Research Laboratories, LLC.

Formulation of the control and reference chemical stock solutions will be conducted by the Sponsor following the standard operating procedures of the Sponsor and in accordance with GLPs. Quality assurance monitoring of these activities for SOP and GLP compliance is the responsibility of the Sponsor. Upon completion of the prescribed activities the Sponsor will provide a signed Quality Assurance statement that will be included in the Battelle Chemical Repository Chemistry Report and included in the final report as an appendix.

This study will be included on the WIL master list of regulated studies.

11 RECORDS TO BE MAINTAINED:

All specimens and original raw data records, as defined by WIL SOPs and the applicable GLPs, will be stored as described in Section 12 in the Archives at WIL Research Laboratories, LLC.

Raw data records generated by the Sponsor will be stored as defined by the Sponsor's applicable standard operating procedures.

12 WORK PRODUCT:

The Sponsor will have title to all documentation records, raw data, specimens and other work product generated during the performance of the study. All work product, including raw paper data, pertinent electronic storage media and specimens, will be retained at no charge for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, LLC. Thereafter, WIL Research Laboratories, LLC will charge a monthly archiving fee for retention of all work product. Appropriate supporting documentation for statistical analyses conducted and reported by BioSTAT Consultants, Inc. will be maintained in the Archives at WIL Research Laboratories, LLC. All work product will be stored in compliance with regulatory requirements.

Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, LLC to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, LLC will not be responsible for shipment following delivery to the common carrier.

13 REPORTS:

An interim data set, in the form of a spreadsheet and data summary, will be submitted to the Sponsor. The spreadsheets will be submitted within 14 calendar days of completing the incubations/analyses. This interim data submission will not be audited by the Quality Assurance Unit and will be identified as "unaudited preliminary data." The data will be checked for accuracy by the technical staff.

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 and reference 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 reference 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 9 of this document.

Draft final and final reports will be written. The format for the draft final report will be provided by the Sponsor. The draft final report will be submitted to the Sponsor. One revision of the full report will be permitted as part of the cost of the study, from which the Sponsor's reasonable revisions and suggestions will be incorporated into the final report, as appropriate. Additional changes or revisions, requiring a new report, may be made, at extra cost. It is expected that the Sponsor will review the draft report and provide comments to WIL within a two-month time frame following submission. WIL will submit the final report in a timely manner following receipt of comments. If the Sponsor's comments and/or authorization to finalize the report have not been received by WIL within one year following submission of the draft report, WIL may elect to finalize the report following appropriate written notification to the Sponsor. One electronic copy (PDF format) will be provided; requests for paper copies of the final report may result in additional charges.

14 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the written permission (electronic email or paper document) of the Sponsor. In the event that the Sponsor requests or approves a change in the protocol, such changes will be made by appropriate documentation in the form of a protocol amendment. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

15 REFERENCES:

Bowman, C.J. Validation of the Placental Microsomal Aromatase Assay: Positive Control Study (WA 4-16, Task 4) (Study No. WIL-431006), WIL Research Laboratories, LLC, Ashland, OH, **Draft**.

Thomas-Wohlever, J. Validation of the Placental Aromatase Assay for Endocrine Disruptor Screening (WA 4-16, Task 5) (Study No. WIL-431007), WIL Research Laboratories, LLC, Ashland, OH, **Draft**.

Thomas-Wohlever, J. Validation of the Placental Aromatase Assay Using Prepared Microsomes (WA 4-16, Task 6) (Study No. WIL-431008), WIL Research Laboratories, LLC, Ashland, OH, **Draft.**

16 PROTOCOL APPROVAL:

Sponsor approval received via email on 7/7/05
Date

Jennifer Thomas-Wohlever 7/7/05
Jennifer Thomas-Wohlever, PhD Date
Study Director
WIL Research Laboratories, LLC

for DWS 13 July 2005
Daniel W. Sved, PhD Date
Director, Metabolism and
Analytical Chemistry
WIL Research Laboratories, LLC

Jerry D Johnson 7-8-05
Jerry D. Johnson, PhD, DABT Date
Work Assignment Leader/Study Monitor
Endocrine Disruptor Screening Program
Battelle Memorial Institute

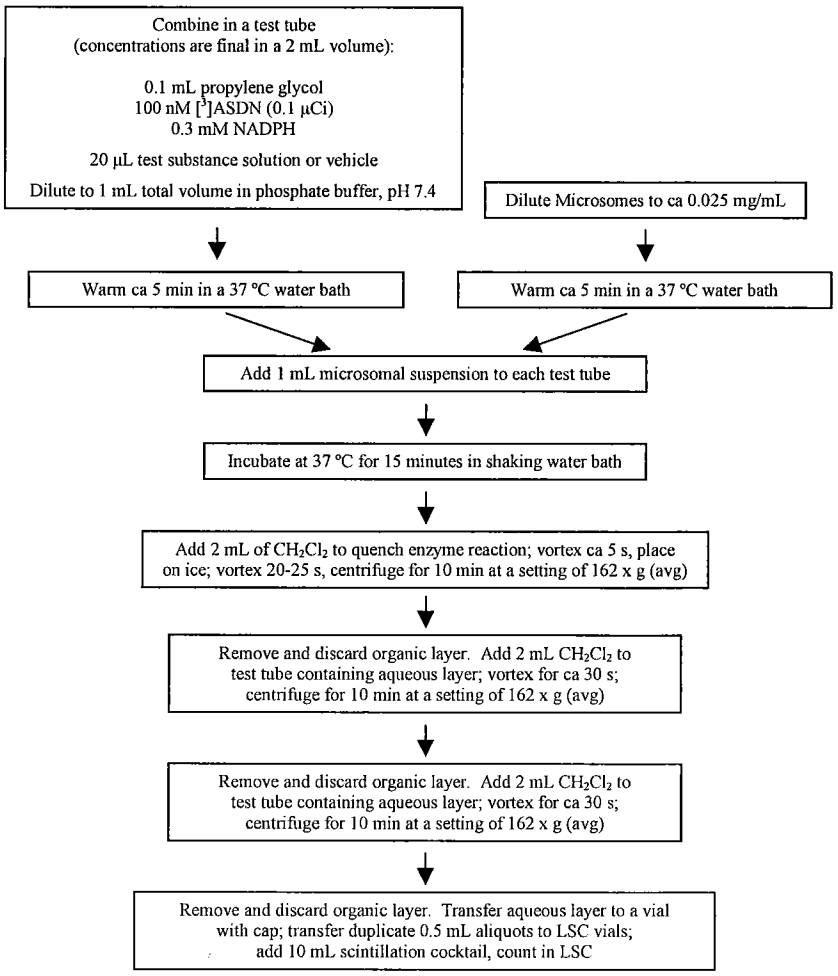
David P. Houchens 7/11/05
David P. Houchens, PhD Date
Program Manager
Endocrine Disruptor Screening Program
Battelle Memorial Institute

17 PROTOCOL REVIEW:

Heather L Osborn 7/12/05
Heather L. Osborn, BS, RQAP-GLP Date
Manager, Quality Assurance
WIL Research Laboratories, LLC

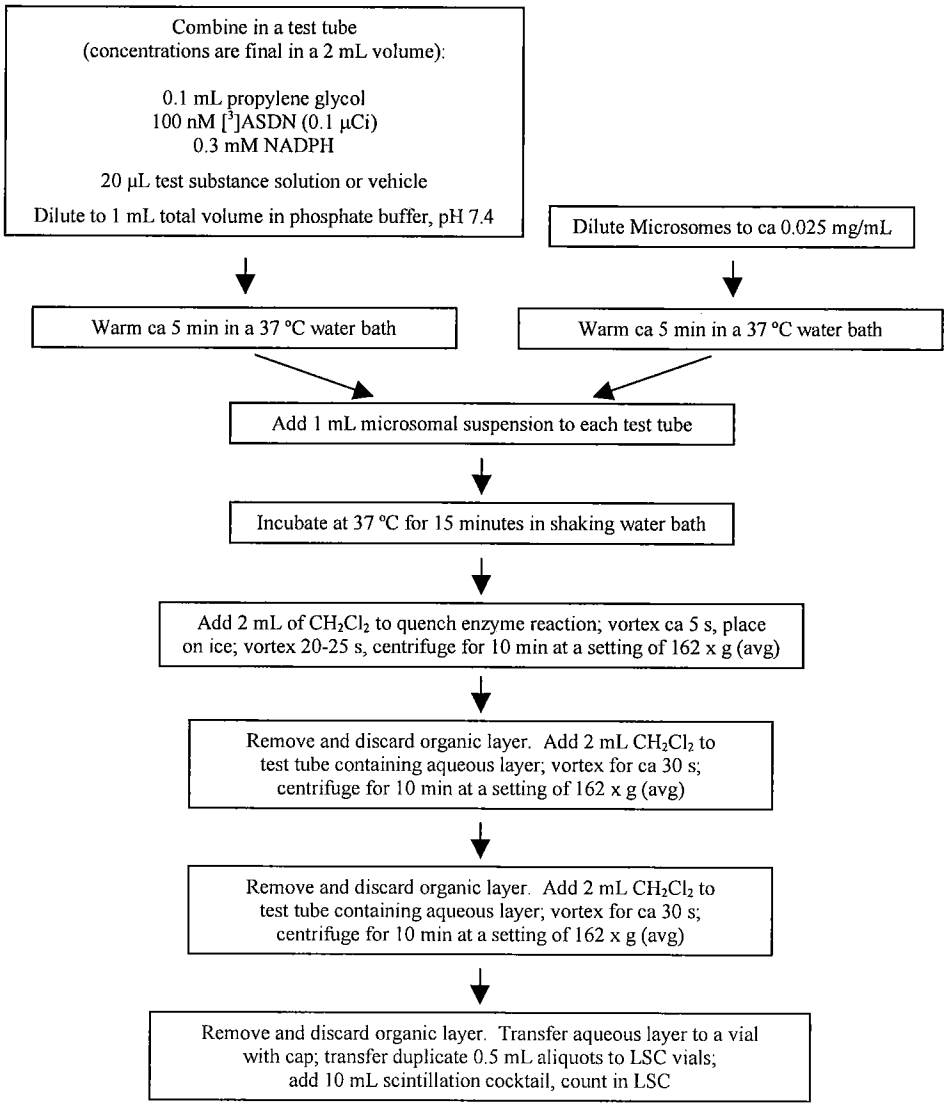
Terri L Pollock 7-11-05
Terri L. Pollock, BA Date
Quality Assurance Manager
Endocrine Disruptor Screening Program
Battelle Memorial Institute

Appendix A


Combine in a test tube
(concentrations are final in a 2 mL volume):
0.1 mL propylene glycol
100 nM [3]ASDN (0.1 µCi)
0.3 mM NADPH
20 µL test substance solution or vehicle
Dilute to 1 mL total volume in phosphate buffer, pH 7.4
Dilute Microsomes to ca 0.025 mg/mL
Warm ca 5 min in a 37 °C water bath
Warm ca 5 min in a 37 °C water bath
Add 1 mL microsomal suspension to each test tube
Incubate at 37 °C for 15 minutes in shaking water bath
Add 2 mL of CH2Cl2 to quench enzyme reaction; vortex ca 5 s, place on ice; vortex 20-25 s, centrifuge for 10 min at a setting of 162 x g (avg)
Remove and discard organic layer. Add 2 mL CH2Cl2 to test tube containing aqueous layer; vortex for ca 30 s; centrifuge for 10 min at a setting of 162 x g (avg)
Remove and discard organic layer. Add 2 mL CH2Cl2 to test tube containing aqueous layer; vortex for ca 30 s; centrifuge for 10 min at a setting of 162 x g (avg)
Remove and discard organic layer. Transfer aqueous layer to a vial with cap; transfer duplicate 0.5 mL aliquots to LSC vials; add 10 mL scintillation cocktail, count in LSC

Appendix B

Assay Procedure


Combine in a test tube
(concentrations are final in a 2 mL volume):
0.1 mL propylene glycol
100 nM [3]ASDN (0.1 µCi)
0.3 mM NADPH
20 µL test substance solution or vehicle
Dilute to 1 mL total volume in phosphate buffer, pH 7.4
Dilute Microsomes to ca 0.025 mg/mL
Warm ca 5 min in a 37 °C water bath
Warm ca 5 min in a 37 °C water bath
Add 1 mL microsomal suspension to each test tube
Incubate at 37 °C for 15 minutes in shaking water bath
Add 2 mL of CH2Cl2 to quench enzyme reaction; vortex ca 5 s, place on ice; vortex 20-25 s, centrifuge for 10 min at a setting of 162 x g (avg)
Remove and discard organic layer. Add 2 mL CH2Cl2 to test tube containing aqueous layer; vortex for ca 30 s; centrifuge for 10 min at a setting of 162 x g (avg)
Remove and discard organic layer. Add 2 mL CH2Cl2 to test tube containing aqueous layer; vortex for ca 30 s; centrifuge for 10 min at a setting of 162 x g (avg)
Remove and discard organic layer. Transfer aqueous layer to a vial with cap; transfer duplicate 0.5 mL aliquots to LSC vials; add 10 mL scintillation cocktail, count in LSC

Appendix C

[^{3}H]ASDN Purity Assessment Report (RTI International)

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:

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).

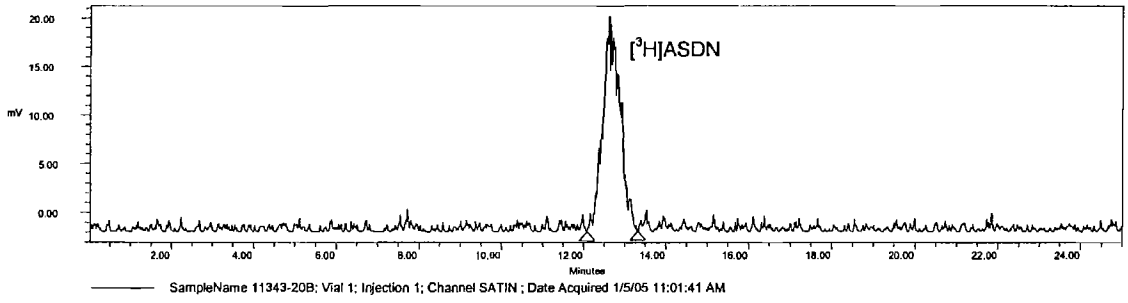
The radiochemical purity of the [^{3}H]ASDN (1:100 dilution in ethanol) was determined using high performance liquid chromatography (HPLC) and liquid scintillation counting. The HPLC system consists of a Waters 2690 Separations Module, a Waters 2487 Dual λ Absorbance Detector and a β-RAM Model 3 flow-through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

Individual Replicate Spreadsheets

Aromatase Assay Spreadsheet

Assay Date	7/20/2005	Test Chemical ID	Chem 1	# Concentrations tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Placental	Microsome ID IVT	

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	28105.1	1405255	
2	0.0198	29726.77	1501352	
3	0.0200	30277.09	1513855	
4	0.0200	30802.76	1540138	
5	0.0202	30821.5	1525817	
			Average DPM/g soln	1497283
			SD	53411
			CV	3.57
			µCi/g soln	0.674

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

ASDN solution	mg ASDN added	(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	8.2218 g
Mass of dilution B used in substrate prep	4.6028 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.571025 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00763 µg/g soln.
µg/g soln.

a. µCi/g soln	0.674
b. Specific activity of [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.

= 0.571025 + 0.00763
= 0.578660 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.166 µCi/µg ASDN

741060 dpm/nmol

Test

Assay Date 7/20/2005 Chemical ID Chem 1 tested 8

ID JG Replicate # Rep 1 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.534	0.323	0.150	0.090	0.054	0.040	0.048	25	100	
	0.540	0.328	0.155	0.096	0.055	0.048	0.067			
	0.548	0.317	0.152	0.098	0.052		0.029			
Samples:										
	0.053	0.281	0.092							
	0.054	0.284	0.092							
			0.092							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.541	0.493	0.0486
0.125	100	200	0.00013	200	0.0250	0.323	0.275	0.0273
0.05	200	1000	0.00005	200	0.0100	0.152	0.104	0.0107
0.025	100	1000	0.00003	200	0.0050	0.095	0.047	0.0051
0.01	40	1000	0.00001	200	0.0020	0.054	0.006	0.0011
0.005	20	1000	0.00001	200	0.0010	0.044	-0.004	0.0002

Blank 0.048

r^2= 0.995
m= 0.097
b= 0.001

Variables	Regression results	
m, b	0.097	0.001
se_m, se_b	0.004	0.001
r , se_y	0.995	0.002
F, df	769	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	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
0.053	0.005	0.001	200	1	1	0.000	0.000	0.005
0.054	0.006	0.001	200	1	1	0.000		
				1	1			
0.281	0.233	0.023	200	1	1	0.000	0.000	0.117
0.284	0.236	0.024	200	1	1	0.000		
0.092	0.044	0.005	200	218	70018	0.008	0.008	7.801
0.092	0.044	0.005	200	218	70018	0.008		
0.092	0.044	0.005	200	218	70018	0.008		

Assay Date	7/20/2005	Test Chemical ID	Chem 1	tested	8
ID	JG	Replicate #	Rep 1	Microsome type	Placental

Microsome ID IVT

									Total volume of	
Standards:		0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
		0.323	0.150	0.090	0.054	0.040	0.048	25	100	
		0.328	0.155	0.096	0.055	0.048	0.067			
		0.317	0.152	0.098	0.052		0.029			

Samples:	10	100	microsomes
	0.053	0.281	0.092
	0.054	0.284	0.092
			0.092

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0	200	200	0.00025	200	0.0500			
0.125	100	200	0.00013	200	0.0250	0.323	0.275	0.0249
0.05	200	1000	0.00005	200	0.0100	0.152	0.104	0.0102
0.025	100	1000	0.00003	200	0.0050	0.095	0.047	0.0053
0.01	40	1000	0.00001	200	0.0020	0.054	0.006	0.0017
0.005	20	1000	0.00001	200	0.0010	0.044	-0.004	0.0009

Variables		Regression results		
m, b		0.086	0.001	
se_m, se_b		0.001	0.000	
r , se_y		0.999	0.000	
F, df		5274	3	
ss_{reg}, ss_{resid}		0.000	0.000	

Regression results are calculated using the function LINEST

Blank	0.048	r^2=	0.999
		m=	0.086
		b=	0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol. microsome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.053	0.005	0.002	200	1	1	0.000	0.000	0.008
10	0.054	0.006	0.002	200	1	1	0.000		
10					1	1			
100	0.281	0.233	0.021	200	1	1	0.000	0.000	0.107
100	0.284	0.236	0.022	200	1	1	0.000		
100									
	0.092	0.044	0.005	200	218	70018	0.008	0.008	8.089
	0.092	0.044	0.005	200	218	70018	0.008		
	0.092	0.044	0.005	200	218	70018	0.008		

Assay Date	7/20/2005	Test Chemical ID	Chem 1	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	IVT

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID
Samples:									

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	7/20/2005	Chemical ID	Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.183486	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.183486	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.089
Protein Concentration (dilution added to assay, mg/mL):	0.025185

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	7/20/2005	Test Chemical ID	Chem 1	# Concentrations tested		S Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	2587.15	5174.3	5168.4	10336.8	0.1	149728	6.90	10231	0.0138	1	0.013	15	0.0365
			0.5	2	2581.25	5162.5			0.1					1		15	
	2	2	0.5	1	2417.75	4835.5	4854.97	9709.94	0.1	149728	6.49	9604	0.0130	1	0.013	15	0.0343
			0.5	2	2437.22	4874.44			0.1					1		15	
	3	2	0.5	1	2216.52	4433.04	4416.5	8833	0.1	149728	5.90	8727	0.0118	1	0.013	15	0.0312
			0.5	2	2199.98	4399.96			0.1					1		15	
	4	2	0.5	1	2117.94	4235.88	4277.32	8554.64	0.1	149728	5.71	8449	0.0114	1	0.013	15	0.0302
			0.5	2	2159.38	4318.76			0.1					1		15	
Background control	1	2	0.5	1	22.67	45.34	48.85	97.7	0.1	149728	0.07	-8	0.0000	1	0.013	15	0.0000
			0.5	2	26.18	52.36			0.1					1		15	
	2	2	0.5	1	22.31	44.62	45.03	90.06	0.1	149728	0.06	-15	0.0000	1	0.013	15	-0.0001
			0.5	2	22.72	45.44			0.1					1		15	
	3	2	0.5	1	22.12	44.24	57.18	114.36	0.1	149728	0.08	9	0.0000	1	0.013	15	0.0000
			0.5	2	35.06	70.12			0.1					1		15	
	4	2	0.5	1	32.86	65.72	60	120	0.1	149728	0.08	14	0.0000	1	0.013	15	0.0001
			0.5	2	27.14	54.28			0.1					1		15	
Positive control	1	2	0.5	1	1276.8	2553.6	2566.96	5133.92	0.1	149728	3.43	5028	0.0068	1	0.013	15	0.0180
			0.5	2	1290.16	2580.32			0.1					1		15	
	2	2	0.5	1	1209.76	2419.52	2454.79	4909.58	0.1	149728	3.28	4804	0.0065	1	0.013	15	0.0172
			0.5	2	1245.03	2490.06			0.1					1		15	
	3	2	0.5	1	1158.28	2316.56	2339.86	4679.72	0.1	149728	3.13	4574	0.0062	1	0.013	15	0.0163
			0.5	2	1181.58	2363.16			0.1					1		15	
	4	2	0.5	1	1178.1	2356.2	2324.13	4648.26	0.1	149728	3.10	4543	0.0061	1	0.013	15	0.0162
			0.5	2	1146.03	2292.06			0.1					1		15	
Negative Control	1	2	0.5	1	2373.88	4747.76	4783.82	9567.64	0.1	149728	6.39	9462	0.0128	1	0.013	15	0.0338
			0.5	2	2409.94	4819.88			0.1					1		15	
	2	2	0.5	1	2350.63	4701.26	4622.15	9244.3	0.1	149728	6.17	9139	0.0123	1	0.013	15	0.0326
			0.5	2	2271.52	4543.04			0.1					1		15	
	3	2	0.5	1	2197.45	4394.9	4407.36	8814.72	0.1	149728	5.89	8709	0.0118	1	0.013	15	0.0311
			0.5	2	2209.91	4419.82			0.1					1		15	
	4	2	0.5	1	2110.34	4220.68	4227.35	8454.7	0.1	149728	5.65	8349	0.0113	1	0.013	15	0.0298
			0.5	2	2117.01	4234.02			0.1					1		15	
Chem 1	1-1	2	0.5	1	81.91	163.82	187.55	375.1	0.1	149728	0.25	270	0.0004	1	0.013	15	0.0010
			0.5	2	105.64	211.28			0.1					1		15	
	1-2	2	0.5	1	109.9	219.8	215.83	431.66	0.1	149728	0.29	326	0.0004	1	0.013	15	0.0012
			0.5	2	105.93	211.86			0.1					1		15	
	1-3	2	0.5	1	111.22	222.44	219.96	439.92	0.1	149728	0.29	334	0.0005	1	0.013	15	0.0012
			0.5	2	108.74	217.48			0.1					1		15	
	2-1	2	0.5	1	898.94	1797.88	1805.62	3611.24	0.1	149728	2.41	3506	0.0047	1	0.013	15	0.0125
			0.5	2	906.68	1813.36			0.1					1		15	
	2-2	2	0.5	1	901.63	1803.26	1802.66	3605.32	0.1	149728	2.41	3500	0.0047	1	0.013	15	0.0125
			0.5	2	901.03	1802.06			0.1					1		15	
	2-3	2	0.5	1	908.04	1816.08	1790.25	3580.5	0.1	149728	2.39	3475	0.0047	1	0.013	15	0.0124
			0.5	2	882.21	1764.42			0.1					1		15	
	3-1	2	0.5	1	2058.49	4116.98	4104.36	8208.72	0.1	149728	5.48	8103	0.0109	1	0.013	15	0.0289
			0.5	2	2045.87	4091.74			0.1					1		15	
	3-2	2	0.5	1	2007.23	4014.46	4050.13	8100.26	0.1	149728	5.41	7995	0.0108	1	0.013	15	0.0286
			0.5	2	2042.9	4085.8			0.1					1		15	
	3-3	2	0.5	1	1994.72	3989.44	4013	8026	0.1	149728	5.36	7920	0.0107	1	0.013	15	0.0283
			0.5	2	2018.28	4036.56			0.1					1		15	
	4-1	2	0.5	1	2290.21	4580.42	4466.66	8933.32	0.1	149728	5.97	8828	0.0119	1	0.013	15	0.0315
			0.5	2	2176.45	4352.9			0.1					1		15	
	4-2	2	0.5	1	2210.76	4421.52	4563.16	9126.32	0.1	149728	6.10	9021	0.0122	1	0.013	15	0.0322
			0.5	2	2352.4	4704.8			0.1					1		15	
	4-3	2	0.5	1	2306.8	4613.6	4687.84	9375.68	0.1	149728	6.26	9270	0.0125	1	0.013	15	0.0331
			0.5	2	2381.04	4762.08			0.1					1		15	
	5-1	2	0.5	1	2306.93	4613.86	4623.18	9246.36	0.1	149728	6.18	9141	0.0123	1	0.013	15	0.0327
			0.5	2	2316.25	4632.5			0.1					1		15	
	5-2	2	0.5	1	2434.95	4869.9	4813.98	9627.96	0.1	149728	6.43	9522	0.0128	1	0.013	15	0.0340
			0.5	2	2379.03	4758.06			0.1					1		15	
	5-3	2	0.5	1	2389	4778	4820.5	9641	0.1	149728	6.44	9535	0.0129	1	0.013	15	0.0341
			0.5	2	2431.5	4863			0.1					1		15	
	6-1	2	0.5	1	2499.03	4999.06	5005.21	10010.42	0.1	149728	6.69	9905	0.0134	1	0.013	15	0.0354
			0.5	2	2506.18	5012.36			0.1					1		15	
	6-2	2	0.5	1	2509.68	5019.36	4977.74	9955.48	0.1	149728	6.65	9850	0.0133	1	0.013	15	0.0352
			0.5	2	2468.06	4936.12			0.1					1		15	
	6-3	2	0.5	1	2422.87	4845.74	4880.69	9761.38	0.1	149728	6.52	9656	0.0130	1	0.013	15	0.0345
			0.5	2	2457.82	4915.64			0.1					1		15	
	7-1	2	0.5	1	2505.36	5010.72	5013.74	10027.48	0.1	149728	6.70	9922	0.0134	1	0.013	15	0.0354
			0.5	2	2508.38	5016.76			0.1					1		15	
	7-2	2	0.5	1	2450.2	4900.4	4855.34	9710.68	0.1	149728	6.49	9605	0.0130	1	0.013	15	0.0343
			0.5	2	2405.14	4810.28			0.1					1		15	
	7-3	2	0.5	1	2426.76	4853.52	4765.23	9530.46	0.1	149728	6.37	9425	0.0127	1	0.013	15	0.0337
			0.5	2	2338.47	4676.94			0.1					1		15	
	8-1	2	0.5	1	2493.05	4986.1	4926.78	9853.56	0.1	149728	6.58	9748	0.0132	1	0.013	15	0.0348
			0.5	2	2433.73	4867.46			0.1					1		15	
	8-2	2	0.5	1	2488.3	4976.6	5003.25	10006.5	0.1	149728	6.68	9901	0.0134	1	0.013	15	0.0354
			0.5	2	2514.95	5029.9			0.1					1		15	
	8-3	2	0.5	1	2377.06	4754.12	4745	9490	0.1	149728	6.34	9384	0.0127	1	0.013	15	0.0335
			0.5	2	2367.94	4735.88			0.1					1		15	

Assay Date	7/20/2005	ID	Chem 1	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0354	0.0016
Full activity	End	0.0307	0.0007
Full activity	Overall	0.0331	0.0029
Background	Beginning	0.0000	1.92971E-05
Background	End	0.0000	1.42455E-05
Background	Overall	0.0000	5.00074E-05
Positive	Beginning	0.0176	0.0006
Positive	End	0.0163	0.0001
Positive	Overall	0.0169	0.0008
Negative	Beginning	0.0332	0.0008
Negative	End	0.0305	0.0009
Negative	Overall	0.0318	0.0017

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Chem 1	1	1	1.00E-03	-3.00	0.0010
Chem 1	1	2	1.00E-03	-3.00	0.0012
Chem 1	1	3	1.00E-03	-3.00	0.0012
Chem 1	2	1	1.00E-04	-4.00	0.0125
Chem 1	2	2	1.00E-04	-4.00	0.0125
Chem 1	2	3	1.00E-04	-4.00	0.0124
Chem 1	3	1	1.00E-05	-5.00	0.0289
Chem 1	3	2	1.00E-05	-5.00	0.0286
Chem 1	3	3	1.00E-05	-5.00	0.0283
Chem 1	4	1	1.00E-06	-6.00	0.0315
Chem 1	4	2	1.00E-06	-6.00	0.0322
Chem 1	4	3	1.00E-06	-6.00	0.0331
Chem 1	5	1	1.00E-07	-7.00	0.0327
Chem 1	5	2	1.00E-07	-7.00	0.0340
Chem 1	5	3	1.00E-07	-7.00	0.0341
Chem 1	6	1	1.00E-08	-8.00	0.0354
Chem 1	6	2	1.00E-08	-8.00	0.0352
Chem 1	6	3	1.00E-08	-8.00	0.0345
Chem 1	7	1	1.00E-09	-9.00	0.0354
Chem 1	7	2	1.00E-09	-9.00	0.0343
Chem 1	7	3	1.00E-09	-9.00	0.0337
Chem 1	8	1	1.00E-10	-10.00	0.0348
Chem 1	8	2	1.00E-10	-10.00	0.0354
Chem 1	8	3	1.00E-10	-10.00	0.0335

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-3.00	2.91	3.52	3.61
2	-4.00	37.89	37.82	37.55
3	-5.00	87.57	86.40	85.60
4	-6.00	95.40	97.49	100.18
5	-7.00	98.79	102.91	103.05
6	-8.00	107.04	106.45	104.35
7	-9.00	107.23	103.81	101.86
8	-10.00	105.35	107.00	101.42

Aromatase Assay Spreadsheet

Assay Date	7/22/2005	Test Chemical ID	Chem 1	# Concentrations tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0197	26770.4	1358904	
2	0.0201	27781.05	1382142	
3	0.0200	28602.94	1430147	
4	0.0204	29119.98	1427450	
5	0.0202	29126.51	1441906	
			Average DPM/g soln	1408110
			SD	35698
			CV	2.54
			µCi/g soln	0.634

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

ASDN solution	mg ASDN added	(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	8.2025 g
Mass of dilution B used in substrate prep	4.5916 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.582172 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00718 µg/g soln.
µg/g soln.

a. µCi/g soln	0.634
b. Specific activity of [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.

= 0.582172 + 0.00718
= 0.589352 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.076 µCi/µg ASDN

684281 dpm/nmol

Assay Date 7/22/2005 Test Chemical ID Chem 1 tested 8

ID JG Replicate # Rep 2 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.575	0.346	0.163	0.104	0.053	0.044	0.033	25		
	0.583	0.352	0.168	0.099	0.053	0.044	0.032			
	0.583	0.352	0.168	0.102	0.059	0.046	0.033			

Samples:	10	100	microsomes
	0.058	0.291	0.101
	0.059	0.292	0.101
			0.101

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.580	0.548	0.0483
0.125	100	200	0.00013	200	0.0250	0.350	0.317	0.0275
0.05	200	1000	0.00005	200	0.0100	0.166	0.134	0.0110
0.025	100	1000	0.00003	200	0.0050	0.102	0.070	0.0052
0.01	40	1000	0.00001	200	0.0020	0.055	0.023	0.0009
0.005	20	1000	0.00001	200	0.0010	0.044	0.012	0.0000
		Blank		0.032				

Variables	Regression results	
m, b	0.090	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	583	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

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/µL Prep.	average mg/µL	mg/mL
10	0.058	0.025	0.001	200	1	1	0.000	0.000	0.006
10	0.059	0.027	0.001	200	1	1	0.000		
10					1	1			
100	0.291	0.259	0.022	200	1	1	0.000	0.000	0.111
100	0.292	0.260	0.022	200	1	1	0.000		
100									
	0.101	0.069	0.005	200	218	70018	0.008	0.008	8.180
	0.101	0.068	0.005	200	218	70018	0.008		
	0.101	0.068	0.005	200	218	70018	0.008		

Assay Date 7/22/2005 Test Chemical ID Chem 1 tested 8

ID JG Replicate # Rep 2 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.575	0.346	0.163	0.104	0.053	0.044	0.033	25	100	
	0.583	0.352	0.168	0.099	0.053	0.044	0.032			
	0.583	0.352	0.168	0.102	0.059	0.046	0.033			

Samples:	10	100	microsomes
	0.058	0.291	0.101
	0.059	0.292	0.101
			0.101

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.580	0.548	0.0429
0.125	100	200	0.00013	200	0.0250	0.350	0.317	0.0248
0.05	200	1000	0.00005	200	0.0100	0.166	0.134	0.0104
0.025	100	1000	0.00003	200	0.0050	0.102	0.070	0.0053
0.01	40	1000	0.00001	200	0.0020	0.055	0.023	0.0016
0.005	20	1000	0.00001	200	0.0010	0.044	0.012	0.0008

Variables	Regression results		
m, b	0.079	0.000	
se_m, se_b	0.002	0.000	
r , se_y	0.999	0.000	
F, df	2437	3	
ss_{reg}, ss_{resid}	0.000	0.000	

Regression results are calculated using the function LINEST

Blank 0.032

r^2= 0.999
m= 0.079
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.058	0.025	0.002	200	1	1	0.000	0.000	0.010
10	0.059	0.027	0.002	200	1	1	0.000		
10					1	1			
100	0.291	0.259	0.020	200	1	1	0.000	0.000	0.101
100	0.292	0.260	0.020	200	1	1	0.000		
100									
	0.101	0.069	0.005	200	218	70018	0.008	0.008	8.442
	0.101	0.068	0.005	200	218	70018	0.008		
	0.101	0.068	0.005	200	218	70018	0.008		

Assay Date	7/22/2005	Test Chemical ID	Chem 1	tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	IVT

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID
Samples:									

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	7/22/2005	Chemical ID	Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 2

Microsome Dilution Details

Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.183486	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.183486	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.442
Protein Concentration (dilution added to assay, mg/mL):	0.026284

Test Chemical Concentrations

Level	Final Concentration (M)
1	1.00E-03
2	5.00E-04
3	1.00E-04
4	5.00E-05
5	1.00E-05
6	1.00E-06
7	1.00E-07
8	1.00E-09

Assay Date	7/22/2005	Test Chemical ID	Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3778.35	7556.7	7554.11	15108.22	0.1	140811	10.73	14990	0.0219	1	0.013	15	0.0556
			0.5	2	3775.76	7551.52			0.1					1		15	
	2	2	0.5	1	3543.42	7086.84	7151.86	14303.72	0.1	140811	10.16	14186	0.0207	1	0.013	15	0.0526
			0.5	2	3608.44	7216.88			0.1					1		15	
	3	2	0.5	1	3316.91	6633.82	6668.09	13336.18	0.1	140811	9.47	13218	0.0193	1	0.013	15	0.0490
			0.5	2	3351.18	6702.36			0.1					1		15	
	4	2	0.5	1	3207.09	6414.18	6468.91	12933.82	0.1	140811	9.19	12816	0.0187	1	0.013	15	0.0475
			0.5	2	3259.82	6519.64			0.1					1		15	
Background control	1	2	0.5	1	32.36	64.72	60.34	120.68	0.1	140811	0.09	3	0.0000	1	0.013	15	0.0000
			0.5	2	27.98	55.96			0.1					1		15	
	2	2	0.5	1	27.53	55.06	50.77	101.54	0.1	140811	0.07	-17	0.0000	1	0.013	15	-0.0001
			0.5	2	23.24	46.48			0.1					1		15	
	3	2	0.5	1	30.51	61.02	60.17	120.34	0.1	140811	0.09	2	0.0000	1	0.013	15	0.0000
			0.5	2	29.66	59.32			0.1					1		15	
	4	2	0.5	1	28.95	57.9	65.01	130.02	0.1	140811	0.09	12	0.0000	1	0.013	15	0.0000
			0.5	2	36.06	72.12			0.1					1		15	
Positive control	1	2	0.5	1	1696.7	3393.4	3409.85	6817.7	0.1	140811	4.84	6700	0.0098	1	0.013	15	0.0248
			0.5	2	1712.15	3424.3			0.1					1		15	
	2	2	0.5	1	1680.62	3361.24	3391.87	6783.74	0.1	140811	4.82	6666	0.0097	1	0.013	15	0.0247
			0.5	2	1711.25	3422.5			0.1					1		15	
	3	2	0.5	1	1550.59	3101.18	3079.12	6158.24	0.1	140811	4.37	6040	0.0088	1	0.013	15	0.0224
			0.5	2	1528.53	3057.06			0.1					1		15	
	4	2	0.5	1	1480.78	2961.56	2969.24	5938.48	0.1	140811	4.22	5820	0.0085	1	0.013	15	0.0216
			0.5	2	1488.46	2976.92			0.1					1		15	
Negative Control	1	2	0.5	1	3739.26	7478.52	7435.19	14870.38	0.1	140811	10.56	14752	0.0216	1	0.013	15	0.0547
			0.5	2	3695.93	7391.86			0.1					1		15	
	2	2	0.5	1	3656.63	7313.26	7336.54	14673.08	0.1	140811	10.42	14555	0.0213	1	0.013	15	0.0540
			0.5	2	3679.91	7359.82			0.1					1		15	
	3	2	0.5	1	3452.83	6905.66	6827	13654	0.1	140811	9.70	13536	0.0198	1	0.013	15	0.0502
			0.5	2	3374.17	6748.34			0.1					1		15	
	4	2	0.5	1	3309.79	6619.58	6591	13182	0.1	140811	9.36	13064	0.0191	1	0.013	15	0.0484
			0.5	2	3281.21	6562.42			0.1					1		15	
Chem 1	1-1	2	0.5	1	255.59	511.18	499.1	998.2	0.1	140811	0.71	880	0.0013	1	0.013	15	0.0033
			0.5	2	243.51	487.02			0.1					1		15	
	1-2	2	0.5	1	241.1	482.2	641.97	1283.94	0.1	140811	0.91	1166	0.0017	1	0.013	15	0.0043
			0.5	2	400.87	801.74			0.1					1		15	
	1-3	2	0.5	1	239.79	479.58	470.09	940.16	0.1	140811	0.67	822	0.0012	1	0.013	15	0.0030
			0.5	2	230.3	460.6			0.1					1		15	
	2-1	2	0.5	1	479.7	959.4	984.49	1968.98	0.1	140811	1.40	1851	0.0027	1	0.013	15	0.0069
			0.5	2	504.79	1009.58			0.1					1		15	
	2-2	2	0.5	1	471.42	942.84	947.28	1894.56	0.1	140811	1.35	1776	0.0026	1	0.013	15	0.0066
			0.5	2	475.86	951.72			0.1					1		15	
	2-3	2	0.5	1	472.25	944.5	952.49	1904.98	0.1	140811	1.35	1787	0.0026	1	0.013	15	0.0066
			0.5	2	480.24	960.48			0.1					1		15	
	3-1	2	0.5	1	1511.98	3023.96	3038.46	6076.92	0.1	140811	4.32	5959	0.0087	1	0.013	15	0.0221
			0.5	2	1526.48	3052.96			0.1					1		15	
	3-2	2	0.5	1	1494.42	2988.84	2999.8	5999.6	0.1	140811	4.26	5881	0.0086	1	0.013	15	0.0218
			0.5	2	1505.38	3010.76			0.1					1		15	
	3-3	2	0.5	1	1469.86	2939.72	2934.11	5868.22	0.1	140811	4.17	5750	0.0084	1	0.013	15	0.0213
			0.5	2	1464.25	2928.5			0.1					1		15	
	4-1	2	0.5	1	2159.64	4319.28	4326.83	8653.66	0.1	140811	6.15	8536	0.0125	1	0.013	15	0.0316
			0.5	2	2167.19	4334.38			0.1					1		15	
	4-2	2	0.5	1	2073.08	4146.16	4108.29	8216.58	0.1	140811	5.84	8098	0.0118	1	0.013	15	0.0300
			0.5	2	2035.21	4070.42			0.1					1		15	
	4-3	2	0.5	1	2138.97	4277.94	4304.87	8609.74	0.1	140811	6.11	8492	0.0124	1	0.013	15	0.0315
			0.5	2	2165.9	4331.8			0.1					1		15	
	5-1	2	0.5	1	3162.67	6325.34	6328.8	12657.6	0.1	140811	8.99	12539	0.0183	1	0.013	15	0.0465
			0.5	2	3166.13	6332.26			0.1					1		15	
	5-2	2	0.5	1	3158.15	6316.3	6400.05	12800.1	0.1	140811	9.09	12682	0.0185	1	0.013	15	0.0470
			0.5	2	3241.9	6483.8			0.1					1		15	
	5-3	2	0.5	1	3220.24	6440.48	6487.86	12975.72	0.1	140811	9.21	12858	0.0188	1	0.013	15	0.0477
			0.5	2	3267.62	6535.24			0.1					1		15	
	6-1	2	0.5	1	3659.61	7319.22	7477.87	14955.74	0.1	140811	10.62	14838	0.0217	1	0.013	15	0.0550
			0.5	2	3818.26	7636.52			0.1					1		15	
	6-2	2	0.5	1	3541.2	7082.4	7099.34	14198.68	0.1	140811	10.08	14081	0.0206	1	0.013	15	0.0522
			0.5	2	3558.14	7116.28			0.1					1		15	
	6-3	2	0.5	1	3592.08	7184.16	7169.41	14338.82	0.1	140811	10.18	14221	0.0208	1	0.013	15	0.0527
			0.5	2	3577.33	7154.66			0.1					1		15	
	7-1	2	0.5	1	3660.41	7320.82	7331.05	14662.1	0.1	140811	10.41	14544	0.0213	1	0.013	15	0.0539
			0.5	2	3670.64	7341.28			0.1					1		15	
	7-2	2	0.5	1	3763.07	7526.14	7538.62	15077.24	0.1	140811	10.71	14959	0.0219	1	0.013	15	0.0554
			0.5	2	3775.55	7551.1			0.1					1		15	
	7-3	2	0.5	1	3705.8	7411.6	7334.3	14668.6	0.1	140811	10.42	14550	0.0213	1	0.013	15	0.0539
			0.5	2	3628.5	7257			0.1					1		15	
	8-1	2	0.5	1	3547.11	7094.22	7100.89	14201.78	0.1	140811	10.09	14084	0.0206	1	0.013	15	0.0522
			0.5	2	3553.78	7107.56			0.1					1		15	
	8-2	2	0.5	1	3580.42	7160.84	7193.39	14386.78	0.1	140811	10.22	14269	0.0209	1	0.013	15	0.0529
			0.5	2	3612.97	7225.94			0.1					1		15	
	8-3	2	0.5	1	3169.98	6379.96	6366.97	12733.94	0.1	140811	9.04	12616	0.0184	1	0.013	15	0.0468
			0.5	2	3177.29	6354.58			0.1					1		15	

Assay Date	7/22/2005	ID	Chem 1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0541	0.0021
Full activity	End	0.0482	0.0011
Full activity	Overall	0.0512	0.0036
Background	Beginning	0.0000	5.01659E-05
Background	End	0.0000	2.53713E-05
Background	Overall	0.0000	4.42727E-05
Positive	Beginning	0.0248	0.0001
Positive	End	0.0220	0.0006
Positive	Overall	0.0234	0.0016
Negative	Beginning	0.0543	0.0005
Negative	End	0.0493	0.0012
Negative	Overall	0.0518	0.0030

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Chem 1	1	1	1.00E-03	-3.00	0.0033
Chem 1	1	2	1.00E-03	-3.00	0.0043
Chem 1	1	3	1.00E-03	-3.00	0.0030
Chem 1	2	1	5.00E-04	-3.30	0.0069
Chem 1	2	2	5.00E-04	-3.30	0.0066
Chem 1	2	3	5.00E-04	-3.30	0.0066
Chem 1	3	1	1.00E-04	-4.00	0.0221
Chem 1	3	2	1.00E-04	-4.00	0.0218
Chem 1	3	3	1.00E-04	-4.00	0.0213
Chem 1	4	1	5.00E-05	-4.30	0.0316
Chem 1	4	2	5.00E-05	-4.30	0.0300
Chem 1	4	3	5.00E-05	-4.30	0.0315
Chem 1	5	1	1.00E-05	-5.00	0.0465
Chem 1	5	2	1.00E-05	-5.00	0.0470
Chem 1	5	3	1.00E-05	-5.00	0.0477
Chem 1	6	1	1.00E-06	-6.00	0.0550
Chem 1	6	2	1.00E-06	-6.00	0.0522
Chem 1	6	3	1.00E-06	-6.00	0.0527
Chem 1	7	1	1.00E-07	-7.00	0.0539
Chem 1	7	2	1.00E-07	-7.00	0.0554
Chem 1	7	3	1.00E-07	-7.00	0.0539
Chem 1	8	1	1.00E-09	-9.00	0.0522
Chem 1	8	2	1.00E-09	-9.00	0.0529
Chem 1	8	3	1.00E-09	-9.00	0.0468

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-3.00	6.38	8.45	5.96
2	-3.30	13.41	12.87	12.95
3	-4.00	43.17	42.61	41.66
4	-4.30	61.84	58.67	61.52
5	-5.00	90.85	91.88	93.16
6	-6.00	107.50	102.02	103.03
7	-7.00	105.37	108.38	105.42
8	-9.00	102.04	103.38	91.40

Aromatase Assay Spreadsheet

Assay Date	7/26/2005	Test Chemical ID	RC1	# Concentrations tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	29133.43	1471385	
2	0.0200	30022.34	1501117	
3	0.0199	31077.71	1561694	
4	0.0200	31032.9	1551645	
5	0.0200	31207.44	1560372	
			Average DPM/g soln	1529243
			SD	40812
			CV	2.67
			µCi/g soln	0.689

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

ASDN solution	mg ASDN added	(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	8.2243 g
Mass of dilution B used in substrate prep	4.6253 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.573642 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00780 µg/g soln.
µg/g soln.

a. µCi/g soln 0.689
b. Specific activity of [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.

= 0.573642 + 0.00780
= 0.581440 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.185 µCi/µg ASDN

753259 dpm/nmol

Test

Assay Date 7/26/2005 Chemical ID RC1 tested 8

ID JG Replicate # Rep 3 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.477	0.320	0.148	0.091	0.049	0.040	0.034	25		
	0.532	0.326	0.148	0.092	0.048	0.036	0.029			
	0.524	0.319	0.148	0.094	0.054	0.036	0.029			
Samples:	10	100	microsomes							
	0.053	0.250	0.091							
	0.053	0.241	0.088							
			0.092							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.511	0.480	0.0477
0.125	100	200	0.00013	200	0.0250	0.322	0.291	0.0285
0.05	200	1000	0.00005	200	0.0100	0.148	0.117	0.0109
0.025	100	1000	0.00003	200	0.0050	0.093	0.062	0.0053
0.01	40	1000	0.00001	200	0.0020	0.050	0.019	0.0010
0.005	20	1000	0.00001	200	0.0010	0.037	0.006	-0.0003

Blank 0.031

r^2= 0.988
m= 0.101
b= -0.001

Variables	Regression results	
m, b	0.101	-0.001
se_m, se_b	0.006	0.001
r , se_y	0.988	0.002
F, df	336	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	µSOMES prep. (µL)	Final vol. Diluted uscmes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.053	0.022	0.001	200	1	1	0.000	0.000	0.006
10	0.053	0.022	0.001	200	1	1	0.000		
10					1	1			
100	0.250	0.219	0.021	200	1	1	0.000	0.000	0.104
100	0.241	0.210	0.020	200	1	1	0.000		
100									
	0.091	0.060	0.005	200	218	70018	0.008	0.008	8.135
	0.088	0.058	0.005	200	218	70018	0.008		
	0.092	0.061	0.005	200	218	70018	0.008		

Test

Assay Date 7/26/2005 Chemical ID RC1 tested 8

ID JG Replicate # Rep 3 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.477	0.320	0.148	0.091	0.049	0.040	0.034	25	100	
	0.532	0.326	0.148	0.092	0.048	0.036	0.029			
	0.524	0.319	0.148	0.094	0.054	0.036	0.029			

Samples:	10	100	microsomes
	0.053	0.250	0.091
	0.053	0.241	0.088
			0.092

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.511	0.480	0.0409
0.125	100	200	0.00013	200	0.0250	0.322	0.291	0.0249
0.05	200	1000	0.00005	200	0.0100	0.148	0.117	0.0101
0.025	100	1000	0.00003	200	0.0050	0.093	0.062	0.0054
0.01	40	1000	0.00001	200	0.0020	0.050	0.019	0.0018
0.005	20	1000	0.00001	200	0.0010	0.037	0.006	0.0007

Blank 0.031

r^2= 0.999
m= 0.085
b= 0.000

Variables	Regression results	
m, b	0.085	0.000
se_m, se_b	0.001	0.000
r , se_y	0.999	0.000
F, df	3586	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	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.053	0.022	0.002	200	1	1	0.000	0.000	0.010
10	0.053	0.022	0.002	200	1	1	0.000		
10					1	1			
100	0.250	0.219	0.019	200	1	1	0.000	0.000	0.092
100	0.241	0.210	0.018	200	1	1	0.000		
100									
	0.091	0.060	0.005	200	218	70018	0.008	0.008	8.436
	0.088	0.058	0.005	200	218	70018	0.008		
	0.092	0.061	0.005	200	218	70018	0.009		

Assay Date	7/26/2005	Test Chemical ID	RC1	tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	IVT

								Protein stock (mg/10 mL)	Protein stock ID
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk		

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	7/26/2005	Chemical ID	RC1	# Concentrations tested	8	Microsome type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 3

Microsome Dilution Details

Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.1835	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.1835	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.436
Protein Concentration (dilution added to assay, mg/mL):	0.026265

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	5.00E-04
3	1.00E-04
4	5.00E-05
5	1.00E-05
6	1.00E-06
7	1.00E-07
8	1.00E-09

Assay Date	7/26/2005	Test Chemical ID	RC1	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3993.49	7986.98	8156.66	16313.3	0.1	152924	10.67	16189	0.0215	1	0.013	15	0.0545
			0.5	2	4163.16	8326.32			0.1					1		15	
	2	2	0.5	1	3881.95	7763.9	7738.56	15477.12	0.1	152924	10.12	15352	0.0204	1	0.013	15	0.0517
			0.5	2	3856.61	7713.22			0.1					1		15	
	3	2	0.5	1	3898.39	7796.78	7841.1	15682.2	0.1	152924	10.25	15558	0.0207	1	0.013	15	0.0524
			0.5	2	3942.71	7885.42			0.1					1		15	
	4	2	0.5	1	3640.61	7281.22	7329.44	14658.88	0.1	152924	9.59	14534	0.0193	1	0.013	15	0.0490
			0.5	2	3688.83	7377.66			0.1					1		15	
Background control	1	2	0.5	1	30.41	60.82	62.49	124.98	0.1	152924	0.08	0	0.0000	1	0.013	15	0.0000
			0.5	2	32.08	64.16			0.1					1		15	
	2	2	0.5	1	34.78	69.56	58.59	117.18	0.1	152924	0.08	-7	0.0000	1	0.013	15	0.0000
			0.5	2	23.81	47.62			0.1					1		15	
	3	2	0.5	1	29.35	58.7	62.09	124.18	0.1	152924	0.08	0	0.0000	1	0.013	15	0.0000
			0.5	2	32.74	65.48			0.1					1		15	
	4	2	0.5	1	32.24	64.48	66.18	132.36	0.1	152924	0.09	8	0.0000	1	0.013	15	0.0000
			0.5	2	33.94	67.88			0.1					1		15	
Positive control	1	2	0.5	1	1864.04	3728.08	3718.36	7436.72	0.1	152924	4.86	7312	0.0097	1	0.013	15	0.0246
			0.5	2	1854.32	3708.64			0.1					1		15	
	2	2	0.5	1	1763.23	3526.46	3601.01	7202.02	0.1	152924	4.71	7077	0.0094	1	0.013	15	0.0238
			0.5	2	1837.78	3675.56			0.1					1		15	
	3	2	0.5	1	1764.24	3528.48	3539.77	7079.54	0.1	152924	4.63	6955	0.0092	1	0.013	15	0.0234
			0.5	2	1775.53	3551.06			0.1					1		15	
	4	2	0.5	1	1681.04	3362.08	3291.01	6582.02	0.1	152924	4.30	6457	0.0086	1	0.013	15	0.0218
			0.5	2	1609.97	3219.94			0.1					1		15	
Negative Control	1	2	0.5	1	4029.26	8058.52	8050.41	16100.82	0.1	152924	10.53	15976	0.0212	1	0.013	15	0.0538
			0.5	2	4021.15	8042.3			0.1					1		15	
	2	2	0.5	1	3884.72	7769.44	7892.63	15785.26	0.1	152924	10.32	15661	0.0208	1	0.013	15	0.0528
			0.5	2	4007.81	8015.82			0.1					1		15	
	3	2	0.5	1	3491.77	6983.54	6987.1	13974.2	0.1	152924	9.14	13850	0.0184	1	0.013	15	0.0467
			0.5	2	3495.33	6990.66			0.1					1		15	
	4	2	0.5	1	3376.31	6752.62	6913.57	13827.14	0.1	152924	9.04	13702	0.0182	1	0.013	15	0.0462
			0.5	2	3537.26	7074.52			0.1					1		15	
RC1	1-1	2	0.5	1	238.16	476.32	463.22	926.44	0.1	152924	0.61	802	0.0011	1	0.013	15	0.0027
			0.5	2	225.06	450.12			0.1					1		15	
	1-2	2	0.5	1	246.55	493.1	490.04	980.08	0.1	152924	0.64	855	0.0011	1	0.013	15	0.0029
			0.5	2	243.49	486.98			0.1					1		15	
	1-3	2	0.5	1	273.03	546.06	540.78	1081.56	0.1	152924	0.71	957	0.0013	1	0.013	15	0.0032
			0.5	2	267.75	535.5			0.1					1		15	
	2-1	2	0.5	1	508.10	1016.2	1029.14	2058.28	0.1	152924	1.35	1934	0.0026	1	0.013	15	0.0065
			0.5	2	521.04	1042.08			0.1					1		15	
	2-2	2	0.5	1	521.03	1042.06	1035.26	2070.52	0.1	152924	1.35	1946	0.0026	1	0.013	15	0.0066
			0.5	2	514.23	1028.46			0.1					1		15	
	2-3	2	0.5	1	466.44	932.88	921.04	1842.08	0.1	152924	1.20	1717	0.0023	1	0.013	15	0.0058
			0.5	2	454.60	909.2			0.1					1		15	
	3-1	2	0.5	1	1512.43	3024.86	3059.45	6118.9	0.1	152924	4.00	5994	0.0080	1	0.013	15	0.0202
			0.5	2	1547.02	3094.04			0.1					1		15	
	3-2	2	0.5	1	1597.17	3194.34	3199.78	6399.56	0.1	152924	4.18	6275	0.0083	1	0.013	15	0.0211
			0.5	2	1602.61	3205.22			0.1					1		15	
	3-3	2	0.5	1	1584.19	3168.38	3167.86	6335.72	0.1	152924	4.14	6211	0.0082	1	0.013	15	0.0209
			0.5	2	1583.67	3167.34			0.1					1		15	
	4-1	2	0.5	1	2164.11	4328.22	4325.79	8651.58	0.1	152924	5.66	8527	0.0113	1	0.013	15	0.0287
			0.5	2	2161.68	4323.36			0.1					1		15	
	4-2	2	0.5	1	2277.89	4555.78	4397.39	8794.78	0.1	152924	5.75	8670	0.0115	1	0.013	15	0.0292
			0.5	2	2119.50	4239			0.1					1		15	
	4-3	2	0.5	1	2219.33	4438.66	4496.52	8993.04	0.1	152924	5.88	8868	0.0118	1	0.013	15	0.0299
			0.5	2	2277.19	4554.38			0.1					1		15	
	5-1	2	0.5	1	3416.34	6832.68	6841.91	13683.62	0.1	152924	8.95	13559	0.0180	1	0.013	15	0.0457
			0.5	2	3425.47	6850.94			0.1					1		15	
	5-2	2	0.5	1	3482.80	6965.6	6954.89	13909.78	0.1	152924	9.10	13785	0.0183	1	0.013	15	0.0465
			0.5	2	3472.09	6944.18			0.1					1		15	
	5-3	2	0.5	1	3464.76	6929.52	6878.95	13757.9	0.1	152924	9.00	13633	0.0181	1	0.013	15	0.0459
			0.5	2	3414.19	6828.38			0.1					1		15	
	6-1	2	0.5	1	3895.70	7791.4	7800.87	15601.74	0.1	152924	10.20	15477	0.0205	1	0.013	15	0.0522
			0.5	2	3905.17	7810.34			0.1					1		15	
	6-2	2	0.5	1	3979.75	7959.5	8045.61	16091.22	0.1	152924	10.52	15967	0.0212	1	0.013	15	0.0538
			0.5	2	4065.86	8131.72			0.1					1		15	
	6-3	2	0.5	1	3939.81	7879.62	7860.51	15721.02	0.1	152924	10.28	15596	0.0207	1	0.013	15	0.0526
			0.5	2	3920.70	7841.4			0.1					1		15	
	7-1	2	0.5	1	4018.61	8037.22	8039.84	16079.68	0.1	152924	10.51	15955	0.0212	1	0.013	15	0.0538
			0.5	2	4021.23	8042.46			0.1					1		15	
	7-2	2	0.5	1	4070.12	8140.24	8143.45	16286.9	0.1	152924	10.65	16162	0.0215	1	0.013	15	0.0545
			0.5	2	4073.33	8146.66			0.1					1		15	
	7-3	2	0.5	1	3740.61	7481.22	7565.37	15130.74	0.1	152924	9.89	15006	0.0199	1	0.013	15	0.0506
			0.5	2	3824.76	7649.52			0.1					1		15	
	8-1	2	0.5	1	3799.42	7598.84	7647.24	15294.48	0.1	152924	10.00	15170	0.0201	1	0.013	15	0.0511
			0.5	2	3847.82	7695.64			0.1					1		15	
	8-2	2	0.5	1	4047.45	8094.9	8077.41	16154.82	0.1	152924	10.56	16030	0.0213	1	0.013	15	0.0540
			0.5	2	4029.96	8059.92			0.1					1		15	
	8-3	2	0.5	1	3985.76	7971.52	8014.74	16029.48	0.1	152924	10.48	15905	0.0211	1	0.013	15	0.0536
			0.5	2	4028.96	8057.96			0.1					1		15	

Assay Date	7/26/2005	ID	RC1	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0531	0.0020
Full activity	End	0.0507	0.0024
Full activity	Overall	0.0519	0.0023
Background	Beginning	0.0000	1.85849E-05
Background	End	0.0000	1.94903E-05
Background	Overall	0.0000	2.09145E-05
Positive	Beginning	0.0242	0.0006
Positive	End	0.0226	0.0012
Positive	Overall	0.0234	0.0012
Negative	Beginning	0.0533	0.0008
Negative	End	0.0464	0.0004
Negative	Overall	0.0499	0.0040

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC1	1	1	1.00E-03	-3.00	0.0027
RC1	1	2	1.00E-03	-3.00	0.0029
RC1	1	3	1.00E-03	-3.00	0.0032
RC1	2	1	5.00E-04	-3.30	0.0065
RC1	2	2	5.00E-04	-3.30	0.0066
RC1	2	3	5.00E-04	-3.30	0.0058
RC1	3	1	1.00E-04	-4.00	0.0202
RC1	3	2	1.00E-04	-4.00	0.0211
RC1	3	3	1.00E-04	-4.00	0.0209
RC1	4	1	5.00E-05	-4.30	0.0287
RC1	4	2	5.00E-05	-4.30	0.0292
RC1	4	3	5.00E-05	-4.30	0.0299
RC1	5	1	1.00E-05	-5.00	0.0457
RC1	5	2	1.00E-05	-5.00	0.0465
RC1	5	3	1.00E-05	-5.00	0.0459
RC1	6	1	1.00E-06	-6.00	0.0522
RC1	6	2	1.00E-06	-6.00	0.0538
RC1	6	3	1.00E-06	-6.00	0.0526
RC1	7	1	1.00E-07	-7.00	0.0538
RC1	7	2	1.00E-07	-7.00	0.0545
RC1	7	3	1.00E-07	-7.00	0.0506
RC1	8	1	1.00E-09	-9.00	0.0511
RC1	8	2	1.00E-09	-9.00	0.0540
RC1	8	3	1.00E-09	-9.00	0.0536

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-3.00	5.20	5.55	6.21
2	-3.30	12.55	12.63	11.15
3	-4.00	38.90	40.72	40.31
4	-4.30	55.34	56.27	57.56
5	-5.00	88.00	89.47	88.48
6	-6.00	100.45	103.62	101.22
7	-7.00	103.55	104.89	97.39
8	-9.00	98.45	104.04	103.22

Aromatase Assay Spreadsheet

Assay Date	7/20/2005	Test Chemical ID	Chem 2	# Concentrations tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.
1	0.0200	28105.1	1405255
2	0.0198	29726.77	1501352
3	0.0200	30277.09	1513855
4	0.0200	30802.76	1540138
5	0.0202	30821.5	1525817
		Average DPM/g soln	1497283
		SD	53411
		CV	3.57
		µCi/g soln	0.674

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

ASDN solution	mg ASDN added	(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	8.2218 g
Mass of dilution B used in substrate prep	4.6028 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.571025 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00763 µg/g soln.
µg/g soln.

a. µCi/g soln	0.674
b. Specific activity of [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.

= 0.571025 + 0.00763
= 0.578660 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.166 µCi/µg ASDN

741060 dpm/nmol

Assay Date	7/20/2005	Test Chemical ID	Chem 2	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.534	0.323	0.150	0.090	0.054	0.040	0.048	25		
	0.540	0.328	0.155	0.096	0.055	0.048	0.067			
	0.548	0.317	0.152	0.098	0.052		0.029			
Samples:										
	0.053	0.281	0.092							
	0.054	0.284	0.092							
			0.092							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.541	0.493	0.0486
0.125	100	200	0.00013	200	0.0250	0.323	0.275	0.0273
0.05	200	1000	0.00005	200	0.0100	0.152	0.104	0.0107
0.025	100	1000	0.00003	200	0.0050	0.095	0.047	0.0051
0.01	40	1000	0.00001	200	0.0020	0.054	0.006	0.0011
0.005	20	1000	0.00001	200	0.0010	0.044	-0.004	0.0002

Variables	Regression results	
m, b	0.097	0.001
se_m, se_b	0.004	0.001
r , se_y	0.995	0.002
F, df	769	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank 0.048

r^2= 0.995
m= 0.097
b= 0.001

A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol. usomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
0.053	0.005	0.001	200	1	1	0.000	0.000	0.005
0.054	0.006	0.001	200	1	1	0.000		
				1	1			
0.281	0.233	0.023	200	1	1	0.000	0.000	0.117
0.284	0.236	0.024	200	1	1	0.000		
0.092	0.044	0.005	200	218	70018	0.008	0.008	7.801
0.092	0.044	0.005	200	218	70018	0.008		
0.092	0.044	0.005	200	218	70018	0.008		

Assay Date 7/20/2005 Test Chemical ID Chem 2 tested 8

ID JG Replicate # Rep 1 Microsome type Placental Microsome ID IVT

Standards:	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.323	0.150	0.090	0.054	0.040	0.048	25	100	
	0.328	0.155	0.096	0.055	0.048	0.067			
	0.317	0.152	0.096	0.052		0.029			

Samples:	10	100	microsomes
	0.053	0.281	0.092
	0.054	0.284	0.092
			0.092

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0	200	200	0.00025	200	0.0500			
0.125	100	200	0.00013	200	0.0250	0.323	0.275	0.0249
0.05	200	1000	0.00005	200	0.0100	0.152	0.104	0.0102
0.025	100	1000	0.00003	200	0.0050	0.095	0.047	0.0053
0.01	40	1000	0.00001	200	0.0020	0.054	0.006	0.0017
0.005	20	1000	0.00001	200	0.0010	0.044	-0.004	0.0009

Blank 0.048

r^2= 0.999
m= 0.086
b= 0.001

Variables	Regression results	
m, b	0.086	0.001
se_m, se_b	0.001	0.000
r , se_y	0.999	0.000
F, df	5274	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	µL microsome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.053	0.005	0.002	200	1	1	0.000	0.000	0.008
10	0.054	0.006	0.002	200	1	1	0.000		
10					1	1			
100	0.281	0.233	0.021	200	1	1	0.000	0.000	0.107
100	0.284	0.236	0.022	200	1	1	0.000		
100									
	0.092	0.044	0.005	200	218	70018	0.008	0.008	8.089
	0.092	0.044	0.005	200	218	70018	0.008		
	0.092	0.044	0.005	200	218	70018	0.008		

Assay Date 7/20/2005 Test Chemical ID Chem 2 tested 8

ID JG Replicate # Rep 1 Microsome type Placental Microsome ID IVT

Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	7/20/2005	Chemical ID	Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.1835	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.1835	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.089
Protein Concentration (dilution added to assay, mg/mL):	0.025185

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	7/20/2005	Test Chemical ID	Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	2587.15	5174.3	5168.4	10336.8	0.1	149728	6.90	10231	0.0136	1	0.013	15	0.0365
			0.5	2	2581.25	5162.5			0.1					1		15	
	2	2	0.5	1	2417.75	4835.5	4854.97	9709.94	0.1	149728	6.49	9604	0.0130	1	0.013	15	0.0343
			0.5	2	2437.22	4874.44			0.1					1		15	
	3	2	0.5	1	2216.52	4433.04	4416.5	8833	0.1	149728	5.90	8727	0.0118	1	0.013	15	0.0312
			0.5	2	2199.98	4399.96			0.1					1		15	
	4	2	0.5	1	2117.94	4235.88	4277.32	8554.64	0.1	149728	5.71	8449	0.0114	1	0.013	15	0.0302
			0.5	2	2159.38	4318.76			0.1					1		15	
Background control	1	2	0.5	1	22.67	45.34	48.85	97.7	0.1	149728	0.07	-8	0.0000	1	0.013	15	0.0000
			0.5	2	26.18	52.36			0.1					1		15	
	2	2	0.5	1	22.31	44.62	45.03	90.06	0.1	149728	0.06	-15	0.0000	1	0.013	15	-0.0001
			0.5	2	22.72	45.44			0.1					1		15	
	3	2	0.5	1	22.12	44.24	57.18	114.36	0.1	149728	0.08	9	0.0000	1	0.013	15	0.0000
			0.5	2	35.06	70.12			0.1					1		15	
	4	2	0.5	1	32.86	65.72	60	120	0.1	149728	0.08	14	0.0000	1	0.013	15	0.0001
			0.5	2	27.14	54.28			0.1					1		15	
Positive control	1	2	0.5	1	1276.8	2553.6	2566.96	5133.92	0.1	149728	3.43	5028	0.0068	1	0.013	15	0.0180
			0.5	2	1290.16	2580.32			0.1					1		15	
	2	2	0.5	1	1209.76	2419.52	2454.79	4909.58	0.1	149728	3.28	4804	0.0065	1	0.013	15	0.0172
			0.5	2	1245.03	2490.06			0.1					1		15	
	3	2	0.5	1	1158.28	2316.56	2339.86	4679.72	0.1	149728	3.13	4574	0.0062	1	0.013	15	0.0163
			0.5	2	1181.58	2363.16			0.1					1		15	
	4	2	0.5	1	1178.1	2356.2	2324.13	4648.26	0.1	149728	3.10	4543	0.0061	1	0.013	15	0.0162
			0.5	2	1146.03	2292.06			0.1					1		15	
Negative Control	1	2	0.5	1	2373.88	4747.76	4783.82	9567.64	0.1	149728	6.39	9462	0.0126	1	0.013	15	0.0338
			0.5	2	2409.94	4819.88			0.1					1		15	
	2	2	0.5	1	2350.63	4701.26	4622.15	9244.3	0.1	149728	6.17	9139	0.0123	1	0.013	15	0.0326
			0.5	2	2271.52	4543.04			0.1					1		15	
	3	2	0.5	1	2197.45	4394.9	4407.36	8814.72	0.1	149728	5.89	8709	0.0118	1	0.013	15	0.0311
			0.5	2	2209.91	4419.82			0.1					1		15	
	4	2	0.5	1	2110.34	4220.68	4227.35	8454.7	0.1	149728	5.65	8349	0.0113	1	0.013	15	0.0298
			0.5	2	2117.01	4234.02			0.1					1		15	
Chem 2	1-1	2	0.5	1	28.91	57.82	58.29	116.58	0.1	149728	0.08	11	0.0000	1	0.013	15	0.0000
			0.5	2	29.38	58.76			0.1					1		15	
	1-2	2	0.5	1	37.23	74.46	64.44	128.88	0.1	149728	0.09	23	0.0000	1	0.013	15	0.0001
			0.5	2	27.21	54.42			0.1					1		15	
	1-3	2	0.5	1	29.26	58.52	57.74	115.48	0.1	149728	0.08	10	0.0000	1	0.013	15	0.0000
			0.5	2	28.48	56.96			0.1					1		15	
	2-1	2	0.5	1	95.97	191.94	197.85	395.7	0.1	149728	0.26	290	0.0004	1	0.013	15	0.0010
			0.5	2	101.88	203.76			0.1					1		15	
	2-2	2	0.5	1	102.39	204.78	209.14	418.28	0.1	149728	0.28	313	0.0004	1	0.013	15	0.0011
			0.5	2	106.75	213.5			0.1					1		15	
	2-3	2	0.5	1	117.44	234.88	224.29	448.58	0.1	149728	0.30	343	0.0005	1	0.013	15	0.0012
			0.5	2	106.85	213.7			0.1					1		15	
	3-1	2	0.5	1	186.07	372.14	383.49	766.98	0.1	149728	0.51	681	0.0009	1	0.013	15	0.0024
			0.5	2	197.42	394.84			0.1					1		15	
	3-2	2	0.5	1	201.68	403.36	380.18	760.36	0.1	149728	0.51	655	0.0009	1	0.013	15	0.0023
			0.5	2	178.5	357			0.1					1		15	
	3-3	2	0.5	1	200.26	400.52	408.14	816.28	0.1	149728	0.55	711	0.0010	1	0.013	15	0.0025
			0.5	2	207.88	415.76			0.1					1		15	
	4-1	2	0.5	1	654.3	1308.6	1319.69	2639.38	0.1	149728	1.78	2534	0.0034	1	0.013	15	0.0091
			0.5	2	665.39	1330.78			0.1					1		15	
	4-2	2	0.5	1	690.6	1381.2	1381.4	2762.8	0.1	149728	1.85	2657	0.0036	1	0.013	15	0.0095
			0.5	2	690.8	1381.6			0.1					1		15	
	4-3	2	0.5	1	661.67	1323.74	1294.37	2588.74	0.1	149728	1.73	2483	0.0034	1	0.013	15	0.0089
			0.5	2	632.5	1265			0.1					1		15	
	5-1	2	0.5	1	1782	3564	3577.9	7155.8	0.1	149728	4.78	7050	0.0095	1	0.013	15	0.0252
			0.5	2	1795.9	3591.8			0.1					1		15	
	5-2	2	0.5	1	1776.94	3553.88	3548.61	7097.22	0.1	149728	4.74	6992	0.0094	1	0.013	15	0.0250
			0.5	2	1771.67	3543.34			0.1					1		15	
	5-3	2	0.5	1	1816.06	3632.12	3637.41	7274.82	0.1	149728	4.86	7169	0.0097	1	0.013	15	0.0256
			0.5	2	1821.35	3642.7			0.1					1		15	
	6-1	2	0.5	1	2214.21	4428.42	4422.66	8845.32	0.1	149728	5.91	8740	0.0118	1	0.013	15	0.0312
			0.5	2	2208.45	4416.9			0.1					1		15	
	6-2	2	0.5	1	2299.35	4598.7	4564.67	9129.34	0.1	149728	6.10	9024	0.0122	1	0.013	15	0.0322
			0.5	2	2265.32	4530.64			0.1					1		15	
	6-3	2	0.5	1	2213.71	4427.42	4425.68	8851.36	0.1	149728	5.91	8746	0.0118	1	0.013	15	0.0312
			0.5	2	2211.97	4423.94			0.1					1		15	
	7-1	2	0.5	1	2294.18	4588.36	4543.69	9087.38	0.1	149728	6.07	8982	0.0121	1	0.013	15	0.0321
			0.5	2	2249.51	4499.02			0.1					1		15	
	7-2	2	0.5	1	2268.78	4537.56	4520	9040	0.1	149728	6.04	8934	0.0121	1	0.013	15	0.0319
			0.5	2	2251.22	4502.44			0.1					1		15	
	7-3	2	0.5	1	2286.74	4573.48	4609.03	9218.06	0.1	149728	6.16	9113	0.0123	1	0.013	15	0.0326
			0.5	2	2322.29	4644.58			0.1					1		15	
	8-1	2	0.5	1	2261.78	4523.56	4559.41	9118.82	0.1	149728	6.09	9013	0.0122	1	0.013	15	0.0322
			0.5	2	2297.63	4595.26			0.1					1		15	
	8-2	2	0.5	1	2258.92	4517.84	4458.69	8917.38	0.1	149728	5.96	8812	0.0119	1	0.013	15	0.0315
			0.5	2	2199.77	4399.54			0.1					1		15	
	8-3	2	0.5	1	2276.37	4552.74	4481.19	8962.38	0.1	149728	5.99	8857	0.0120	1	0.013	15	0.0316
			0.5	2	2204.82	4409.64			0.1					1		15	

Assay Date	7/20/2005	ID	Chem 2	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0354	0.0016
Full activity	End	0.0307	0.0007
Full activity	Overall	0.0331	0.0029
Background	Beginning	0.0000	1.92971E-05
Background	End	0.0000	1.42455E-05
Background	Overall	0.0000	5.00074E-05
Positive	Beginning	0.0176	0.0006
Positive	End	0.0163	0.0001
Positive	Overall	0.0169	0.0008
Negative	Beginning	0.0332	0.0008
Negative	End	0.0305	0.0009
Negative	Overall	0.0318	0.0017

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Chem 2	1	1	1.00E-04	-4.00	0.0000
Chem 2	1	2	1.00E-04	-4.00	0.0001
Chem 2	1	3	1.00E-04	-4.00	0.0000
Chem 2	2	1	1.00E-05	-5.00	0.0010
Chem 2	2	2	1.00E-05	-5.00	0.0011
Chem 2	2	3	1.00E-05	-5.00	0.0012
Chem 2	3	1	5.00E-06	-5.30	0.0024
Chem 2	3	2	5.00E-06	-5.30	0.0023
Chem 2	3	3	5.00E-06	-5.30	0.0025
Chem 2	4	1	1.00E-06	-6.00	0.0091
Chem 2	4	2	1.00E-06	-6.00	0.0095
Chem 2	4	3	1.00E-06	-6.00	0.0089
Chem 2	5	1	1.00E-07	-7.00	0.0252
Chem 2	5	2	1.00E-07	-7.00	0.0250
Chem 2	5	3	1.00E-07	-7.00	0.0256
Chem 2	6	1	1.00E-08	-8.00	0.0312
Chem 2	6	2	1.00E-08	-8.00	0.0322
Chem 2	6	3	1.00E-08	-8.00	0.0312
Chem 2	7	1	1.00E-09	-9.00	0.0321
Chem 2	7	2	1.00E-09	-9.00	0.0319
Chem 2	7	3	1.00E-09	-9.00	0.0326
Chem 2	8	1	1.00E-10	-10.00	0.0322
Chem 2	8	2	1.00E-10	-10.00	0.0315
Chem 2	8	3	1.00E-10	-10.00	0.0316

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-4.00	0.12	0.25	0.11
2	-5.00	3.14	3.38	3.71
3	-5.30	7.15	7.08	7.68
4	-6.00	27.38	28.72	26.84
5	-7.00	76.19	75.56	77.48
6	-8.00	94.45	97.52	94.52
7	-9.00	97.07	96.56	98.48
8	-10.00	97.41	95.23	95.72

Aromatase Assay Spreadsheet

Assay Date	7/22/2005	Test Chemical ID	Chem 2	# Concentrations tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0197	26770.4	1358904	
2	0.0201	27781.05	1382142	
3	0.0200	28602.94	1430147	
4	0.0204	29119.98	1427450	
5	0.0202	29126.51	1441906	
			Average DPM/g soln	1408110
			SD	35698
			CV	2.54
			µCi/g soln	0.634

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

ASDN solution	mg ASDN added	(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	8.2025 g
Mass of dilution B used in substrate prep	4.5916 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.582172 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00718 µg/g soln.
µg/g soln.

a. µCi/g soln	0.634
b. Specific activity of [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.

= 0.582172 + 0.00718
= 0.589352 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.076 µCi/µg ASDN

684281 dpm/nmol

Test

Assay Date 7/22/2005 Chemical ID Chem 2 tested 8

ID JG Replicate # Rep 2 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.575	0.346	0.163	0.104	0.053	0.044	0.033	25		
	0.583	0.352	0.168	0.099	0.053	0.044	0.032			
	0.583	0.352	0.168	0.102	0.059	0.046	0.033			
Samples:	10	100	microsomes							
	0.058	0.291	0.101							
	0.059	0.292	0.101							
			0.101							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.580	0.548	0.0483
0.125	100	200	0.00013	200	0.0250	0.350	0.317	0.0275
0.05	200	1000	0.00005	200	0.0100	0.166	0.134	0.0110
0.025	100	1000	0.00003	200	0.0050	0.102	0.070	0.0052
0.01	40	1000	0.00001	200	0.0020	0.055	0.023	0.0009
0.005	20	1000	0.00001	200	0.0010	0.044	0.012	0.0000

Blank 0.032

r^2= 0.993
m= 0.090
b= -0.001

Variables	Regression results	
m, b	0.090	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	583	4
ss_{regr}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	µL µsomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.058	0.025	0.001	200	1	1	0.000	0.000	0.006
10	0.059	0.027	0.001	200	1	1	0.000		
10					1	1			
100	0.291	0.259	0.022	200	1	1	0.000	0.000	0.111
100	0.292	0.260	0.022	200	1	1	0.000		
100									
	0.101	0.069	0.005	200	218	70018	0.008	0.008	8.180
	0.101	0.068	0.005	200	218	70018	0.008		
	0.101	0.068	0.005	200	218	70018	0.008		

Assay Date 7/22/2005 Test Chemical ID Chem 2 tested 8

ID JG Replicate # Rep 2 Microsome type Placental Microsome ID IVT

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.575	0.346	0.163	0.104	0.053	0.044	0.033	25	100	
	0.583	0.352	0.168	0.099	0.053	0.044	0.032			
	0.583	0.352	0.168	0.102	0.059	0.046	0.033			

Samples:	10	100	microsomes
	0.058	0.291	0.101
	0.059	0.292	0.101
			0.101

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.580	0.548	0.0429
0.125	100	200	0.00013	200	0.0250	0.350	0.317	0.0248
0.05	200	1000	0.00005	200	0.0100	0.166	0.134	0.0104
0.025	100	1000	0.00003	200	0.0050	0.102	0.070	0.0053
0.01	40	1000	0.00001	200	0.0020	0.055	0.023	0.0016
0.005	20	1000	0.00001	200	0.0010	0.044	0.012	0.0008

Blank 0.032

Variables		
	Regression results	
m, b	0.079	0.000
se_m, se_b	0.002	0.000
r , se_y	0.999	0.000
F, df	2437	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.999
m= 0.079
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.058	0.025	0.002	200	1	1	0.000	0.000	0.010
10	0.059	0.027	0.002	200	1	1	0.000		
10					1	1			
100	0.291	0.259	0.020	200	1	1	0.000	0.000	0.101
100	0.292	0.260	0.020	200	1	1	0.000		
100									
	0.101	0.069	0.005	200	218	70018	0.008	0.008	8.442
	0.101	0.068	0.005	200	218	70018	0.008		
	0.101	0.068	0.005	200	218	70018	0.008		

Assay Date	7/22/2005	Test Chemical ID	Chem 2	tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	IVT

								Protein stock (mg/10 mL)	Protein stock ID
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk		

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	7/22/2005	Chemical ID	Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.183486	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.183486	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.442
Protein Concentration (dilution added to assay, mg/mL):	0.026284

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	7/22/2005	Test Chemical ID	Chem 2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3778.35	7556.7	7554.11	15108.22	0.1	140811	10.73	14990	0.0219	1	0.013	15	0.0556
			0.5	2	3775.76	7551.52			0.1					1		15	
	2	2	0.5	1	3543.42	7086.84	7151.86	14303.72	0.1	140811	10.16	14186	0.0207	1	0.013	15	0.0526
			0.5	2	3608.44	7216.88			0.1					1		15	
	3	2	0.5	1	3316.91	6633.82	6668.09	13336.18	0.1	140811	9.47	13218	0.0193	1	0.013	15	0.0490
			0.5	2	3351.18	6702.36			0.1					1		15	
	4	2	0.5	1	3207.09	6414.18	6466.91	12933.82	0.1	140811	9.19	12816	0.0187	1	0.013	15	0.0475
			0.5	2	3259.82	6519.64			0.1					1		15	
Background control	1	2	0.5	1	32.36	64.72	60.34	120.68	0.1	140811	0.09	3	0.0000	1	0.013	15	0.0000
			0.5	2	27.98	55.96			0.1					1		15	
	2	2	0.5	1	27.53	55.06	50.77	101.54	0.1	140811	0.07	-17	0.0000	1	0.013	15	-0.0001
			0.5	2	23.24	46.48			0.1					1		15	
	3	2	0.5	1	30.51	61.02	60.17	120.34	0.1	140811	0.09	2	0.0000	1	0.013	15	0.0000
			0.5	2	29.66	59.32			0.1					1		15	
	4	2	0.5	1	28.95	57.9	65.01	130.02	0.1	140811	0.09	12	0.0000	1	0.013	15	0.0000
			0.5	2	36.06	72.12			0.1					1		15	
Positive control	1	2	0.5	1	1696.7	3393.4	3408.85	6817.7	0.1	140811	4.84	6700	0.0098	1	0.013	15	0.0248
			0.5	2	1712.15	3424.3			0.1					1		15	
	2	2	0.5	1	1680.62	3361.24	3391.87	6783.74	0.1	140811	4.82	6666	0.0097	1	0.013	15	0.0247
			0.5	2	1711.25	3422.5			0.1					1		15	
	3	2	0.5	1	1550.59	3101.18	3079.12	6158.24	0.1	140811	4.37	6040	0.0088	1	0.013	15	0.0224
			0.5	2	1528.53	3057.06			0.1					1		15	
	4	2	0.5	1	1480.76	2961.36	2969.24	5938.48	0.1	140811	4.22	5820	0.0085	1	0.013	15	0.0216
			0.5	2	1488.46	2976.92			0.1					1		15	
Negative Control	1	2	0.5	1	3739.26	7478.52	7435.19	14870.38	0.1	140811	10.56	14752	0.0216	1	0.013	15	0.0547
			0.5	2	3695.93	7391.86			0.1					1		15	
	2	2	0.5	1	3656.63	7313.26	7336.54	14673.08	0.1	140811	10.42	14555	0.0213	1	0.013	15	0.0540
			0.5	2	3679.91	7359.82			0.1					1		15	
	3	2	0.5	1	3452.83	6905.66	6827	13654	0.1	140811	9.70	13536	0.0198	1	0.013	15	0.0502
			0.5	2	3374.17	6748.34			0.1					1		15	
	4	2	0.5	1	3309.79	6619.58	6591	13182	0.1	140811	9.36	13064	0.0191	1	0.013	15	0.0484
			0.5	2	3281.21	6562.42			0.1					1		15	
Chem 2	1-1	2	0.5	1	36.32	72.64	76.69	153.38	0.1	140811	0.11	35	0.0001	1	0.013	15	0.0001
			0.5	2	40.37	80.74			0.1					1		15	
	1-2	2	0.5	1	30.62	61.24	76.68	153.36	0.1	140811	0.11	35	0.0001	1	0.013	15	0.0001
			0.5	2	46.06	92.12			0.1					1		15	
	1-3	2	0.5	1	40.66	81.32	73.2	146.4	0.1	140811	0.10	28	0.0000	1	0.013	15	0.0001
			0.5	2	32.54	65.08			0.1					1		15	
	2-1	2	0.5	1	187.42	374.84	389.78	779.56	0.1	140811	0.55	661	0.0010	1	0.013	15	0.0025
			0.5	2	202.36	404.72			0.1					1		15	
	2-2	2	0.5	1	175.37	350.74	364.99	729.96	0.1	140811	0.52	612	0.0009	1	0.013	15	0.0023
			0.5	2	189.61	379.22			0.1					1		15	
	2-3	2	0.5	1	191.99	383.98	387.47	774.94	0.1	140811	0.55	657	0.0010	1	0.013	15	0.0024
			0.5	2	195.48	390.96			0.1					1		15	
	3-1	2	0.5	1	364.31	728.62	741.73	1483.46	0.1	140811	1.05	1365	0.0020	1	0.013	15	0.0051
			0.5	2	377.42	754.84			0.1					1		15	
	3-2	2	0.5	1	330.96	661.92	660.45	1320.9	0.1	140811	0.94	1203	0.0018	1	0.013	15	0.0045
			0.5	2	329.49	658.98			0.1					1		15	
	3-3	2	0.5	1	327.88	655.76	677.15	1354.3	0.1	140811	0.96	1236	0.0018	1	0.013	15	0.0046
			0.5	2	349.27	698.54			0.1					1		15	
	4-1	2	0.5	1	1096.18	2192.36	2171.1	4342.2	0.1	140811	3.08	4224	0.0062	1	0.013	15	0.0157
			0.5	2	1074.92	2149.84			0.1					1		15	
	4-2	2	0.5	1	1120.48	2240.96	2269.18	4538.36	0.1	140811	3.22	4420	0.0065	1	0.013	15	0.0164
			0.5	2	1148.7	2297.4			0.1					1		15	
	4-3	2	0.5	1	1181.01	2362.02	2346.33	4692.66	0.1	140811	3.33	4575	0.0067	1	0.013	15	0.0170
			0.5	2	1165.32	2330.64			0.1					1		15	
	5-1	2	0.5	1	2988.43	5976.86	5889.69	11779.38	0.1	140811	8.37	11661	0.0170	1	0.013	15	0.0432
			0.5	2	2901.26	5802.52			0.1					1		15	
	5-2	2	0.5	1	2802.83	5605.66	5605.66	11211.32	0.1	140811	7.96	11093	0.0162	1	0.013	15	0.0411
			0.5	2	2802.83	5605.66			0.1					1		15	
	5-3	2	0.5	1	2824.58	5649.16	5671.55	11343.1	0.1	140811	8.06	11225	0.0164	1	0.013	15	0.0416
			0.5	2	2846.97	5693.94			0.1					1		15	
	6-1	2	0.5	1	3566.28	7132.56	7218.64	14437.28	0.1	140811	10.25	14319	0.0209	1	0.013	15	0.0531
			0.5	2	3652.36	7304.72			0.1					1		15	
	6-2	2	0.5	1	3389.41	6778.82	6812.18	13624.36	0.1	140811	9.68	13506	0.0197	1	0.013	15	0.0501
			0.5	2	3422.77	6845.54			0.1					1		15	
	6-3	2	0.5	1	3384	6768	6784.97	13569.94	0.1	140811	9.64	13452	0.0197	1	0.013	15	0.0499
			0.5	2	3400.97	6801.94			0.1					1		15	
	7-1	2	0.5	1	3567.83	7135.66	7196.49	14392.98	0.1	140811	10.22	14275	0.0209	1	0.013	15	0.0529
			0.5	2	3628.66	7257.32			0.1					1		15	
	7-2	2	0.5	1	3437.92	6875.84	6914.6	13829.2	0.1	140811	9.82	13711	0.0200	1	0.013	15	0.0508
			0.5	2	3476.68	6953.36			0.1					1		15	
	7-3	2	0.5	1	3482.44	6964.88	6904.5	13809	0.1	140811	9.81	13691	0.0200	1	0.013	15	0.0507
			0.5	2	3422.06	6844.12			0.1					1		15	
	8-1	2	0.5	1	3571.33	7142.66	7083.66	14167.32	0.1	140811	10.06	14049	0.0205	1	0.013	15	0.0521
			0.5	2	3512.33	7024.66			0.1					1		15	
	8-2	2	0.5	1	3307.47	6614.94	6670.69	13341.38	0.1	140811	9.47	13223	0.0193	1	0.013	15	0.0490
			0.5	2	3363.22	6726.44			0.1					1		15	
	8-3	2	0.5	1	3367.07	6734.14	6792.71	13585.42	0.1	140811	9.65	13467	0.0197	1	0.013	15	0.0499
			0.5	2	3425.64	6851.28			0.1					1		15	

Assay Date	7/22/2005	ID	Chem 2	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0541	0.0021
Full activity	End	0.0482	0.0011
Full activity	Overall	0.0512	0.0036
Background	Beginning	0.0000	5.01659E-05
Background	End	0.0000	2.53713E-05
Background	Overall	0.0000	4.42727E-05
Positive	Beginning	0.0248	0.0001
Positive	End	0.0220	0.0006
Positive	Overall	0.0234	0.0016
Negative	Beginning	0.0543	0.0005
Negative	End	0.0493	0.0012
Negative	Overall	0.0518	0.0030

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Chem 2	1	1	1.00E-04	-4.00	0.0001
Chem 2	1	2	1.00E-04	-4.00	0.0001
Chem 2	1	3	1.00E-04	-4.00	0.0001
Chem 2	2	1	1.00E-05	-5.00	0.0025
Chem 2	2	2	1.00E-05	-5.00	0.0023
Chem 2	2	3	1.00E-05	-5.00	0.0024
Chem 2	3	1	5.00E-06	-5.30	0.0051
Chem 2	3	2	5.00E-06	-5.30	0.0045
Chem 2	3	3	5.00E-06	-5.30	0.0046
Chem 2	4	1	1.00E-06	-6.00	0.0157
Chem 2	4	2	1.00E-06	-6.00	0.0164
Chem 2	4	3	1.00E-06	-6.00	0.0170
Chem 2	5	1	1.00E-07	-7.00	0.0432
Chem 2	5	2	1.00E-07	-7.00	0.0411
Chem 2	5	3	1.00E-07	-7.00	0.0416
Chem 2	6	1	1.00E-08	-8.00	0.0531
Chem 2	6	2	1.00E-08	-8.00	0.0501
Chem 2	6	3	1.00E-08	-8.00	0.0499
Chem 2	7	1	1.00E-09	-9.00	0.0529
Chem 2	7	2	1.00E-09	-9.00	0.0508
Chem 2	7	3	1.00E-09	-9.00	0.0507
Chem 2	8	1	1.00E-10	-10.00	0.0521
Chem 2	8	2	1.00E-10	-10.00	0.0490
Chem 2	8	3	1.00E-10	-10.00	0.0499

Percent of control values				
	Log[test	Replicate		
Level	bstance]	1	2	3
1	-4.00	0.26	0.26	0.20
2	-5.00	4.79	4.43	4.76
3	-5.30	9.89	8.71	8.96
4	-6.00	30.60	32.03	33.14
5	-7.00	84.49	80.37	81.33
6	-8.00	103.74	97.85	97.46
7	-9.00	103.42	99.34	99.19
8	-10.00	101.79	95.80	97.57

Aromatase Assay Spreadsheet

Assay Date	7/26/2005	Test Chemical ID	RC2	# Concentrations tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	29133.43	1471385	
2	0.0200	30022.34	1501117	
3	0.0199	31077.71	1561694	
4	0.0200	31032.9	1551645	
5	0.0200	31207.44	1560372	
			Average DPM/g soln	1529243
			SD	40812
			CV	2.67
			µCi/g soln	0.689

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

ASDN solution	mg ASDN added	(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	8.2243 g
Mass of dilution B used in substrate prep	4.6253 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.573642 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00780 µg/g soln.
µg/g soln.

a. µCi/g soln	0.689
b. Specific activity of [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.

= 0.573642 + 0.00780
= 0.581440 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.185 µCi/µg ASDN

753259 dpm/nmol

Assay Date 7/26/2005 Test Chemical ID RC2 tested 8

ID JG Replicate # Rep 3 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.477	0.320	0.148	0.091	0.049	0.040	0.034	25	100	
	0.532	0.326	0.148	0.092	0.048	0.036	0.029			
	0.524	0.319	0.148	0.094	0.054	0.036	0.029			

Samples:	10	100	microsomes
	0.053	0.250	0.091
	0.053	0.241	0.088
			0.092

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.511	0.480	0.0477
0.125	100	200	0.00013	200	0.0250	0.322	0.291	0.0285
0.05	200	1000	0.00005	200	0.0100	0.148	0.117	0.0109
0.025	100	1000	0.00003	200	0.0050	0.093	0.062	0.0053
0.01	40	1000	0.00001	200	0.0020	0.050	0.019	0.0010
0.005	20	1000	0.00001	200	0.0010	0.037	0.006	-0.0003

Blank 0.031

r^2= 0.988
m= 0.101
b= -0.001

Variables	Regression results	
m, b	0.101	-0.001
se_m, se_b	0.006	0.001
r , se_y	0.988	0.002
F, df	336	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	µL µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.053	0.022	0.001	200	1	1	0.000	0.000	0.006
10	0.053	0.022	0.001	200	1	1	0.000		
10					1	1			
100	0.250	0.219	0.021	200	1	1	0.000	0.000	0.104
100	0.241	0.210	0.020	200	1	1	0.000		
100									
	0.091	0.060	0.005	200	218	70018	0.008	0.008	8.135
	0.088	0.058	0.005	200	218	70018	0.008		
	0.092	0.061	0.005	200	218	70018	0.008		

Assay Date 7/26/2005 | Test Chemical ID RC2 | tested 8

ID JG | Replicate # Rep 3 | Microsome type Placental | Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.477	0.320	0.148	0.091	0.049	0.040	0.034	25	100	
	0.532	0.326	0.148	0.092	0.048	0.036	0.029			
	0.524	0.319	0.148	0.094	0.054	0.036	0.029			

Samples:	10	100	microsomes
	0.053	0.250	0.091
	0.053	0.241	0.088
			0.092

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.511	0.480	0.0409
0.125	100	200	0.00013	200	0.0250	0.322	0.291	0.0249
0.05	200	1000	0.00005	200	0.0100	0.148	0.117	0.0101
0.025	100	1000	0.00003	200	0.0050	0.093	0.062	0.0054
0.01	40	1000	0.00001	200	0.0020	0.050	0.019	0.0018
0.005	20	1000	0.00001	200	0.0010	0.037	0.006	0.0007

Blank 0.031

r^2= 0.999
m= 0.085
b= 0.000

Variables	Regression results	
m, b	0.085	0.000
se_m, se_b	0.001	0.000
r , se_y	0.999	0.000
F, df	3586	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. used in prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.053	0.022	0.002	200	1	1	0.000	0.000	0.010
10	0.053	0.022	0.002	200	1	1	0.000		
10					1	1			
100	0.250	0.219	0.019	200	1	1	0.000	0.000	0.092
100	0.241	0.210	0.018	200	1	1	0.000		
100									
	0.091	0.060	0.005	200	218	70018	0.008	0.008	8.436
	0.088	0.058	0.005	200	218	70018	0.008		
	0.092	0.061	0.005	200	218	70018	0.009		

Test
Assay Date 7/26/2005 Chemical ID RC2 tested 8

ID JG Replicate # Rep 3 Microsome type Placental Microsome ID IVT

Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank r^2= Regression results are calculated using the function LINEST
m=
b=

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	7/26/2005	Chemical ID	RC2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 3

Microsome Dilution Details		
Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.1835	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.1835	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.436
Protein Concentration (dilution added to assay, mg/mL):	0.026265

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	7/26/2005	Test Chemical ID	RC2	# Concentrations tested		8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3993.49	7986.98	8156.65	16313.3	0.1	152924	10.67	16189	0.0215	1	0.013	15	0.0545
			0.5	2	4163.16	8326.32			0.1					1		15	
	2	2	0.5	1	3881.95	7763.9	7738.56	15477.12	0.1	152924	10.12	15352	0.0204	1	0.013	15	0.0517
			0.5	2	3856.61	7713.22			0.1					1		15	
	3	2	0.5	1	3898.39	7796.78	7641.1	15682.2	0.1	152924	10.25	15558	0.0207	1	0.013	15	0.0524
			0.5	2	3942.71	7885.42			0.1					1		15	
	4	2	0.5	1	3640.61	7281.22	7329.44	14658.88	0.1	152924	9.59	14534	0.0193	1	0.013	15	0.0490
			0.5	2	3688.83	7377.66			0.1					1		15	
Background control	1	2	0.5	1	30.41	60.82	62.49	124.98	0.1	152924	0.08	0	0.0000	1	0.013	15	0.0000
			0.5	2	32.08	64.16			0.1					1		15	
	2	2	0.5	1	34.78	69.56	58.59	117.18	0.1	152924	0.08	7	0.0000	1	0.013	15	0.0000
			0.5	2	23.81	47.62			0.1					1		15	
	3	2	0.5	1	29.35	58.7	62.09	124.18	0.1	152924	0.08	0	0.0000	1	0.013	15	0.0000
			0.5	2	32.74	65.48			0.1					1		15	
	4	2	0.5	1	32.24	64.48	66.18	132.36	0.1	152924	0.09	8	0.0000	1	0.013	15	0.0000
			0.5	2	33.94	67.88			0.1					1		15	
Positive control	1	2	0.5	1	1864.04	3728.08	3718.36	7436.72	0.1	152924	4.85	7312	0.0097	1	0.013	15	0.0246
			0.5	2	1854.32	3708.64			0.1					1		15	
	2	2	0.5	1	1763.23	3526.46	3601.01	7202.02	0.1	152924	4.71	7077	0.0094	1	0.013	15	0.0238
			0.5	2	1837.78	3675.56			0.1					1		15	
	3	2	0.5	1	1764.24	3528.48	3539.77	7079.54	0.1	152924	4.63	6955	0.0092	1	0.013	15	0.0234
			0.5	2	1775.53	3551.06			0.1					1		15	
	4	2	0.5	1	1681.04	3362.08	3291.01	6582.02	0.1	152924	4.30	6457	0.0086	1	0.013	15	0.0218
			0.5	2	1609.97	3219.94			0.1					1		15	
Negative Control	1	2	0.5	1	4029.26	8058.52	8050.41	16100.82	0.1	152924	10.53	15976	0.0212	1	0.013	15	0.0538
			0.5	2	4021.15	8042.3			0.1					1		15	
	2	2	0.5	1	3884.72	7769.44	7892.63	15785.26	0.1	152924	10.32	15661	0.0208	1	0.013	15	0.0528
			0.5	2	4007.91	8015.82			0.1					1		15	
	3	2	0.5	1	3491.77	6983.54	6987.1	13974.2	0.1	152924	9.14	13850	0.0184	1	0.013	15	0.0467
			0.5	2	3495.33	6990.66			0.1					1		15	
	4	2	0.5	1	3376.31	6752.62	6913.57	13827.14	0.1	152924	9.04	13702	0.0182	1	0.013	15	0.0462
			0.5	2	3537.26	7074.52			0.1					1		15	
RC2	1-1	2	0.5	1	51.83	103.66	94.75	189.5	0.1	152924	0.12	65	0.0001	1	0.013	15	0.0002
			0.5	2	42.92	85.84			0.1					1		15	
	1-2	2	0.5	1	34.90	69.8	77.97	155.94	0.1	152924	0.10	31	0.0000	1	0.013	15	0.0001
			0.5	2	43.07	86.14			0.1					1		15	
	1-3	2	0.5	1	43.94	87.88	78.27	156.54	0.1	152924	0.10	32	0.0000	1	0.013	15	0.0001
			0.5	2	34.33	68.66			0.1					1		15	
	2-1	2	0.5	1	196.18	392.36	392.4	784.8	0.1	152924	0.51	660	0.0009	1	0.013	15	0.0022
			0.5	2	196.22	392.44			0.1					1		15	
	2-2	2	0.5	1	199.19	398.38	384.48	768.96	0.1	152924	0.50	644	0.0009	1	0.013	15	0.0022
			0.5	2	185.29	370.58			0.1					1		15	
	2-3	2	0.5	1	182.20	364.4	368	736	0.1	152924	0.48	611	0.0008	1	0.013	15	0.0021
			0.5	2	185.80	371.6			0.1					1		15	
	3-1	2	0.5	1	353.22	706.44	700.71	1401.42	0.1	152924	0.92	1277	0.0017	1	0.013	15	0.0043
			0.5	2	347.49	694.98			0.1					1		15	
	3-2	2	0.5	1	347.37	694.74	697.21	1394.42	0.1	152924	0.91	1270	0.0017	1	0.013	15	0.0043
			0.5	2	349.84	699.68			0.1					1		15	
	3-3	2	0.5	1	350.15	700.3	701.55	1403.1	0.1	152924	0.92	1278	0.0017	1	0.013	15	0.0043
			0.5	2	351.40	702.8			0.1					1		15	
	4-1	2	0.5	1	999.93	1999.86	2019.3	4038.6	0.1	152924	2.64	3914	0.0052	1	0.013	15	0.0132
			0.5	2	1019.37	2038.74			0.1					1		15	
	4-2	2	0.5	1	989.57	1979.14	1990.35	3980.7	0.1	152924	2.60	3856	0.0051	1	0.013	15	0.0130
			0.5	2	1000.78	2001.56			0.1					1		15	
	4-3	2	0.5	1	1015.09	2030.18	1997.53	3995.06	0.1	152924	2.61	3870	0.0051	1	0.013	15	0.0130
			0.5	2	982.44	1964.88			0.1					1		15	
	5-1	2	0.5	1	3269.23	6538.46	6536.18	13072.36	0.1	152924	8.55	12948	0.0172	1	0.013	15	0.0436
			0.5	2	3266.95	6533.9			0.1					1		15	
	5-2	2	0.5	1	3303.02	6606.04	6611.59	13223.18	0.1	152924	8.65	13099	0.0174	1	0.013	15	0.0441
			0.5	2	3308.57	6617.14			0.1					1		15	
	5-3	2	0.5	1	3193.12	6386.24	6466.93	12933.86	0.1	152924	8.46	12809	0.0170	1	0.013	15	0.0432
			0.5	2	3273.81	6547.62			0.1					1		15	
	6-1	2	0.5	1	3860.23	7720.46	7663.13	15326.26	0.1	152924	10.02	15202	0.0202	1	0.013	15	0.0512
			0.5	2	3802.90	7605.8			0.1					1		15	
	6-2	2	0.5	1	3770.98	7541.96	7197.08	14394.16	0.1	152924	9.41	14269	0.0189	1	0.013	15	0.0481
			0.5	2	3426.10	6852.2			0.1					1		15	
	6-3	2	0.5	1	3841.95	7683.9	7737.05	15474.1	0.1	152924	10.12	15349	0.0204	1	0.013	15	0.0517
			0.5	2	3895.10	7790.2			0.1					1		15	
	7-1	2	0.5	1	3962.78	7925.56	7872.32	15744.64	0.1	152924	10.30	15620	0.0207	1	0.013	15	0.0526
			0.5	2	3909.54	7819.08			0.1					1		15	
	7-2	2	0.5	1	3951.97	7903.94	7826.62	15653.24	0.1	152924	10.24	15529	0.0206	1	0.013	15	0.0523
			0.5	2	3874.65	7749.3			0.1					1		15	
	7-3	2	0.5	1	4069.33	8138.66	8119.78	16239.56	0.1	152924	10.62	16115	0.0214	1	0.013	15	0.0543
			0.5	2	4050.45	8100.9			0.1					1		15	
	8-1	2	0.5	1	4048.55	8097.1	8157.91	16315.82	0.1	152924	10.67	16191	0.0215	1	0.013	15	0.0546
			0.5	2	4109.36	8218.72			0.1					1		15	
	8-2	2	0.5	1	3947.41	7894.82	7872.78	15745.56	0.1	152924	10.30	15621	0.0207	1	0.013	15	0.0526
			0.5	2	3925.37	7850.74			0.1					1		15	
	8-3	2	0.5	1	3811.59	7623.18	7647.17	15294.34	0.1	152924	10.00	15170	0.0201	1	0.013	15	0.0511
			0.5	2	3835.59	7671.16			0.1					1		15	

Assay Date	7/26/2005	ID	RC2	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0531	0.0020
Full activity	End	0.0507	0.0024
Full activity	Overall	0.0519	0.0023
Background	Beginning	0.0000	1.85849E-05
Background	End	0.0000	1.94903E-05
Background	Overall	0.0000	2.09145E-05
Positive	Beginning	0.0242	0.0006
Positive	End	0.0226	0.0012
Positive	Overall	0.0234	0.0012
Negative	Beginning	0.0533	0.0008
Negative	End	0.0464	0.0004
Negative	Overall	0.0499	0.0040

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC2	1	1	1.00E-04	-4.00	0.0002
RC2	1	2	1.00E-04	-4.00	0.0001
RC2	1	3	1.00E-04	-4.00	0.0001
RC2	2	1	1.00E-05	-5.00	0.0022
RC2	2	2	1.00E-05	-5.00	0.0022
RC2	2	3	1.00E-05	-5.00	0.0021
RC2	3	1	5.00E-06	-5.30	0.0043
RC2	3	2	5.00E-06	-5.30	0.0043
RC2	3	3	5.00E-06	-5.30	0.0043
RC2	4	1	1.00E-06	-6.00	0.0132
RC2	4	2	1.00E-06	-6.00	0.0130
RC2	4	3	1.00E-06	-6.00	0.0130
RC2	5	1	1.00E-07	-7.00	0.0436
RC2	5	2	1.00E-07	-7.00	0.0441
RC2	5	3	1.00E-07	-7.00	0.0432
RC2	6	1	1.00E-08	-8.00	0.0512
RC2	6	2	1.00E-08	-8.00	0.0481
RC2	6	3	1.00E-08	-8.00	0.0517
RC2	7	1	1.00E-09	-9.00	0.0526
RC2	7	2	1.00E-09	-9.00	0.0523
RC2	7	3	1.00E-09	-9.00	0.0543
RC2	8	1	1.00E-10	-10.00	0.0546
RC2	8	2	1.00E-10	-10.00	0.0526
RC2	8	3	1.00E-10	-10.00	0.0511

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-4.00	0.42	0.20	0.21
2	-5.00	4.28	4.18	3.97
3	-5.30	8.29	8.24	8.30
4	-6.00	25.40	25.03	25.12
5	-7.00	84.03	85.01	83.13
6	-8.00	98.66	92.61	99.62
7	-9.00	101.37	100.78	104.59
8	-10.00	105.08	101.38	98.45

Aromatase Assay Spreadsheet

Assay Date	8/3/2005	Test Chemical ID	RC3	# Concentrations tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	35272.34	1763617	
2	0.0199	35027.1	1760156	
3	0.0202	35557.43	1760269	
4	0.0202	35088.79	1737069	
5	0.0202	35770.76	1770830	
			Average DPM/g soln	1758388
			SD	12682
			CV	0.72
			µCi/g soln	0.792

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

ASDN solution	mg ASDN added	(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	8.0728 g
Mass of dilution B used in substrate prep	4.5296 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.583538 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00897 µg/g soln.
µg/g soln.

a. µCi/g soln 0.792
b. Specific activity of [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.

= 0.583538 + 0.00897
= 0.592504 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.337 µCi/µg ASDN

849956 dpm/nmol

Test

Assay Date 8/3/2005 Chemical ID RC3 tested 8

ID TNB Replicate # Rep 1 Microsome type Placental Microsome ID Battelle

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.541	0.329	0.153	0.102	0.053	0.046	0.032	25	100	
	0.566	0.337	0.157	0.101	0.053	0.043	0.032			
	0.566	0.349	0.154	0.103	0.058	0.038	0.027			

Samples:	10	100	microsomes
	0.078	0.280	0.100
	0.060	0.295	0.095
			0.099

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.557	0.527	0.0483
0.125	100	200	0.00013	200	0.0250	0.338	0.308	0.0277
0.05	200	1000	0.00005	200	0.0100	0.154	0.124	0.0105
0.025	100	1000	0.00003	200	0.0050	0.102	0.072	0.0055
0.01	40	1000	0.00001	200	0.0020	0.055	0.024	0.0011
0.005	20	1000	0.00001	200	0.0010	0.042	0.012	-0.0001

Blank 0.030

r^2= 0.993
m= 0.094
b= -0.001

Variables	Regression results	
m, b	0.094	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	567	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. usomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.078	0.048	0.003	200	1	1	0.000	0.000	0.012
10	0.060	0.030	0.002	200	1	1	0.000		
10					1	1			
100	0.280	0.250	0.022	200	1	1	0.000	0.000	0.115
100	0.295	0.265	0.024	200	1	1	0.000		
100									
	0.100	0.069	0.005	200	83	69983	0.022	0.022	21.636
	0.095	0.064	0.005	200	83	69983	0.020		
	0.099	0.069	0.005	200	83	69983	0.022		

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Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.541	0.329	0.153	0.102	0.053	0.046	0.032	25	100	
	0.566	0.337	0.157	0.101	0.053	0.043	0.032			
	0.566	0.349	0.154	0.103	0.058	0.038	0.027			

Samples:	10	100	microsomes
	0.078	0.280	0.100
	0.060	0.295	0.095
			0.099

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.557	0.527	0.0428
0.125	100	200	0.00013	200	0.0250	0.338	0.308	0.0249
0.05	200	1000	0.00005	200	0.0100	0.154	0.124	0.0099
0.025	100	1000	0.00003	200	0.0050	0.102	0.072	0.0056
0.01	40	1000	0.00001	200	0.0020	0.055	0.024	0.0018
0.005	20	1000	0.00001	200	0.0010	0.042	0.012	0.0008

Blank 0.030

r^2= 0.999
m= 0.082
b= 0.000

Variables	Regression results	
m, b	0.082	0.000
se_m, se_b	0.002	0.000
r , se_y	0.999	0.000
F, df	2233	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. used in prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.078	0.048	0.004	200	1	1	0.000	0.000	0.015
10	0.060	0.030	0.002	200	1	1	0.000		
10					1	1			
100	0.280	0.250	0.020	200	1	1	0.000	0.000	0.104
100	0.295	0.265	0.021	200	1	1	0.000		
100									
	0.100	0.069	0.005	200	83	69983	0.023	0.022	22.309
	0.095	0.064	0.005	200	83	69983	0.021		
	0.099	0.069	0.005	200	83	69983	0.023		

Assay Date	8/3/2005	Test Chemical ID	RC3	tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	Battelle

								Protein stock (mg/10 mL)	Protein stock ID
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk		

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank — r^2= m= b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/3/2005	Chemical ID	RC3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Microsome Dilution Details

Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.16867	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.16867	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	22.309
Protein Concentration (dilution added to assay, mg/mL):	0.026459

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	8/3/2005	Test Chemical ID	RC3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5595.55	11191.1	11177.3	22354.6	0.1	175839	12.71	22194	0.0261	1	0.013	15	0.0656
			0.5	2	5581.75	11163.5			0.1					1		15	
	2	2	0.5	1	5853.35	11706.7	11688.47	23376.94	0.1	175839	13.29	23217	0.0273	1	0.013	15	0.0688
			0.5	2	5835.12	11670.24			0.1					1		15	
	3	2	0.5	1	4383.57	8767.14	8737.9	17475.8	0.1	175839	9.94	17315	0.0204	1	0.013	15	0.0513
			0.5	2	4354.33	8708.66			0.1					1		15	
	4	2	0.5	1	5024.5	10049	9966.14	19932.28	0.1	175839	11.34	19772	0.0233	1	0.013	15	0.0586
			0.5	2	4941.64	9883.28			0.1					1		15	
Background control	1	2	0.5	1	25.39	50.78	47.47	94.94	0.1	175839	0.05	-65	-0.0001	1	0.013	15	-0.0002
			0.5	2	22.08	44.16			0.1					1		15	
	2	2	0.5	1	14.3	28.6	37.29	74.58	0.1	175839	0.04	-86	-0.0001	1	0.013	15	-0.0003
			0.5	2	22.99	45.98			0.1					1		15	
	3	2	0.5	1	64.87	129.74	120.06	240.12	0.1	175839	0.14	80	0.0001	1	0.013	15	0.0002
			0.5	2	55.19	110.38			0.1					1		15	
	4	2	0.5	1	64.46	128.92	115.91	231.82	0.1	175839	0.13	71	0.0001	1	0.013	15	0.0002
			0.5	2	51.45	102.9			0.1					1		15	
Positive control	1	2	0.5	1	2246.46	4492.92	4548.23	9096.46	0.1	175839	5.17	8936	0.0105	1	0.013	15	0.0265
			0.5	2	2301.77	4603.54			0.1					1		15	
	2	2	0.5	1	2354.94	4709.88	4749.07	9498.14	0.1	175839	5.40	9338	0.0110	1	0.013	15	0.0277
			0.5	2	2394.13	4788.26			0.1					1		15	
	3	2	0.5	1	2100.89	4201.78	4236.1	8472.2	0.1	175839	4.82	8312	0.0098	1	0.013	15	0.0246
			0.5	2	2135.21	4270.42			0.1					1		15	
	4	2	0.5	1	694.44	1388.88	1319.41	2639.82	0.1	175839	1.50	2478	0.0029	1	0.013	15	0.0073
			0.5	2	624.97	1249.94			0.1					1		15	
Negative Control	1	2	0.5	1	5680.93	11361.86	11332.81	22665.62	0.1	175839	12.89	22505	0.0265	1	0.013	15	0.0667
			0.5	2	5651.88	11303.76			0.1					1		15	
	2	2	0.5	1	5511.77	11023.54	10954.8	21909.6	0.1	175839	12.46	21749	0.0256	1	0.013	15	0.0645
			0.5	2	5443.03	10886.06			0.1					1		15	
	3	2	0.5	1	4901.84	9803.68	9737.46	19474.92	0.1	175839	11.08	19315	0.0227	1	0.013	15	0.0573
			0.5	2	4835.62	9671.24			0.1					1		15	
	4	2	0.5	1	5129.62	10259.24	10309.03	20618.06	0.1	175839	11.73	20458	0.0241	1	0.013	15	0.0606
			0.5	2	5179.41	10358.82			0.1					1		15	
RC3	1-1	2	0.5	1	77.87	155.74	148.09	296.18	0.1	175839	0.17	136	0.0002	1	0.013	15	0.0004
			0.5	2	70.22	140.44			0.1					1		15	
	1-2	2	0.5	1	32.02	64.04	61.77	123.54	0.1	175839	0.07	-37	0.0000	1	0.013	15	-0.0001
			0.5	2	29.75	59.5			0.1					1		15	
	1-3	2	0.5	1	23.75	47.5	50.36	100.72	0.1	175839	0.06	-60	-0.0001	1	0.013	15	-0.0002
			0.5	2	26.61	53.22			0.1					1		15	
	2-1	2	0.5	1	38.2	76.4	63.9	127.8	0.1	175839	0.07	-33	0.0000	1	0.013	15	-0.0001
			0.5	2	25.7	51.4			0.1					1		15	
	2-2	2	0.5	1	20.5	41	50.64	101.28	0.1	175839	0.06	-59	-0.0001	1	0.013	15	-0.0002
			0.5	2	30.14	60.28			0.1					1		15	
	2-3	2	0.5	1	30.62	61.24	62.99	125.98	0.1	175839	0.07	-34	0.0000	1	0.013	15	-0.0001
			0.5	2	32.37	64.74			0.1					1		15	
	3-1	2	0.5	1	39.99	79.98	84.45	168.9	0.1	175839	0.10	9	0.0000	1	0.013	15	0.0000
			0.5	2	44.48	88.92			0.1					1		15	
	3-2	2	0.5	1	28.36	56.72	66.09	132.18	0.1	175839	0.08	-28	0.0000	1	0.013	15	-0.0001
			0.5	2	37.73	75.46			0.1					1		15	
	3-3	2	0.5	1	40.81	81.62	79.58	159.16	0.1	175839	0.09	-1	0.0000	1	0.013	15	0.0000
			0.5	2	38.77	77.54			0.1					1		15	
	4-1	2	0.5	1	151.81	303.62	315.89	631.78	0.1	175839	0.36	471	0.0006	1	0.013	15	0.0014
			0.5	2	164.08	328.16			0.1					1		15	
	4-2	2	0.5	1	160.71	321.42	321.33	642.66	0.1	175839	0.37	482	0.0006	1	0.013	15	0.0014
			0.5	2	160.62	321.24			0.1					1		15	
	4-3	2	0.5	1	183.66	367.32	354.59	709.18	0.1	175839	0.40	549	0.0006	1	0.013	15	0.0016
			0.5	2	170.93	341.86			0.1					1		15	
	5-1	2	0.5	1	1101.05	2202.1	2315.39	4630.78	0.1	175839	2.63	4470	0.0053	1	0.013	15	0.0133
			0.5	2	1214.34	2428.68			0.1					1		15	
	5-2	2	0.5	1	1156.57	2313.14	2339.8	4679.6	0.1	175839	2.66	4519	0.0053	1	0.013	15	0.0134
			0.5	2	1183.23	2366.46			0.1					1		15	
	5-3	2	0.5	1	1102.08	2204.16	2227.75	4455.5	0.1	175839	2.53	4295	0.0051	1	0.013	15	0.0127
			0.5	2	1125.67	2251.34			0.1					1		15	
	6-1	2	0.5	1	4098.24	8196.48	8143.66	16287.32	0.1	175839	9.26	16127	0.0190	1	0.013	15	0.0478
			0.5	2	4045.42	8090.84			0.1					1		15	
	6-2	2	0.5	1	3855.49	7710.98	7760.16	15520.32	0.1	175839	8.83	15360	0.0181	1	0.013	15	0.0455
			0.5	2	3904.67	7809.34			0.1					1		15	
	6-3	2	0.5	1	3926.81	7853.62	7833.31	15666.62	0.1	175839	8.91	15506	0.0182	1	0.013	15	0.0460
			0.5	2	3906.5	7813			0.1					1		15	
	7-1	2	0.5	1	5819.96	11639.92	11546.09	23092.18	0.1	175839	13.13	22932	0.0270	1	0.013	15	0.0680
			0.5	2	5726.13	11452.26			0.1					1		15	
	7-2	2	0.5	1	5382.76	10765.52	10729.31	21458.62	0.1	175839	12.20	21298	0.0251	1	0.013	15	0.0631
			0.5	2	5346.55	10693.1			0.1					1		15	
	7-3	2	0.5	1	5166.79	10333.58	10403.74	20807.48	0.1	175839	11.83	20647	0.0243	1	0.013	15	0.0612
			0.5	2	5236.95	10473.9			0.1					1		15	
	8-1	2	0.5	1	6032.59	12065.18	11917.59	23835.18	0.1	175839	13.56	23675	0.0279	1	0.013	15	0.0702
			0.5	2	5885	11770			0.1					1		15	
	8-2	2	0.5	1	4981.81	9963.62	9930.05	19860.1	0.1	175839	11.29	19700	0.0232	1	0.013	15	0.0584
			0.5	2	4948.24	9896.48			0.1					1		15	
	8-3	2	0.5	1	5832	11664	11628.59	23257.18	0.1	175839	13.23	23097	0.0272	1	0.013	15	0.0685
			0.5	2	5796.59	11593.18			0.1					1		15	

Assay Date	8/3/2005	ID	RC3	# Concentrations tested	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0673	0.0021
Full activity	End	0.0550	0.0051
Full activity	Overall	0.0611	0.0078
Background	Beginning	-0.0002	4.26785E-05
Background	End	0.0002	1.73984E-05
Background	Overall	0.0000	0.000260166
Positive	Beginning	0.0271	0.0008
Positive	End	0.0160	0.0122
Positive	Overall	0.0215	0.0095
Negative	Beginning	0.0656	0.0016
Negative	End	0.0590	0.0024
Negative	Overall	0.0623	0.0042

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC3	1	1	1.00E-03	-3.00	0.0004
RC3	1	2	1.00E-03	-3.00	-0.0001
RC3	1	3	1.00E-03	-3.00	-0.0002
RC3	2	1	1.00E-04	-4.00	-0.0001
RC3	2	2	1.00E-04	-4.00	-0.0002
RC3	2	3	1.00E-04	-4.00	-0.0001
RC3	3	1	1.00E-05	-5.00	0.0000
RC3	3	2	1.00E-05	-5.00	-0.0001
RC3	3	3	1.00E-05	-5.00	0.0000
RC3	4	1	1.00E-06	-6.00	0.0014
RC3	4	2	1.00E-06	-6.00	0.0014
RC3	4	3	1.00E-06	-6.00	0.0016
RC3	5	1	1.00E-07	-7.00	0.0133
RC3	5	2	1.00E-07	-7.00	0.0134
RC3	5	3	1.00E-07	-7.00	0.0127
RC3	6	1	1.00E-08	-8.00	0.0478
RC3	6	2	1.00E-08	-8.00	0.0455
RC3	6	3	1.00E-08	-8.00	0.0460
RC3	7	1	1.00E-09	-9.00	0.0680
RC3	7	2	1.00E-09	-9.00	0.0631
RC3	7	3	1.00E-09	-9.00	0.0612
RC3	8	1	1.00E-10	-10.00	0.0702
RC3	8	2	1.00E-10	-10.00	0.0584
RC3	8	3	1.00E-10	-10.00	0.0685

Percent of control values				
	Log[test	Replicate		
Level	bstance]	1	2	3
1	-3.00	0.66	-0.18	-0.29
2	-4.00	-0.16	-0.29	-0.17
3	-5.00	0.04	-0.14	-0.01
4	-6.00	2.29	2.34	2.66
5	-7.00	21.68	21.91	20.83
6	-8.00	78.19	74.47	75.18
7	-9.00	111.19	103.27	100.11
8	-10.00	114.79	95.52	111.99

Aromatase Assay Spreadsheet

Assay Date	8/5/2005	Test Chemical ID	RC 3	# Concentrations tested	8	
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	32083.34	1604167	
2	0.0201	32014.95	1592784	
3	0.0202	33169.23	1642041	
4	0.0198	32366.64	1634679	
5	0.0202	32127.45	1590468	
			Average DPM/g soln	1612828
			SD	24019
			CV	1.49
			µCi/g soln	0.726

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

ASDN solution	mg ASDN added	(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	8.1781 g
Mass of dilution B used in substrate prep	4.61 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.574975 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00822 µg/g soln.
µg/g soln.

a. µCi/g soln	0.726
b. Specific activity of [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.

= 0.574975 + 0.00822
= 0.583199 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.246 µCi/µg ASDN

792035 dpm/nmol

Assay Date 8/5/2005 Test Chemical ID RC 3 tested 8

ID TNB Replicate # Rep 2 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.564	0.339	0.165	0.096	0.051	0.037	0.027	25		
	0.587	0.346	0.169	0.098	0.055	0.039	0.030			
	0.570	0.356	0.163	0.103	0.057	0.039	0.027			

Samples:	10	100	microsomes
	0.051	0.272	0.089
	0.047	0.277	0.090
			0.090

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.574	0.546	0.0482
0.125	100	200	0.00013	200	0.0250	0.347	0.319	0.0277
0.05	200	1000	0.00005	200	0.0100	0.166	0.138	0.0112
0.025	100	1000	0.00003	200	0.0050	0.099	0.071	0.0052
0.01	40	1000	0.00001	200	0.0020	0.054	0.026	0.0011
0.005	20	1000	0.00001	200	0.0010	0.038	0.010	-0.0003

Blank 0.028

r^2= 0.992
m= 0.091
b= -0.001

Variables	Regression results	
m, b	0.091	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.992	0.002
F, df	496	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. microsome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.051	0.023	0.001	200	1	1	0.000	0.000	0.003
10	0.047	0.019	0.000	200	1	1	0.000		
10					1	1			
100	0.272	0.244	0.021	200	1	1	0.000	0.000	0.105
100	0.277	0.249	0.021	200	1	1	0.000		
100									
	0.089	0.061	0.004	200	83	69983	0.018	0.018	18.268
	0.090	0.062	0.004	200	83	69983	0.018		
	0.090	0.062	0.004	200	83	69983	0.018		

Assay Date 8/5/2005 Test Chemical ID RC 3 tested 8

ID TNB Replicate # Rep 2 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.564	0.339	0.165	0.096	0.051	0.037	0.027	25		
	0.587	0.346	0.169	0.098	0.055	0.039	0.030			
	0.570	0.356	0.163	0.103	0.057	0.039	0.027			
Samples:	10	100	microsomes							
	0.051	0.272	0.089							
	0.047	0.277	0.090							
			0.090							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
0.25	200	200	0.00025	200	0.0500	0.574	0.546	0.0424	m, b	0.078	0.000
0.125	100	200	0.00013	200	0.0250	0.347	0.319	0.0247	se_m, se_b	0.002	0.000
0.05	200	1000	0.00005	200	0.0100	0.166	0.138	0.0106	r , se_y	0.998	0.000
0.025	100	1000	0.00003	200	0.0050	0.099	0.071	0.0053	F, df	1593	3
0.01	40	1000	0.00001	200	0.0020	0.054	0.026	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.038	0.010	0.0006			

Blank 0.028

r^2= 0.998
m= 0.078
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.051	0.023	0.002	200	1	1	0.000	0.000	0.007
10	0.047	0.019	0.001	200	1	1	0.000		
10					1	1			
100	0.272	0.244	0.019	200	1	1	0.000	0.000	0.095
100	0.277	0.249	0.019	200	1	1	0.000		
100									
	0.089	0.061	0.005	200	83	69983	0.019	0.019	19.426
	0.090	0.062	0.005	200	83	69983	0.020		
	0.090	0.062	0.005	200	83	69983	0.020		

Assay Date	8/5/2005	Test Chemical ID	RC 3	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle

Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/5/2005	Chemical ID	RC 3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.16867	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.16867	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	19.426
Protein Concentration (dilution added to assay, mg/mL):	0.023039

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	3.00E-08
5	1.00E-08
6	3.00E-09
7	1.00E-09
8	1.00E-10

Assay Date	8/5/2005	Test Chemical ID	RC 3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4612.96	9225.92	9268.03	18536.06	0.1	161283	11.49	15489	0.0196	1	0.012	15	0.0566
			0.5	2	4655.07	9310.14			0.1					1		15	
	2	2	0.5	1	5050.47	10100.94	10027.3	20054.6	0.1	161283	12.43	17007	0.0215	1	0.012	15	0.0621
			0.5	2	4976.83	9953.66			0.1					1		15	
	3	2	0.5	1	3711.48	7422.96	7452.47	14904.94	0.1	161283	9.24	11858	0.0150	1	0.012	15	0.0433
			0.5	2	3740.99	7481.98			0.1					1		15	
	4	2	0.5	1	3802.39	7604.78	7570.93	15141.86	0.1	161283	9.39	12095	0.0153	1	0.012	15	0.0442
			0.5	2	3768.54	7537.08			0.1					1		15	
Background control	1	2	0.5	1	902.48	1804.96	1821.39	3642.78	0.1	161283	2.26	596	0.0008	1	0.012	15	0.0022
			0.5	2	918.91	1837.82			0.1					1		15	
	2	2	0.5	1	859.65	1717.3	1766.4	3532.8	0.1	161283	2.19	486	0.0006	1	0.012	15	0.0018
			0.5	2	907.75	1815.5			0.1					1		15	
	3	2	0.5	1	626.1	1252.2	1267.41	2534.82	0.1	161283	1.57	-512	-0.0006	1	0.012	15	-0.0019
			0.5	2	641.31	1282.62			0.1					1		15	
	4	2	0.5	1	628.54	1257.08	1239.12	2478.24	0.1	161283	1.54	-569	-0.0007	1	0.012	15	-0.0021
			0.5	2	610.59	1221.16			0.1					1		15	
Positive control	1	2	0.5	1	2101.37	4202.74	4427.3	8854.6	0.1	161283	5.49	5807	0.0073	1	0.012	15	0.0212
			0.5	2	2325.93	4651.86			0.1					1		15	
	2	2	0.5	1	2116.69	4233.38	4274.41	8548.82	0.1	161283	5.30	5502	0.0069	1	0.012	15	0.0201
			0.5	2	2157.72	4315.44			0.1					1		15	
	3	2	0.5	1	1608.22	3216.44	3222.78	6445.56	0.1	161283	4.00	3398	0.0043	1	0.012	15	0.0124
			0.5	2	1614.56	3229.12			0.1					1		15	
	4	2	0.5	1	1591.23	3182.46	3250.95	6501.9	0.1	161283	4.03	3455	0.0044	1	0.012	15	0.0126
			0.5	2	1659.72	3319.44			0.1					1		15	
Negative Control	1	2	0.5	1	4768.81	9537.62	9490.42	18980.84	0.1	161283	11.77	15934	0.0201	1	0.012	15	0.0582
			0.5	2	4721.61	9443.22			0.1					1		15	
	2	2	0.5	1	4624.85	9249.7	9296.47	18592.94	0.1	161283	11.53	15546	0.0196	1	0.012	15	0.0568
			0.5	2	4671.62	9343.24			0.1					1		15	
	3	2	0.5	1	3491.85	6983.7	7047.53	14095.06	0.1	161283	8.74	11048	0.0139	1	0.012	15	0.0404
			0.5	2	3555.68	7111.36			0.1					1		15	
	4	2	0.5	1	3498.36	6996.72	7042.78	14085.56	0.1	161283	8.73	11038	0.0139	1	0.012	15	0.0403
			0.5	2	3544.42	7088.84			0.1					1		15	
RC 3	1-1	2	0.5	1	41.71	83.42	94.22	188.44	0.1	161283	0.12	-2859	-0.0036	1	0.012	15	-0.0104
			0.5	2	52.51	105.02			0.1					1		15	
	1-2	2	0.5	1	33.73	67.46	70.1	140.2	0.1	161283	0.09	-2907	-0.0037	1	0.012	15	-0.0106
			0.5	2	36.37	72.74			0.1					1		15	
	1-3	2	0.5	1	16.83	84.15	42.075	84.15	0.1	161283	0.05	-2963	-0.0037	1	0.012	15	-0.0108
			0.2	2	0	0			0.1					1		15	
	2-1	2	0.5	1	144.76	289.52	291.34	582.68	0.1	161283	0.36	-2464	-0.0031	1	0.012	15	-0.0090
			0.5	2	146.58	293.16			0.1					1		15	
	2-2	2	0.5	1	144.83	289.66	285.64	571.28	0.1	161283	0.35	-2476	-0.0031	1	0.012	15	-0.0090
			0.5	2	140.81	281.62			0.1					1		15	
	2-3	2	0.2	1	56.35	281.75	275	550	0.1	161283	0.34	-2497	-0.0032	1	0.012	15	-0.0091
			0.2	2	53.65	268.25			0.1					1		15	
	3-1	2	0.5	1	949.48	1898.96	1888.06	3776.12	0.1	161283	2.34	729	0.0009	1	0.012	15	0.0027
			0.5	2	938.58	1877.16			0.1					1		15	
	3-2	2	0.5	1	988.69	1977.38	1995.34	3990.68	0.1	161283	2.47	944	0.0012	1	0.012	15	0.0034
			0.5	2	1006.65	2013.3			0.1					1		15	
	3-3	2	0.2	1	340.59	1702.9	1788.25	3576.5	0.1	161283	2.22	529	0.0007	1	0.012	15	0.0019
			0.2	2	374.72	1873.6			0.1					1		15	
	4-1	2	0.5	1	2013.13	4026.26	4022.43	8044.86	0.1	161283	4.99	4998	0.0063	1	0.012	15	0.0183
			0.5	2	2009.3	4018.6			0.1					1		15	
	4-2	2	0.5	1	2206.23	4412.46	4468.4	8936.8	0.1	161283	5.54	5890	0.0074	1	0.012	15	0.0215
			0.5	2	2262.17	4524.34			0.1					1		15	
	4-3	2	0.2	1	812.93	4064.65	4071.375	8142.75	0.1	161283	5.05	5096	0.0064	1	0.012	15	0.0186
			0.2	2	815.62	4078.1			0.1					1		15	
	5-1	2	0.5	1	3220.5	6441	6344.1	12688.2	0.1	161283	7.87	9641	0.0122	1	0.012	15	0.0352
			0.5	2	3123.6	6247.2			0.1					1		15	
	5-2	2	0.5	1	3511.77	7023.54	7023.76	14047.52	0.1	161283	8.71	11000	0.0139	1	0.012	15	0.0402
			0.5	2	3511.99	7023.98			0.1					1		15	
	5-3	2	0.2	1	1179.47	5897.35	6071.225	12142.45	0.1	161283	7.53	9095	0.0115	1	0.012	15	0.0332
			0.2	2	1249.02	6245.1			0.1					1		15	
	6-1	2	0.5	1	3969.17	7938.34	7994	15988	0.1	161283	9.91	12941	0.0163	1	0.012	15	0.0473
			0.5	2	4024.83	8049.66			0.1					1		15	
	6-2	2	0.5	1	4196.66	8393.32	8377	16754	0.1	161283	10.39	13707	0.0173	1	0.012	15	0.0501
			0.5	2	4180.34	8360.68			0.1					1		15	
	6-3	2	0.2	1	1576.39	7881.95	7862.8	15725.6	0.1	161283	9.75	12678	0.0160	1	0.012	15	0.0463
			0.2	2	1568.73	7843.65			0.1					1		15	
	7-1	2	0.5	1	4300.72	8601.44	8696.68	17393.36	0.1	161283	10.78	14346	0.0181	1	0.012	15	0.0524
			0.5	2	4395.96	8791.92			0.1					1		15	
	7-2	2	0.5	1	4668.94	9337.88	9248.96	18497.92	0.1	161283	11.47	15451	0.0195	1	0.012	15	0.0564
			0.5	2	4580.02	9160.04			0.1					1		15	
	7-3	2	0.2	1	954.38	4771.9	6703.175	13406.35	0.1	161283	8.31	10359	0.0131	1	0.012	15	0.0378
			0.2	2	1726.89	8634.45			0.1					1		15	
	8-1	2	0.5	1	4209.63	8419.26	8517.85	17035.7	0.1	161283	10.56	13989	0.0177	1	0.012	15	0.0511
			0.5	2	4308.22	8616.44			0.1					1		15	
	8-2	2	0.5	1	4447.17	8894.34	9023.3	18046.6	0.1	161283	11.19	14999	0.0189	1	0.012	15	0.0548
			0.5	2	4576.13	9152.26			0.1					1		15	
	8-3	2	0.2	1	1678.2	8391	8463.65	16927.3	0.1	161283	10.50	13880	0.0175	1	0.012	15	0.0507
			0.2	2	1707.26	8536.3			0.1					1		15	

Assay Date	8/5/2005	ID	RC 3	# Concentrations tested	Microsome 8 type	Placental	Microsome ID Battelle	Technician ID TNB	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0594	0.0039
Full activity	End	0.0438	0.0006
Full activity	Overall	0.0516	0.0093
Background	Beginning	0.0020	0.000284115
Background	End	-0.0020	0.000146165
Background	Overall	0.0000	0.002288131
Positive	Beginning	0.0207	0.0008
Positive	End	0.0125	0.0001
Positive	Overall	0.0166	0.0047
Negative	Beginning	0.0575	0.0010
Negative	End	0.0403	0.0000
Negative	Overall	0.0489	0.0099

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC 3	1	1	1.00E-05	-5.00	-0.0104
RC 3	1	2	1.00E-05	-5.00	-0.0106
RC 3	1	3	1.00E-05	-5.00	-0.0108
RC 3	2	1	1.00E-06	-6.00	-0.0090
RC 3	2	2	1.00E-06	-6.00	-0.0090
RC 3	2	3	1.00E-06	-6.00	-0.0091
RC 3	3	1	1.00E-07	-7.00	0.0027
RC 3	3	2	1.00E-07	-7.00	0.0034
RC 3	3	3	1.00E-07	-7.00	0.0019
RC 3	4	1	3.00E-08	-7.52	0.0183
RC 3	4	2	3.00E-08	-7.52	0.0215
RC 3	4	3	3.00E-08	-7.52	0.0186
RC 3	5	1	1.00E-08	-8.00	0.0352
RC 3	5	2	1.00E-08	-8.00	0.0402
RC 3	5	3	1.00E-08	-8.00	0.0332
RC 3	6	1	3.00E-09	-8.52	0.0473
RC 3	6	2	3.00E-09	-8.52	0.0501
RC 3	6	3	3.00E-09	-8.52	0.0463
RC 3	7	1	1.00E-09	-9.00	0.0524
RC 3	7	2	1.00E-09	-9.00	0.0564
RC 3	7	3	1.00E-09	-9.00	0.0378
RC 3	8	1	1.00E-10	-10.00	0.0511
RC 3	8	2	1.00E-10	-10.00	0.0548
RC 3	8	3	1.00E-10	-10.00	0.0507

	Percent of control values			
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-5.00	-20.26	-20.60	-21.00
2	-6.00	-17.46	-17.54	-17.70
3	-7.00	5.17	6.69	3.75
4	-7.52	35.41	41.73	36.11
5	-8.00	68.32	77.95	64.45
6	-8.52	91.70	97.13	89.84
7	-9.00	101.66	109.49	73.41
8	-10.00	99.12	106.29	98.36

Aromatase Assay Spreadsheet

Assay Date	8/9/2005	Test Chemical ID	RC3	# Concentrations tested	8	
ID	TNB	Replicate #	3-Jan	Microsome type	Placental	Microsome ID Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	32352.68	1609586	
2	0.0201	31891.58	1586646	
3	0.0193	30889.07	1600470	
4	0.0199	32486.51	1632488	
5	0.0200	32306.54	1615327	
			Average DPM/g soln	1608903
			SD	17062
			CV	1.06
			µCi/g soln	0.725

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

ASDN solution	mg ASDN added	(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	8.1505 g
Mass of dilution B used in substrate prep	4.6215 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56702 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00820 µg/g soln.
µg/g soln.

a. µCi/g soln 0.725
b. Specific activity of [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.

= 0.567020 + 0.00820
= 0.575225 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.260 µCi/µg ASDN

801061 dpm/nmol

Assay Date	8/9/2005	Test Chemical ID	RC3	tested	8				
ID	TNB	Replicate #	3	Microsome type	Placental	Microsome ID	Battelle		

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.556	0.348	0.159	0.087	0.049	0.034	0.033	25	100	
	0.578	0.340	0.156	0.095	0.049	0.038	0.030			
	0.577	0.341	0.155	0.092	0.047	0.041	0.030			

Samples:	10	100	microsomes
	0.054	0.304	0.095
	0.054	0.259	0.096
			0.099

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
0.25	200	200	0.00025	200	0.0500	0.570	0.539	0.0483	m, b	0.090	0.000
0.125	100	200	0.00013	200	0.0250	0.343	0.312	0.0277	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.157	0.126	0.0109	r , se_y	0.993	0.002
0.025	100	1000	0.00003	200	0.0050	0.092	0.060	0.0050	F, df	566	4
0.01	40	1000	0.00001	200	0.0020	0.048	0.017	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.038	0.006	0.0001			

Regression results are calculated using the function LINEST

Blank 0.031

r^2= 0.993
m= 0.090
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.054	0.023	0.002	200	1	1	0.000	0.000	0.008
10	0.054	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.304	0.273	0.024	200	1	1	0.000	0.000	0.111
100	0.259	0.227	0.020	200	1	1	0.000		
100									
	0.095	0.064	0.005	200	83	69983	0.022	0.023	23.003
	0.096	0.065	0.005	200	83	69983	0.023		
	0.099	0.068	0.006	200	83	69983	0.024		

Assay Date	8/9/2005	Test Chemical ID	RC3	tested	8				
ID	TNB	Replicate #	3	Microsome type	Placental	Microsome ID	Battelle		

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.556	0.348	0.159	0.087	0.049	0.034	0.033	25		
	0.578	0.340	0.156	0.095	0.049	0.038	0.030			
	0.577	0.341	0.155	0.092	0.047	0.041	0.030			
Samples:	10	100	microsomes							
	0.054	0.304	0.095							
	0.054	0.259	0.096							
			0.099							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
0.25	200	200	0.00025	200	0.0500	0.570	0.539	0.0427	m, b	0.078	0.000
0.125	100	200	0.00013	200	0.0250	0.343	0.312	0.0249	se_m, se_b	0.001	0.000
0.05	200	1000	0.00005	200	0.0100	0.157	0.126	0.0103	r , se_y	1.000	0.000
0.025	100	1000	0.00003	200	0.0050	0.092	0.060	0.0052	F, df	6782	3
0.01	40	1000	0.00001	200	0.0020	0.048	0.017	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.038	0.006	0.0009			

Regression results are calculated using the function LINEST

Blank 0.031

r^2= 1.000
m= 0.078
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol. usomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.054	0.023	0.002	200	1	1	0.000	0.000	0.011
10	0.054	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.304	0.273	0.022	200	1	1	0.000	0.000	0.100
100	0.259	0.227	0.018	200	1	1	0.000		
100									
	0.095	0.064	0.005	200	83	69983	0.023	0.023	23.498
	0.096	0.065	0.006	200	83	69983	0.023		
	0.099	0.068	0.006	200	83	69983	0.024		

Assay Date	8/9/2005	Test Chemical ID	RC3	tested	8		
ID	TNB	Replicate #	3	Microsome type	Placental	Microsome ID	Battelle

Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/9/2005	Chemical ID	RC3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	3

Microsome Dilution Details

Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.1687	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.1687	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	23.498
Protein Concentration (dilution added to assay, mg/mL):	0.027869

Test Chemical Concentrations

Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	3.00E-08
5	1.00E-08
6	3.00E-09
7	1.00E-09
8	1.00E-10

Assay Date	8/9/2005	Test Chemical ID	RC3	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5914.52	11829.04	11916.21	23832.42	0.1	160890	14.81	23703	0.0296	1	0.014	15	0.0708
			0.5	2	6001.69	12003.38			0.1					1		15	
	2	2	0.5	1	5773.86	11547.72	11536.83	23073.66	0.1	160890	14.34	22944	0.0286	1	0.014	15	0.0685
			0.5	2	5762.97	11525.94			0.1					1		15	
	3	2	0.5	1	5484.06	10968.12	12747.35	25494.7	0.1	160890	15.85	25365	0.0317	1	0.014	15	0.0757
			0.5	2	7263.29	14526.58			0.1					1		15	
	4	2	0.5	1	5437.45	10874.9	10947.56	21895.12	0.1	160890	13.61	21765	0.0272	1	0.014	15	0.0650
			0.5	2	5510.11	11020.22			0.1					1		15	
Background control	1	2	0.5	1	30.86	61.72	61.64	123.28	0.1	160890	0.08	-7	0.0000	1	0.014	15	0.0000
			0.5	2	30.78	61.56			0.1					1		15	
	2	2	0.5	1	25.99	51.98	57.41	114.82	0.1	160890	0.07	-15	0.0000	1	0.014	15	0.0000
			0.5	2	31.42	62.84			0.1					1		15	
	3	2	0.5	1	25.67	51.34	59.11	118.22	0.1	160890	0.07	-12	0.0000	1	0.014	15	0.0000
			0.5	2	33.44	66.88			0.1					1		15	
	4	2	0.5	1	42.75	85.5	81.47	162.94	0.1	160890	0.10	33	0.0000	1	0.014	15	0.0001
			0.5	2	38.72	77.44			0.1					1		15	
Positive control	1	2	0.5	1	2344.85	4689.7	4613.05	9226.1	0.1	160890	5.73	9096	0.0114	1	0.014	15	0.0272
			0.5	2	2268.2	4536.4			0.1					1		15	
	2	2	0.5	1	2230.4	4460.8	4504.96	9009.92	0.1	160890	5.60	8880	0.0111	1	0.014	15	0.0265
			0.5	2	2274.56	4549.12			0.1					1		15	
	3	2	0.5	1	2231.78	4463.56	5220.26	10440.52	0.1	160890	6.49	10311	0.0129	1	0.014	15	0.0308
			0.5	2	2988.48	5976.96			0.1					1		15	
	4	2	0.5	1	2108.68	4217.36	4373.24	8746.48	0.1	160890	5.44	8617	0.0108	1	0.014	15	0.0257
			0.5	2	2264.56	4529.12			0.1					1		15	
Negative Control	1	2	0.5	1	5858.32	11716.64	11575.14	23150.28	0.1	160890	14.39	23020	0.0287	1	0.014	15	0.0687
			0.5	2	5716.82	11433.64			0.1					1		15	
	2	2	0.5	1	5674.21	11348.42	11469.73	22939.46	0.1	160890	14.26	22810	0.0285	1	0.014	15	0.0681
			0.5	2	5795.52	11591.04			0.1					1		15	
	3	2	0.5	1	5317.88	10635.76	10651.9	21303.8	0.1	160890	13.24	21174	0.0264	1	0.014	15	0.0632
			0.5	2	5334.02	10668.04			0.1					1		15	
	4	2	0.5	1	5331.42	10662.84	10744.08	21488.16	0.1	160890	13.36	21358	0.0267	1	0.014	15	0.0638
			0.5	2	5412.66	10825.32			0.1					1		15	
RC3	1-1	2	0.5	1	56.22	112.44	111.59	223.18	0.1	160890	0.14	93	0.0001	1	0.014	15	0.0003
			0.5	2	55.37	110.74			0.1					1		15	
	1-2	2	0.5	1	41.73	83.46	91.07	182.14	0.1	160890	0.11	52	0.0001	1	0.014	15	0.0002
			0.5	2	49.34	98.68			0.1					1		15	
	1-3	2	0.5	1	43.4	86.8	85.43	170.86	0.1	160890	0.11	41	0.0001	1	0.014	15	0.0001
			0.5	2	42.03	84.06			0.1					1		15	
	2-1	2	0.5	1	206.94	413.88	400.72	801.44	0.1	160890	0.50	672	0.0008	1	0.014	15	0.0020
			0.5	2	193.78	387.56			0.1					1		15	
	2-2	2	0.5	1	190.11	380.22	377.49	754.98	0.1	160890	0.47	625	0.0008	1	0.014	15	0.0019
			0.5	2	187.38	374.76			0.1					1		15	
	2-3	2	0.5	1	193.66	387.32	374.63	749.26	0.1	160890	0.47	619	0.0008	1	0.014	15	0.0018
			0.5	2	180.97	361.94			0.1					1		15	
	3-1	2	0.5	1	1284.72	2569.44	2536.2	5072.4	0.1	160890	3.15	4943	0.0062	1	0.014	15	0.0148
			0.5	2	1251.48	2502.96			0.1					1		15	
	3-2	2	0.5	1	1225.55	2451.1	2501.23	5002.46	0.1	160890	3.11	4873	0.0061	1	0.014	15	0.0146
			0.5	2	1275.68	2551.36			0.1					1		15	
	3-3	2	0.5	1	1191.22	2382.44	2365.04	4730.08	0.1	160890	2.94	4600	0.0057	1	0.014	15	0.0137
			0.5	2	1173.82	2347.64			0.1					1		15	
	4-1	2	0.5	1	2697.09	5394.18	5418.04	10836.08	0.1	160890	6.74	10706	0.0134	1	0.014	15	0.0320
			0.5	2	2720.95	5441.9			0.1					1		15	
	4-2	2	0.5	1	2888.63	5777.26	5725.45	11450.9	0.1	160890	7.12	11321	0.0141	1	0.014	15	0.0338
			0.5	2	2836.82	5673.64			0.1					1		15	
	4-3	2	0.5	1	2747.53	5495.06	5465.98	10931.96	0.1	160890	6.79	10802	0.0135	1	0.014	15	0.0323
			0.5	2	2718.45	5436.9			0.1					1		15	
	5-1	2	0.5	1	4236.28	8472.56	8462.65	16925.3	0.1	160890	10.52	16795	0.0210	1	0.014	15	0.0502
			0.5	2	4226.37	8452.74			0.1					1		15	
	5-2	2	0.5	1	4479.13	8958.26	8969.58	17939.16	0.1	160890	11.15	17809	0.0222	1	0.014	15	0.0532
			0.5	2	4490.45	8980.9			0.1					1		15	
	5-3	2	0.5	1	4130.87	8261.74	8276.71	16553.42	0.1	160890	10.29	16424	0.0205	1	0.014	15	0.0490
			0.5	2	4145.84	8291.68			0.1					1		15	
	6-1	2	0.5	1	5405.32	10810.64	10660.57	21321.14	0.1	160890	13.25	21191	0.0265	1	0.014	15	0.0633
			0.5	2	5255.25	10510.5			0.1					1		15	
	6-2	2	0.5	1	5043.35	10086.7	10085.15	20170.3	0.1	160890	12.54	20040	0.0250	1	0.014	15	0.0598
			0.5	2	5041.8	10083.6			0.1					1		15	
	6-3	2	0.5	1	5036.32	10072.64	10178.75	20357.5	0.1	160890	12.65	20228	0.0253	1	0.014	15	0.0604
			0.5	2	5142.43	10284.86			0.1					1		15	
	7-1	2	0.5	1	6051.19	12102.38	11946.63	23893.26	0.1	160890	14.85	23763	0.0297	1	0.014	15	0.0710
			0.5	2	5895.44	11790.88			0.1					1		15	
	7-2	2	0.5	1	5811.84	11623.68	11652.02	23304.04	0.1	160890	14.48	23174	0.0289	1	0.014	15	0.0692
			0.5	2	5840.18	11680.36			0.1					1		15	
	7-3	2	0.5	1	5620.84	11241.68	11227.18	22454.36	0.1	160890	13.96	22325	0.0279	1	0.014	15	0.0667
			0.5	2	5606.34	11212.68			0.1					1		15	
	8-1	2	0.5	1	6290.25	12580.5	12427.64	24855.28	0.1	160890	15.45	24725	0.0309	1	0.014	15	0.0738
			0.5	2	6137.39	12274.78			0.1					1		15	
	8-2	2	0.5	1	5889.68	11779.36	11848.36	23696.72	0.1	160890	14.73	23567	0.0294	1	0.014	15	0.0704
			0.5	2	5958.68	11917.36			0.1					1		15	
	8-3	2	0.5	1	5690.06	11380.12	11478.28	22956.56	0.1	160890	14.27	22827	0.0285	1	0.014	15	0.0682
			0.5	2	5788.22	11576.44			0.1					1		15	

Assay Date	8/9/2005	ID	RC3	# Concentrations tested	Microsome 8 type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0696	0.0016
Full activity	End	0.0704	0.0076
Full activity	Overall	0.0700	0.0045
Background	Beginning	0.0000	1.78641E-05
Background	End	0.0000	9.44308E-05
Background	Overall	0.0000	6.67583E-05
Positive	Beginning	0.0268	0.0005
Positive	End	0.0283	0.0036
Positive	Overall	0.0276	0.0022
Negative	Beginning	0.0684	0.0004
Negative	End	0.0635	0.0004
Negative	Overall	0.0660	0.0029

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC3	1	1	1.00E-05	-5.00	0.0003
RC3	1	2	1.00E-05	-5.00	0.0002
RC3	1	3	1.00E-05	-5.00	0.0001
RC3	2	1	1.00E-06	-6.00	0.0020
RC3	2	2	1.00E-06	-6.00	0.0019
RC3	2	3	1.00E-06	-6.00	0.0018
RC3	3	1	1.00E-07	-7.00	0.0148
RC3	3	2	1.00E-07	-7.00	0.0146
RC3	3	3	1.00E-07	-7.00	0.0137
RC3	4	1	3.00E-08	-7.52	0.0320
RC3	4	2	3.00E-08	-7.52	0.0338
RC3	4	3	3.00E-08	-7.52	0.0323
RC3	5	1	1.00E-08	-8.00	0.0502
RC3	5	2	1.00E-08	-8.00	0.0532
RC3	5	3	1.00E-08	-8.00	0.0490
RC3	6	1	3.00E-09	-8.52	0.0633
RC3	6	2	3.00E-09	-8.52	0.0598
RC3	6	3	3.00E-09	-8.52	0.0604
RC3	7	1	1.00E-09	-9.00	0.0710
RC3	7	2	1.00E-09	-9.00	0.0692
RC3	7	3	1.00E-09	-9.00	0.0667
RC3	8	1	1.00E-10	-10.00	0.0738
RC3	8	2	1.00E-10	-10.00	0.0704
RC3	8	3	1.00E-10	-10.00	0.0682

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-5.00	0.40	0.22	0.18
2	-6.00	2.86	2.67	2.64
3	-7.00	21.08	20.78	19.62
4	-7.52	45.67	48.29	46.08
5	-8.00	71.64	75.96	70.05
6	-8.52	90.39	85.48	86.28
7	-9.00	101.36	98.85	95.22
8	-10.00	105.47	100.52	97.37

Aromatase Assay Spreadsheet

Assay Date	9/19/2005	Test Chemical ID	RC4	# Concentrations tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	33431.13	1688441	
2	0.0199	34707.17	1744079	
3	0.0199	35182.46	1767963	
4	0.0199	36963.08	1857441	
5	0.0199	34675.65	1742495	
			Average DPM/g soln	1760084
			SD	61723
			CV	3.51
			µCi/g soln	0.793

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

ASDN solution	mg ASDN added	(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	8.1968 g
Mass of dilution B used in substrate prep	4.6021 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.57268 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00897 µg/g soln.
µg/g soln.

a. µCi/g soln	0.793
b. Specific activity of [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.

= 0.572680 + 0.00897
= 0.581655 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.363 µCi/µg ASDN

866645 dpm/nmol

Test

Assay Date 9/19/2005 Chemical ID RC4 tested 8

ID JG Replicate # Rep 1 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.414	0.261	0.132	0.087	0.049	0.038	0.032	25	100	
	0.426	0.294	0.132	0.088	0.049	0.037	0.034			
	0.432	0.271	0.132	0.084	0.051	0.037	0.032			

Samples:	10	100	microsomes
	0.057	0.213	0.101
	0.059	0.217	0.096
			0.093

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.424	0.391	0.0473
0.125	100	200	0.00013	200	0.0250	0.275	0.243	0.0289
0.05	200	1000	0.00005	200	0.0100	0.132	0.099	0.0111
0.025	100	1000	0.00003	200	0.0050	0.086	0.054	0.0054
0.01	40	1000	0.00001	200	0.0020	0.050	0.017	0.0009
0.005	20	1000	0.00001	200	0.0010	0.037	0.005	-0.0006

Variables	Regression results			
m, b	0.124	-0.001		
se_m, se_b	0.008	0.001		
r , se_y	0.985	0.003		
F, df	259	4		
ss_{reg}, ss_{resid}	0.002	0.000		

Regression results are calculated using the function LINEST

Blank 0.033

r^2= 0.985
m= 0.124
b= -0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol. usomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.057	0.024	0.002	200	1	1	0.000	0.000	0.010
10	0.059	0.026	0.002	200	1	1	0.000		
10					1	1			
100	0.213	0.180	0.021	200	1	1	0.000	0.000	0.107
100	0.217	0.184	0.022	200	1	1	0.000		
100									
	0.101	0.068	0.007	200	83	69983	0.031	0.028	28.337
	0.096	0.063	0.007	200	83	69983	0.028		
	0.093	0.060	0.006	200	83	69983	0.026		

Assay Date 9/19/2005 Test Chemical ID RC4 tested 8

ID JG Replicate # Rep 1 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.414	0.261	0.132	0.087	0.049	0.038	0.032	25		
	0.426	0.294	0.132	0.088	0.049	0.037	0.034			
	0.432	0.271	0.132	0.084	0.051	0.037	0.032			
Samples:	10	100	microsomes							
	0.057	0.213	0.101							
	0.059	0.217	0.096							
			0.093							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.424	0.391	0.0399
0.125	100	200	0.00013	200	0.0250	0.275	0.243	0.0248
0.05	200	1000	0.00005	200	0.0100	0.132	0.099	0.0102
0.025	100	1000	0.00003	200	0.0050	0.086	0.054	0.0056
0.01	40	1000	0.00001	200	0.0020	0.050	0.017	0.0018
0.005	20	1000	0.00001	200	0.0010	0.037	0.005	0.0006

Blank 0.033

r^2= 0.998
m= 0.102
b= 0.000

Variables	Regression results	
m, b	0.102	0.000
se_m, se_b	0.002	0.000
r , se_y	0.998	0.000
F, df	1891	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	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.057	0.024	0.003	200	1	1	0.000	0.000	0.013
10	0.059	0.026	0.003	200	1	1	0.000		
10					1	1			
100	0.213	0.180	0.018	200	1	1	0.000	0.000	0.093
100	0.217	0.184	0.019	200	1	1	0.000		
100									
	0.101	0.068	0.007	200	83	69983	0.030	0.028	27.902
	0.096	0.063	0.007	200	83	69983	0.028		
	0.093	0.060	0.006	200	83	69983	0.026		

Assay Date	9/19/2005	Test Chemical ID	RC4	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	Battelle

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Regression results are calculated using the function LINEST

Blank

r^2=
m=
b=

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/19/2005	Chemical ID	RC4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	JG	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.1687	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.1687	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	27.902
Protein Concentration (dilution added to assay, mg/mL):	0.033092

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	9/19/2005	Test Chemical ID	RC4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	JG	Replicate #	Rep 1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5867.14	11734.28	11543.2	23086.4	0.1	176008	13.12	22968	0.0265	1	0.017	15	0.0534
			0.5	2	5676.06	11352.12			0.1					1		15	
	2	2	0.5	1	5732	11464	11593.06	23186.12	0.1	176008	13.17	23068	0.0266	1	0.017	15	0.0536
			0.5	2	5861.06	11722.12			0.1					1		15	
	3	2	0.5	1	5309.55	10619.1	10605.03	21210.06	0.1	176008	12.05	21092	0.0243	1	0.017	15	0.0490
			0.5	2	5295.48	10590.96			0.1					1		15	
	4	2	0.5	1	5367.62	10735.24	10673.64	21347.28	0.1	176008	12.13	21229	0.0245	1	0.017	15	0.0493
			0.5	2	5306.02	10612.04			0.1					1		15	
Background control	1	2	0.5	1	25.52	51.04	48.55	97.1	0.1	176008	0.06	-21	0.0000	1	0.017	15	0.0000
			0.5	2	23.03	46.06			0.1					1		15	
	2	2	0.5	1	33.41	66.82	59.49	118.98	0.1	176008	0.07	1	0.0000	1	0.017	15	0.0000
			0.5	2	26.08	52.16			0.1					1		15	
	3	2	0.5	1	28.59	57.18	63.62	127.24	0.1	176008	0.07	9	0.0000	1	0.017	15	0.0000
			0.5	2	35.03	70.06			0.1					1		15	
	4	2	0.5	1	32.62	65.24	64.92	129.84	0.1	176008	0.07	12	0.0000	1	0.017	15	0.0000
			0.5	2	32.3	64.6			0.1					1		15	
Positive control	1	2	0.5	1	2513.74	5027.48	5056.3	10112.6	0.1	176008	5.75	9994	0.0115	1	0.017	15	0.0232
			0.5	2	2542.56	5085.12			0.1					1		15	
	2	2	0.5	1	2395.89	4791.78	4878.94	9757.88	0.1	176008	5.54	9840	0.0111	1	0.017	15	0.0224
			0.5	2	2483.05	4966.1			0.1					1		15	
	3	2	0.5	1	2340.24	4680.48	4652.62	9305.24	0.1	176008	5.29	9187	0.0106	1	0.017	15	0.0214
			0.5	2	2312.38	4624.76			0.1					1		15	
	4	2	0.5	1	2203.48	4406.96	4478.81	8957.62	0.1	176008	5.09	8839	0.0102	1	0.017	15	0.0205
			0.5	2	2275.33	4550.66			0.1					1		15	
Negative Control	1	2	0.5	1	5800.48	11600.96	11631.05	23262.1	0.1	176008	13.22	23144	0.0267	1	0.017	15	0.0538
			0.5	2	5830.57	11661.14			0.1					1		15	
	2	2	0.5	1	5967.33	11934.66	11970.08	23940.16	0.1	176008	13.60	23822	0.0275	1	0.017	15	0.0554
			0.5	2	6002.75	12005.5			0.1					1		15	
	3	2	0.5	1	5415.69	10831.38	10758.9	21517.8	0.1	176008	12.23	21400	0.0247	1	0.017	15	0.0497
			0.5	2	5343.21	10686.42			0.1					1		15	
	4	2	0.5	1	5413.55	10827.1	10871.09	21742.18	0.1	176008	12.35	21624	0.0250	1	0.017	15	0.0503
			0.5	2	5457.54	10915.08			0.1					1		15	
RC4	1-1	2	0.5	1	62.2	124.4	121.37	242.74	0.1	176008	0.14	124	0.0001	1	0.017	15	0.0003
			0.5	2	59.17	118.34			0.1					1		15	
	1-2	2	0.5	1	51.85	103.7	94.85	189.7	0.1	176008	0.11	71	0.0001	1	0.017	15	0.0002
			0.5	2	43	86			0.1					1		15	
	1-3	2	0.5	1	39.7	79.4	88.01	176.02	0.1	176008	0.10	58	0.0001	1	0.017	15	0.0001
			0.5	2	48.31	96.62			0.1					1		15	
	2-1	2	0.5	1	467.12	934.24	928.43	1856.86	0.1	176008	1.05	1739	0.0020	1	0.017	15	0.0040
			0.5	2	461.31	922.62			0.1					1		15	
	2-2	2	0.5	1	482.3	964.6	931.8	1863.6	0.1	176008	1.06	1745	0.0020	1	0.017	15	0.0041
			0.5	2	449.5	899			0.1					1		15	
	2-3	2	0.5	1	435.72	871.44	859.98	1719.96	0.1	176008	0.98	1602	0.0018	1	0.017	15	0.0037
			0.5	2	424.26	848.52			0.1					1		15	
	3-1	2	0.5	1	4839.06	9678.12	9742.65	19485.3	0.1	176008	11.07	19367	0.0223	1	0.017	15	0.0450
			0.5	2	4903.59	9807.18			0.1					1		15	
	3-2	2	0.5	1	4811.77	9623.54	9858.05	19716.1	0.1	176008	11.20	19598	0.0226	1	0.017	15	0.0456
			0.5	2	5046.28	10092.56			0.1					1		15	
	3-3	2	0.5	1	4943.66	9887.32	9895.83	19791.66	0.1	176008	11.24	19673	0.0227	1	0.017	15	0.0457
			0.5	2	4952.17	9904.34			0.1					1		15	
	4-1	2	0.5	1	5812.55	11625.1	11764.72	23529.44	0.1	176008	13.37	23411	0.0270	1	0.017	15	0.0544
			0.5	2	5952.17	11904.34			0.1					1		15	
	4-2	2	0.5	1	5845.12	11690.24	11436.04	22872.08	0.1	176008	12.99	22754	0.0263	1	0.017	15	0.0529
			0.5	2	5590.92	11181.84			0.1					1		15	
	4-3	2	0.5	1	5878.75	11757.5	11613.43	23226.86	0.1	176008	13.20	23109	0.0267	1	0.017	15	0.0537
			0.5	2	5734.68	11469.36			0.1					1		15	
	5-1	2	0.5	1	5668.36	11336.72	11591.03	23182.06	0.1	176008	13.17	23064	0.0266	1	0.017	15	0.0536
			0.5	2	5922.67	11845.34			0.1					1		15	
	5-2	2	0.5	1	5728.81	11457.62	11485.38	22970.76	0.1	176008	13.05	22852	0.0264	1	0.017	15	0.0531
			0.5	2	5756.57	11513.14			0.1					1		15	
	5-3	2	0.5	1	5817.42	11634.84	11559.71	23119.42	0.1	176008	13.14	23001	0.0265	1	0.017	15	0.0535
			0.5	2	5742.29	11484.58			0.1					1		15	
	6-1	2	0.5	1	5768.81	11537.62	11656.54	23313.08	0.1	176008	13.25	23195	0.0268	1	0.017	15	0.0539
			0.5	2	5887.73	11775.46			0.1					1		15	
	6-2	2	0.5	1	5787.73	11575.46	11577.19	23154.38	0.1	176008	13.16	23036	0.0266	1	0.017	15	0.0535
			0.5	2	5789.46	11578.92			0.1					1		15	
	6-3	2	0.5	1	5744.85	11489.7	11537.57	23075.14	0.1	176008	13.11	22957	0.0265	1	0.017	15	0.0534
			0.5	2	5792.72	11585.44			0.1					1		15	
	7-1	2	0.5	1	5914.25	11828.5	11818.47	23636.94	0.1	176008	13.43	23519	0.0271	1	0.017	15	0.0547
			0.5	2	5904.22	11808.44			0.1					1		15	
	7-2	2	0.5	1	5791.52	11583.04	11641.37	23282.74	0.1	176008	13.23	23164	0.0267	1	0.017	15	0.0538
			0.5	2	5849.85	11699.7			0.1					1		15	
	7-3	2	0.5	1	5787.26	11574.52	11496.69	22993.38	0.1	176008	13.06	22875	0.0264	1	0.017	15	0.0532
			0.5	2	5709.43	11418.86			0.1					1		15	
	8-1	2	0.5	1	5810.69	11621.38	11729.93	23459.86	0.1	176008	13.33	23342	0.0269	1	0.017	15	0.0543
			0.5	2	5919.24	11838.48			0.1					1		15	
	8-2	2	0.5	1	5817.41	11634.82	11659.5	23319	0.1	176008	13.25	23201	0.0268	1	0.017	15	0.0539
			0.5	2	5842.09	11684.18			0.1					1		15	
	8-3	2	0.5	1	5714.64	11949.28	11428.58	22857.16	0.1	176008	12.99	22739	0.0262	1	0.017	15	0.0529
			0.5	2	5653.94	11307.88			0.1					1		15	

Assay Date	9/19/2005	ID	RC4	# Concentrations tested	Microsome 8 type	Placental	Microsome ID	Battelle	Technician ID	JG	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0535	0.0002
Full activity	End	0.0492	0.0002
Full activity	Overall	0.0513	0.0025
Background	Beginning	0.0000	3.59649E-05
Background	End	0.0000	4.27371E-06
Background	Overall	0.0000	3.45575E-05
Positive	Beginning	0.0228	0.0006
Positive	End	0.0210	0.0006
Positive	Overall	0.0219	0.0012
Negative	Beginning	0.0546	0.0011
Negative	End	0.0500	0.0004
Negative	Overall	0.0523	0.0027

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC4	1	1	1.00E-03	-3.00	0.0003
RC4	1	2	1.00E-03	-3.00	0.0002
RC4	1	3	1.00E-03	-3.00	0.0001
RC4	2	1	1.00E-04	-4.00	0.0040
RC4	2	2	1.00E-04	-4.00	0.0041
RC4	2	3	1.00E-04	-4.00	0.0037
RC4	3	1	1.00E-05	-5.00	0.0450
RC4	3	2	1.00E-05	-5.00	0.0456
RC4	3	3	1.00E-05	-5.00	0.0457
RC4	4	1	1.00E-06	-6.00	0.0544
RC4	4	2	1.00E-06	-6.00	0.0529
RC4	4	3	1.00E-06	-6.00	0.0537
RC4	5	1	1.00E-07	-7.00	0.0536
RC4	5	2	1.00E-07	-7.00	0.0531
RC4	5	3	1.00E-07	-7.00	0.0535
RC4	6	1	1.00E-08	-8.00	0.0539
RC4	6	2	1.00E-08	-8.00	0.0535
RC4	6	3	1.00E-08	-8.00	0.0534
RC4	7	1	1.00E-09	-9.00	0.0547
RC4	7	2	1.00E-09	-9.00	0.0538
RC4	7	3	1.00E-09	-9.00	0.0532
RC4	8	1	1.00E-10	-10.00	0.0543
RC4	8	2	1.00E-10	-10.00	0.0539
RC4	8	3	1.00E-10	-10.00	0.0529

Percent of control values				
	Log[test	Replicate		
_evel	bstance]	1	2	3
1	-3.00	0.56	0.32	0.26
2	-4.00	7.87	7.90	7.25
3	-5.00	87.68	88.72	89.06
4	-6.00	105.98	103.01	104.61
5	-7.00	104.41	103.46	104.13
6	-8.00	105.01	104.29	103.93
7	-9.00	106.47	104.87	103.56
8	-10.00	105.67	105.03	102.94

Aromatase Assay Spreadsheet

Assay Date	9/22/2005	Test Chemical ID	RC4	# Concentrations tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	34353.11	1717656	
2	0.0197	33191.65	1684855	
3	0.0199	34297.95	1723515	
4	0.0199	35433.68	1780587	
5	0.0199	35153.98	1766532	
			Average DPM/g soln	1734629
			SD	38791
			CV	2.24
			µCi/g soln	0.781

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

ASDN solution	mg ASDN added	(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	8.1975 g
Mass of dilution B used in substrate prep	4.6012 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.561293 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00885 µg/g soln.
µg/g soln.

a. µCi/g soln	0.781
b. Specific activity of [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.

= 0.561293 + 0.00885
= 0.570138 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.370 µCi/µg ASDN

871364 dpm/nmol

Assay Date 9/22/2005 Test Chemical ID RC4 tested 8

ID JG Replicate # Rep 2 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.559	0.332	0.145	0.085	0.055	0.044	0.036	25		
	0.559	0.337	0.151	0.088	0.054	0.044	0.036			
	0.546	0.341	0.153	0.089	0.053	0.045	0.035			
Samples:	10	100	microsomes							
	0.055	0.282	0.099							
	0.053	0.282	0.098							
			0.098							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.555	0.519	0.0483
0.125	100	200	0.00013	200	0.0250	0.337	0.301	0.0279
0.05	200	1000	0.00005	200	0.0100	0.150	0.114	0.0104
0.025	100	1000	0.00003	200	0.0050	0.087	0.052	0.0045
0.01	40	1000	0.00001	200	0.0020	0.054	0.018	0.0014
0.005	20	1000	0.00001	200	0.0010	0.044	0.009	0.0005

Variables	Regression results	
m, b	0.094	0.000
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	593	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank 0.036

r^2= 0.993
m= 0.094
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.055	0.019	0.001	200	1	1	0.000	0.000	0.007
10	0.053	0.017	0.001	200	1	1	0.000		
10					1	1			
100	0.282	0.246	0.023	200	1	1	0.000	0.000	0.114
100	0.282	0.246	0.023	200	1	1	0.000		
100									
	0.099	0.063	0.006	200	83	69983	0.024	0.023	23.424
	0.098	0.062	0.006	200	83	69983	0.023		
	0.098	0.062	0.006	200	83	69983	0.023		

Assay Date 9/22/2005 Test Chemical ID RC4 tested 8

ID JG Replicate # Rep 2 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.559	0.332	0.145	0.085	0.055	0.044	0.036	25	100	
	0.559	0.337	0.151	0.088	0.054	0.044	0.036			
	0.548	0.341	0.153	0.089	0.053	0.045	0.035			
Samples:	10	100	microsomes							
	0.055	0.282	0.099							
	0.053	0.282	0.098							
			0.098							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.555	0.519	0.0429
0.125	100	200	0.00013	200	0.0250	0.337	0.301	0.0251
0.05	200	1000	0.00005	200	0.0100	0.150	0.114	0.0098
0.025	100	1000	0.00003	200	0.0050	0.087	0.052	0.0048
0.01	40	1000	0.00001	200	0.0020	0.054	0.018	0.0020
0.005	20	1000	0.00001	200	0.0010	0.044	0.009	0.0013

Blank 0.036

r^2= 1.000
m= 0.082
b= 0.001

Variables	Regression results	
m, b	0.082	0.001
se_m, se_b	0.001	0.000
r , se_y	1.000	0.000
F, df	7372	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	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.055	0.019	0.002	200	1	1	0.000	0.000	0.010
10	0.053	0.017	0.002	200	1	1	0.000		
10					1	1			
100	0.282	0.246	0.021	200	1	1	0.000	0.000	0.103
100	0.282	0.246	0.021	200	1	1	0.000		
100									
	0.099	0.063	0.006	200	83	69983	0.024	0.024	23.861
	0.098	0.062	0.006	200	83	69983	0.024		
	0.098	0.062	0.006	200	83	69983	0.024		

Assay Date	9/22/2005	Test Chemical ID	RC4	tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/22/2005	Chemical ID	RC4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	JG	Replicate #	Rep 2

Microsome Dilution Details

Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.1687	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.1687	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	23.861
Protein Concentration (dilution added to assay, mg/mL):	0.028299

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	9/22/2005	Test Chemical ID	RC4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	JG	Replicate #	Rep 2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5309.7	10619.4	10663.1	21326.2	0.1	173463	12.29	21196	0.0243	1	0.014	15	0.0573
			0.5	2	5353.4	10706.8			0.1					1		15	
	2	2	0.5	1	5621.08	11242.16	11368.35	22732.7	0.1	173463	13.11	22603	0.0259	1	0.014	15	0.0611
			0.5	2	5745.27	11490.54			0.1					1		15	
	3	2	0.5	1	5190.44	10380.88	10641.11	21282.22	0.1	173463	12.27	21152	0.0243	1	0.014	15	0.0572
			0.5	2	5450.67	10901.34			0.1					1		15	
	4	2	0.5	1	5352.2	10704.4	10684.43	21368.86	0.1	173463	12.32	21239	0.0244	1	0.014	15	0.0574
			0.5	2	5332.23	10664.46			0.1					1		15	
Background control	1	2	0.5	1	40.83	81.66	88.53	177.06	0.1	173463	0.10	47	0.0001	1	0.014	15	0.0001
			0.5	2	47.7	95.4			0.1					1		15	
	2	2	0.5	1	35.99	71.98	60.42	120.84	0.1	173463	0.07	-9	0.0000	1	0.014	15	0.0000
			0.5	2	24.43	48.86			0.1					1		15	
	3	2	0.5	1	28.35	56.7	62.03	124.06	0.1	173463	0.07	-6	0.0000	1	0.014	15	0.0000
			0.5	2	33.68	67.36			0.1					1		15	
	4	2	0.5	1	28.39	56.78	49.25	98.5	0.1	173463	0.06	-32	0.0000	1	0.014	15	-0.0001
			0.5	2	20.86	41.72			0.1					1		15	
Positive control	1	2	0.5	1	2390.27	4780.54	4797.5	9595	0.1	173463	5.53	9465	0.0109	1	0.014	15	0.0256
			0.5	2	2407.23	4814.46			0.1					1		15	
	2	2	0.5	1	2384.32	4768.64	4809.31	9618.62	0.1	173463	5.55	9489	0.0109	1	0.014	15	0.0257
			0.5	2	2424.99	4849.98			0.1					1		15	
	3	2	0.5	1	2208.8	4417.6	4439.86	8879.72	0.1	173463	5.12	8750	0.0100	1	0.014	15	0.0237
			0.5	2	2231.06	4462.12			0.1					1		15	
	4	2	0.5	1	2198.71	4397.42	4383.68	8767.36	0.1	173463	5.05	8637	0.0099	1	0.014	15	0.0234
			0.5	2	2184.97	4369.94			0.1					1		15	
Negative Control	1	2	0.5	1	5443.72	10887.44	10985.58	21971.16	0.1	173463	12.67	21841	0.0251	1	0.014	15	0.0590
			0.5	2	5541.86	11083.72			0.1					1		15	
	2	2	0.5	1	5610.93	11221.86	11231.6	22463.2	0.1	173463	12.95	22333	0.0256	1	0.014	15	0.0604
			0.5	2	5620.67	11241.34			0.1					1		15	
	3	2	0.5	1	5120.64	10241.28	10281.78	20563.56	0.1	173463	11.85	20433	0.0234	1	0.014	15	0.0552
			0.5	2	5161.14	10322.28			0.1					1		15	
	4	2	0.5	1	5232.34	10464.68	10452.76	20905.52	0.1	173463	12.05	20775	0.0238	1	0.014	15	0.0562
			0.5	2	5220.42	10440.84			0.1					1		15	
RC4	1-1	2	0.5	1	66.93	133.86	135.07	270.14	0.1	173463	0.16	140	0.0002	1	0.014	15	0.0004
			0.5	2	68.14	136.28			0.1					1		15	
	1-2	2	0.5	1	55.05	110.1	108.37	216.74	0.1	173463	0.12	87	0.0001	1	0.014	15	0.0002
			0.5	2	53.32	106.64			0.1					1		15	
	1-3	2	0.5	1	48.48	96.96	87.41	174.82	0.1	173463	0.10	45	0.0001	1	0.014	15	0.0001
			0.5	2	38.93	77.86			0.1					1		15	
	2-1	2	0.5	1	437.84	875.68	887.73	1775.46	0.1	173463	1.02	1645	0.0019	1	0.014	15	0.0044
			0.5	2	449.89	899.78			0.1					1		15	
	2-2	2	0.5	1	498.06	996.12	972.21	1944.42	0.1	173463	1.12	1814	0.0021	1	0.014	15	0.0049
			0.5	2	474.15	948.3			0.1					1		15	
	2-3	2	0.5	1	420.26	840.52	870.98	1741.96	0.1	173463	1.00	1612	0.0018	1	0.014	15	0.0044
			0.5	2	450.72	901.44			0.1					1		15	
	3-1	2	0.5	1	1281.67	2563.34	2539.63	5079.26	0.1	173463	2.93	4949	0.0057	1	0.014	15	0.0134
			0.5	2	1257.96	2515.92			0.1					1		15	
	3-2	2	0.5	1	1232.14	2464.28	2465.24	4930.48	0.1	173463	2.84	4800	0.0055	1	0.014	15	0.0130
			0.5	2	1233.1	2466.2			0.1					1		15	
	3-3	2	0.5	1	1215.03	2430.06	2437.5	4875	0.1	173463	2.81	4745	0.0054	1	0.014	15	0.0128
			0.5	2	1222.47	2444.94			0.1					1		15	
	4-1	2	0.5	1	4802.74	9605.48	9640.6	19281.2	0.1	173463	11.12	19151	0.0220	1	0.014	15	0.0518
			0.5	2	4837.86	9675.72			0.1					1		15	
	4-2	2	0.5	1	4728.94	9457.88	9465.9	18931.8	0.1	173463	10.91	18802	0.0216	1	0.014	15	0.0508
			0.5	2	4736.96	9473.92			0.1					1		15	
	4-3	2	0.5	1	4716.03	9432.06	9456.88	18913.76	0.1	173463	10.90	18784	0.0216	1	0.014	15	0.0508
			0.5	2	4740.85	9481.7			0.1					1		15	
	5-1	2	0.5	1	5616.09	11232.18	11260.15	22520.3	0.1	173463	12.98	22390	0.0257	1	0.014	15	0.0605
			0.5	2	5644.06	11288.12			0.1					1		15	
	5-2	2	0.5	1	5546.85	11093.7	11080.66	22161.32	0.1	173463	12.78	22031	0.0253	1	0.014	15	0.0596
			0.5	2	5533.81	11067.62			0.1					1		15	
	5-3	2	0.5	1	5383.66	10767.32	10864.13	21728.26	0.1	173463	12.53	21596	0.0248	1	0.014	15	0.0584
			0.5	2	5480.47	10960.94			0.1					1		15	
	6-1	2	0.5	1	4764.64	9529.28	9783.43	19566.86	0.1	173463	11.28	19437	0.0223	1	0.014	15	0.0525
			0.5	2	5018.79	10037.58			0.1					1		15	
	6-2	2	0.5	1	5485	10970	11022.43	22044.86	0.1	173463	12.71	21915	0.0251	1	0.014	15	0.0592
			0.5	2	5537.43	11074.86			0.1					1		15	
	6-3	2	0.5	1	5649.54	11299.08	11314.97	22629.94	0.1	173463	13.05	22500	0.0258	1	0.014	15	0.0608
			0.5	2	5665.43	11330.86			0.1					1		15	
	7-1	2	0.5	1	5527.71	11055.42	11068.61	22137.22	0.1	173463	12.76	22007	0.0253	1	0.014	15	0.0595
			0.5	2	5540.9	11081.8			0.1					1		15	
	7-2	2	0.5	1	5470.25	10940.5	11005.16	22010.32	0.1	173463	12.69	21880	0.0251	1	0.014	15	0.0592
			0.5	2	5534.91	11069.82			0.1					1		15	
	7-3	2	0.5	1	5405.85	10811.7	10862.56	21725.12	0.1	173463	12.52	21595	0.0248	1	0.014	15	0.0584
			0.5	2	5456.71	10913.42			0.1					1		15	
	8-1	2	0.5	1	5388.13	10776.26	10764.64	21529.28	0.1	173463	12.41	21399	0.0246	1	0.014	15	0.0579
			0.5	2	5376.51	10753.02			0.1					1		15	
	8-2	2	0.5	1	5457.23	10914.46	10871.56	21743.12	0.1	173463	12.53	21613	0.0248	1	0.014	15	0.0584
			0.5	2	5414.33	10828.66			0.1					1		15	
	8-3	2	0.5	1	5277.74	10555.48	10767.02	21534.04	0.1	173463	12.41	21404	0.0246	1	0.014	15	0.0579
			0.5	2	5489.28	10978.56			0.1					1		15	

Assay Date	9/22/2005	ID	RC4	# Concentrations tested	Microsome 8 type	Placental	Microsome ID Battelle	Technician ID JG	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0592	0.0027
Full activity	End	0.0573	0.0002
Full activity	Overall	0.0583	0.0019
Background	Beginning	0.0001	0.000107476
Background	End	-0.0001	4.88631E-05
Background	Overall	0.0000	9.00197E-05
Positive	Beginning	0.0256	0.0000
Positive	End	0.0235	0.0002
Positive	Overall	0.0246	0.0012
Negative	Beginning	0.0597	0.0009
Negative	End	0.0557	0.0007
Negative	Overall	0.0577	0.0024

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC4	1	1	1.00E-03	-3.00	0.0004
RC4	1	2	1.00E-03	-3.00	0.0002
RC4	1	3	1.00E-03	-3.00	0.0001
RC4	2	1	1.00E-04	-4.00	0.0044
RC4	2	2	1.00E-04	-4.00	0.0049
RC4	2	3	1.00E-04	-4.00	0.0044
RC4	3	1	5.00E-05	-4.30	0.0134
RC4	3	2	5.00E-05	-4.30	0.0130
RC4	3	3	5.00E-05	-4.30	0.0128
RC4	4	1	1.00E-05	-5.00	0.0518
RC4	4	2	1.00E-05	-5.00	0.0508
RC4	4	3	1.00E-05	-5.00	0.0508
RC4	5	1	1.00E-06	-6.00	0.0605
RC4	5	2	1.00E-06	-6.00	0.0596
RC4	5	3	1.00E-06	-6.00	0.0584
RC4	6	1	1.00E-07	-7.00	0.0525
RC4	6	2	1.00E-07	-7.00	0.0592
RC4	6	3	1.00E-07	-7.00	0.0608
RC4	7	1	1.00E-08	-8.00	0.0595
RC4	7	2	1.00E-08	-8.00	0.0592
RC4	7	3	1.00E-08	-8.00	0.0584
RC4	8	1	1.00E-09	-9.00	0.0579
RC4	8	2	1.00E-09	-9.00	0.0584
RC4	8	3	1.00E-09	-9.00	0.0579

Percent of control values				
	Log[test	Replicate		
_evel	bstance]	1	2	3
1	-3.00	0.65	0.40	0.21
2	-4.00	7.64	8.42	7.48
3	-4.30	22.97	22.28	22.02
4	-5.00	88.88	87.26	87.17
5	-6.00	103.91	102.25	100.24
6	-7.00	90.20	101.70	104.42
7	-8.00	102.13	101.54	100.22
8	-9.00	99.31	100.30	99.33

Aromatase Assay Spreadsheet

Assay Date	9/26/2005	Test Chemical ID	RC4	# Concentrations tested	8	
ID	JG	Replicate #	Rep 3	Microsome type	Placental	Microsome ID Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	33809.76	1698983	
2	0.0202	34394.46	1702696	
3	0.0201	34276.69	1705308	
4	0.0200	35031.75	1751588	
5	0.0200	34985.33	1749267	
			Average DPM/g soln	1721568
			SD	26453
			CV	1.54
			µCi/g soln	0.775

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

ASDN solution	mg ASDN added	(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	8.2205 g
Mass of dilution B used in substrate prep	4.6194 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.584414 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00878 µg/g soln.
µg/g soln.

a. µCi/g soln	0.775
b. Specific activity of [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.

= 0.584414 + 0.00878
= 0.593193 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.307 µCi/µg ASDN

831192 dpm/nmol

Test

Assay Date 9/26/2005 Chemical ID RC4 tested 8

ID JG Replicate # Rep 3 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.578	0.342	0.161	0.098	0.059	0.045	0.036	25		
	0.585	0.358	0.153	0.095	0.059	0.047	0.036			
	0.581	0.347	0.147	0.097	0.060	0.045	0.036			
Samples:	10	100	microsomes							
	0.059	0.279	0.102							
	0.057	0.289	0.102							
			0.104							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
0.25	200	200	0.00025	200	0.0500	0.581	0.545	0.0485	m, b	0.090	-0.001
0.125	100	200	0.00013	200	0.0250	0.349	0.313	0.0276	se_m, se_b	0.003	0.001
0.05	200	1000	0.00005	200	0.0100	0.154	0.118	0.0100	r , se_y	0.995	0.002
0.025	100	1000	0.00003	200	0.0050	0.097	0.061	0.0049	F, df	744	4
0.01	40	1000	0.00001	200	0.0020	0.059	0.023	0.0016	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.046	0.010	0.0003			

Regression results are calculated using the function LINEST

Blank 0.036

r^2= 0.995
m= 0.090
b= -0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.059	0.023	0.002	200	1	1	0.000	0.000	0.007
10	0.057	0.021	0.001	200	1	1	0.000		
10					1	1			
100	0.279	0.243	0.021	200	1	1	0.000	0.000	0.109
100	0.289	0.253	0.022	200	1	1	0.000		
100									
	0.102	0.066	0.005	200	83	69983	0.023	0.023	23.010
	0.102	0.066	0.005	200	83	69983	0.023		
	0.104	0.068	0.006	200	83	69983	0.024		

Assay Date 9/26/2005 Test Chemical ID RC4 tested 8

ID JG Replicate # Rep 3 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.578	0.342	0.161	0.098	0.059	0.045	0.036	25		
	0.585	0.358	0.153	0.095	0.059	0.047	0.036			
	0.581	0.347	0.147	0.097	0.060	0.045	0.036			
Samples:	10	100	microsomes							
	0.059	0.279	0.102							
	0.057	0.289	0.102							
			0.104							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.581	0.545	0.0436
0.125	100	200	0.00013	200	0.0250	0.349	0.313	0.0251
0.05	200	1000	0.00005	200	0.0100	0.154	0.118	0.0096
0.025	100	1000	0.00003	200	0.0050	0.097	0.061	0.0051
0.01	40	1000	0.00001	200	0.0020	0.059	0.023	0.0021
0.005	20	1000	0.00001	200	0.0010	0.046	0.010	0.0010

Blank 0.036

r^2= 1.000
m= 0.079
b= 0.000

Variables	Regression results	
m, b	0.079	0.000
se_m, se_b	0.001	0.000
r , se_y	1.000	0.000
F, df	6121	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	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.059	0.023	0.002	200	1	1	0.000	0.000	0.010
10	0.057	0.021	0.002	200	1	1	0.000		
10					1	1			
100	0.279	0.243	0.020	200	1	1	0.000	0.000	0.100
100	0.289	0.253	0.020	200	1	1	0.000		
100									
	0.102	0.066	0.006	200	83	69983	0.023	0.023	23.477
	0.102	0.066	0.006	200	83	69983	0.023		
	0.104	0.068	0.006	200	83	69983	0.024		

Assay Date	9/26/2005	Test Chemical ID	RC4	tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	Battelle

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/26/2005	Chemical ID	RC4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	JG	Replicate #	Rep 3

Microsome Dilution Details		
Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.1687	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.1687	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	23.477
Protein Concentration (dilution added to assay, mg/mL):	0.027844

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	9/26/2005	Test Chemical ID	RC4	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	JG	Replicate #	Rep 3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	6149.11	12298.22	12193.88	24387.76	0.1	172157	14.17	24243	0.0292	1	0.014	15	0.0698
			0.5	2	6044.77	12089.54			0.1					1		15	
	2	2	0.5	1	5889.61	11779.22	11776.4	23552.8	0.1	172157	13.68	23408	0.0282	1	0.014	15	0.0674
			0.5	2	5886.79	11773.58			0.1					1		15	
	3	2	0.5	1	5633.29	11266.58	11319.14	22638.28	0.1	172157	13.15	22494	0.0271	1	0.014	15	0.0648
			0.5	2	5685.85	11371.7			0.1					1		15	
	4	2	0.5	1	5577.17	11154.34	11211.14	22422.28	0.1	172157	13.02	22278	0.0268	1	0.014	15	0.0642
			0.5	2	5633.97	11267.94			0.1					1		15	
Background control	1	2	0.5	1	20.24	40.48	58.62	117.24	0.1	172157	0.07	-27	0.0000	1	0.014	15	-0.0001
			0.5	2	38.38	76.76			0.1					1		15	
	2	2	0.5	1	36.53	73.06	71.78	143.56	0.1	172157	0.08	-1	0.0000	1	0.014	15	0.0000
			0.5	2	35.25	70.5			0.1					1		15	
	3	2	0.5	1	36.32	72.64	85.92	171.84	0.1	172157	0.10	27	0.0000	1	0.014	15	0.0001
			0.5	2	49.6	99.2			0.1					1		15	
	4	2	0.5	1	29.87	59.74	72.43	144.86	0.1	172157	0.08	0	0.0000	1	0.014	15	0.0000
			0.5	2	42.56	85.12			0.1					1		15	
Positive control	1	2	0.5	1	2495.15	4990.3	5048.54	10097.08	0.1	172157	5.87	9953	0.0120	1	0.014	15	0.0287
			0.5	2	2553.38	5106.76			0.1					1		15	
	2	2	0.5	1	2572.2	5144.4	5118.81	10237.62	0.1	172157	5.95	10093	0.0121	1	0.014	15	0.0291
			0.5	2	2546.61	5093.22			0.1					1		15	
	3	2	0.5	1	2496.62	4993.24	4962.09	9924.18	0.1	172157	5.76	9780	0.0118	1	0.014	15	0.0282
			0.5	2	2465.47	4930.94			0.1					1		15	
	4	2	0.5	1	2451.48	4902.96	4830.94	9661.88	0.1	172157	5.61	9518	0.0115	1	0.014	15	0.0274
			0.5	2	2379.46	4758.92			0.1					1		15	
Negative Control	1	2	0.5	1	5824.04	11648.08	11581.34	23162.68	0.1	172157	13.45	23018	0.0277	1	0.014	15	0.0663
			0.5	2	5757.3	11514.6			0.1					1		15	
	2	2	0.5	1	5913.34	11826.68	11867.49	23734.98	0.1	172157	13.79	23591	0.0284	1	0.014	15	0.0680
			0.5	2	5954.15	11908.3			0.1					1		15	
	3	2	0.5	1	5568.26	11136.52	11178.7	22359.4	0.1	172157	12.99	22215	0.0267	1	0.014	15	0.0640
			0.5	2	5610.44	11220.88			0.1					1		15	
	4	2	0.5	1	5389.66	10779.32	10710.18	21420.36	0.1	172157	12.44	21276	0.0256	1	0.014	15	0.0613
			0.5	2	5320.52	10641.04			0.1					1		15	
RC4	1-1	2	0.5	1	34.32	68.64	77.41	154.82	0.1	172157	0.09	10	0.0000	1	0.014	15	0.0000
			0.5	2	43.09	86.18			0.1					1		15	
	1-2	2	0.5	1	48.35	96.7	97.26	194.52	0.1	172157	0.11	50	0.0001	1	0.014	15	0.0001
			0.5	2	48.91	97.82			0.1					1		15	
	1-3	2	0.5	1	48.09	96.18	98.88	197.76	0.1	172157	0.11	53	0.0001	1	0.014	15	0.0002
			0.5	2	50.79	101.58			0.1					1		15	
	2-1	2	0.5	1	464.84	929.68	944.03	1888.06	0.1	172157	1.10	1744	0.0021	1	0.014	15	0.0050
			0.5	2	479.19	958.38			0.1					1		15	
	2-2	2	0.5	1	497.77	995.54	993.56	1987.12	0.1	172157	1.15	1843	0.0022	1	0.014	15	0.0053
			0.5	2	495.79	991.58			0.1					1		15	
	2-3	2	0.5	1	527.69	1055.38	1053.3	2108.6	0.1	172157	1.22	1962	0.0024	1	0.014	15	0.0057
			0.5	2	525.61	1051.22			0.1					1		15	
	3-1	2	0.5	1	1412.1	2824.2	2802.42	5604.84	0.1	172157	3.26	5460	0.0068	1	0.014	15	0.0157
			0.5	2	1390.32	2780.64			0.1					1		15	
	3-2	2	0.5	1	1393.5	2787	2790.29	5580.58	0.1	172157	3.24	5438	0.0065	1	0.014	15	0.0157
			0.5	2	1396.79	2793.58			0.1					1		15	
	3-3	2	0.5	1	1202.56	2405.12	2416.9	4833.8	0.1	172157	2.81	4689	0.0056	1	0.014	15	0.0135
			0.5	2	1214.34	2428.68			0.1					1		15	
	4-1	2	0.5	1	5051.65	10103.3	10219	20438	0.1	172157	11.87	20294	0.0244	1	0.014	15	0.0585
			0.5	2	5167.35	10334.7			0.1					1		15	
	4-2	2	0.5	1	5126.77	10253.54	10262.89	20525.78	0.1	172157	11.92	20381	0.0245	1	0.014	15	0.0587
			0.5	2	5136.12	10272.24			0.1					1		15	
	4-3	2	0.5	1	5020.39	10040.78	10112.06	20224.12	0.1	172157	11.75	20080	0.0242	1	0.014	15	0.0578
			0.5	2	5091.67	10183.34			0.1					1		15	
	5-1	2	0.5	1	5641.44	11282.88	11394.7	22789.4	0.1	172157	13.24	22645	0.0272	1	0.014	15	0.0652
			0.5	2	5753.26	11506.52			0.1					1		15	
	5-2	2	0.5	1	5630.29	11260.58	11458.19	22916.38	0.1	172157	13.31	22772	0.0274	1	0.014	15	0.0656
			0.5	2	5827.9	11655.8			0.1					1		15	
	5-3	2	0.5	1	5656.75	11313.5	11401.86	22803.72	0.1	172157	13.25	22659	0.0273	1	0.014	15	0.0653
			0.5	2	5745.11	11490.22			0.1					1		15	
	6-1	2	0.5	1	5705.71	11411.42	11500.69	23001.38	0.1	172157	13.36	22857	0.0275	1	0.014	15	0.0658
			0.5	2	5794.96	11589.96			0.1					1		15	
	6-2	2	0.5	1	5777.96	11555.96	11593.37	23186.74	0.1	172157	13.47	23042	0.0277	1	0.014	15	0.0664
			0.5	2	5815.39	11630.78			0.1					1		15	
	6-3	2	0.5	1	5688.12	11376.24	11419.5	22839	0.1	172157	13.27	22695	0.0273	1	0.014	15	0.0654
			0.5	2	5731.38	11462.76			0.1					1		15	
	7-1	2	0.5	1	5622.01	11244.02	11318.2	22636.4	0.1	172157	13.15	22492	0.0271	1	0.014	15	0.0648
			0.5	2	5696.19	11392.38			0.1					1		15	
	7-2	2	0.5	1	5743.11	11486.22	11540.98	23081.96	0.1	172157	13.41	22938	0.0276	1	0.014	15	0.0661
			0.5	2	5797.87	11595.74			0.1					1		15	
	7-3	2	0.5	1	5654.3	11308.6	11473.72	22947.44	0.1	172157	13.33	22803	0.0274	1	0.014	15	0.0657
			0.5	2	5819.42	11638.84			0.1					1		15	
	8-1	2	0.5	1	5799.36	11598.72	11588.67	23177.34	0.1	172157	13.46	23033	0.0277	1	0.014	15	0.0663
			0.5	2	5789.31	11578.62			0.1					1		15	
	8-2	2	0.5	1	5746.07	11492.14	11426.45	22852.9	0.1	172157	13.27	22709	0.0273	1	0.014	15	0.0654
			0.5	2	5680.38	11360.76			0.1					1		15	
	8-3	2	0.5	1	5674.46	11348.92	11320.3	22640.6	0.1	172157	13.15	22496	0.0271	1	0.014	15	0.0648
			0.5	2	5645.84	11291.68			0.1					1		15	

Assay Date	9/26/2005	ID	RC4	# Concentrations tested	Microsome 8 type	Placental	Microsome ID Battelle	Technician ID JG	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0686	0.0017
Full activity	End	0.0645	0.0004
Full activity	Overall	0.0666	0.0026
Background	Beginning	0.0000	5.36105E-05
Background	End	0.0000	5.49549E-05
Background	Overall	0.0000	6.42296E-05
Positive	Beginning	0.0289	0.0003
Positive	End	0.0278	0.0005
Positive	Overall	0.0283	0.0007
Negative	Beginning	0.0671	0.0012
Negative	End	0.0626	0.0019
Negative	Overall	0.0649	0.0029

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC4	1	1	1.00E-03	-3.00	0.0000
RC4	1	2	1.00E-03	-3.00	0.0001
RC4	1	3	1.00E-03	-3.00	0.0002
RC4	2	1	1.00E-04	-4.00	0.0050
RC4	2	2	1.00E-04	-4.00	0.0053
RC4	2	3	1.00E-04	-4.00	0.0057
RC4	3	1	5.00E-05	-4.30	0.0157
RC4	3	2	5.00E-05	-4.30	0.0157
RC4	3	3	5.00E-05	-4.30	0.0135
RC4	4	1	1.00E-05	-5.00	0.0585
RC4	4	2	1.00E-05	-5.00	0.0587
RC4	4	3	1.00E-05	-5.00	0.0578
RC4	5	1	1.00E-06	-6.00	0.0652
RC4	5	2	1.00E-06	-6.00	0.0656
RC4	5	3	1.00E-06	-6.00	0.0653
RC4	6	1	1.00E-07	-7.00	0.0658
RC4	6	2	1.00E-07	-7.00	0.0664
RC4	6	3	1.00E-07	-7.00	0.0654
RC4	7	1	1.00E-08	-8.00	0.0648
RC4	7	2	1.00E-08	-8.00	0.0661
RC4	7	3	1.00E-08	-8.00	0.0657
RC4	8	1	1.00E-09	-9.00	0.0663
RC4	8	2	1.00E-09	-9.00	0.0654
RC4	8	3	1.00E-09	-9.00	0.0648

Percent of control values				
	Log[test	Replicate		
_evel	bstance]	1	2	3
1	-3.00	0.05	0.22	0.23
2	-4.00	7.55	7.98	8.49
3	-4.30	23.63	23.53	20.30
4	-5.00	87.83	88.21	86.90
5	-6.00	98.01	98.55	98.07
6	-7.00	98.92	99.73	98.22
7	-8.00	97.34	99.27	98.69
8	-9.00	99.68	98.28	97.36

Aromatase Assay Spreadsheet

Assay Date	9/28/2005	Test Chemical ID	RC5	# Concentrations tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0202	35924.7	1778450		
2	0.0204	36569.46	1792621		
3	0.0203	35586.75	1753042		
4	0.0204	35368.41	1733746		
5	0.0201	36350.35	1808475		
			Average DPM/g soln		1773267
			SD		30050
			CV		1.69
			µCi/g soln		0.799

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

ASDN solution	mg ASDN added	(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	8.1588 g
Mass of dilution B used in substrate prep	4.6181 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.577347 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00904 µg/g soln.
µg/g soln.

a. µCi/g soln	0.799
b. Specific activity of [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.

= 0.577347 + 0.00904
= 0.586390 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.362 µCi/µg ASDN

866086 dpm/nmol

Assay Date 9/28/2005 Test Chemical ID RC5 tested 8

ID TNB Replicate # Rep 1 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.549	0.333	0.148	0.081	0.052	0.043	0.036	25	100	
	0.561	0.332	0.152	0.085	0.052	0.043	0.035			
	0.552	0.339	0.149	0.088	0.053	0.042	0.034			

Samples:	10	100	microsomes
	0.057	0.282	0.099
	0.054	0.289	0.097
			0.094

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.554	0.519	0.0483
0.125	100	200	0.00013	200	0.0250	0.335	0.300	0.0278
0.05	200	1000	0.00005	200	0.0100	0.150	0.115	0.0105
0.025	100	1000	0.00003	200	0.0050	0.085	0.050	0.0044
0.01	40	1000	0.00001	200	0.0020	0.052	0.017	0.0014
0.005	20	1000	0.00001	200	0.0010	0.043	0.008	0.0005

Variables	Regression results	
m, b	0.094	0.000
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	606	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank 0.035

r^2= 0.993
m= 0.094
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µL microsomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.057	0.022	0.002	200	1	1	0.000	0.000	0.008
10	0.054	0.019	0.002	200	1	1	0.000		
10					1	1			
100	0.282	0.247	0.023	200	1	1	0.000	0.000	0.116
100	0.289	0.254	0.024	200	1	1	0.000		
100									
	0.099	0.064	0.006	200	218	70018	0.009	0.009	8.914
	0.097	0.062	0.006	200	218	70018	0.009		
	0.094	0.059	0.005	200	218	70018	0.009		

Assay Date 9/28/2005 Test Chemical ID RC5 tested 8

ID TNB Replicate # Rep 1 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.549	0.333	0.148	0.081	0.052	0.043	0.036	25	100	
	0.561	0.332	0.152	0.085	0.052	0.043	0.035			
	0.552	0.339	0.149	0.088	0.053	0.042	0.034			
Samples:	10	100	microsomes							
	0.057	0.282	0.099							
	0.054	0.289	0.097							
			0.094							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.554	0.519	0.0483
0.125	100	200	0.00013	200	0.0250	0.335	0.300	0.0278
0.05	200	1000	0.00005	200	0.0100	0.150	0.115	0.0105
0.025	100	1000	0.00003	200	0.0050	0.085	0.050	0.0044
0.01	40	1000	0.00001	200	0.0020	0.052	0.017	0.0014
0.005	20	1000	0.00001	200	0.0010	0.043	0.008	0.0005

Blank 0.035

r^2= 0.993
m= 0.094
b= 0.000

Variables	Regression results	
m, b	0.094	0.000
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	606	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	vol. usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.057	0.022	0.002	200	1	1	0.000	0.000	0.008
10	0.054	0.019	0.002	200	1	1	0.000		
10					1	1			
100	0.282	0.247	0.023	200	1	1	0.000	0.000	0.116
100	0.289	0.254	0.024	200	1	1	0.000		
100									
	0.099	0.064	0.006	200	218	70018	0.009	0.009	8.914
	0.097	0.062	0.006	200	218	70018	0.009		
	0.094	0.059	0.005	200	218	70018	0.009		

Test

Assay Date 9/28/2005 Chemical ID RC5 tested 8

ID TNB Replicate # Rep 1 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.549	0.333	0.148	0.081	0.052	0.043	0.036	25	100	
	0.561	0.332	0.152	0.085	0.052	0.043	0.035			
	0.552	0.339	0.149	0.088	0.053	0.042	0.034			

Samples:	10	100	microsomes
	0.057	0.282	0.099
	0.054	0.289	0.097
			0.094

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.554	0.519	0.0429
0.125	100	200	0.00013	200	0.0250	0.335	0.300	0.0251
0.05	200	1000	0.00005	200	0.0100	0.150	0.115	0.0100
0.025	100	1000	0.00003	200	0.0050	0.085	0.050	0.0047
0.01	40	1000	0.00001	200	0.0020	0.052	0.017	0.0020
0.005	20	1000	0.00001	200	0.0010	0.043	0.008	0.0013

Blank 0.035

r^2= 1.000
m= 0.082
b= 0.001

Variables	Regression results	
m, b	0.082	0.001
se_m, se_b	0.001	0.000
r , se_y	1.000	0.000
F, df	6641	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	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.057	0.022	0.002	200	1	1	0.000	0.000	0.011
10	0.054	0.019	0.002	200	1	1	0.000		
10					1	1			
100	0.282	0.247	0.021	200	1	1	0.000	0.000	0.105
100	0.289	0.254	0.021	200	1	1	0.000		
100									
	0.099	0.064	0.006	200	218	70018	0.009	0.009	9.081
	0.097	0.062	0.006	200	218	70018	0.009		
	0.094	0.059	0.005	200	218	70018	0.009		

Assay Date	9/28/2005	Test Chemical ID	RC5	tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	IVT

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID
Samples:									

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/28/2005	Chemical ID	RC5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	TNB	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.1835	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.1835	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	9.081
Protein Concentration (dilution added to assay, mg/mL):	0.028274

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/28/2005	Test Chemical ID	RC5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	TNB	Replicate #	Rep 1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.2	1	1821.2	9105	8900.45	17800.9	0.1	177327	10.04	17607	0.0203	1	0.014	15	0.0479
			0.2	2	1738.98	8694.9			0.1					1		15	
	2	2	0.5	1	4484.17	8968.34	8996.37	17992.74	0.1	177327	10.15	17799	0.0206	1	0.014	15	0.0485
			0.5	2	4512.2	9024.4			0.1					1		15	
	3	2	0.5	1	4612.54	9225.08	9156.41	18312.82	0.1	177327	10.33	18119	0.0209	1	0.014	15	0.0493
			0.5	2	4543.87	9087.74			0.1					1		15	
	4	2	0.5	1	4527.72	9055.44	9233.66	18467.32	0.1	177327	10.41	18273	0.0211	1	0.014	15	0.0497
			0.5	2	4705.94	9411.88			0.1					1		15	
Background control	1	2	0.2	1	22.19	110.95	111.15	222.3	0.1	177327	0.13	28	0.0000	1	0.014	15	0.0001
			0.2	2	22.27	111.35			0.1					1		15	
	2	2	0.5	1	46.57	93.14	88.42	176.84	0.1	177327	0.10	-17	0.0000	1	0.014	15	0.0000
			0.5	2	41.85	83.7			0.1					1		15	
	3	2	0.5	1	53.18	106.36	95.27	190.54	0.1	177327	0.11	-4	0.0000	1	0.014	15	0.0000
			0.5	2	42.09	84.18			0.1					1		15	
	4	2	0.5	1	43.63	87.26	93.33	186.66	0.1	177327	0.11	-7	0.0000	1	0.014	15	0.0000
			0.5	2	49.7	99.4			0.1					1		15	
Positive control	1	2	0.5	1	1916.8	3833.6	3814.79	7629.58	0.1	177327	4.30	7435	0.0086	1	0.014	15	0.0202
			0.5	2	1897.99	3795.98			0.1					1		15	
	2	2	0.5	1	1829.94	3659.88	3716.94	7433.88	0.1	177327	4.19	7240	0.0084	1	0.014	15	0.0197
			0.5	2	1887	3774			0.1					1		15	
	3	2	0.5	1	2007.13	4014.26	4039.48	8078.96	0.1	177327	4.56	7885	0.0091	1	0.014	15	0.0215
			0.5	2	2032.35	4064.7			0.1					1		15	
	4	2	0.5	1	2000.05	4000.1	4047.06	8094.12	0.1	177327	4.56	7900	0.0091	1	0.014	15	0.0215
			0.5	2	2047.01	4094.02			0.1					1		15	
Negative Control	1	2	0.5	1	4379.53	8759.06	8930.47	17860.94	0.1	177327	10.07	17667	0.0204	1	0.014	15	0.0481
			0.5	2	4550.94	9101.88			0.1					1		15	
	2	2	0.5	1	4740.12	9480.24	9429.2	18858.4	0.1	177327	10.63	18664	0.0216	1	0.014	15	0.0508
			0.5	2	4689.08	9378.16			0.1					1		15	
	3	2	0.5	1	2879.59	5759.18	5778.13	11556.26	0.1	177327	6.52	11362	0.0131	1	0.014	15	0.0309
			0.5	2	2898.54	5797.08			0.1					1		15	
	4	2	0.5	1	4481.45	8962.9	8879.19	17758.38	0.1	177327	10.01	17564	0.0203	1	0.014	15	0.0478
			0.5	2	4397.74	8795.48			0.1					1		15	
RC5	1-1	2	0.5	1	4273.9	8547.8	8677.61	17355.22	0.1	177327	9.79	17161	0.0198	1	0.014	15	0.0467
			0.5	2	4403.71	8807.42			0.1					1		15	
	1-2	2	0.5	1	4462.13	8924.26	8898.37	17796.74	0.1	177327	10.04	17603	0.0203	1	0.014	15	0.0479
			0.5	2	4436.24	8872.48			0.1					1		15	
	1-3	2	0.5	1	4508.33	9016.66	9043.57	18087.14	0.1	177327	10.20	17893	0.0207	1	0.014	15	0.0487
			0.5	2	4535.24	9070.48			0.1					1		15	
	2-1	2	0.5	1	4309.71	8619.42	8525.99	17051.98	0.1	177327	9.62	16858	0.0195	1	0.014	15	0.0459
			0.5	2	4216.28	8432.56			0.1					1		15	
	2-2	2	0.5	1	4494.21	8988.42	9098.7	18197.4	0.1	177327	10.26	18003	0.0208	1	0.014	15	0.0490
			0.5	2	4604.49	9208.98			0.1					1		15	
	2-3	2	0.5	1	4494.24	8988.48	9015.61	18031.22	0.1	177327	10.17	17837	0.0206	1	0.014	15	0.0486
			0.5	2	4521.37	9042.74			0.1					1		15	
	3-1	2	0.5	1	4409.22	8818.44	8917.53	17835.06	0.1	177327	10.06	17641	0.0204	1	0.014	15	0.0480
			0.5	2	4508.31	9016.62			0.1					1		15	
	3-2	2	0.5	1	4491.85	8983.7	9052.3	18104.6	0.1	177327	10.21	17911	0.0207	1	0.014	15	0.0488
			0.5	2	4560.45	9120.9			0.1					1		15	
	3-3	2	0.5	1	4406.53	8813.06	8746.23	17492.46	0.1	177327	9.86	17298	0.0200	1	0.014	15	0.0471
			0.5	2	4339.7	8679.4			0.1					1		15	
	4-1	2	0.5	1	4597.59	9195.15	9076.01	18152.02	0.1	177327	10.24	17956	0.0207	1	0.014	15	0.0489
			0.5	2	4478.43	8956.86			0.1					1		15	
	4-2	2	0.5	1	4511.07	9022.14	9071.09	18142.18	0.1	177327	10.23	17948	0.0207	1	0.014	15	0.0489
			0.5	2	4560.02	9120.04			0.1					1		15	
	4-3	2	0.5	1	4472.16	8944.32	9018.86	18037.72	0.1	177327	10.17	17844	0.0206	1	0.014	15	0.0486
			0.5	2	4546.7	9093.4			0.1					1		15	
	5-1	2	0.5	1	4550.69	9101.38	9074.39	18148.78	0.1	177327	10.23	17955	0.0207	1	0.014	15	0.0489
			0.5	2	4523.7	9047.4			0.1					1		15	
	5-2	2	0.5	1	4437.2	8874.4	8925.08	17850.16	0.1	177327	10.07	17656	0.0204	1	0.014	15	0.0481
			0.5	2	4487.88	8975.76			0.1					1		15	
	5-3	2	0.5	1	4577.69	9155.38	9000.95	18001.9	0.1	177327	10.15	17808	0.0206	1	0.014	15	0.0485
			0.5	2	4423.26	8846.52			0.1					1		15	
	6-1	2	0.5	1	4461.5	8923	9043.26	18086.52	0.1	177327	10.20	17892	0.0207	1	0.014	15	0.0487
			0.5	2	4581.76	9163.52			0.1					1		15	
	6-2	2	0.5	1	4611.29	9222.58	9183.39	18366.78	0.1	177327	10.36	18173	0.0210	1	0.014	15	0.0495
			0.5	2	4572.1	9144.2			0.1					1		15	
	6-3	2	0.5	1	4677.33	9354.66	9332.66	18665.32	0.1	177327	10.53	18471	0.0213	1	0.014	15	0.0503
			0.5	2	4655.33	9310.66			0.1					1		15	
	7-1	2	0.5	1	4656.96	9313.92	9396.06	18792.12	0.1	177327	10.60	18598	0.0215	1	0.014	15	0.0506
			0.5	2	4739.1	9478.2			0.1					1		15	
	7-2	2	0.5	1	4720.48	9440.92	9376.97	18753.94	0.1	177327	10.58	18560	0.0214	1	0.014	15	0.0505
			0.5	2	4656.51	9313.02			0.1					1		15	
	7-3	2	0.5	1	4650.19	9300.38	9319.11	18638.22	0.1	177327	10.51	18444	0.0213	1	0.014	15	0.0502
			0.5	2	4668.92	9337.84			0.1					1		15	
	8-1	2	0.5	1	4675.11	9350.22	9425.31	18850.62	0.1	177327	10.63	18657	0.0215	1	0.014	15	0.0508
			0.5	2	4750.2	9500.4			0.1					1		15	
	8-2	2	0.5	1	4560.03	9120.06	9104.58	18209.16	0.1	177327	10.27	18015	0.0208	1	0.014	15	0.0490
			0.5	2	4544.55	9089.1			0.1					1		15	
	8-3	2	0.5	1	4356.45	8712.9	8736.92	17473.84	0.1	177327	9.85	17280	0.0200	1	0.014	15	0.0470
			0.5	2	4380.47	8760.94			0.1					1		15	

Assay Date	9/28/2005	ID	RC5	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID TNB	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0482	0.0004
Full activity	End	0.0495	0.0003
Full activity	Overall	0.0489	0.0008
Background	Beginning	0.0000	8.75149E-05
Background	End	0.0000	7.46937E-06
Background	Overall	0.0000	5.35618E-05
Positive	Beginning	0.0200	0.0004
Positive	End	0.0215	0.0000
Positive	Overall	0.0207	0.0009
Negative	Beginning	0.0495	0.0019
Negative	End	0.0394	0.0119
Negative	Overall	0.0444	0.0091

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC5	1	1	1.00E-04	-4.00	0.0467
RC5	1	2	1.00E-04	-4.00	0.0479
RC5	1	3	1.00E-04	-4.00	0.0487
RC5	2	1	1.00E-05	-5.00	0.0459
RC5	2	2	1.00E-05	-5.00	0.0490
RC5	2	3	1.00E-05	-5.00	0.0486
RC5	3	1	5.00E-06	-5.30	0.0480
RC5	3	2	5.00E-06	-5.30	0.0488
RC5	3	3	5.00E-06	-5.30	0.0471
RC5	4	1	1.00E-06	-6.00	0.0489
RC5	4	2	1.00E-06	-6.00	0.0489
RC5	4	3	1.00E-06	-6.00	0.0486
RC5	5	1	1.00E-07	-7.00	0.0489
RC5	5	2	1.00E-07	-7.00	0.0481
RC5	5	3	1.00E-07	-7.00	0.0485
RC5	6	1	1.00E-08	-8.00	0.0487
RC5	6	2	1.00E-08	-8.00	0.0495
RC5	6	3	1.00E-08	-8.00	0.0503
RC5	7	1	1.00E-09	-9.00	0.0506
RC5	7	2	1.00E-09	-9.00	0.0505
RC5	7	3	1.00E-09	-9.00	0.0502
RC5	8	1	1.00E-10	-10.00	0.0508
RC5	8	2	1.00E-10	-10.00	0.0490
RC5	8	3	1.00E-10	-10.00	0.0470

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-4.00	95.61	98.07	99.69
2	-5.00	93.92	100.30	99.37
3	-5.30	98.28	99.78	96.37
4	-6.00	100.05	99.99	99.41
5	-7.00	100.03	98.37	99.21
6	-8.00	99.68	101.24	102.91
7	-9.00	103.61	103.40	102.76
8	-10.00	103.94	100.37	96.27

Aromatase Assay Spreadsheet

Assay Date	9/30/2005	Test Chemical ID	RC5	# Concentrations tested	8	
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0203	36832.94	1814431	
2	0.0203	35400.34	1743859	
3	0.0202	36333.49	1798688	
4	0.0206	37242.77	1807901	
5	0.0206	36431.73	1768531	
			Average DPM/g soln	1786682
			SD	29697
			CV	1.66
			µCi/g soln	0.805

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

ASDN solution	mg ASDN added	(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	8.2249 g
Mass of dilution B used in substrate prep	4.6126 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.560809 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 [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.

= 0.560809 + 0.00911
= 0.569920 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.412 µCi/µg ASDN

897856 dpm/nmol

Assay Date 9/30/2005 Test Chemical ID RC5 tested 8

ID TNB Replicate # Rep 2 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.590	0.344	0.149	0.093	0.053	0.041	0.038	25	100	
	0.591	0.358	0.154	0.094	0.048	0.043	0.037			
	0.575	0.352	0.148	0.090	0.051	0.044	0.035			

Samples:	10	100	microsomes
	0.059	0.286	0.095
	0.061	0.291	0.092
			0.094

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.585	0.549	0.0484
0.125	100	200	0.00013	200	0.0250	0.351	0.315	0.0278
0.05	200	1000	0.00005	200	0.0100	0.150	0.114	0.0101
0.025	100	1000	0.00003	200	0.0050	0.092	0.056	0.0049
0.01	40	1000	0.00001	200	0.0020	0.051	0.014	0.0013
0.005	20	1000	0.00001	200	0.0010	0.043	0.006	0.0006

Blank 0.037

r^2= 0.994
m= 0.088
b= 0.000

Variables	Regression results	
m, b	0.088	0.000
se_m, se_b	0.003	0.001
r , se_y	0.994	0.002
F, df	657	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	µL usomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.059	0.022	0.002	200	1	1	0.000	0.000	0.010
10	0.061	0.024	0.002	200	1	1	0.000		
10					1	1			
100	0.286	0.249	0.022	200	1	1	0.000	0.000	0.111
100	0.291	0.254	0.022	200	1	1	0.000		
100									
	0.095	0.058	0.005	200	150	60150	0.010	0.010	10.127
	0.092	0.055	0.005	200	150	60150	0.010		
	0.094	0.057	0.005	200	150	60150	0.010		

Assay Date 9/30/2005 Test Chemical ID RC5 tested 8

ID TNB Replicate # Rep 2 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.590	0.344	0.149	0.093	0.053	0.041	0.038	25	100
	0.591	0.358	0.154	0.094	0.048	0.043	0.037		
	0.575	0.352	0.148	0.090	0.051	0.044	0.035		

Protein stock ID

Samples:	10	100
	0.059	0.286
	0.061	0.291

microsomes
0.095
0.092
0.094

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.585	0.549	0.0432
0.125	100	200	0.00013	200	0.0250	0.351	0.315	0.0251
0.05	200	1000	0.00005	200	0.0100	0.150	0.114	0.0096
0.025	100	1000	0.00003	200	0.0050	0.092	0.056	0.0051
0.01	40	1000	0.00001	200	0.0020	0.051	0.014	0.0019
0.005	20	1000	0.00001	200	0.0010	0.043	0.006	0.0013

Blank 0.037

r^2= 0.999
m= 0.077
b= 0.001

Variables	Regression results	
m, b	0.077	0.001
se_m, se_b	0.001	0.000
r , se_y	0.999	0.000
F, df	4123	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	µL usomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.059	0.022	0.003	200	1	1	0.000	0.000	0.013
10	0.061	0.024	0.003	200	1	1	0.000		
10					1	1			
100	0.286	0.249	0.020	200	1	1	0.000	0.000	0.101
100	0.291	0.254	0.020	200	1	1	0.000		
100									
	0.095	0.058	0.005	200	150	60150	0.011	0.010	10.457
	0.092	0.055	0.005	200	150	60150	0.010		
	0.094	0.057	0.005	200	150	60150	0.011		

Assay Date	9/30/2005	Test Chemical ID	RC5	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	IVT

								Protein stock (mg/10 mL)	Protein stock ID
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk		

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

A_{raw}	$A_{adj.}$	mg protein measured	µ µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/30/2005	Chemical ID	RC5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	TNB	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.15	mL microsome Stock used
	60.15	mL total volume
	401	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	401	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	10.457
Protein Concentration (dilution added to assay, mg/mL):	0.026077

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	9/30/2005	Test Chemical ID	RCS	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	TNB	Replicate #	Rep 2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3636.9	7273.8	7274.78	14549.56	0.1	178668	8.14	14316	0.0159	1	0.013	15	0.0408
			0.5	2	3637.88	7275.76			0.1					1		15	
	2	2	0.5	1	2591.7	5183.4	5128.89	10257.78	0.1	178668	5.74	10024	0.0112	1	0.013	15	0.0285
			0.5	2	2537.19	5074.38			0.1					1		15	
	3	2	0.5	1	3578.75	7157.5	7147.22	14294.44	0.1	178668	8.00	14061	0.0157	1	0.013	15	0.0400
			0.5	2	3568.47	7136.94			0.1					1		15	
	4	2	0.5	1	3634.38	7268.76	7270.12	14540.24	0.1	178668	8.14	14307	0.0159	1	0.013	15	0.0407
			0.5	2	3635.74	7271.48			0.1					1		15	
Background control	1	2	0.5	1	54.78	109.56	122.42	244.84	0.1	178668	0.14	11	0.0000	1	0.013	15	0.0000
			0.5	2	67.64	135.28			0.1					1		15	
	2	2	0.5	1	59.48	118.96	115.69	231.38	0.1	178668	0.13	-2	0.0000	1	0.013	15	0.0000
			0.5	2	56.21	112.42			0.1					1		15	
	3	2	0.5	1	53.68	107.36	110.18	220.36	0.1	178668	0.12	-13	0.0000	1	0.013	15	0.0000
			0.5	2	56.5	113			0.1					1		15	
	4	2	0.5	1	61.41	122.82	118.75	237.5	0.1	178668	0.13	4	0.0000	1	0.013	15	0.0000
			0.5	2	57.34	114.68			0.1					1		15	
Positive control	1	2	0.5	1	1548.56	3097.12	3118.53	6237.06	0.1	178668	3.49	6004	0.0067	1	0.013	15	0.0171
			0.5	2	1569.97	3139.94			0.1					1		15	
	2	2	0.5	1	1547.81	3095.62	3110.11	6220.22	0.1	178668	3.48	5987	0.0067	1	0.013	15	0.0170
			0.5	2	1562.3	3124.6			0.1					1		15	
	3	2	0.5	1	1547.1	3094.2	3052.3	6104.6	0.1	178668	3.42	5871	0.0065	1	0.013	15	0.0167
			0.5	2	1505.2	3010.4			0.1					1		15	
	4	2	0.5	1	1471.02	2942.04	2552.65	5105.3	0.1	178668	2.86	4872	0.0054	1	0.013	15	0.0139
			0.5	2	1081.63	2163.26			0.1					1		15	
Negative Control	1	2	0.5	1	3506.12	7012.24	7021.49	14042.98	0.1	178668	7.86	13809	0.0154	1	0.013	15	0.0393
			0.5	2	3515.37	7030.74			0.1					1		15	
	2	2	0.5	1	3796.5	7593	7718.55	15437.1	0.1	178668	8.64	15204	0.0169	1	0.013	15	0.0433
			0.5	2	3922.05	7844.1			0.1					1		15	
	3	2	0.5	1	3477.42	6954.84	6900.37	13800.74	0.1	178668	7.72	13567	0.0151	1	0.013	15	0.0386
			0.5	2	3422.95	6845.9			0.1					1		15	
	4	2	0.5	1	3552.47	7104.94	7109.29	14218.58	0.1	178668	7.96	13985	0.0156	1	0.013	15	0.0398
			0.5	2	3556.82	7113.64			0.1					1		15	
RCS	1-1	2	0.5	1	3594.93	7189.86	7273.44	14546.88	0.1	178668	8.14	14313	0.0159	1	0.013	15	0.0408
			0.5	2	3678.51	7357.02			0.1					1		15	
	1-2	2	0.5	1	3751.01	7502.02	7347.05	14694.1	0.1	178668	8.22	14461	0.0161	1	0.013	15	0.0412
			0.5	2	3596.04	7192.08			0.1					1		15	
	1-3	2	0.5	1	3532.89	7065.78	7179.79	14359.58	0.1	178668	8.04	14126	0.0157	1	0.013	15	0.0402
			0.5	2	3646.9	7293.8			0.1					1		15	
	2-1	2	0.5	1	3986.41	7972.82	8048.38	16096.76	0.1	178668	9.01	15863	0.0177	1	0.013	15	0.0452
			0.5	2	4061.97	8123.94			0.1					1		15	
	2-2	2	0.5	1	3568.74	7137.48	7091.81	14183.62	0.1	178668	7.94	13950	0.0155	1	0.013	15	0.0397
			0.5	2	3523.07	7046.14			0.1					1		15	
	2-3	2	0.5	1	3746.6	7493.2	7420	14840	0.1	178668	8.31	14606	0.0163	1	0.013	15	0.0416
			0.5	2	3673.4	7346.8			0.1					1		15	
	3-1	2	0.5	1	3828.89	7657.78	7682.33	15364.66	0.1	178668	8.60	15131	0.0169	1	0.013	15	0.0431
			0.5	2	3853.44	7706.88			0.1					1		15	
	3-2	2	0.5	1	3171.01	6342.02	6365.05	12730.1	0.1	178668	7.12	12497	0.0139	1	0.013	15	0.0356
			0.5	2	3194.04	6388.08			0.1					1		15	
	3-3	2	0.5	1	3739.71	7479.42	7532.48	15064.96	0.1	178668	8.43	14831	0.0165	1	0.013	15	0.0422
			0.5	2	3792.77	7585.54			0.1					1		15	
	4-1	2	0.5	1	3839.65	7679.3	7516.45	15032.9	0.1	178668	8.41	14799	0.0165	1	0.013	15	0.0421
			0.5	2	3676.8	7353.6			0.1					1		15	
	4-2	2	0.5	1	3620.1	7240.2	7234.8	14469.6	0.1	178668	8.10	14236	0.0159	1	0.013	15	0.0405
			0.5	2	3614.7	7229.4			0.1					1		15	
	4-3	2	0.5	1	3650.24	7300.48	7258.6	14517.2	0.1	178668	8.13	14284	0.0159	1	0.013	15	0.0407
			0.5	2	3608.36	7216.72			0.1					1		15	
	5-1	2	0.5	1	3818.36	7636.72	7645.12	15290.24	0.1	178668	8.56	15057	0.0168	1	0.013	15	0.0429
			0.5	2	3826.76	7653.52			0.1					1		15	
	5-2	2	0.5	1	3673.69	7347.38	7399.57	14799.14	0.1	178668	8.28	14566	0.0162	1	0.013	15	0.0415
			0.5	2	3725.88	7451.76			0.1					1		15	
	5-3	2	0.5	1	3671.22	7342.44	7308.72	14617.44	0.1	178668	8.18	14384	0.0160	1	0.013	15	0.0410
			0.5	2	3637.5	7275			0.1					1		15	
	6-1	2	0.5	1	2800.11	5600.22	5701.04	11402.08	0.1	178668	6.38	11169	0.0124	1	0.013	15	0.0318
			0.5	2	2900.93	5801.86			0.1					1		15	
	6-2	2	0.5	1	3734.79	7469.58	7174.62	14349.24	0.1	178668	8.03	14116	0.0157	1	0.013	15	0.0402
			0.5	2	3439.83	6879.66			0.1					1		15	
	6-3	2	0.5	1	3768.04	7536.08	7486.7	14973.4	0.1	178668	8.38	14740	0.0164	1	0.013	15	0.0420
			0.5	2	3718.66	7437.32			0.1					1		15	
	7-1	2	0.5	1	3875.82	7751.64	7731.28	15462.56	0.1	178668	8.65	15229	0.0170	1	0.013	15	0.0434
			0.5	2	3855.46	7710.92			0.1					1		15	
	7-2	2	0.5	1	3632.9	7265.8	7341.16	14682.32	0.1	178668	8.22	14449	0.0161	1	0.013	15	0.0411
			0.5	2	3708.26	7416.52			0.1					1		15	
	7-3	2	0.5	1	3812.63	7625.26	7756.01	15512.02	0.1	178668	8.68	15279	0.0170	1	0.013	15	0.0435
			0.5	2	3943.38	7886.76			0.1					1		15	
	8-1	2	0.5	1	3570.06	7140.12	7339.72	14679.44	0.1	178668	8.22	14446	0.0161	1	0.013	15	0.0411
			0.5	2	3769.66	7539.32			0.1					1		15	
	8-2	2	0.5	1	3620.61	7241.22	7234.69	14469.38	0.1	178668	8.10	14236	0.0159	1	0.013	15	0.0405
			0.5	2	3614.08	7228.16			0.1					1		15	
	8-3	2	0.5	1	3707.3	7414.6	7453.24	14906.48	0.1	178668	8.34	14673	0.0163	1	0.013	15	0.0418
			0.5	2	3745.94	7491.88			0.1					1		15	

Assay Date	9/30/2005	ID	RC5	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID TNB	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0347	0.0086
Full activity	End	0.0404	0.0005
Full activity	Overall	0.0375	0.0060
Background	Beginning	0.0000	2.71E-05
Background	End	0.0000	3.45092E-05
Background	Overall	0.0000	2.94874E-05
Positive	Beginning	0.0171	0.0000
Positive	End	0.0153	0.0020
Positive	Overall	0.0162	0.0015
Negative	Beginning	0.0413	0.0028
Negative	End	0.0392	0.0008
Negative	Overall	0.0403	0.0021

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC5	1	1	1.00E-04	-4.00	0.0408
RC5	1	2	1.00E-04	-4.00	0.0412
RC5	1	3	1.00E-04	-4.00	0.0402
RC5	2	1	1.00E-05	-5.00	0.0452
RC5	2	2	1.00E-05	-5.00	0.0397
RC5	2	3	1.00E-05	-5.00	0.0416
RC5	3	1	5.00E-06	-5.30	0.0431
RC5	3	2	5.00E-06	-5.30	0.0356
RC5	3	3	5.00E-06	-5.30	0.0422
RC5	4	1	1.00E-06	-6.00	0.0421
RC5	4	2	1.00E-06	-6.00	0.0405
RC5	4	3	1.00E-06	-6.00	0.0407
RC5	5	1	1.00E-07	-7.00	0.0429
RC5	5	2	1.00E-07	-7.00	0.0415
RC5	5	3	1.00E-07	-7.00	0.0410
RC5	6	1	1.00E-08	-8.00	0.0318
RC5	6	2	1.00E-08	-8.00	0.0402
RC5	6	3	1.00E-08	-8.00	0.0420
RC5	7	1	1.00E-09	-9.00	0.0434
RC5	7	2	1.00E-09	-9.00	0.0411
RC5	7	3	1.00E-09	-9.00	0.0435
RC5	8	1	1.00E-10	-10.00	0.0411
RC5	8	2	1.00E-10	-10.00	0.0405
RC5	8	3	1.00E-10	-10.00	0.0418

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-4.00	108.62	109.74	107.20
2	-5.00	120.39	105.87	110.85
3	-5.30	114.83	94.84	112.56
4	-6.00	112.31	108.04	108.40
5	-7.00	114.27	110.54	109.16
6	-8.00	84.76	107.12	111.86
7	-9.00	115.57	109.65	115.95
8	-10.00	109.63	108.04	111.35

Aromatase Assay Spreadsheet

Assay Date	10/4/2005	Test Chemical ID	RC5	# Concentrations tested	8		
ID	TNB	Replicate #	3	Microsome type	Placental	Microsome ID	IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0208	38817.88	1866244	
2	0.0204	38769.92	1900486	
3	0.0200	36230.74	1811537	
4	0.0200	37745.71	1887286	
5	0.0201	36549.39	1818378	
			Average DPM/g soln	1856786
			SD	40162
			CV	2.16
			µCi/g soln	0.836

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

ASDN solution	mg ASDN added	(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	8.133 g
Mass of dilution B used in substrate prep	4.5908 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.5814 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00947 µg/g soln.
µg/g soln.

a. µCi/g soln	0.836
b. Specific activity of [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.

= 0.581400 + 0.00947
= 0.590868 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.416 µCi/µg ASDN

900004 dpm/nmol

Test

Assay Date 10/4/2005 Chemical ID RC5 tested 8

ID TNB Replicate # 3 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.582	0.340	0.156	0.091	0.050	0.041	0.031	25	100	
	0.577	0.351	0.157	0.083	0.052	0.038	0.032			
	0.567	0.349	0.155	0.087	0.052	0.040	0.032			

Samples:	10	100	microsomes
	0.054	0.283	0.107
	0.054	0.286	0.105
			0.101

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.575	0.544	0.0483
0.125	100	200	0.00013	200	0.0250	0.347	0.315	0.0278
0.05	200	1000	0.00005	200	0.0100	0.156	0.124	0.0107
0.025	100	1000	0.00003	200	0.0050	0.087	0.055	0.0045
0.01	40	1000	0.00001	200	0.0020	0.051	0.020	0.0013
0.005	20	1000	0.00001	200	0.0010	0.040	0.008	0.0003

Blank 0.032

r^2= 0.993
m= 0.090
b= 0.000

Variables	Regression results	
m, b	0.090	0.000
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	579	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	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.054	0.022	0.002	200	1	1	0.000	0.000	0.008
10	0.054	0.022	0.002	200	1	1	0.000		
10									
100	0.283	0.251	0.022	200	1	1	0.000	0.000	0.111
100	0.286	0.254	0.022	200	1	1	0.000		
100									
	0.107	0.075	0.006	200	218	70018	0.010	0.010	9.784
	0.105	0.073	0.006	200	218	70018	0.010		
	0.101	0.069	0.006	200	218	70018	0.009		

Assay Date 10/4/2005 Test Chemical ID RC5 tested 8

ID TNB Replicate # 3 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.582	0.340	0.156	0.091	0.050	0.041	0.031	25	100
	0.577	0.351	0.157	0.083	0.052	0.038	0.032		
	0.567	0.349	0.155	0.087	0.052	0.040	0.032		

Protein stock ID

Samples:	10	100	microsomes
	0.054	0.283	0.107
	0.054	0.286	0.105
			0.101

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.575	0.544	0.0428
0.125	100	200	0.00013	200	0.0250	0.347	0.315	0.0250
0.05	200	1000	0.00005	200	0.0100	0.156	0.124	0.0101
0.025	100	1000	0.00003	200	0.0050	0.087	0.055	0.0048
0.01	40	1000	0.00001	200	0.0020	0.051	0.020	0.0020
0.005	20	1000	0.00001	200	0.0010	0.040	0.008	0.0011

Blank 0.032

r^2= 1.000
m= 0.078
b= 0.000

Variables	Regression results	
m, b	0.078	0.000
se_m, se_b	0.001	0.000
r , se_y	1.000	0.000
F, df	14410	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	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.054	0.022	0.002	200	1	1	0.000	0.000	0.011
10	0.054	0.022	0.002	200	1	1	0.000		
10									
100	0.283	0.251	0.020	200	1	1	0.000	0.000	0.101
100	0.286	0.254	0.020	200	1	1	0.000		
100									
	0.107	0.075	0.006	200	218	70018	0.010	0.010	9.838
	0.105	0.073	0.006	200	218	70018	0.010		
	0.101	0.069	0.006	200	218	70018	0.009		

Assay Date	10/4/2005	Test Chemical ID	RC5	tested	8		
ID	TNB	Replicate #	3	Microsome type	Placental	Microsome ID	IVT

Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	10/4/2005	Chemical ID	RC5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	TNB	Replicate #	3

Microsome Dilution Details

Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.1835	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.1835	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	9.838
Protein Concentration (dilution added to assay, mg/mL):	0.03063

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Assay Date	10/4/2005	Test Chemical ID	RC5	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	TNB	Replicate #	3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2		1					0.1	185679		#VALUE!	#VALUE!		0.000		#VALUE!
				2					0.1					1		15	
	2	2	0.5	1	4084.88	8169.76	8148.63	16297.26	0.1	185679	8.78	16152	0.0179		0.015		0.0391
			0.5	2	4063.75	8127.5			0.1					1		15	
	3	2	0.5	1	4410.33	8820.66	8826.22	17652.44	0.1	185679	9.51	17507	0.0195		0.015		0.0423
			0.5	2	4415.89	8831.78			0.1					1		15	
	4	2	0.5	1	4537.15	9074.3	9115.94	18231.88	0.1	185679	9.82	18087	0.0201		0.015		0.0437
			0.5	2	4578.79	9157.58			0.1					1		15	
Background control	1	2	0.5	1	36.51	73.02	72.82	145.64	0.1	185679	0.08	0	0.0000		0.015		0.0000
			0.5	2	36.31	72.62			0.1					1		15	
	2	2	0.5	1	42.82	85.64	82.45	164.9	0.1	185679	0.09	20	0.0000		0.015		0.0000
			0.5	2	39.83	79.26			0.1					1		15	
	3	2	0.5	1	29.40	58.8	66.11	132.22	0.1	185679	0.07	-13	0.0000		0.015		0.0000
			0.5	2	36.71	73.42			0.1					1		15	
	4	2	0.5	1	27.61	55.22	69.06	138.12	0.1	185679	0.07	-7	0.0000		0.015		0.0000
			0.5	2	41.45	82.9			0.1					1		15	
Positive control	1	2	0.5	1	1428.86	2857.72	2852.83	5705.66	0.1	185679	3.07	5560	0.0062		0.015		0.0134
			0.5	2	1423.97	2847.94			0.1					1		15	
	2	2	0.5	1	1642.91	3285.82	3349.61	6699.22	0.1	185679	3.61	6554	0.0073		0.015		0.0158
			0.5	2	1706.70	3413.4			0.1					1		15	
	3	2	0.5	1	1971.79	3943.58	3944.04	7888.08	0.1	185679	4.25	7743	0.0086		0.015		0.0187
			0.5	2	1972.25	3944.5			0.1					1		15	
	4	2	0.5	1	2026.07	4052.14	4111.68	8223.36	0.1	185679	4.43	8078	0.0090		0.015		0.0195
			0.5	2	2085.61	4171.22			0.1					1		15	
Negative Control	1	2	0.5	1	4760.67	9521.34	9503.31	19006.62	0.1	185679	10.24	18861	0.0210		0.015		0.0456
			0.5	2	4742.64	9485.28			0.1					1		15	
	2	2	0.5	1	4748.77	9497.54	9442.46	18884.92	0.1	185679	10.17	18740	0.0208		0.015		0.0453
			0.5	2	4693.69	9387.38			0.1					1		15	
	3	2	0.5	1	4589.04	9178.08	9229.66	18459.32	0.1	185679	9.94	18314	0.0203		0.015		0.0443
			0.5	2	4640.62	9281.24			0.1					1		15	
	4	2	0.5	1	4641.25	9282.5	9153.6	18307.2	0.1	185679	9.86	18162	0.0202		0.015		0.0439
			0.5	2	4512.35	9024.7			0.1					1		15	
RC5	1-1	2	0.5	1	4437.83	8875.66	8985.15	17970.3	0.1	185679	9.68	17825	0.0198		0.015		0.0431
			0.5	2	4547.32	9094.64			0.1					1		15	
	1-2	2	0.5	1	4654.50	9309	9345.61	18691.22	0.1	185679	10.07	18546	0.0206		0.015		0.0448
			0.5	2	4691.11	9382.22			0.1					1		15	
	1-3	2	0.5	1	4519.09	9038.18	9064.92	18129.84	0.1	185679	9.76	17985	0.0200		0.015		0.0435
			0.5	2	4545.83	9091.66			0.1					1		15	
	2-1	2	0.5	1	4598.80	9197.6	9169.23	18338.46	0.1	185679	9.88	18193	0.0202		0.015		0.0440
			0.5	2	4570.43	9140.86			0.1					1		15	
	2-2	2	0.5	1	4796.96	9593.92	9641.44	19282.88	0.1	185679	10.39	19138	0.0213		0.015		0.0463
			0.5	2	4844.48	9688.96			0.1					1		15	
	2-3	2	0.5	1	4796.40	9592.8	9646.35	19292.7	0.1	185679	10.39	19147	0.0213		0.015		0.0463
			0.5	2	4849.95	9699.9			0.1					1		15	
	3-1	2	0.5	1	4821.15	9642.3	9481.87	18963.74	0.1	185679	10.21	18819	0.0209		0.015		0.0455
			0.5	2	4660.72	9321.44			0.1					1		15	
	3-2	2	0.5	1	4797.24	9594.48	9690.8	19381.6	0.1	185679	10.44	19236	0.0214		0.015		0.0465
			0.5	2	4693.56	9787.12			0.1					1		15	
	3-3	2	0.5	1	4677.05	9354.1	9394.86	18789.72	0.1	185679	10.12	18645	0.0207		0.015		0.0451
			0.5	2	4717.81	9435.62			0.1					1		15	
	4-1	2	0.5	1	4588.59	9177.18	9217.32	18434.64	0.1	185679	9.93	18289	0.0203		0.015		0.0442
			0.5	2	4628.73	9257.46			0.1					1		15	
	4-2	2	0.5	1	4830.71	9661.42	9622.72	19245.44	0.1	185679	10.36	19100	0.0212		0.015		0.0462
			0.5	2	4792.01	9584.02			0.1					1		15	
	4-3	2	0.5	1	3783.08	7566.16	7567.49	15134.98	0.1	185679	8.15	14990	0.0167		0.015		0.0362
			0.5	2	3784.41	7568.82			0.1					1		15	
	5-1	2	0.5	1	4711.38	9422.76	9353.79	18707.58	0.1	185679	10.08	18562	0.0206		0.015		0.0449
			0.5	2	4642.41	9284.82			0.1					1		15	
	5-2	2	0.5	1	4645.92	9291.84	9340.02	18680.04	0.1	185679	10.06	18535	0.0206		0.015		0.0448
			0.5	2	4694.10	9388.2			0.1					1		15	
	5-3	2	0.5	1	4760.80	9521.6	9503.76	19007.52	0.1	185679	10.24	18862	0.0210		0.015		0.0456
			0.5	2	4742.96	9485.92			0.1					1		15	
	6-1	2	0.5	1	4713.87	9427.74	9440.25	18880.5	0.1	185679	10.17	18735	0.0208		0.015		0.0453
			0.5	2	4726.38	9452.76			0.1					1		15	
	6-2	2	0.5	1	4587.33	9174.66	9293.25	18586.5	0.1	185679	10.01	18441	0.0205		0.015		0.0446
			0.5	2	4705.92	9411.84			0.1					1		15	
	6-3	2	0.5	1	4619.16	9638.32	9612.35	19224.7	0.1	185679	10.35	19079	0.0212		0.015		0.0461
			0.5	2	4793.19	9586.38			0.1					1		15	
	7-1	2	0.5	1	4768.16	9536.32	9644.1	19288.2	0.1	185679	10.39	19143	0.0213		0.015		0.0463
			0.5	2	4875.94	9751.88			0.1					1		15	
	7-2	2	0.5	1	4606.89	9213.76	9310.2	18620.4	0.1	185679	10.03	18475	0.0205		0.015		0.0447
			0.5	2	4703.31	9406.62			0.1					1		15	
	7-3	2	0.5	1	4830.89	9661.78	9629.7	19259.4	0.1	185679	10.37	19114	0.0212		0.015		0.0462
			0.5	2	4798.81	9597.62			0.1					1		15	
	8-1	2	0.5	1	4619.81	9239.62	9265.24	18530.48	0.1	185679	9.98	18385	0.0204		0.015		0.0445
			0.5	2	4645.43	9290.86			0.1					1		15	
	8-2	2	0.5	1	4729.79	9459.58	9360.43	18720.86	0.1	185679	10.08	18576	0.0206		0.015		0.0449
			0.5	2	4630.64	9261.28			0.1					1		15	
	8-3	2	0.5	1	4768.85	9537.7	9465.73	18931.46	0.1	185679	10.20	18786	0.0209		0.015		0.0454
			0.5	2	4696.88	9393.76			0.1					1		15	

Assay Date	10/4/2005	ID	RC5	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID TNB	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0391	#DIV/0!
Full activity	End	0.0430	0.0010
Full activity	Overall	0.0417	0.0024
Background	Beginning	0.0000	3.29346E-05
Background	End	0.0000	1.0089E-05
Background	Overall	0.0000	3.43957E-05
Positive	Beginning	0.0146	0.0017
Positive	End	0.0191	0.0006
Positive	Overall	0.0169	0.0028
Negative	Beginning	0.0455	0.0002
Negative	End	0.0441	0.0003
Negative	Overall	0.0448	0.0008

Note: In this version of the spreadsheet, the formulas for calculation of average control values and their standard deviations are open.

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC5	1	1	1.00E-04	-4.00	0.0431
RC5	1	2	1.00E-04	-4.00	0.0448
RC5	1	3	1.00E-04	-4.00	0.0435
RC5	2	1	1.00E-05	-5.00	0.0440
RC5	2	2	1.00E-05	-5.00	0.0463
RC5	2	3	1.00E-05	-5.00	0.0463
RC5	3	1	5.00E-06	-5.30	0.0455
RC5	3	2	5.00E-06	-5.30	0.0465
RC5	3	3	5.00E-06	-5.30	0.0451
RC5	4	1	1.00E-06	-6.00	0.0442
RC5	4	2	1.00E-06	-6.00	0.0462
RC5	4	3	1.00E-06	-6.00	0.0362
RC5	5	1	1.00E-07	-7.00	0.0449
RC5	5	2	1.00E-07	-7.00	0.0448
RC5	5	3	1.00E-07	-7.00	0.0456
RC5	6	1	1.00E-08	-8.00	0.0453
RC5	6	2	1.00E-08	-8.00	0.0446
RC5	6	3	1.00E-08	-8.00	0.0461
RC5	7	1	1.00E-09	-9.00	0.0463
RC5	7	2	1.00E-09	-9.00	0.0447
RC5	7	3	1.00E-09	-9.00	0.0462
RC5	8	1	1.00E-10	-10.00	0.0445
RC5	8	2	1.00E-10	-10.00	0.0449
RC5	8	3	1.00E-10	-10.00	0.0454

Percent of control values				
	Log[test	Replicate		
Level	bstance]	1	2	3
1	-4.00	103.34	107.52	104.27
2	-5.00	105.48	110.95	111.01
3	-5.30	109.10	111.52	108.09
4	-6.00	106.03	110.73	86.90
5	-7.00	107.62	107.46	109.36
6	-8.00	108.62	106.91	110.61
7	-9.00	110.98	107.11	110.82
8	-10.00	106.59	107.69	108.91

Aromatase Assay Spreadsheet

Assay Date	8/3/2005	Test Chemical ID	RC6	# Concentrations tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	35272.34	1763617	
2	0.0199	35027.1	1760156	
3	0.0202	35557.43	1760269	
4	0.0202	35088.79	1737069	
5	0.0202	35770.76	1770830	
			Average DPM/g soln	1758388
			SD	12682
			CV	0.72
			µCi/g soln	0.792

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

ASDN solution	mg ASDN added	(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	8.0728 g
Mass of dilution B used in substrate prep	4.5296 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.583538 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00897 µg/g soln.
µg/g soln.

a. µCi/g soln	0.792
b. Specific activity of [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.

= 0.583538 + 0.00897
= 0.592504 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.337 µCi/µg ASDN

849956 dpm/nmol

Test

Assay Date 8/3/2005 Chemical ID RC6 tested 8

ID TNB Replicate # Rep 1 Microsome type Placental Microsome ID Battelle

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.541	0.329	0.153	0.102	0.053	0.046	0.032	25	100	
	0.566	0.337	0.157	0.101	0.053	0.043	0.032			
	0.566	0.349	0.154	0.103	0.058	0.038	0.027			

Samples:	10	100	microsomes
	0.078	0.280	0.100
	0.060	0.295	0.095
			0.099

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.557	0.527	0.0483
0.125	100	200	0.00013	200	0.0250	0.338	0.308	0.0277
0.05	200	1000	0.00005	200	0.0100	0.154	0.124	0.0105
0.025	100	1000	0.00003	200	0.0050	0.102	0.072	0.0055
0.01	40	1000	0.00001	200	0.0020	0.055	0.024	0.0011
0.005	20	1000	0.00001	200	0.0010	0.042	0.012	-0.0001

Blank 0.030

r^2= 0.993
m= 0.094
b= -0.001

Variables	Regression results	
m, b	0.094	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	567	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	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.078	0.048	0.003	200	1	1	0.000	0.000	0.012
10	0.060	0.030	0.002	200	1	1	0.000		
10					1	1			
100	0.280	0.250	0.022	200	1	1	0.000	0.000	0.115
100	0.295	0.265	0.024	200	1	1	0.000		
100									
	0.100	0.069	0.005	200	83	69983	0.022	0.022	21.636
	0.095	0.064	0.005	200	83	69983	0.020		
	0.099	0.069	0.005	200	83	69983	0.022		

Assay Date 8/3/2005 Test Chemical ID RC6 tested 8

ID TNB Replicate # Rep 1 Microsome type Placental Microsome ID Battelle

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.541	0.329	0.153	0.102	0.053	0.046	0.032	25	100	
	0.566	0.337	0.157	0.101	0.053	0.043	0.032			
	0.566	0.349	0.154	0.103	0.058	0.038	0.027			

Samples:	10	100	microsomes
	0.078	0.280	0.100
	0.060	0.295	0.095
			0.099

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.557	0.527	0.0428
0.125	100	200	0.00013	200	0.0250	0.338	0.308	0.0249
0.05	200	1000	0.00005	200	0.0100	0.154	0.124	0.0099
0.025	100	1000	0.00003	200	0.0050	0.102	0.072	0.0056
0.01	40	1000	0.00001	200	0.0020	0.055	0.024	0.0018
0.005	20	1000	0.00001	200	0.0010	0.042	0.012	0.0008

Blank 0.030

r^2= 0.999
m= 0.082
b= 0.000

Variables	Regression results	
m, b	0.082	0.000
se_m, se_b	0.002	0.000
r , se_y	0.999	0.000
F, df	2233	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	µL microsomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.078	0.048	0.004	200	1	1	0.000	0.000	0.015
10	0.060	0.030	0.002	200	1	1	0.000		
10					1	1			
100	0.280	0.250	0.020	200	1	1	0.000	0.000	0.104
100	0.295	0.265	0.021	200	1	1	0.000		
100									
	0.100	0.069	0.005	200	83	69983	0.023	0.022	22.309
	0.095	0.064	0.005	200	83	69983	0.021		
	0.099	0.069	0.005	200	83	69983	0.023		

Assay Date	8/3/2005	Test Chemical ID	RC6	tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	Battelle

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID
Samples:									

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/3/2005	Chemical ID	RC6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.1687	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.1687	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	22.309
Protein Concentration (dilution added to assay, mg/mL):	0.026459

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	8/3/2005	Test Chemical ID	RC6	# Concentrations tested		a Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5595.59	11191.1	11177.3	22354.6	0.1	175839	12.71	22194	0.0261	1	0.013	15	0.0658
			0.5	2	5581.75	11163.5			0.1					1		15	
	2	2	0.5	1	5853.35	11706.7	11688.47	23376.94	0.1	175839	13.29	23217	0.0273	1	0.013	15	0.0688
			0.5	2	5835.12	11670.24			0.1					1		15	
	3	2	0.5	1	4383.57	8767.14	8737.9	17475.8	0.1	175839	9.94	17315	0.0204	1	0.013	15	0.0513
			0.5	2	4354.33	8708.66			0.1					1		15	
	4	2	0.5	1	5024.5	10049	9966.14	19932.28	0.1	175839	11.34	19772	0.0233	1	0.013	15	0.0586
			0.5	2	4941.64	9883.28			0.1					1		15	
Background control	1	2	0.5	1	25.39	50.78	47.47	94.94	0.1	175839	0.05	-65	-0.0001	1	0.013	15	-0.0002
			0.5	2	22.08	44.16			0.1					1		15	
	2	2	0.5	1	14.3	28.6	37.29	74.58	0.1	175839	0.04	-86	-0.0001	1	0.013	15	-0.0003
			0.5	2	22.99	45.98			0.1					1		15	
	3	2	0.5	1	64.87	129.74	120.06	240.12	0.1	175839	0.14	80	0.0001	1	0.013	15	0.0002
			0.5	2	55.19	110.38			0.1					1		15	
	4	2	0.5	1	64.46	128.92	115.91	231.82	0.1	175839	0.13	71	0.0001	1	0.013	15	0.0002
			0.5	2	51.45	102.9			0.1					1		15	
Positive control	1	2	0.5	1	2246.46	4492.92	4548.23	9096.46	0.1	175839	5.17	8936	0.0105	1	0.013	15	0.0265
			0.5	2	2301.77	4603.54			0.1					1		15	
	2	2	0.5	1	2354.94	4709.88	4749.07	9498.14	0.1	175839	5.40	9338	0.0110	1	0.013	15	0.0277
			0.5	2	2394.13	4788.26			0.1					1		15	
	3	2	0.5	1	2100.89	4201.78	4236.1	8472.2	0.1	175839	4.82	8312	0.0098	1	0.013	15	0.0246
			0.5	2	2135.21	4270.42			0.1					1		15	
	4	2	0.5	1	694.44	1388.88	1319.41	2638.82	0.1	175839	1.50	2478	0.0029	1	0.013	15	0.0073
			0.5	2	624.97	1249.94			0.1					1		15	
Negative Control	1	2	0.5	1	5680.93	11361.86	11332.81	22665.62	0.1	175839	12.89	22505	0.0265	1	0.013	15	0.0667
			0.5	2	5651.88	11303.76			0.1					1		15	
	2	2	0.5	1	5511.77	11023.54	10954.8	21909.6	0.1	175839	12.46	21749	0.0256	1	0.013	15	0.0645
			0.5	2	5443.03	10886.06			0.1					1		15	
	3	2	0.5	1	4901.84	9803.68	9737.46	19474.92	0.1	175839	11.08	19315	0.0227	1	0.013	15	0.0573
			0.5	2	4835.62	9671.24			0.1					1		15	
	4	2	0.5	1	5129.62	10259.24	10309.03	20618.06	0.1	175839	11.73	20458	0.0241	1	0.013	15	0.0606
			0.5	2	5179.41	10358.82			0.1					1		15	
RC6	1-1	2	0.5	1	89.12	178.24	178.64	357.28	0.1	175839	0.20	197	0.0002	1	0.013	15	0.0006
			0.5	2	89.52	179.04			0.1					1		15	
	1-2	2	0.5	1	75.47	150.94	152.84	305.68	0.1	175839	0.17	145	0.0002	1	0.013	15	0.0004
			0.5	2	77.37	154.74			0.1					1		15	
	1-3	2	0.5	1	84.87	169.74	169.53	339.06	0.1	175839	0.19	179	0.0002	1	0.013	15	0.0005
			0.5	2	84.66	169.32			0.1					1		15	
	2-1	2	0.5	1	338.42	676.84	675.82	1351.64	0.1	175839	0.77	1191	0.0014	1	0.013	15	0.0035
			0.5	2	337.4	674.8			0.1					1		15	
	2-2	2	0.5	1	312.37	624.74	631.2	1262.4	0.1	175839	0.72	1102	0.0013	1	0.013	15	0.0033
			0.5	2	318.83	637.66			0.1					1		15	
	2-3	2	0.5	1	314.43	628.86	632.92	1265.84	0.1	175839	0.72	1105	0.0013	1	0.013	15	0.0033
			0.5	2	318.49	636.98			0.1					1		15	
	3-1	2	0.5	1	2190.66	4381.32	4325.36	8650.72	0.1	175839	4.92	8490	0.0100	1	0.013	15	0.0252
			0.5	2	2134.7	4269.4			0.1					1		15	
	3-2	2	0.5	1	2056.79	4113.58	4071.42	8142.84	0.1	175839	4.63	7982	0.0094	1	0.013	15	0.0237
			0.5	2	2014.63	4029.26			0.1					1		15	
	3-3	2	0.5	1	1988.49	3976.98	4016.99	8033.98	0.1	175839	4.57	7874	0.0093	1	0.013	15	0.0233
			0.5	2	2028.5	4057			0.1					1		15	
	4-1	2	0.5	1	4950.35	9900.7	9813.85	19627.7	0.1	175839	11.16	19467	0.0229	1	0.013	15	0.0577
			0.5	2	4863.5	9727			0.1					1		15	
	4-2	2	0.5	1	4475.55	8951.1	8925.01	17850.02	0.1	175839	10.15	17690	0.0208	1	0.013	15	0.0524
			0.5	2	4449.46	8898.92			0.1					1		15	
	4-3	2	0.5	1	4370.24	8740.48	8823.7	17647.4	0.1	175839	10.04	17487	0.0206	1	0.013	15	0.0518
			0.5	2	4453.46	8906.92			0.1					1		15	
	5-1	2	0.5	1	5090.04	10180.08	10139.66	20279.32	0.1	175839	11.53	20119	0.0237	1	0.013	15	0.0596
			0.5	2	5049.62	10099.24			0.1					1		15	
	5-2	2	0.5	1	5280.9	10561.8	10611.04	21222.08	0.1	175839	12.07	21062	0.0248	1	0.013	15	0.0624
			0.5	2	5330.14	10660.28			0.1					1		15	
	5-3	2	0.5	1	5175.94	10351.88	10277.81	20555.62	0.1	175839	11.69	20395	0.0240	1	0.013	15	0.0605
			0.5	2	5101.87	10203.74			0.1					1		15	
	6-1	2	0.5	1	5678.53	11357.06	11295.37	22590.74	0.1	175839	12.85	22430	0.0264	1	0.013	15	0.0665
			0.5	2	5616.84	11233.68			0.1					1		15	
	6-2	2	0.5	1	5429.61	10859.22	10846.24	21692.48	0.1	175839	12.34	21532	0.0253	1	0.013	15	0.0638
			0.5	2	5416.63	10833.26			0.1					1		15	
	6-3	2	0.5	1	5405.1	10810.2	10722.34	21444.68	0.1	175839	12.20	21284	0.0250	1	0.013	15	0.0631
			0.5	2	5317.24	10634.48			0.1					1		15	
	7-1	2	0.5	1	5580.58	11161.16	11113.6	22227.2	0.1	175839	12.64	22067	0.0260	1	0.013	15	0.0654
			0.5	2	5533.02	11066.04			0.1					1		15	
	7-2	2	0.5	1	5337.39	10674.78	10662.02	21324.04	0.1	175839	12.13	21164	0.0249	1	0.013	15	0.0627
			0.5	2	5324.63	10649.26			0.1					1		15	
	7-3	2	0.5	1	5011.58	10023.16	10180.1	20360.2	0.1	175839	11.58	20200	0.0238	1	0.013	15	0.0599
			0.5	2	5168.52	10337.04			0.1					1		15	
	8-1	2	0.5	1	5416.1	10832.2	10857.06	21714.12	0.1	175839	12.35	21554	0.0254	1	0.013	15	0.0639
			0.5	2	5440.96	10881.92			0.1					1		15	
	8-2	2	0.5	1	4749.9	9499.8	9402.26	18804.52	0.1	175839	10.69	18644	0.0219	1	0.013	15	0.0553
			0.5	2	4652.36	9304.72			0.1					1		15	
	8-3	2	0.5	1	5103.96	10207.92	10180.82	20361.64	0.1	175839	11.58	20201	0.0238	1	0.013	15	0.0599
			0.5	2	5076.86	10153.72			0.1					1		15	

Assay Date	8/3/2005	ID	RC6	# Concentrations tested	Microsome 8 type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0673	0.0021
Full activity	End	0.0550	0.0051
Full activity	Overall	0.0611	0.0078
Background	Beginning	-0.0002	4.26785E-05
Background	End	0.0002	1.73984E-05
Background	Overall	0.0000	0.000260166
Positive	Beginning	0.0271	0.0008
Positive	End	0.0160	0.0122
Positive	Overall	0.0215	0.0095
Negative	Beginning	0.0656	0.0016
Negative	End	0.0590	0.0024
Negative	Overall	0.0623	0.0042

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC6	1	1	1.00E-03	-3.00	0.0006
RC6	1	2	1.00E-03	-3.00	0.0004
RC6	1	3	1.00E-03	-3.00	0.0005
RC6	2	1	1.00E-04	-4.00	0.0035
RC6	2	2	1.00E-04	-4.00	0.0033
RC6	2	3	1.00E-04	-4.00	0.0033
RC6	3	1	1.00E-05	-5.00	0.0252
RC6	3	2	1.00E-05	-5.00	0.0237
RC6	3	3	1.00E-05	-5.00	0.0233
RC6	4	1	1.00E-06	-6.00	0.0577
RC6	4	2	1.00E-06	-6.00	0.0524
RC6	4	3	1.00E-06	-6.00	0.0518
RC6	5	1	1.00E-07	-7.00	0.0596
RC6	5	2	1.00E-07	-7.00	0.0624
RC6	5	3	1.00E-07	-7.00	0.0605
RC6	6	1	1.00E-08	-8.00	0.0665
RC6	6	2	1.00E-08	-8.00	0.0638
RC6	6	3	1.00E-08	-8.00	0.0631
RC6	7	1	1.00E-09	-9.00	0.0654
RC6	7	2	1.00E-09	-9.00	0.0627
RC6	7	3	1.00E-09	-9.00	0.0599
RC6	8	1	1.00E-10	-10.00	0.0639
RC6	8	2	1.00E-10	-10.00	0.0553
RC6	8	3	1.00E-10	-10.00	0.0599

Percent of control values				
	Log[test	Replicate		
_evel	bstance]	1	2	3
1	-3.00	0.95	0.70	0.87
2	-4.00	5.78	5.34	5.36
3	-5.00	41.17	38.70	38.18
4	-6.00	94.39	85.77	84.79
5	-7.00	97.55	102.12	98.89
6	-8.00	108.76	104.40	103.20
7	-9.00	106.99	102.61	97.94
8	-10.00	104.51	90.40	97.95

Aromatase Assay Spreadsheet

Assay Date	8/5/2005	Test Chemical ID	RC 6	# Concentrations tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	32083.34	1604167	
2	0.0201	32014.95	1592784	
3	0.0202	33169.23	1642041	
4	0.0198	32366.64	1634679	
5	0.0202	32127.45	1590468	
			Average DPM/g soln	1612828
			SD	24019
			CV	1.49
			µCi/g soln	0.726

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

ASDN solution	mg ASDN added	(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	8.1781 g
Mass of dilution B used in substrate prep	4.61 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.574975 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00822 µg/g soln.
µg/g soln.

a. µCi/g soln	0.726
b. Specific activity of [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.

= 0.574975 + 0.00822
= 0.583199 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.246 µCi/µg ASDN

792035 dpm/nmol

Assay Date 8/5/2005 Test Chemical ID RC 6 tested 8

ID TNB Replicate # Rep 2 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.564	0.339	0.165	0.096	0.051	0.037	0.027	25		
	0.587	0.346	0.169	0.098	0.055	0.039	0.030			
	0.570	0.356	0.163	0.103	0.057	0.039	0.027			
Samples:	10	100	microsomes							
	0.051	0.272	0.089							
	0.047	0.277	0.090							
			0.090							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.574	0.546	0.0482
0.125	100	200	0.00013	200	0.0250	0.347	0.319	0.0277
0.05	200	1000	0.00005	200	0.0100	0.166	0.138	0.0112
0.025	100	1000	0.00003	200	0.0050	0.099	0.071	0.0052
0.01	40	1000	0.00001	200	0.0020	0.054	0.026	0.0011
0.005	20	1000	0.00001	200	0.0010	0.038	0.010	-0.0003

Blank 0.028

r^2= 0.992
m= 0.091
b= -0.001

Variables	Regression results	
m, b	0.091	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.992	0.002
F, df	496	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	µsomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.051	0.023	0.001	200	1	1	0.000	0.000	0.003
10	0.047	0.019	0.000	200	1	1	0.000		
10					1	1			
100	0.272	0.244	0.021	200	1	1	0.000	0.000	0.105
100	0.277	0.249	0.021	200	1	1	0.000		
100									
	0.089	0.061	0.004	200	83	69983	0.018	0.018	18.268
	0.090	0.062	0.004	200	83	69983	0.018		
	0.090	0.062	0.004	200	83	69983	0.018		

Assay Date	8/5/2005	Test Chemical ID	RC 6	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.564	0.339	0.165	0.096	0.051	0.037	0.027	25	100	
	0.587	0.346	0.169	0.098	0.055	0.039	0.030			
	0.570	0.356	0.163	0.103	0.057	0.039	0.027			

Samples:	10	100	microsomes
	0.051	0.272	0.089
	0.047	0.277	0.090
			0.090

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.574	0.546	0.0424
0.125	100	200	0.00013	200	0.0250	0.347	0.319	0.0247
0.05	200	1000	0.00005	200	0.0100	0.166	0.138	0.0106
0.025	100	1000	0.00003	200	0.0050	0.099	0.071	0.0053
0.01	40	1000	0.00001	200	0.0020	0.054	0.026	0.0018
0.005	20	1000	0.00001	200	0.0010	0.038	0.010	0.0006

Blank	0.028	r^2=	0.998	
		m=	0.078	
		b=	0.000	

Variables	Regression results	
m, b	0.078	0.000
se_m, se_b	0.002	0.000
r , se_y	0.998	0.000
F, df	1593	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	µL microsome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.051	0.023	0.002	200	1	1	0.000	0.000	0.007
10	0.047	0.019	0.001	200	1	1	0.000		
10					1	1			
100	0.272	0.244	0.019	200	1	1	0.000	0.000	0.095
100	0.277	0.249	0.019	200	1	1	0.000		
100									
	0.089	0.061	0.005	200	83	69983	0.019	0.019	19.426
	0.090	0.062	0.005	200	83	69983	0.020		
	0.090	0.062	0.005	200	83	69983	0.020		

Assay Date	8/5/2005	Test Chemical ID	RC 6	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle

Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/5/2005	Chemical ID	RC 6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 2

Microsome Dilution Details

Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.16867	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.16867	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	19.426
Protein Concentration (dilution added to assay, mg/mL):	0.023039

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	3.00E-05
4	1.00E-05
5	3.00E-06
6	1.00E-06
7	1.00E-07
8	1.00E-09

Assay Date	8/5/2005	Test Chemical ID	RC 6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4612.98	9225.92	9268.03	18536.06	0.1	161283	11.49	15489	0.0196	1	0.012	15	0.0566
			0.5	2	4655.07	9310.14			0.1					1		15	
	2	2	0.5	1	5050.47	10100.94	10027.3	20054.6	0.1	161283	12.43	17007	0.0215	1	0.012	15	0.0621
			0.5	2	4976.83	9953.66			0.1					1		15	
	3	2	0.5	1	3711.48	7422.96	7452.47	14904.94	0.1	161283	9.24	11858	0.0150	1	0.012	15	0.0433
			0.5	2	3740.99	7481.98			0.1					1		15	
	4	2	0.5	1	3802.39	7604.78	7570.93	15141.86	0.1	161283	9.39	12095	0.0153	1	0.012	15	0.0442
			0.5	2	3768.54	7537.08			0.1					1		15	
Background control	1	2	0.5	1	902.48	1804.96	1821.39	3642.78	0.1	161283	2.26	596	0.0008	1	0.012	15	0.0022
			0.5	2	918.91	1837.82			0.1					1		15	
	2	2	0.5	1	858.65	1717.3	1766.4	3532.8	0.1	161283	2.19	486	0.0006	1	0.012	15	0.0018
			0.5	2	907.75	1815.5			0.1					1		15	
	3	2	0.5	1	626.1	1252.2	1267.41	2534.82	0.1	161283	1.57	-512	-0.0006	1	0.012	15	-0.0019
			0.5	2	641.31	1282.62			0.1					1		15	
	4	2	0.5	1	628.54	1257.08	1239.12	2478.24	0.1	161283	1.54	-569	-0.0007	1	0.012	15	-0.0021
			0.5	2	610.58	1221.16			0.1					1		15	
Positive control	1	2	0.5	1	2101.37	4202.74	4427.3	8854.6	0.1	161283	5.49	5807	0.0073	1	0.012	15	0.0212
			0.5	2	2325.93	4651.86			0.1					1		15	
	2	2	0.5	1	2116.69	4233.38	4274.41	8548.82	0.1	161283	5.30	5502	0.0069	1	0.012	15	0.0201
			0.5	2	2157.72	4315.44			0.1					1		15	
	3	2	0.5	1	1608.22	3216.44	3222.78	6445.56	0.1	161283	4.00	3398	0.0043	1	0.012	15	0.0124
			0.5	2	1614.56	3229.12			0.1					1		15	
	4	2	0.5	1	1591.23	3182.46	3250.95	6501.9	0.1	161283	4.03	3455	0.0044	1	0.012	15	0.0126
			0.5	2	1659.72	3319.44			0.1					1		15	
Negative Control	1	2	0.5	1	4768.81	9537.62	9490.42	18980.84	0.1	161283	11.77	15934	0.0201	1	0.012	15	0.0582
			0.5	2	4721.61	9443.22			0.1					1		15	
	2	2	0.5	1	4624.85	9249.7	9296.47	18592.94	0.1	161283	11.53	15548	0.0196	1	0.012	15	0.0568
			0.5	2	4671.62	9343.24			0.1					1		15	
	3	2	0.5	1	3491.85	6983.7	7047.53	14095.06	0.1	161283	8.74	11048	0.0139	1	0.012	15	0.0404
			0.5	2	3555.68	7111.36			0.1					1		15	
	4	2	0.5	1	3498.36	6996.72	7042.78	14085.56	0.1	161283	8.73	11038	0.0139	1	0.012	15	0.0403
			0.5	2	3544.42	7088.84			0.1					1		15	
RC 6	1-1	2	0.5	1	57.64	115.28	108.77	217.54	0.1	161283	0.13	-2830	-0.0036	1	0.012	15	-0.0103
			0.5	2	51.13	102.26			0.1					1		15	
	1-2	2	0.5	1	88.45	176.9	163.3	326.6	0.1	161283	0.20	-2721	-0.0034	1	0.012	15	-0.0099
			0.5	2	74.85	149.7			0.1					1		15	
	1-3	2	0.2	1	15.08	75.4	100.85	201.7	0.1	161283	0.13	-2845	-0.0036	1	0.012	15	-0.0104
			0.2	2	25.26	126.3			0.1					1		15	
	2-1	2	0.5	1	268.27	536.54	523.97	1047.94	0.1	161283	0.65	-1999	-0.0025	1	0.012	15	-0.0073
			0.5	2	255.7	511.4			0.1					1		15	
	2-2	2	0.5	1	284.62	569.24	567.34	1134.68	0.1	161283	0.70	-1912	-0.0024	1	0.012	15	-0.0070
			0.5	2	282.72	565.44			0.1					1		15	
	2-3	2	0.2	1	75.64	378.2	375.025	750.05	0.1	161283	0.47	-2297	-0.0029	1	0.012	15	-0.0084
			0.2	2	74.37	371.85			0.1					1		15	
	3-1	2	0.5	1	689.02	1378.04	1434.31	2868.62	0.1	161283	1.78	-179	-0.0002	1	0.012	15	-0.0007
			0.5	2	745.29	1490.58			0.1					1		15	
	3-2	2	0.5	1	742.7	1485.4	1511.43	3022.86	0.1	161283	1.87	-24	0.0000	1	0.012	15	-0.0001
			0.5	2	768.73	1537.46			0.1					1		15	
	3-3	2	0.2	1	363.82	1819.1	1793.975	3587.95	0.1	161283	2.22	541	0.0007	1	0.012	15	0.0020
			0.2	2	353.77	1768.85			0.1					1		15	
	4-1	2	0.5	1	1468.44	2936.88	2938.6	5877.2	0.1	161283	3.64	2830	0.0036	1	0.012	15	0.0103
			0.5	2	1470.16	2940.32			0.1					1		15	
	4-2	2	0.5	1	1503.76	3007.52	3038.68	6077.36	0.1	161283	3.77	3030	0.0038	1	0.012	15	0.0111
			0.5	2	1534.92	3069.84			0.1					1		15	
	4-3	2	0.2	1	578.47	2892.35	2934.225	5868.45	0.1	161283	3.64	2821	0.0036	1	0.012	15	0.0103
			0.2	2	595.22	2976.1			0.1					1		15	
	5-1	2	0.5	1	2422.42	4844.84	4905.76	9811.52	0.1	161283	6.08	6764	0.0085	1	0.012	15	0.0247
			0.5	2	2483.34	4966.68			0.1					1		15	
	5-2	2	0.5	1	2542.62	5085.24	5367.41	10734.82	0.1	161283	6.66	7688	0.0097	1	0.012	15	0.0281
			0.5	2	2824.79	5649.58			0.1					1		15	
	5-3	2	0.2	1	934.22	4671.1	4787.2	9574.4	0.1	161283	5.94	6527	0.0082	1	0.012	15	0.0238
			0.2	2	980.66	4903.3			0.1					1		15	
	6-1	2	0.5	1	3218.7	6437.4	6619.64	13239.28	0.1	161283	8.21	10192	0.0129	1	0.012	15	0.0372
			0.5	2	3400.94	6801.88			0.1					1		15	
	6-2	2	0.5	1	3531.15	7062.3	7042.97	14085.94	0.1	161283	8.73	11039	0.0139	1	0.012	15	0.0403
			0.5	2	3511.82	7023.64			0.1					1		15	
	6-3	2	0.2	1	1246.4	6232	6311.5	12623	0.1	161283	7.83	9576	0.0121	1	0.012	15	0.0350
			0.2	2	1278.2	6391			0.1					1		15	
	7-1	2	0.5	1	3437.83	6875.66	6981.84	13963.68	0.1	161283	8.66	10917	0.0138	1	0.012	15	0.0399
			0.5	2	3544.01	7088.02			0.1					1		15	
	7-2	2	0.5	1	3546.94	7093.88	7084.97	14169.94	0.1	161283	8.79	11123	0.0140	1	0.012	15	0.0406
			0.5	2	3538.03	7076.06			0.1					1		15	
	7-3	2	0.2	1	1311.79	6558.95	6518.05	13036.1	0.1	161283	8.08	9989	0.0126	1	0.012	15	0.0365
			0.2	2	1295.43	6477.15			0.1					1		15	
	8-1	2	0.5	1	3662	7324	7515.32	15030.64	0.1	161283	9.32	11963	0.0151	1	0.012	15	0.0438
			0.5	2	3853.32	7706.64			0.1					1		15	
	8-2	2	0.5	1	3760.64	7521.28	7399.84	14799.68	0.1	161283	9.18	11753	0.0148	1	0.012	15	0.0429
			0.5	2	3639.2	7278.4			0.1					1		15	
	8-3	2	0.2	1					0.1	161283		#VALUE!	#VALUE!	1	0.012	15	#VALUE!
			0.2	2					0.1					1		15	

Assay Date	8/5/2005	ID	RC 6	# Concentrations tested	Microsome 8 type	Placental	Microsome ID Battelle	Technician ID TNB	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0594	0.0039
Full activity	End	0.0438	0.0006
Full activity	Overall	0.0516	0.0093
Background	Beginning	0.0020	0.000284115
Background	End	-0.0020	0.000146165
Background	Overall	0.0000	0.002288131
Positive	Beginning	0.0207	0.0008
Positive	End	0.0125	0.0001
Positive	Overall	0.0166	0.0047
Negative	Beginning	0.0575	0.0010
Negative	End	0.0403	0.0000
Negative	Overall	0.0489	0.0099

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC 6	1	1	1.00E-03	-3.00	-0.0103
RC 6	1	2	1.00E-03	-3.00	-0.0099
RC 6	1	3	1.00E-03	-3.00	-0.0104
RC 6	2	1	1.00E-04	-4.00	-0.0073
RC 6	2	2	1.00E-04	-4.00	-0.0070
RC 6	2	3	1.00E-04	-4.00	-0.0084
RC 6	3	1	3.00E-05	-4.52	-0.0007
RC 6	3	2	3.00E-05	-4.52	-0.0001
RC 6	3	3	3.00E-05	-4.52	0.0020
RC 6	4	1	1.00E-05	-5.00	0.0103
RC 6	4	2	1.00E-05	-5.00	0.0111
RC 6	4	3	1.00E-05	-5.00	0.0103
RC 6	5	1	3.00E-06	-5.52	0.0247
RC 6	5	2	3.00E-06	-5.52	0.0281
RC 6	5	3	3.00E-06	-5.52	0.0238
RC 6	6	1	1.00E-06	-6.00	0.0372
RC 6	6	2	1.00E-06	-6.00	0.0403
RC 6	6	3	1.00E-06	-6.00	0.0350
RC 6	7	1	1.00E-07	-7.00	0.0399
RC 6	7	2	1.00E-07	-7.00	0.0406
RC 6	7	3	1.00E-07	-7.00	0.0365
RC 6	8	1	1.00E-09	-9.00	0.0438
RC 6	8	2	1.00E-09	-9.00	0.0429
RC 6	8	3	1.00E-09	-9.00	#VALUE!

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-3.00	-20.05	-19.28	-20.16
2	-4.00	-14.17	-13.55	-16.28
3	-4.52	-1.27	-0.17	3.83
4	-5.00	20.05	21.47	19.99
5	-5.52	47.93	54.48	46.25
6	-6.00	72.22	78.22	67.86
7	-7.00	77.36	78.82	70.78
8	-9.00	84.92	83.28	#VALUE!

Aromatase Assay Spreadsheet

Assay Date	8/9/2005	Test Chemical ID	RC6	# Concentrations tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	32352.68	1609586	
2	0.0201	31891.58	1586646	
3	0.0193	30889.07	1600470	
4	0.0199	32486.51	1632488	
5	0.0200	32306.54	1615327	
			Average DPM/g soln	1608903
			SD	17062
			CV	1.06
			µCi/g soln	0.725

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

ASDN solution	mg ASDN added	(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	8.1505 g
Mass of dilution B used in substrate prep	4.6215 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56702 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00820 µg/g soln.
µg/g soln.

a. µCi/g soln	0.725
b. Specific activity of [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.

= 0.567020 + 0.00820
= 0.575225 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.260 µCi/µg ASDN

801061 dpm/nmol

Test

Assay Date 8/9/2005 Chemical ID RC6 tested 8

ID TNB Replicate # Rep 3 Microsome type Placental Microsome ID Battelle

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.556	0.348	0.159	0.087	0.049	0.034	0.033	25	100	
	0.578	0.340	0.156	0.095	0.049	0.038	0.030			
	0.577	0.341	0.155	0.092	0.047	0.041	0.030			

Samples:	10	100	microsomes
	0.054	0.304	0.095
	0.054	0.259	0.096
			0.099

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.570	0.539	0.0483
0.125	100	200	0.00013	200	0.0250	0.343	0.312	0.0277
0.05	200	1000	0.00005	200	0.0100	0.157	0.126	0.0109
0.025	100	1000	0.00003	200	0.0050	0.092	0.060	0.0050
0.01	40	1000	0.00001	200	0.0020	0.048	0.017	0.0011
0.005	20	1000	0.00001	200	0.0010	0.038	0.006	0.0001

Variables	Regression results	
m, b	0.090	0.000
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	566	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank 0.031

r^2= 0.993
m= 0.090
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.054	0.023	0.002	200	1	1	0.000	0.000	0.008
10	0.054	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.304	0.273	0.024	200	1	1	0.000	0.000	0.111
100	0.259	0.227	0.020	200	1	1	0.000		
100									
	0.095	0.064	0.005	200	83	69983	0.022	0.023	23.003
	0.096	0.065	0.005	200	83	69983	0.023		
	0.099	0.068	0.006	200	83	69983	0.024		

Assay Date 8/9/2005 Test Chemical ID RC6 tested 8

ID TNB Replicate # Rep 3 Microsome type Placental Microsome ID Battelle

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.556	0.348	0.159	0.087	0.049	0.034	0.033	25	100	
	0.578	0.340	0.156	0.095	0.049	0.038	0.030			
	0.577	0.341	0.155	0.092	0.047	0.041	0.030			

Samples:	10	100	microsomes
	0.054	0.304	0.095
	0.054	0.259	0.096
			0.099

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
0.25	200	200	0.00025	200	0.0500	0.570	0.539	0.0427	m, b	0.078	0.000
0.125	100	200	0.00013	200	0.0250	0.343	0.312	0.0249	se_m, se_b	0.001	0.000
0.05	200	1000	0.00005	200	0.0100	0.157	0.126	0.0103	r , se_y	1.000	0.000
0.025	100	1000	0.00003	200	0.0050	0.092	0.060	0.0052	F, df	6782	3
0.01	40	1000	0.00001	200	0.0020	0.048	0.017	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.038	0.006	0.0009			

Regression results are calculated using the function LINEST

Blank 0.031

r^2= 1.000
m= 0.078
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.054	0.023	0.002	200	1	1	0.000	0.000	0.011
10	0.054	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.304	0.273	0.022	200	1	1	0.000	0.000	0.100
100	0.259	0.227	0.018	200	1	1	0.000		
100									
	0.095	0.064	0.005	200	83	69983	0.023	0.023	23.498
	0.096	0.065	0.006	200	83	69983	0.023		
	0.099	0.068	0.006	200	83	69983	0.024		

Assay Date	8/9/2005	Test Chemical ID	RC6	tested	8			
ID	TNB	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	Battelle Protein stock (mg/10 mL)	Protein stock ID
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/9/2005	Chemical ID	RC6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 3

Microsome Dilution Details		
Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.16867	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.16867	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	23.498
Protein Concentration (dilution added to assay, mg/mL):	0.027869

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	3.00E-05
4	1.00E-05
5	3.00E-06
6	1.00E-06
7	1.00E-07
8	1.00E-09

Assay Date	8/9/2005	Test Chemical ID	RC6	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5914.52	11829.04	11916.21	23832.42	0.1	160890	14.81	23703	0.0296	1	0.014	15	0.0708
			0.5	2	6001.69	12003.38			0.1					1		15	
	2	2	0.5	1	5773.86	11547.72	11536.83	23073.66	0.1	160890	14.34	22944	0.0286	1	0.014	15	0.0685
			0.5	2	5762.97	11525.94			0.1					1		15	
	3	2	0.5	1	5484.06	10968.12	12747.35	25494.7	0.1	160890	15.85	25365	0.0317	1	0.014	15	0.0757
			0.5	2	7263.29	14526.58			0.1					1		15	
	4	2	0.5	1	5437.45	10874.9	10947.56	21895.12	0.1	160890	13.61	21765	0.0272	1	0.014	15	0.0650
			0.5	2	5510.11	11020.22			0.1					1		15	
Background control	1	2	0.5	1	30.86	61.72	61.64	123.28	0.1	160890	0.08	-7	0.0000	1	0.014	15	0.0000
			0.5	2	30.78	61.56			0.1					1		15	
	2	2	0.5	1	25.99	51.98	57.41	114.82	0.1	160890	0.07	-15	0.0000	1	0.014	15	0.0000
			0.5	2	31.42	62.84			0.1					1		15	
	3	2	0.5	1	25.67	51.34	59.11	118.22	0.1	160890	0.07	-12	0.0000	1	0.014	15	0.0000
			0.5	2	33.44	66.88			0.1					1		15	
	4	2	0.5	1	42.75	85.5	81.47	162.94	0.1	160890	0.10	33	0.0000	1	0.014	15	0.0001
			0.5	2	38.72	77.44			0.1					1		15	
Positive control	1	2	0.5	1	2344.85	4689.7	4613.05	9226.1	0.1	160890	5.73	9096	0.0114	1	0.014	15	0.0272
			0.5	2	2268.2	4536.4			0.1					1		15	
	2	2	0.5	1	2230.4	4460.8	4504.96	9009.92	0.1	160890	5.60	8880	0.0111	1	0.014	15	0.0265
			0.5	2	2274.56	4549.12			0.1					1		15	
	3	2	0.5	1	2231.78	4463.56	5220.28	10440.52	0.1	160890	6.49	10311	0.0129	1	0.014	15	0.0308
			0.5	2	2988.48	5976.96			0.1					1		15	
	4	2	0.5	1	2108.68	4217.36	4373.24	8746.48	0.1	160890	5.44	8617	0.0108	1	0.014	15	0.0257
			0.5	2	2264.56	4529.12			0.1					1		15	
Negative Control	1	2	0.5	1	5858.32	11716.64	11575.14	23150.28	0.1	160890	14.39	23020	0.0287	1	0.014	15	0.0687
			0.5	2	5716.82	11433.64			0.1					1		15	
	2	2	0.5	1	5674.21	11348.42	11469.73	22939.46	0.1	160890	14.26	22810	0.0285	1	0.014	15	0.0681
			0.5	2	5795.52	11591.04			0.1					1		15	
	3	2	0.5	1	5317.88	10635.76	10651.9	21303.8	0.1	160890	13.24	21174	0.0264	1	0.014	15	0.0632
			0.5	2	5334.02	10668.04			0.1					1		15	
	4	2	0.5	1	5331.42	10662.84	10744.08	21488.16	0.1	160890	13.36	21358	0.0267	1	0.014	15	0.0638
			0.5	2	5412.66	10825.32			0.1					1		15	
RC6	1-1	2	0.5	1	111.92	223.84	214.09	428.18	0.1	160890	0.27	298	0.0004	1	0.014	15	0.0009
			0.5	2	102.17	204.34			0.1					1		15	
	1-2	2	0.5	1	104.29	208.58	222.79	445.58	0.1	160890	0.28	316	0.0004	1	0.014	15	0.0009
			0.5	2	118.5	237			0.1					1		15	
	1-3	2	0.5	1	99.53	199.06	200.6	401.2	0.1	160890	0.25	271	0.0003	1	0.014	15	0.0008
			0.5	2	101.07	202.14			0.1					1		15	
	2-1	2	0.5	1	384.54	769.08	790.25	1580.5	0.1	160890	0.98	1451	0.0018	1	0.014	15	0.0043
			0.5	2	405.71	811.42			0.1					1		15	
	2-2	2	0.5	1	372.51	745.02	745.56	1491.12	0.1	160890	0.93	1361	0.0017	1	0.014	15	0.0041
			0.5	2	373.05	746.1			0.1					1		15	
	2-3	2	0.5	1	366.54	733.08	715.2	1430.4	0.1	160890	0.89	1301	0.0016	1	0.014	15	0.0039
			0.5	2	348.66	697.32			0.1					1		15	
	3-1	2	0.5	1	1069.81	2139.62	2124.96	4249.92	0.1	160890	2.64	4120	0.0051	1	0.014	15	0.0123
			0.5	2	1055.15	2110.3			0.1					1		15	
	3-2	2	0.5	1	1170.36	2340.72	2316.28	4632.56	0.1	160890	2.88	4503	0.0056	1	0.014	15	0.0134
			0.5	2	1145.92	2291.84			0.1					1		15	
	3-3	2	0.5	1	1041.2	2082.4	2114.67	4229.34	0.1	160890	2.63	4100	0.0051	1	0.014	15	0.0122
			0.5	2	1073.47	2146.94			0.1					1		15	
	4-1	2	0.5	1	2295.66	4591.32	4610.98	9221.96	0.1	160890	5.73	9092	0.0114	1	0.014	15	0.0272
			0.5	2	2315.32	4630.64			0.1					1		15	
	4-2	2	0.5	1	2233.53	4467.06	4443.69	8887.38	0.1	160890	5.52	8758	0.0109	1	0.014	15	0.0262
			0.5	2	2210.16	4420.32			0.1					1		15	
	4-3	2	0.5	1	2151.29	4302.58	4317.59	8635.18	0.1	160890	5.37	8505	0.0106	1	0.014	15	0.0254
			0.5	2	2166.3	4332.6			0.1					1		15	
	5-1	2	0.5	1	3880.28	7760.56	7787.64	15575.28	0.1	160890	9.68	15445	0.0193	1	0.014	15	0.0461
			0.5	2	3907.36	7814.72			0.1					1		15	
	5-2	2	0.5	1	3819.03	7638.06	7605.52	15211.04	0.1	160890	9.45	15081	0.0188	1	0.014	15	0.0450
			0.5	2	3786.49	7572.98			0.1					1		15	
	5-3	2	0.5	1	3786.03	7572.06	7622.53	15245.06	0.1	160890	9.48	15115	0.0189	1	0.014	15	0.0451
			0.5	2	3836.5	7673			0.1					1		15	
	6-1	2	0.5	1	4826.11	9652.22	9644.81	19289.62	0.1	160890	11.99	19160	0.0239	1	0.014	15	0.0572
			0.5	2	4818.7	9637.4			0.1					1		15	
	6-2	2	0.5	1	4585.03	9170.06	9166.39	18332.78	0.1	160890	11.39	18203	0.0227	1	0.014	15	0.0544
			0.5	2	4581.36	9162.72			0.1					1		15	
	6-3	2	0.5	1	4469.03	8938.06	9042.5	18085	0.1	160890	11.24	17955	0.0224	1	0.014	15	0.0536
			0.5	2	4573.47	9146.94			0.1					1		15	
	7-1	2	0.5	1	5075.18	10150.36	9993.45	19986.9	0.1	160890	12.42	19857	0.0248	1	0.014	15	0.0593
			0.5	2	4918.27	9836.54			0.1					1		15	
	7-2	2	0.5	1	4804.97	9609.94	9992.94	19985.88	0.1	160890	12.42	19856	0.0248	1	0.014	15	0.0593
			0.5	2	5187.97	10375.94			0.1					1		15	
	7-3	2	0.5	1	4846.88	9693.76	9718.77	19437.54	0.1	160890	12.08	19308	0.0241	1	0.014	15	0.0577
			0.5	2	4871.89	9743.78			0.1					1		15	
	8-1	2	0.5	1	4851.97	9703.94	9726.93	19453.86	0.1	160890	12.09	19324	0.0241	1	0.014	15	0.0577
			0.5	2	4874.96	9749.92			0.1					1		15	
	8-2	2	0.5	1	5433.17	10866.34	10977.55	21955.1	0.1	160890	13.65	21825	0.0272	1	0.014	15	0.0652
			0.5	2	5544.38	11088.76			0.1					1		15	
	8-3	2	0.5	1	5609.11	11218.22	11295.62	22591.24	0.1	160890	14.04	22461	0.0280	1	0.014	15	0.0671
			0.5	2	5686.51	11373.02			0.1					1		15	

Assay Date	8/9/2005	ID	RC6	# Concentrations tested	Microsome 8 type	Placental	Microsome ID Battelle	Technician ID TNB	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0696	0.0016
Full activity	End	0.0704	0.0076
Full activity	Overall	0.0700	0.0045
Background	Beginning	0.0000	1.78641E-05
Background	End	0.0000	9.44308E-05
Background	Overall	0.0000	6.67583E-05
Positive	Beginning	0.0268	0.0005
Positive	End	0.0283	0.0036
Positive	Overall	0.0276	0.0022
Negative	Beginning	0.0684	0.0004
Negative	End	0.0635	0.0004
Negative	Overall	0.0660	0.0029

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC6	1	1	1.00E-03	-3.00	0.0009
RC6	1	2	1.00E-03	-3.00	0.0009
RC6	1	3	1.00E-03	-3.00	0.0008
RC6	2	1	1.00E-04	-4.00	0.0043
RC6	2	2	1.00E-04	-4.00	0.0041
RC6	2	3	1.00E-04	-4.00	0.0039
RC6	3	1	3.00E-05	-4.52	0.0123
RC6	3	2	3.00E-05	-4.52	0.0134
RC6	3	3	3.00E-05	-4.52	0.0122
RC6	4	1	1.00E-05	-5.00	0.0272
RC6	4	2	1.00E-05	-5.00	0.0262
RC6	4	3	1.00E-05	-5.00	0.0254
RC6	5	1	3.00E-06	-5.52	0.0461
RC6	5	2	3.00E-06	-5.52	0.0450
RC6	5	3	3.00E-06	-5.52	0.0451
RC6	6	1	1.00E-06	-6.00	0.0572
RC6	6	2	1.00E-06	-6.00	0.0544
RC6	6	3	1.00E-06	-6.00	0.0536
RC6	7	1	1.00E-07	-7.00	0.0593
RC6	7	2	1.00E-07	-7.00	0.0593
RC6	7	3	1.00E-07	-7.00	0.0577
RC6	8	1	1.00E-09	-9.00	0.0577
RC6	8	2	1.00E-09	-9.00	0.0652
RC6	8	3	1.00E-09	-9.00	0.0671

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-3.00	1.27	1.35	1.16
2	-4.00	6.19	5.81	5.55
3	-4.52	17.57	19.21	17.49
4	-5.00	38.78	37.35	36.28
5	-5.52	65.88	64.33	64.47
6	-6.00	81.73	77.64	76.59
7	-7.00	84.70	84.70	82.36
8	-9.00	82.43	93.09	95.81

Aromatase Assay Spreadsheet

Assay Date	8/15/2005	Test Chemical ID	RC 7	# Concentrations tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	31483.41	1582081	
2	0.0200	31966.14	1598307	
3	0.0201	31992.18	1591651	
4	0.0202	31979.68	1583152	
5	0.0201	32440.45	1613953	
			Average DPM/g soln	1593829
			SD	13055
			CV	0.82
			µCi/g soln	0.718

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

ASDN solution	mg ASDN added	(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	8.2352 g
Mass of dilution B used in substrate prep	4.6261 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.567365 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 [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.

= 0.567365 + 0.00813
= 0.575492 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.248 µCi/µg ASDN

793187 dpm/nmol

Assay Date 8/15/2005 Test Chemical ID RC 7 tested 8

ID JG Replicate # Rep 1 Microsome type Placental Microsome ID IVT

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.522	0.355	0.189	0.116	0.055	0.032	0.025	25	100	
	0.534	0.320	0.192	0.106	0.052	0.033	0.027			
	0.557	0.314	0.192	0.108	0.051	0.034	0.017			

Samples:	10	100	microsomes
	0.040	0.273	0.093
	0.042	0.278	0.090
			0.094

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.537	0.514	0.0474
0.125	100	200	0.00013	200	0.0250	0.330	0.307	0.0272
0.05	200	1000	0.00005	200	0.0100	0.191	0.168	0.0137
0.025	100	1000	0.00003	200	0.0050	0.110	0.088	0.0059
0.01	40	1000	0.00001	200	0.0020	0.053	0.030	0.0003
0.005	20	1000	0.00001	200	0.0010	0.033	0.010	-0.0016

Blank 0.023

r^2= 0.980
m= 0.097
b= -0.003

Variables	Regression results	
m, b	0.097	-0.003
se_m, se_b	0.007	0.002
r , se_y	0.980	0.003
F, df	197	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	µL microsomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.040	0.018	-0.001	200	1	1	0.000	0.000	-0.004
10	0.042	0.020	-0.001	200	1	1	0.000		
10					1	1			
100	0.273	0.250	0.022	200	1	1	0.000	0.000	0.110
100	0.278	0.255	0.022	200	1	1	0.000		
100									
	0.093	0.071	0.004	200	218	70018	0.007	0.007	6.705
	0.090	0.068	0.004	200	218	70018	0.006		
	0.094	0.071	0.004	200	218	70018	0.007		

Assay Date 8/15/2005 Test Chemical ID RC 7 tested 8

ID JG Replicate # Rep 1 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.522	0.355	0.189	0.116	0.055	0.032	0.025	25		
	0.534	0.320	0.192	0.106	0.052	0.033	0.027			
	0.557	0.314	0.192	0.108	0.051	0.034	0.017			
Samples:	10	100	microsomes							
	0.040	0.273	0.093							
	0.042	0.278	0.090							
			0.094							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.537	0.514	0.0401
0.125	100	200	0.00013	200	0.0250	0.330	0.307	0.0235
0.05	200	1000	0.00005	200	0.0100	0.191	0.168	0.0124
0.025	100	1000	0.00003	200	0.0050	0.110	0.088	0.0060
0.01	40	1000	0.00001	200	0.0020	0.053	0.030	0.0013
0.005	20	1000	0.00001	200	0.0010	0.033	0.010	-0.0002
		Blank		0.023				

Variables	Regression results	
m, b	0.080	-0.001
se_m, se_b	0.008	0.001
r , se_y	0.972	0.002
F, df	104	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are caiculated using the function LINEST

r^2= 0.972
m= 0.080
b= -0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µL microsome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.040	0.018	0.000	200	1	1	0.000	0.000	0.002
10	0.042	0.020	0.001	200	1	1	0.000		
10					1	1			
100	0.273	0.250	0.019	200	1	1	0.000	0.000	0.096
100	0.278	0.255	0.019	200	1	1	0.000		
100									
	0.093	0.071	0.005	200	218	70018	0.007	0.007	7.288
	0.090	0.068	0.004	200	218	70018	0.007		
	0.094	0.071	0.005	200	218	70018	0.007		

Assay Date	8/15/2005	Test Chemical ID	RC 7	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	IVT

Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					
Blank		r^2=					Regression results are calculated using the function LINEST
		m=					
		b=					

A_{raw}	A_{adj}	mg protein measured	µ µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/15/2005	Chemical ID	RC 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.1835	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.1835	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	7.288
Protein Concentration (dilution added to assay, mg/mL):	0.022691

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	8/15/2005	Test Chemical ID	RC 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5005.92	10011.84	10115.1	20230.2	0.1	159383	12.69	20072	0.0253	1	0.011	15	0.0743
			0.5	2	5109.18	10218.36			0.1					1		15	
	2	2	0.5	1	4929.32	9856.64	9773.03	19546.06	0.1	159383	12.26	19388	0.0244	1	0.011	15	0.0718
			0.5	2	4843.71	9687.42			0.1					1		15	
	3	2	0.5	1	4887.8	9775.6	9757.17	19514.34	0.1	159383	12.24	19356	0.0244	1	0.011	15	0.0717
			0.5	2	4869.37	9738.74			0.1					1		15	
	4	2	0.5	1	4818.53	9637.06	9816.4	19632.8	0.1	159383	12.32	19475	0.0246	1	0.011	15	0.0721
			0.5	2	4997.87	9995.74			0.1					1		15	
Background control	1	2	0.5	1	29.46	58.92	60.54	121.08	0.1	159383	0.08	-37	0.0000	1	0.011	15	-0.0001
			0.5	2	31.08	62.16			0.1					1		15	
	2	2	0.5	1	26.82	53.64	61.97	123.94	0.1	159383	0.08	-34	0.0000	1	0.011	15	-0.0001
			0.5	2	35.15	70.3			0.1					1		15	
	3	2	0.5	1	38.59	77.18	77.74	155.48	0.1	159383	0.10	-3	0.0000	1	0.011	15	0.0000
			0.5	2	39.15	78.3			0.1					1		15	
	4	2	0.5	1	58.34	116.68	116.21	232.42	0.1	159383	0.15	74	0.0001	1	0.011	15	0.0003
			0.5	2	57.87	115.74			0.1					1		15	
Positive control	1	2	0.5	1	2127.14	4254.28	4173.92	8347.84	0.1	159383	5.24	8190	0.0103	1	0.011	15	0.0303
			0.5	2	2046.78	4093.56			0.1					1		15	
	2	2	0.5	1	1987.01	3974.02	3940.26	7880.52	0.1	159383	4.94	7722	0.0097	1	0.011	15	0.0286
			0.5	2	1953.25	3906.5			0.1					1		15	
	3	2	0.5	1	1914.73	3829.46	3763.88	7527.76	0.1	159383	4.72	7370	0.0093	1	0.011	15	0.0273
			0.5	2	1849.15	3698.3			0.1					1		15	
	4	2	0.5	1	1771.36	3542.72	3588.55	7177.1	0.1	159383	4.50	7019	0.0088	1	0.011	15	0.0260
			0.5	2	1817.19	3634.38			0.1					1		15	
Negative Control	1	2	0.5	1	5199.55	10399.1	10294.73	20589.46	0.1	159383	12.92	20431	0.0258	1	0.011	15	0.0757
			0.5	2	5095.18	10190.36			0.1					1		15	
	2	2	0.5	1	5230.31	10460.62	10446.99	20893.98	0.1	159383	13.11	20736	0.0261	1	0.011	15	0.0768
			0.5	2	5216.68	10433.36			0.1					1		15	
	3	2	0.5	1	4827.6	9655.2	9541.3	19082.6	0.1	159383	11.97	18924	0.0239	1	0.011	15	0.0701
			0.5	2	4713.7	9427.4			0.1					1		15	
	4	2	0.5	1	4722.72	9445.44	9462.77	18925.54	0.1	159383	11.87	18767	0.0237	1	0.011	15	0.0695
			0.5	2	4740.05	9480.1			0.1					1		15	
RC 7	1-1	2	0.5	1	70.44	140.88	138.17	276.34	0.1	159383	0.17	118	0.0001	1	0.011	15	0.0004
			0.5	2	67.73	135.46			0.1					1		15	
	1-2	2	0.5	1	53.07	106.14	102.2	204.4	0.1	159383	0.13	46	0.0001	1	0.011	15	0.0002
			0.5	2	49.13	98.26			0.1					1		15	
	1-3	2	0.5	1	38.08	76.16	74.99	149.98	0.1	159383	0.09	-8	0.0000	1	0.011	15	0.0000
			0.5	2	36.91	73.82			0.1					1		15	
	2-1	2	0.5	1	41.82	83.64	78.55	157.1	0.1	159383	0.10	-1	0.0000	1	0.011	15	0.0000
			0.5	2	36.73	73.46			0.1					1		15	
	2-2	2	0.5	1	25.28	50.56	49.43	98.86	0.1	159383	0.06	-59	-0.0001	1	0.011	15	-0.0002
			0.5	2	24.15	48.3			0.1					1		15	
	2-3	2	0.5	1	29.58	59.16	55.15	110.3	0.1	159383	0.07	-48	-0.0001	1	0.011	15	-0.0002
			0.5	2	25.57	51.14			0.1					1		15	
	3-1	2	0.5	1	30.6	61.2	57.72	115.44	0.1	159383	0.07	-43	-0.0001	1	0.011	15	-0.0002
			0.5	2	27.12	54.24			0.1					1		15	
	3-2	2	0.5	1	47.25	94.5	76.61	153.22	0.1	159383	0.10	-5	0.0000	1	0.011	15	0.0000
			0.5	2	29.36	58.72			0.1					1		15	
	3-3	2	0.5	1	32.34	64.68	55.99	111.98	0.1	159383	0.07	-46	-0.0001	1	0.011	15	-0.0002
			0.5	2	23.65	47.3			0.1					1		15	
	4-1	2	0.5	1	42.14	84.28	87.23	174.46	0.1	159383	0.11	16	0.0000	1	0.011	15	0.0001
			0.5	2	45.09	90.18			0.1					1		15	
	4-2	2	0.5	1	46.03	92.06	100.72	201.44	0.1	159383	0.13	43	0.0001	1	0.011	15	0.0002
			0.5	2	54.69	109.38			0.1					1		15	
	4-3	2	0.5	1	30.42	60.84	61.04	122.08	0.1	159383	0.08	-36	0.0000	1	0.011	15	-0.0001
			0.5	2	30.62	61.24			0.1					1		15	
	5-1	2	0.5	1	88.39	176.78	190.86	381.72	0.1	159383	0.24	223	0.0003	1	0.011	15	0.0008
			0.5	2	102.47	204.94			0.1					1		15	
	5-2	2	0.5	1	133.7	267.4	265.92	531.84	0.1	159383	0.33	374	0.0005	1	0.011	15	0.0014
			0.5	2	132.22	264.44			0.1					1		15	
	5-3	2	0.5	1	122.84	245.68	239.6	479.2	0.1	159383	0.30	321	0.0004	1	0.011	15	0.0012
			0.5	2	116.76	233.52			0.1					1		15	
	6-1	2	0.5	1	632.02	1264.04	1260.75	2521.5	0.1	159383	1.58	2363	0.0030	1	0.011	15	0.0088
			0.5	2	628.73	1257.46			0.1					1		15	
	6-2	2	0.5	1	646.1	1292.2	1300.05	2600.1	0.1	159383	1.63	2442	0.0031	1	0.011	15	0.0090
			0.5	2	653.95	1307.9			0.1					1		15	
	6-3	2	0.5	1	583.66	1167.32	1150.15	2300.3	0.1	159383	1.44	2142	0.0027	1	0.011	15	0.0079
			0.5	2	566.49	1132.98			0.1					1		15	
	7-1	2	0.5	1	3114.92	6229.84	6215.37	12430.74	0.1	159383	7.80	12273	0.0155	1	0.011	15	0.0455
			0.5	2	3100.45	6200.9			0.1					1		15	
	7-2	2	0.5	1	3132.48	6264.96	6276.68	12553.36	0.1	159383	7.88	12395	0.0156	1	0.011	15	0.0459
			0.5	2	3144.2	6288.4			0.1					1		15	
	7-3	2	0.5	1	3073.45	6146.9	6116.75	12233.5	0.1	159383	7.68	12075	0.0152	1	0.011	15	0.0447
			0.5	2	3043.3	6086.6			0.1					1		15	
	8-1	2	0.5	1	4622.64	9245.28	9380.86	18761.72	0.1	159383	11.77	18603	0.0235	1	0.011	15	0.0689
			0.5	2	4758.22	9516.44			0.1					1		15	
	8-2	2	0.5	1	4652.57	9305.14	9325.5	18651	0.1	159383	11.70	18493	0.0233	1	0.011	15	0.0685
			0.5	2	4672.93	9345.86			0.1					1		15	
	8-3	2	0.5	1	4731.42	9462.84	9483.53	18967.06	0.1	159383	11.90	18809	0.0237	1	0.011	15	0.0697
			0.5	2	4752.11	9504.22			0.1					1		15	

Assay Date	8/15/2005	ID	RC 7	# Concentrations tested	Microsome type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0731	0.0018
Full activity	End	0.0719	0.0003
Full activity	Overall	0.0725	0.0012
Background	Beginning	-0.0001	7.49082E-06
Background	End	0.0001	0.000201519
Background	Overall	0.0000	0.000192084
Positive	Beginning	0.0295	0.0012
Positive	End	0.0266	0.0009
Positive	Overall	0.0281	0.0019
Negative	Beginning	0.0762	0.0008
Negative	End	0.0698	0.0004
Negative	Overall	0.0730	0.0038

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC 7	1	1	1.00E-03	-3.00	0.0004
RC 7	1	2	1.00E-03	-3.00	0.0002
RC 7	1	3	1.00E-03	-3.00	0.0000
RC 7	2	1	1.00E-04	-4.00	0.0000
RC 7	2	2	1.00E-04	-4.00	-0.0002
RC 7	2	3	1.00E-04	-4.00	-0.0002
RC 7	3	1	1.00E-05	-5.00	-0.0002
RC 7	3	2	1.00E-05	-5.00	0.0000
RC 7	3	3	1.00E-05	-5.00	-0.0002
RC 7	4	1	1.00E-06	-6.00	0.0001
RC 7	4	2	1.00E-06	-6.00	0.0002
RC 7	4	3	1.00E-06	-6.00	-0.0001
RC 7	5	1	1.00E-07	-7.00	0.0008
RC 7	5	2	1.00E-07	-7.00	0.0014
RC 7	5	3	1.00E-07	-7.00	0.0012
RC 7	6	1	1.00E-08	-8.00	0.0088
RC 7	6	2	1.00E-08	-8.00	0.0090
RC 7	6	3	1.00E-08	-8.00	0.0079
RC 7	7	1	1.00E-09	-9.00	0.0455
RC 7	7	2	1.00E-09	-9.00	0.0459
RC 7	7	3	1.00E-09	-9.00	0.0447
RC 7	8	1	1.00E-10	-10.00	0.0689
RC 7	8	2	1.00E-10	-10.00	0.0685
RC 7	8	3	1.00E-10	-10.00	0.0697

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-3.00	0.60	0.24	-0.04
2	-4.00	-0.01	-0.30	-0.24
3	-5.00	-0.22	-0.03	-0.24
4	-6.00	0.08	0.22	-0.18
5	-7.00	1.14	1.91	1.64
6	-8.00	12.07	12.48	10.94
7	-9.00	62.70	63.33	61.69
8	-10.00	95.05	94.48	96.10

Aromatase Assay Spreadsheet

Assay Date	8/22/2005	Test Chemical ID	RC 7	# Concentrations tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Placental	Microsome ID IVT	

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0197	30370.02	1541625	
2	0.0200	30637.99	1531900	
3	0.0201	30781.96	1531441	
4	0.0202	31260.43	1547546	
5	0.0204	31490.88	1543671	
			Average DPM/g soln	1539236
			SD	7229
			CV	0.47
			µCi/g soln	0.693

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

ASDN solution	mg ASDN added	(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	8.1635 g
Mass of dilution B used in substrate prep	4.5734 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.577032 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00785 µg/g soln.
µg/g soln.

a. µCi/g soln	0.693
b. Specific activity of [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.

= 0.577032 + 0.00785
= 0.584881 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.185 µCi/µg ASDN

753721 dpm/nmol

Assay Date 8/22/2005 Test Chemical ID RC 7 tested 8

ID JG Replicate # Rep 2 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.514	0.313	0.209	0.119	0.056	0.039	0.022	25		
	0.528	0.319	0.210	0.122	0.061	0.036	0.024			
	0.522	0.308	0.221	0.120	0.055	0.035	0.021			
Samples:	10	100	microsomes							
	0.045	0.282	0.091							
	0.045	0.282	0.097							
			0.094							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.521	0.499	0.0472
0.125	100	200	0.00013	200	0.0250	0.313	0.291	0.0260
0.05	200	1000	0.00005	200	0.0100	0.214	0.191	0.0158
0.025	100	1000	0.00003	200	0.0050	0.121	0.098	0.0063
0.01	40	1000	0.00001	200	0.0020	0.058	0.035	-0.0001
0.005	20	1000	0.00001	200	0.0010	0.036	0.014	-0.0022

Blank 0.022

r^2= 0.967
m= 0.102
b= -0.004

Variables	Regression results	
m, b	0.102	-0.004
se_m, se_b	0.009	0.002
r , se_y	0.967	0.004
F, df	119	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	µL of microsomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.045	0.023	-0.001	200	1	1	0.000	0.000	-0.007
10	0.045	0.022	-0.001	200	1	1	0.000		
10					1	1			
100	0.282	0.260	0.023	200	1	1	0.000	0.000	0.114
100	0.282	0.259	0.023	200	1	1	0.000		
100									
	0.091	0.068	0.003	200	218	70018	0.005	0.006	5.802
	0.097	0.075	0.004	200	218	70018	0.006		
	0.094	0.071	0.004	200	218	70018	0.006		

Test

Assay Date 8/22/2005 Chemical ID RC 7 tested 8

ID JG Replicate # Rep 2 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.514	0.313	0.209	0.119	0.056	0.039	0.022	25	100	
	0.528	0.319	0.210	0.122	0.061	0.036	0.024			
	0.522	0.308	0.221	0.120	0.055	0.035	0.021			

Samples:	10	100	microsomes
	0.045	0.282	0.091
	0.045	0.282	0.097
			0.094

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.521	0.499	0.0392
0.125	100	200	0.00013	200	0.0250	0.313	0.291	0.0221
0.05	200	1000	0.00005	200	0.0100	0.214	0.191	0.0139
0.025	100	1000	0.00003	200	0.0050	0.121	0.098	0.0063
0.01	40	1000	0.00001	200	0.0020	0.058	0.035	0.0012
0.005	20	1000	0.00001	200	0.0010	0.036	0.014	-0.0006

Blank 0.022

r^2= 0.925
m= 0.082
b= -0.002

Variables	Regression results	
m, b	0.082	-0.002
se_m, se_b	0.013	0.002
r , se_y	0.925	0.003
F, df	37	3
ss_{regr}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µL microsomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.045	0.023	0.000	200	1	1	0.000	0.000	0.001
10	0.045	0.022	0.000	200	1	1	0.000		
10					1	1			
100	0.282	0.260	0.020	200	1	1	0.000	0.000	0.098
100	0.282	0.259	0.020	200	1	1	0.000		
100									
	0.091	0.068	0.004	200	218	70018	0.006	0.007	6.634
	0.097	0.075	0.004	200	218	70018	0.007		
	0.094	0.071	0.004	200	218	70018	0.007		

Assay Date	8/22/2005	Test Chemical ID	RC 7	tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	IVT

								Protein stock (mg/10 mL)	Protein stock ID
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk		
Samples:									

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/22/2005	Chemical ID	RC 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.1835	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.1835	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.634
Protein Concentration (dilution added to assay, mg/mL):	0.020655

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	1.00E-08
5	3.00E-09
6	1.00E-09
7	3.00E-10
8	1.00E-10

Assay Date	8/22/2005	Test Chemical ID	RC 7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4174.22	8348.44	8386.54	16773.08	0.1	153924	10.90	16639	0.0221	1	0.010	15	0.0713
			0.5	2	4212.32	8424.64			0.1					1		15	
	2	2	0.5	1	4048.66	8097.32	8178.71	16357.42	0.1	153924	10.63	16223	0.0215	1	0.010	15	0.0695
			0.5	2	4130.05	8260.1			0.1					1		15	
	3	2	0.5	1	3483.67	6967.34	7014.12	14028.24	0.1	153924	9.11	13894	0.0184	1	0.010	15	0.0595
			0.5	2	3530.45	7060.9			0.1					1		15	
	4	2	0.5	1	3758.9	7517.8	7506	15012	0.1	153924	9.75	14878	0.0197	1	0.010	15	0.0637
			0.5	2	3747.1	7494.2			0.1					1		15	
Background control	1	2	0.5	1	33.01	66.02	64.8	129.6	0.1	153924	0.08	-5	0.0000	1	0.010	15	0.0000
			0.5	2	31.79	63.58			0.1					1		15	
	2	2	0.5	1	32.36	64.72	66.49	132.98	0.1	153924	0.09	-1	0.0000	1	0.010	15	0.0000
			0.5	2	34.13	68.26			0.1					1		15	
	3	2	0.5	1	31.91	63.82	71.91	143.82	0.1	153924	0.09	10	0.0000	1	0.010	15	0.0000
			0.5	2	40	80			0.1					1		15	
	4	2	0.5	1	35.43	70.86	65.32	130.64	0.1	153924	0.08	-4	0.0000	1	0.010	15	0.0000
			0.5	2	29.89	59.78			0.1					1		15	
Positive control	1	2	0.5	1	1717.11	3434.22	3434.87	6869.74	0.1	153924	4.46	6735	0.0089	1	0.010	15	0.0288
			0.5	2	1717.76	3435.52			0.1					1		15	
	2	2	0.5	1	1741.46	3482.92	3492.68	6985.36	0.1	153924	4.54	6851	0.0091	1	0.010	15	0.0293
			0.5	2	1751.22	3502.44			0.1					1		15	
	3	2	0.5	1	1524.61	3049.22	3055.72	6111.44	0.1	153924	3.97	5977	0.0079	1	0.010	15	0.0256
			0.5	2	1531.11	3062.22			0.1					1		15	
	4	2	0.5	1	1574.54	3149.08	3109.14	6218.28	0.1	153924	4.04	6084	0.0081	1	0.010	15	0.0261
			0.5	2	1534.6	3069.2			0.1					1		15	
Negative Control	1	2	0.5	1	4062.86	8125.72	8158.54	16317.08	0.1	153924	10.60	16183	0.0215	1	0.010	15	0.0693
			0.5	2	4095.68	8191.36			0.1					1		15	
	2	2	0.5	1	3959.37	7918.74	7931.93	15863.86	0.1	153924	10.31	15730	0.0209	1	0.010	15	0.0674
			0.5	2	3972.56	7945.12			0.1					1		15	
	3	2	0.5	1	3478.66	6957.32	6966.52	13933.04	0.1	153924	9.05	13799	0.0183	1	0.010	15	0.0591
			0.5	2	3487.86	6975.72			0.1					1		15	
	4	2	0.5	1	3360.19	6720.38	6710.79	13421.58	0.1	153924	8.72	13287	0.0176	1	0.010	15	0.0569
			0.5	2	3350.6	6701.2			0.1					1		15	
RC 7	1-1	2	0.5	1	86.82	173.64	192.24	384.48	0.1	153924	0.25	250	0.0003	1	0.010	15	0.0011
			0.5	2	105.42	210.84			0.1					1		15	
	1-2	2	0.5	1	63.78	127.56	131.49	262.98	0.1	153924	0.17	129	0.0002	1	0.010	15	0.0006
			0.5	2	67.71	135.42			0.1					1		15	
	1-3	2	0.5	1	53.41	106.82	118.66	237.32	0.1	153924	0.15	103	0.0001	1	0.010	15	0.0004
			0.5	2	65.25	130.5			0.1					1		15	
	2-1	2	0.5	1	65.03	130.06	127.74	255.48	0.1	153924	0.17	121	0.0002	1	0.010	15	0.0005
			0.5	2	62.71	125.42			0.1					1		15	
	2-2	2	0.5	1	26.19	52.38	59.28	118.56	0.1	153924	0.08	-16	0.0000	1	0.010	15	-0.0001
			0.5	2	33.09	66.18			0.1					1		15	
	2-3	2	0.5	1	49.75	99.5	87.74	175.48	0.1	153924	0.11	41	0.0001	1	0.010	15	0.0002
			0.5	2	37.99	75.98			0.1					1		15	
	3-1	2	0.5	1	104.66	209.32	206.36	412.72	0.1	153924	0.27	278	0.0004	1	0.010	15	0.0012
			0.5	2	101.7	203.4			0.1					1		15	
	3-2	2	0.5	1	92.53	185.06	190.53	381.06	0.1	153924	0.25	247	0.0003	1	0.010	15	0.0011
			0.5	2	97.7	195.4			0.1					1		15	
	3-3	2	0.5	1	90.02	180.04	183.09	366.18	0.1	153924	0.24	232	0.0003	1	0.010	15	0.0010
			0.5	2	93.07	186.14			0.1					1		15	
	4-1	2	0.5	1	656.89	1313.78	1272.18	2544.36	0.1	153924	1.65	2410	0.0032	1	0.010	15	0.0103
			0.5	2	615.29	1230.58			0.1					1		15	
	4-2	2	0.5	1	611.21	1222.42	1228.04	2456.08	0.1	153924	1.60	2322	0.0031	1	0.010	15	0.0099
			0.5	2	616.83	1233.66			0.1					1		15	
	4-3	2	0.5	1	622.61	1245.22	1229.26	2458.52	0.1	153924	1.60	2324	0.0031	1	0.010	15	0.0100
			0.5	2	606.65	1213.3			0.1					1		15	
	5-1	2	0.5	1	1451.74	2903.48	2930.36	5860.72	0.1	153924	3.81	5726	0.0076	1	0.010	15	0.0245
			0.5	2	1478.62	2957.24			0.1					1		15	
	5-2	2	0.5	1	1466.24	2932.48	2917.35	5834.7	0.1	153924	3.79	5700	0.0076	1	0.010	15	0.0244
			0.5	2	1451.11	2902.22			0.1					1		15	
	5-3	2	0.5	1	1486.27	2972.54	2997.86	5995.72	0.1	153924	3.90	5861	0.0078	1	0.010	15	0.0251
			0.5	2	1511.59	3023.18			0.1					1		15	
	6-1	2	0.5	1	2654.04	5308.08	5186.06	10372.12	0.1	153924	6.74	10238	0.0136	1	0.010	15	0.0438
			0.5	2	2532.02	5064.04			0.1					1		15	
	6-2	2	0.5	1	2579.67	5159.34	5127.7	10255.4	0.1	153924	6.66	10121	0.0134	1	0.010	15	0.0433
			0.5	2	2548.03	5096.06			0.1					1		15	
	6-3	2	0.5	1	2592.79	5185.58	5231.61	10463.22	0.1	153924	6.80	10329	0.0137	1	0.010	15	0.0442
			0.5	2	2638.82	5277.64			0.1					1		15	
	7-1	2	0.5	1	3645.95	7291.9	7281.24	14562.48	0.1	153924	9.46	14428	0.0191	1	0.010	15	0.0618
			0.5	2	3635.29	7270.58			0.1					1		15	
	7-2	2	0.5	1	3557.47	7114.94	7183.04	14366.08	0.1	153924	9.33	14232	0.0189	1	0.010	15	0.0609
			0.5	2	3625.57	7251.14			0.1					1		15	
	7-3	2	0.5	1	3582.41	7164.82	7152.53	14305.06	0.1	153924	9.29	14171	0.0188	1	0.010	15	0.0607
			0.5	2	3570.12	7140.24			0.1					1		15	
	8-1	2	0.5	1	3814.22	7628.44	7718.99	15437.98	0.1	153924	10.03	15304	0.0203	1	0.010	15	0.0655
			0.5	2	3904.77	7809.54			0.1					1		15	
	8-2	2	0.5	1	3784.07	7568.14	7656.44	15312.88	0.1	153924	9.95	15179	0.0201	1	0.010	15	0.0650
			0.5	2	3862.37	7724.74			0.1					1		15	
	8-3	2	0.5	1	3836.71	7673.42	7680.91	15361.82	0.1	153924	9.98	15228	0.0202	1	0.010	15	0.0652
			0.5	2	3844.2	7688.4			0.1					1		15	

Assay Date	8/22/2005	ID	RC 7	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0704	0.0013
Full activity	End	0.0616	0.0030
Full activity	Overall	0.0660	0.0054
Background	Beginning	0.0000	1.02348E-05
Background	End	0.0000	3.99095E-05
Background	Overall	0.0000	2.79556E-05
Positive	Beginning	0.0291	0.0004
Positive	End	0.0258	0.0003
Positive	Overall	0.0275	0.0019
Negative	Beginning	0.0683	0.0014
Negative	End	0.0580	0.0015
Negative	Overall	0.0632	0.0061

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC 7	1	1	1.00E-05	-5.00	0.0011
RC 7	1	2	1.00E-05	-5.00	0.0006
RC 7	1	3	1.00E-05	-5.00	0.0004
RC 7	2	1	1.00E-06	-6.00	0.0005
RC 7	2	2	1.00E-06	-6.00	-0.0001
RC 7	2	3	1.00E-06	-6.00	0.0002
RC 7	3	1	1.00E-07	-7.00	0.0012
RC 7	3	2	1.00E-07	-7.00	0.0011
RC 7	3	3	1.00E-07	-7.00	0.0010
RC 7	4	1	1.00E-08	-8.00	0.0103
RC 7	4	2	1.00E-08	-8.00	0.0099
RC 7	4	3	1.00E-08	-8.00	0.0100
RC 7	5	1	3.00E-09	-8.52	0.0245
RC 7	5	2	3.00E-09	-8.52	0.0244
RC 7	5	3	3.00E-09	-8.52	0.0251
RC 7	6	1	1.00E-09	-9.00	0.0438
RC 7	6	2	1.00E-09	-9.00	0.0433
RC 7	6	3	1.00E-09	-9.00	0.0442
RC 7	7	1	3.00E-10	-9.52	0.0618
RC 7	7	2	3.00E-10	-9.52	0.0609
RC 7	7	3	3.00E-10	-9.52	0.0607
RC 7	8	1	1.00E-10	-10.00	0.0655
RC 7	8	2	1.00E-10	-10.00	0.0650
RC 7	8	3	1.00E-10	-10.00	0.0652

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-5.00	1.62	0.84	0.67
2	-6.00	0.79	-0.10	0.27
3	-7.00	1.81	1.60	1.51
4	-8.00	15.64	15.07	15.08
5	-8.52	37.16	37.00	38.04
6	-9.00	66.44	65.69	67.03
7	-9.52	93.64	92.36	91.97
8	-10.00	99.32	98.51	98.83

Aromatase Assay Spreadsheet

Assay Date	8/30/2005	Test Chemical ID	RC7	# Concentrations tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	32135	1598756	
2	0.0204	32624.61	1599246	
3	0.0201	32156.54	1599828	
4	0.0202	33028.02	1635050	
5	0.0201	33280.02	1655722	
			Average DPM/g soln	1617721
			SD	26294
			CV	1.63
			µCi/g soln	0.729

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

ASDN solution	mg ASDN added	(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	8.2141 g
Mass of dilution B used in substrate prep	4.6134 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.578493 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00825 µg/g soln.
µg/g soln.

a. µCi/g soln	0.729
b. Specific activity of [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.

= 0.578493 + 0.00825
= 0.586742 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.242 µCi/µg ASDN

789640 dpm/nmol

Assay Date 8/30/2005 Test Chemical ID RC7 tested 8

ID JG Replicate # Rep 3 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.575	0.347	0.173	0.106	0.056	0.045	0.039	25	100
	0.583	0.341	0.162	0.103	0.074	0.047	0.045		
	0.567	0.341	0.170	0.110	0.060	0.045	0.039		

Protein stock ID

Samples:	10	100	microsomes
	0.064	0.301	0.105
	0.067	0.302	0.101
			0.107

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.575	0.534	0.0486
0.125	100	200	0.00013	200	0.0250	0.343	0.302	0.0271
0.05	200	1000	0.00005	200	0.0100	0.168	0.128	0.0110
0.025	100	1000	0.00003	200	0.0050	0.106	0.066	0.0053
0.01	40	1000	0.00001	200	0.0020	0.063	0.023	0.0013
0.005	20	1000	0.00001	200	0.0010	0.046	0.005	-0.0003

Blank 0.041

Variables	Regression results	
m, b	0.092	-0.001
se_m, se_b	0.003	0.001
r , se_y	0.995	0.002
F, df	731	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.995
m= 0.092
b= -0.001

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µL microsome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.064	0.023	0.001	200	1	1	0.000	0.000	0.008
10	0.067	0.026	0.002	200	1	1	0.000		
10					1	1			
100	0.301	0.260	0.023	200	1	1	0.000	0.000	0.117
100	0.302	0.261	0.023	200	1	1	0.000		
100									
	0.105	0.064	0.005	200	218	70018	0.008	0.008	8.202
	0.101	0.060	0.005	200	218	70018	0.008		
	0.107	0.066	0.005	200	218	70018	0.009		

Test

Assay Date 8/30/2005 Chemical ID RC7 tested 8

ID JG Replicate # Rep 3 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.575	0.347	0.173	0.106	0.056	0.045	0.039	25	100	
	0.583	0.341	0.162	0.103	0.074	0.047	0.045			
	0.567	0.341	0.170	0.110	0.060	0.045	0.039			

Samples:	10	100	microsomes
	0.064	0.301	0.105
	0.067	0.302	0.101
			0.107

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.575	0.534	0.0437
0.125	100	200	0.00013	200	0.0250	0.343	0.302	0.0247
0.05	200	1000	0.00005	200	0.0100	0.168	0.128	0.0105
0.025	100	1000	0.00003	200	0.0050	0.106	0.066	0.0054
0.01	40	1000	0.00001	200	0.0020	0.063	0.023	0.0019
0.005	20	1000	0.00001	200	0.0010	0.046	0.005	0.0005

Blank 0.041

r^2= 0.998
m= 0.082
b= 0.000

Variables	Regression results	
m, b	0.082	0.000
se_m, se_b	0.002	0.000
r , se_y	0.998	0.001
F, df	1472	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. microsome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.064	0.023	0.002	200	1	1	0.000	0.000	0.010
10	0.067	0.026	0.002	200	1	1	0.000		
10					1	1			
100	0.301	0.260	0.021	200	1	1	0.000	0.000	0.107
100	0.302	0.261	0.021	200	1	1	0.000		
100									
	0.105	0.064	0.005	200	218	70018	0.009	0.008	8.444
	0.101	0.060	0.005	200	218	70018	0.008		
	0.107	0.066	0.005	200	218	70018	0.009		

Assay Date	8/30/2005	Test Chemical ID	RC7	tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	IVT

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

A_{raw}	$A_{adj.}$	mg protein measured	µ µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/30/2005	Chemical ID	RC7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 3

Microsome Dilution Details

Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.183486	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.183486	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.444
Protein Concentration (dilution added to assay, mg/mL):	0.02629

Test Chemical Concentrations

Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	1.00E-08
5	3.00E-09
6	1.00E-09
7	3.00E-10
8	1.00E-10

Assay Date	8/30/2005	Test Chemical ID	RC7	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3574.7	7149.4	7135.45	14270.9	0.1	161772	8.82	14115	0.0179	1	0.013	15	0.0453
			0.5	2	3560.75	7121.5			0.1					1		15	
	2	2	0.5	1	3921.21	7842.42	7824.47	15648.94	0.1	161772	9.67	15493	0.0196	1	0.013	15	0.0498
			0.5	2	3903.26	7806.52			0.1					1		15	
	3	2	0.5	1	3578.35	7156.66	7247.14	14494.28	0.1	161772	8.96	14339	0.0182	1	0.013	15	0.0460
			0.5	2	3668.81	7337.62			0.1					1		15	
	4	2	0.5	1	3633.26	7266.52	7309.5	14619	0.1	161772	9.04	14463	0.0183	1	0.013	15	0.0464
			0.5	2	3676.24	7352.48			0.1					1		15	
Background control	1	2	0.5	1	42.46	84.92	79.21	158.42	0.1	161772	0.10	3	0.0000	1	0.013	15	0.0000
			0.5	2	36.75	73.5			0.1					1		15	
	2	2	0.5	1	32.99	65.98	63.04	126.08	0.1	161772	0.08	-30	0.0000	1	0.013	15	-0.0001
			0.5	2	30.05	60.1			0.1					1		15	
	3	2	0.5	1	32.73	65.46	82.05	164.1	0.1	161772	0.10	8	0.0000	1	0.013	15	0.0000
			0.5	2	49.32	98.64			0.1					1		15	
	4	2	0.5	1	44.39	88.78	86.98	173.96	0.1	161772	0.11	18	0.0000	1	0.013	15	0.0001
			0.5	2	42.59	85.18			0.1					1		15	
Positive control	1	2	0.5	1	1763.36	3526.72	3471.27	6942.54	0.1	161772	4.29	6787	0.0086	1	0.013	15	0.0218
			0.5	2	1707.91	3415.82			0.1					1		15	
	2	2	0.5	1	1730.63	3461.26	3430.65	6861.3	0.1	161772	4.24	6706	0.0085	1	0.013	15	0.0215
			0.5	2	1700.02	3400.04			0.1					1		15	
	3	2	0.5	1	1572.92	3145.84	3109.7	6219.4	0.1	161772	3.84	6084	0.0077	1	0.013	15	0.0195
			0.5	2	1536.78	3073.56			0.1					1		15	
	4	2	0.5	1	1597.79	3195.56	3181.25	6362.5	0.1	161772	3.93	6207	0.0079	1	0.013	15	0.0199
			0.5	2	1583.47	3166.94			0.1					1		15	
Negative Control	1	2	0.5	1	3850.73	7701.46	7716.67	15433.34	0.1	161772	9.54	15278	0.0193	1	0.013	15	0.0491
			0.5	2	3865.94	7731.88			0.1					1		15	
	2	2	0.5	1	3870.25	7740.5	7678.15	15356.3	0.1	161772	9.49	15201	0.0193	1	0.013	15	0.0488
			0.5	2	3807.9	7615.8			0.1					1		15	
	3	2	0.5	1	3431.12	6862.24	6826.43	13652.86	0.1	161772	8.44	13497	0.0171	1	0.013	15	0.0433
			0.5	2	3395.31	6790.62			0.1					1		15	
	4	2	0.5	1	3161.13	6322.26	6209.47	12418.94	0.1	161772	7.68	12263	0.0155	1	0.013	15	0.0394
			0.5	2	3048.34	6096.68			0.1					1		15	
RC7	1-1	2	0.5	1	44.39	88.78	95.39	190.78	0.1	161772	0.12	35	0.0000	1	0.013	15	0.0001
			0.5	2	51	102			0.1					1		15	
	1-2	2	0.5	1	40.74	81.48	80.05	160.1	0.1	161772	0.10	4	0.0000	1	0.013	15	0.0000
			0.5	2	39.31	78.62			0.1					1		15	
	1-3	2	0.5	1	43.91	87.82	100.92	201.84	0.1	161772	0.12	46	0.0001	1	0.013	15	0.0001
			0.5	2	57.01	114.02			0.1					1		15	
	2-1	2	0.5	1	56.07	112.14	107.77	215.54	0.1	161772	0.13	60	0.0001	1	0.013	15	0.0002
			0.5	2	51.7	103.4			0.1					1		15	
	2-2	2	0.5	1	46.58	93.16	79.33	158.66	0.1	161772	0.10	3	0.0000	1	0.013	15	0.0000
			0.5	2	32.75	65.5			0.1					1		15	
	2-3	2	0.5	1	55.65	111.3	91.7	183.4	0.1	161772	0.11	28	0.0000	1	0.013	15	0.0001
			0.5	2	36.05	72.1			0.1					1		15	
	3-1	2	0.5	1	105.64	211.28	206.49	412.98	0.1	161772	0.26	257	0.0003	1	0.013	15	0.0008
			0.5	2	100.85	201.7			0.1					1		15	
	3-2	2	0.5	1	98.19	196.38	194.32	388.64	0.1	161772	0.24	233	0.0003	1	0.013	15	0.0007
			0.5	2	96.13	192.26			0.1					1		15	
	3-3	2	0.5	1	84.71	169.42	176.76	353.52	0.1	161772	0.22	198	0.0003	1	0.013	15	0.0006
			0.5	2	92.05	184.1			0.1					1		15	
	4-1	2	0.5	1	507.24	1014.48	1016.6	2033.2	0.1	161772	1.26	1878	0.0024	1	0.013	15	0.0060
			0.5	2	509.36	1018.72			0.1					1		15	
	4-2	2	0.5	1	547.77	1095.54	1073.6	2147.2	0.1	161772	1.33	1992	0.0025	1	0.013	15	0.0064
			0.5	2	525.83	1051.66			0.1					1		15	
	4-3	2	0.5	1	550.97	1101.94	1104	2208	0.1	161772	1.36	2052	0.0026	1	0.013	15	0.0066
			0.5	2	553.03	1106.06			0.1					1		15	
	5-1	2	0.5	1	1231.55	2463.1	2446.95	4893.9	0.1	161772	3.03	4738	0.0060	1	0.013	15	0.0152
			0.5	2	1215.4	2430.8			0.1					1		15	
	5-2	2	0.5	1	1278.94	2557.88	2543.04	5086.08	0.1	161772	3.14	4930	0.0062	1	0.013	15	0.0158
			0.5	2	1264.1	2528.2			0.1					1		15	
	5-3	2	0.5	1	1241.98	2483.96	2524.45	5048.9	0.1	161772	3.12	4893	0.0062	1	0.013	15	0.0157
			0.5	2	1282.47	2564.94			0.1					1		15	
	6-1	2	0.5	1	2382.75	4765.5	4831.7	9663.4	0.1	161772	5.97	9508	0.0120	1	0.013	15	0.0305
			0.5	2	2448.95	4897.9			0.1					1		15	
	6-2	2	0.5	1	2332.22	4664.44	4619.16	9238.32	0.1	161772	5.71	9083	0.0115	1	0.013	15	0.0292
			0.5	2	2286.94	4573.88			0.1					1		15	
	6-3	2	0.5	1	2256.37	4512.74	4593.52	9187.04	0.1	161772	5.68	9031	0.0114	1	0.013	15	0.0290
			0.5	2	2337.15	4674.3			0.1					1		15	
	7-1	2	0.5	1	3204.32	6408.64	6409.56	12819.12	0.1	161772	7.92	12663	0.0160	1	0.013	15	0.0407
			0.5	2	3205.24	6410.48			0.1					1		15	
	7-2	2	0.5	1	3234.72	6469.44	6458.76	12917.52	0.1	161772	7.99	12762	0.0162	1	0.013	15	0.0410
			0.5	2	3224.04	6448.08			0.1					1		15	
	7-3	2	0.5	1	3202.07	6404.14	6423.5	12847	0.1	161772	7.94	12691	0.0161	1	0.013	15	0.0408
			0.5	2	3221.43	6442.86			0.1					1		15	
	8-1	2	0.5	1	3263.81	6527.62	6683.18	13366.36	0.1	161772	8.26	13211	0.0167	1	0.013	15	0.0424
			0.5	2	3419.37	6838.74			0.1					1		15	
	8-2	2	0.5	1	3435.03	6870.06	6847.97	13695.94	0.1	161772	8.47	13540	0.0171	1	0.013	15	0.0435
			0.5	2	3412.94	6825.88			0.1					1		15	
	8-3	2	0.5	1	3449.34	6898.68	6928.46	13856.92	0.1	161772	8.57	13701	0.0174	1	0.013	15	0.0440
			0.5	2	3479.12	6958.24			0.1					1		15	

Assay Date	8/30/2005	ID	RC7	# Concentrations tested	Microsome type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0475	0.0031
Full activity	End	0.0462	0.0003
Full activity	Overall	0.0469	0.0020
Background	Beginning	0.0000	7.34361E-05
Background	End	0.0000	2.23896E-05
Background	Overall	0.0000	6.65584E-05
Positive	Beginning	0.0217	0.0002
Positive	End	0.0197	0.0003
Positive	Overall	0.0207	0.0012
Negative	Beginning	0.0489	0.0002
Negative	End	0.0414	0.0028
Negative	Overall	0.0452	0.0047

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC7	1	1	1.00E-05	-5.00	0.0001
RC7	1	2	1.00E-05	-5.00	0.0000
RC7	1	3	1.00E-05	-5.00	0.0001
RC7	2	1	1.00E-06	-6.00	0.0002
RC7	2	2	1.00E-06	-6.00	0.0000
RC7	2	3	1.00E-06	-6.00	0.0001
RC7	3	1	1.00E-07	-7.00	0.0008
RC7	3	2	1.00E-07	-7.00	0.0007
RC7	3	3	1.00E-07	-7.00	0.0006
RC7	4	1	1.00E-08	-8.00	0.0060
RC7	4	2	1.00E-08	-8.00	0.0064
RC7	4	3	1.00E-08	-8.00	0.0066
RC7	5	1	3.00E-09	-8.52	0.0152
RC7	5	2	3.00E-09	-8.52	0.0158
RC7	5	3	3.00E-09	-8.52	0.0157
RC7	6	1	1.00E-09	-9.00	0.0305
RC7	6	2	1.00E-09	-9.00	0.0292
RC7	6	3	1.00E-09	-9.00	0.0290
RC7	7	1	3.00E-10	-9.52	0.0407
RC7	7	2	3.00E-10	-9.52	0.0410
RC7	7	3	3.00E-10	-9.52	0.0408
RC7	8	1	1.00E-10	-10.00	0.0424
RC7	8	2	1.00E-10	-10.00	0.0435
RC7	8	3	1.00E-10	-10.00	0.0440

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-5.00	0.24	0.03	0.32
2	-6.00	0.41	0.02	0.19
3	-7.00	1.76	1.60	1.36
4	-8.00	12.86	13.64	14.05
5	-8.52	32.45	33.76	33.51
6	-9.00	65.11	62.20	61.85
7	-9.52	86.72	87.39	86.91
8	-10.00	90.47	92.73	93.83

Aromatase Assay Spreadsheet

Assay Date	8/15/2005	Test Chemical ID	RC 8	# Concentrations tested	8	
ID	JG	Replicate #	Rep 1	Microsome type	Placental	Microsome ID IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	31483.41	1582081	
2	0.0200	31966.14	1598307	
3	0.0201	31992.18	1591651	
4	0.0202	31979.68	1583152	
5	0.0201	32440.45	1613953	
			Average DPM/g soln	1593829
			SD	13055
			CV	0.82
			µCi/g soln	0.718

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

ASDN solution	mg ASDN added	(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	8.2352 g
Mass of dilution B used in substrate prep	4.6261 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.567365 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 [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.

= 0.567365 + 0.00813
= 0.575492 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.248 µCi/µg ASDN

793187 dpm/nmol

Assay Date 8/15/2005 Test Chemical ID RC 8 tested 8

ID JG Replicate # Rep 1 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.522	0.355	0.189	0.116	0.055	0.032	0.025	25		
	0.534	0.320	0.192	0.106	0.052	0.033	0.027			
	0.557	0.314	0.192	0.108	0.051	0.034	0.017			
Samples:	10	100	microsomes							
	0.040	0.273	0.093							
	0.042	0.278	0.090							
			0.094							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.537	0.514	0.0474
0.125	100	200	0.00013	200	0.0250	0.330	0.307	0.0272
0.05	200	1000	0.00005	200	0.0100	0.191	0.168	0.0137
0.025	100	1000	0.00003	200	0.0050	0.110	0.088	0.0059
0.01	40	1000	0.00001	200	0.0020	0.053	0.030	0.0003
0.005	20	1000	0.00001	200	0.0010	0.033	0.010	-0.0016

Blank 0.023

r^2= 0.980
m= 0.097
b= -0.003

Variables	Regression results	
m, b	0.097	-0.003
se_m, se_b	0.007	0.002
r , se_y	0.980	0.003
F, df	197	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol. usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.040	0.018	-0.001	200	1	1	0.000	0.000	-0.004
10	0.042	0.020	-0.001	200	1	1	0.000		
10					1	1			
100	0.273	0.250	0.022	200	1	1	0.000	0.000	0.110
100	0.278	0.255	0.022	200	1	1	0.000		
100									
	0.093	0.071	0.004	200	218	70018	0.007	0.007	6.705
	0.090	0.068	0.004	200	218	70018	0.006		
	0.094	0.071	0.004	200	218	70018	0.007		

Assay Date 8/15/2005 Test Chemical ID RC 8 tested 8

ID JG Replicate # Rep 1 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.522	0.355	0.189	0.116	0.055	0.032	0.025	25		
	0.534	0.320	0.192	0.106	0.052	0.033	0.027			
	0.557	0.314	0.192	0.108	0.051	0.034	0.017			
Samples:	10	100	microsomes							
	0.040	0.273	0.093							
	0.042	0.278	0.090							
			0.094							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.537	0.514	0.0401
0.125	100	200	0.00013	200	0.0250	0.330	0.307	0.0235
0.05	200	1000	0.00005	200	0.0100	0.191	0.168	0.0124
0.025	100	1000	0.00003	200	0.0050	0.110	0.088	0.0060
0.01	40	1000	0.00001	200	0.0020	0.053	0.030	0.0013
0.005	20	1000	0.00001	200	0.0010	0.033	0.010	-0.0002

Blank 0.023

r^2= 0.972
m= 0.080
b= -0.001

Variables	Regression results	
m, b	0.080	-0.001
se_m, se_b	0.008	0.001
r , se_y	0.972	0.002
F, df	104	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. microsomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.040	0.018	0.000	200	1	1	0.000	0.000	0.002
10	0.042	0.020	0.001	200	1	1	0.000		
10					1	1			
100	0.273	0.250	0.019	200	1	1	0.000	0.000	0.096
100	0.278	0.255	0.019	200	1	1	0.000		
100									
	0.093	0.071	0.005	200	218	70018	0.007	0.007	7.288
	0.090	0.068	0.004	200	218	70018	0.007		
	0.094	0.071	0.005	200	218	70018	0.007		

Assay Date	8/15/2005	Test Chemical ID	RC 8	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	IVT

Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/15/2005	Chemical ID	RC 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.1835	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.1835	total dilution factor

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Protein Concentration (stock microsomes, mg/mL):	7.288
Protein Concentration (dilution added to assay, mg/mL):	0.022691

Assay Date	8/15/2005	Test Chemical ID	RC 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	5005.92	10011.84	10115.1	20230.2	0.1	159383	12.69	20072	0.0253	1	0.011	15	0.0743
			0.5	2	5109.18	10218.36			0.1					1		15	
	2	2	0.5	1	4929.32	9859.64	9773.03	19546.06	0.1	159383	12.26	19388	0.0244	1	0.011	15	0.0718
			0.5	2	4843.71	9687.42			0.1					1		15	
	3	2	0.5	1	4887.8	9775.6	9757.17	19514.34	0.1	159383	12.24	19356	0.0244	1	0.011	15	0.0717
			0.5	2	4869.37	9738.74			0.1					1		15	
	4	2	0.5	1	4818.53	9637.06	9816.4	19632.8	0.1	159383	12.32	19475	0.0246	1	0.011	15	0.0721
			0.5	2	4997.87	9995.74			0.1					1		15	
Background control	1	2	0.5	1	29.46	58.92	60.54	121.08	0.1	159383	0.08	-37	0.0000	1	0.011	15	-0.0001
			0.5	2	31.08	62.16			0.1					1		15	
	2	2	0.5	1	26.82	53.64	61.97	123.94	0.1	159383	0.08	-34	0.0000	1	0.011	15	-0.0001
			0.5	2	35.15	70.3			0.1					1		15	
	3	2	0.5	1	38.59	77.18	77.74	155.48	0.1	159383	0.10	-3	0.0000	1	0.011	15	0.0000
			0.5	2	39.15	78.3			0.1					1		15	
	4	2	0.5	1	58.34	116.68	116.21	232.42	0.1	159383	0.15	74	0.0001	1	0.011	15	0.0003
			0.5	2	57.87	115.74			0.1					1		15	
Positive control	1	2	0.5	1	2127.14	4254.28	4173.92	8347.84	0.1	159383	5.24	8190	0.0103	1	0.011	15	0.0303
			0.5	2	2046.78	4093.56			0.1					1		15	
	2	2	0.5	1	1987.01	3974.02	3940.26	7880.52	0.1	159383	4.94	7722	0.0097	1	0.011	15	0.0286
			0.5	2	1953.25	3906.5			0.1					1		15	
	3	2	0.5	1	1914.73	3829.46	3763.88	7527.76	0.1	159383	4.72	7370	0.0093	1	0.011	15	0.0273
			0.5	2	1849.15	3698.3			0.1					1		15	
	4	2	0.5	1	1771.36	3542.72	3588.55	7177.1	0.1	159383	4.50	7019	0.0088	1	0.011	15	0.0260
			0.5	2	1817.19	3634.38			0.1					1		15	
Negative Control	1	2	0.5	1	5199.55	10399.1	10294.73	20589.46	0.1	159383	12.92	20431	0.0258	1	0.011	15	0.0757
			0.5	2	5095.18	10190.36			0.1					1		15	
	2	2	0.5	1	5230.31	10460.62	10446.99	20893.98	0.1	159383	13.11	20736	0.0261	1	0.011	15	0.0768
			0.5	2	5216.68	10433.36			0.1					1		15	
	3	2	0.5	1	4827.6	9655.2	9541.3	19082.6	0.1	159383	11.97	18924	0.0239	1	0.011	15	0.0701
			0.5	2	4713.7	9427.4			0.1					1		15	
	4	2	0.5	1	4722.72	9445.44	9462.77	18925.54	0.1	159383	11.87	18767	0.0237	1	0.011	15	0.0695
			0.5	2	4740.05	9480.1			0.1					1		15	
RC 8	1-1	2	0.5	1	855.25	1710.5	1708.52	3417.04	0.1	159383	2.14	3259	0.0041	1	0.011	15	0.0121
			0.5	2	853.27	1706.54			0.1					1		15	
	1-2	2	0.5	1	498.84	997.68	993.93	1987.86	0.1	159383	1.25	1830	0.0023	1	0.011	15	0.0068
			0.5	2	495.09	990.18			0.1					1		15	
	1-3	2	0.5	1	485.25	970.5	968.03	1936.06	0.1	159383	1.21	1778	0.0022	1	0.011	15	0.0066
			0.5	2	482.78	965.56			0.1					1		15	
	2-1	2	0.5	1	1191.62	2383.24	2393.33	4786.66	0.1	159383	3.00	4628	0.0058	1	0.011	15	0.0171
			0.5	2	1201.71	2403.42			0.1					1		15	
	2-2	2	0.5	1	1212	2424	2413.96	4827.92	0.1	159383	3.03	4670	0.0059	1	0.011	15	0.0173
			0.5	2	1201.96	2403.92			0.1					1		15	
	2-3	2	0.5	1	1162.84	2325.68	2300.18	4600.36	0.1	159383	2.89	4442	0.0056	1	0.011	15	0.0165
			0.5	2	1137.34	2274.68			0.1					1		15	
	3-1	2	0.5	1	1810	3620	3720.38	7440.76	0.1	159383	4.67	7283	0.0092	1	0.011	15	0.0270
			0.5	2	1910.38	3820.76			0.1					1		15	
	3-2	2	0.5	1	1922.8	3845.6	3873.93	7747.86	0.1	159383	4.86	7590	0.0096	1	0.011	15	0.0281
			0.5	2	1951.13	3902.26			0.1					1		15	
	3-3	2	0.5	1	1873.02	3746.04	3764.24	7528.48	0.1	159383	4.72	7370	0.0093	1	0.011	15	0.0273
			0.5	2	1891.22	3782.44			0.1					1		15	
	4-1	2	0.5	1	3777.23	7554.46	7510.05	15020.1	0.1	159383	9.42	14862	0.0187	1	0.011	15	0.0550
			0.5	2	3732.82	7465.64			0.1					1		15	
	4-2	2	0.5	1	3729.61	7459.22	7418.77	14837.54	0.1	159383	9.31	14679	0.0185	1	0.011	15	0.0544
			0.5	2	3689.16	7378.32			0.1					1		15	
	4-3	2	0.5	1	3799.74	7599.48	7552.89	15105.78	0.1	159383	9.48	14948	0.0188	1	0.011	15	0.0554
			0.5	2	3753.15	7506.3			0.1					1		15	
	5-1	2	0.5	1	4679.17	9358.34	9365.24	18730.48	0.1	159383	11.75	18572	0.0234	1	0.011	15	0.0688
			0.5	2	4686.07	9372.14			0.1					1		15	
	5-2	2	0.5	1	4810.78	9621.56	9673.2	19346.4	0.1	159383	12.14	19188	0.0242	1	0.011	15	0.0711
			0.5	2	4862.42	9724.84			0.1					1		15	
	5-3	2	0.5	1	3305.9	6611.8	6605.81	13211.62	0.1	159383	8.29	13053	0.0165	1	0.011	15	0.0484
			0.5	2	3299.91	6599.82			0.1					1		15	
	6-1	2	0.5	1	5091.56	10183.16	10147.27	20294.54	0.1	159383	12.73	20136	0.0254	1	0.011	15	0.0746
			0.5	2	5055.69	10111.38			0.1					1		15	
	6-2	2	0.5	1	4978.82	9957.64	9921.76	19843.56	0.1	159383	12.45	19685	0.0248	1	0.011	15	0.0729
			0.5	2	4942.96	9885.92			0.1					1		15	
	6-3	2	0.5	1	5053.89	10107.78	10122.32	20244.64	0.1	159383	12.70	20086	0.0253	1	0.011	15	0.0744
			0.5	2	5068.43	10136.86			0.1					1		15	
	7-1	2	0.5	1	5113.62	10227.24	10216	20432	0.1	159383	12.82	20274	0.0256	1	0.011	15	0.0751
			0.5	2	5102.38	10204.76			0.1					1		15	
	7-2	2	0.5	1	4938.1	9876.2	9967.12	19934.24	0.1	159383	12.51	19776	0.0249	1	0.011	15	0.0733
			0.5	2	5029.02	10058.04			0.1					1		15	
	7-3	2	0.5	1	4995.59	9991.18	10023.12	20046.24	0.1	159383	12.58	19888	0.0251	1	0.011	15	0.0737
			0.5	2	5027.53	10055.06			0.1					1		15	
	8-1	2	0.5	1	4810.84	9621.68	9570.67	19141.34	0.1	159383	12.01	18983	0.0239	1	0.011	15	0.0703
			0.5	2	4759.83	9519.66			0.1					1		15	
	8-2	2	0.5	1	4849.14	9698.28	9761.29	19522.58	0.1	159383	12.25	19364	0.0244	1	0.011	15	0.0717
			0.5	2	4912.15	9824.3			0.1					1		15	
	8-3	2	0.5	1	4895.42	9790.84	9901.03	19802.06	0.1	159383	12.42	19644	0.0248	1	0.011	15	0.0728
			0.5	2	5006.61	10011.22			0.1					1		15	

Assay Date	8/15/2005	ID	RC 8	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0731	0.0018
Full activity	End	0.0719	0.0003
Full activity	Overall	0.0725	0.0012
Background	Beginning	-0.0001	7.49082E-06
Background	End	0.0001	0.000201519
Background	Overall	0.0000	0.000192084
Positive	Beginning	0.0295	0.0012
Positive	End	0.0266	0.0009
Positive	Overall	0.0281	0.0019
Negative	Beginning	0.0762	0.0008
Negative	End	0.0698	0.0004
Negative	Overall	0.0730	0.0038

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC 8	1	1	1.00E-04	-4.00	0.0121
RC 8	1	2	1.00E-04	-4.00	0.0068
RC 8	1	3	1.00E-04	-4.00	0.0066
RC 8	2	1	1.00E-05	-5.00	0.0171
RC 8	2	2	1.00E-05	-5.00	0.0173
RC 8	2	3	1.00E-05	-5.00	0.0165
RC 8	3	1	5.00E-06	-5.30	0.0270
RC 8	3	2	5.00E-06	-5.30	0.0281
RC 8	3	3	5.00E-06	-5.30	0.0273
RC 8	4	1	1.00E-06	-6.00	0.0550
RC 8	4	2	1.00E-06	-6.00	0.0544
RC 8	4	3	1.00E-06	-6.00	0.0554
RC 8	5	1	1.00E-07	-7.00	0.0688
RC 8	5	2	1.00E-07	-7.00	0.0711
RC 8	5	3	1.00E-07	-7.00	0.0484
RC 8	6	1	1.00E-08	-8.00	0.0746
RC 8	6	2	1.00E-08	-8.00	0.0729
RC 8	6	3	1.00E-08	-8.00	0.0744
RC 8	7	1	1.00E-09	-9.00	0.0751
RC 8	7	2	1.00E-09	-9.00	0.0733
RC 8	7	3	1.00E-09	-9.00	0.0737
RC 8	8	1	1.00E-10	-10.00	0.0703
RC 8	8	2	1.00E-10	-10.00	0.0717
RC 8	8	3	1.00E-10	-10.00	0.0728

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-4.00	16.65	9.35	9.08
2	-5.00	23.65	23.86	22.70
3	-5.30	37.21	38.78	37.66
4	-6.00	75.93	75.00	76.37
5	-7.00	94.89	98.04	66.69
6	-8.00	102.88	100.58	102.63
7	-9.00	103.58	101.04	101.61
8	-10.00	96.99	98.94	100.36

Aromatase Assay Spreadsheet

Assay Date	8/22/2005	Test Chemical ID	RC 8	# Concentrations tested	8	
ID	JG	Replicate #	Rep 2	Microsome type	Placental	Microsome ID IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0197	30370.02	1541625	
2	0.0200	30637.99	1531900	
3	0.0201	30781.96	1531441	
4	0.0202	31260.43	1547546	
5	0.0204	31490.88	1543671	
			Average DPM/g soln	1539236
			SD	7229
			CV	0.47
			µCi/g soln	0.693

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

ASDN solution	mg ASDN added	(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	8.1635 g
Mass of dilution B used in substrate prep	4.5734 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.577032 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00785 µg/g soln.
µg/g soln.

a. µCi/g soln	0.693
b. Specific activity of [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.

= 0.577032 + 0.00785
= 0.584881 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.185 µCi/µg ASDN

753721 dpm/nmol

Test

Assay Date 8/22/2005 Chemical ID RC 8 tested 8

ID JG Replicate # Rep 2 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.514	0.313	0.209	0.119	0.056	0.039	0.022	25	100	
	0.528	0.319	0.210	0.122	0.061	0.036	0.024			
	0.522	0.308	0.221	0.120	0.055	0.035	0.021			

Samples:	10	100	microsomes
	0.045	0.282	0.091
	0.045	0.282	0.097
			0.094

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.521	0.499	0.0472
0.125	100	200	0.00013	200	0.0250	0.313	0.291	0.0260
0.05	200	1000	0.00005	200	0.0100	0.214	0.191	0.0158
0.025	100	1000	0.00003	200	0.0050	0.121	0.098	0.0063
0.01	40	1000	0.00001	200	0.0020	0.058	0.035	-0.0001
0.005	20	1000	0.00001	200	0.0010	0.036	0.014	-0.0022
			Blank	0.022				

Variables	Regression results	
m, b	0.102	-0.004
se_m, se_b	0.009	0.002
r , se_y	0.967	0.004
F, df	119	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

r^2= 0.967
m= 0.102
b= -0.004

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µL microsomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.045	0.023	-0.001	200	1	1	0.000	0.000	-0.007
10	0.045	0.022	-0.001	200	1	1	0.000		
10					1	1			
100	0.282	0.260	0.023	200	1	1	0.000	0.000	0.114
100	0.282	0.259	0.023	200	1	1	0.000		
100									
	0.091	0.068	0.003	200	218	70018	0.005	0.006	5.802
	0.097	0.075	0.004	200	218	70018	0.006		
	0.094	0.071	0.004	200	218	70018	0.006		

Assay Date 8/22/2005 Test Chemical ID RC 8 tested 8

ID JG Replicate # Rep 2 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.514	0.313	0.209	0.119	0.056	0.039	0.022	25		
	0.528	0.319	0.210	0.122	0.061	0.036	0.024			
	0.522	0.308	0.221	0.120	0.055	0.035	0.021			
Samples:	10	100	microsomes							
	0.045	0.282	0.091							
	0.045	0.282	0.097							
			0.094							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
0.25	200	200	0.00025	200	0.0500	0.521	0.499	0.0392	m, b	0.082	-0.002
0.125	100	200	0.00013	200	0.0250	0.313	0.291	0.0221	se_m, se_b	0.013	0.002
0.05	200	1000	0.00005	200	0.0100	0.214	0.191	0.0139	r , se_y	0.925	0.003
0.025	100	1000	0.00003	200	0.0050	0.121	0.098	0.0063	F, df	37	3
0.01	40	1000	0.00001	200	0.0020	0.058	0.035	0.0012	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.036	0.014	-0.0006			

Regression results are calculated using the function LINEST

Blank 0.022

r^2= 0.925
m= 0.082
b= -0.002

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µL µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.045	0.023	0.000	200	1	1	0.000	0.000	0.001
10	0.045	0.022	0.000	200	1	1	0.000		
10					1	1			
100	0.282	0.260	0.020	200	1	1	0.000	0.000	0.098
100	0.282	0.259	0.020	200	1	1	0.000		
100									
	0.091	0.068	0.004	200	218	70018	0.006	0.007	6.634
	0.097	0.075	0.004	200	218	70018	0.007		
	0.094	0.071	0.004	200	218	70018	0.007		

Assay Date	8/22/2005	Test Chemical ID	RC 8	tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	IVT

Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/22/2005	Chemical ID	RC 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.1835	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.1835	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.634
Protein Concentration (dilution added to assay, mg/mL):	0.020655

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-10

Assay Date	8/22/2005	Test Chemical ID	RC 8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4174.22	8348.44	8386.54	16773.08	0.1	153924	10.90	16639	0.0221	1	0.010	15	0.0713
			0.5	2	4212.32	8424.64			0.1					1		15	
	2	2	0.5	1	4048.66	8097.32	8178.71	16357.42	0.1	153924	10.63	16223	0.0215	1	0.010	15	0.0695
			0.5	2	4130.05	8260.1			0.1					1		15	
	3	2	0.5	1	3483.67	6967.34	7014.12	14028.24	0.1	153924	9.11	13894	0.0184	1	0.010	15	0.0595
			0.5	2	3530.45	7060.9			0.1					1		15	
	4	2	0.5	1	3758.9	7517.8	7506	15012	0.1	153924	9.75	14878	0.0197	1	0.010	15	0.0637
			0.5	2	3747.1	7494.2			0.1					1		15	
Background control	1	2	0.5	1	33.01	66.02	64.8	129.6	0.1	153924	0.08	-5	0.0000	1	0.010	15	0.0000
			0.5	2	31.79	63.58			0.1					1		15	
	2	2	0.5	1	32.36	64.72	66.49	132.98	0.1	153924	0.09	-1	0.0000	1	0.010	15	0.0000
			0.5	2	34.13	68.26			0.1					1		15	
	3	2	0.5	1	31.91	63.82	71.91	143.82	0.1	153924	0.09	10	0.0000	1	0.010	15	0.0000
			0.5	2	40	80			0.1					1		15	
	4	2	0.5	1	35.43	70.86	65.32	130.64	0.1	153924	0.08	-4	0.0000	1	0.010	15	0.0000
			0.5	2	29.89	59.78			0.1					1		15	
Positive control	1	2	0.5	1	1717.11	3434.22	3434.87	6869.74	0.1	153924	4.46	6735	0.0089	1	0.010	15	0.0286
			0.5	2	1717.76	3435.52			0.1					1		15	
	2	2	0.5	1	1741.46	3482.92	3492.68	6985.36	0.1	153924	4.54	6851	0.0091	1	0.010	15	0.0293
			0.5	2	1751.22	3502.44			0.1					1		15	
	3	2	0.5	1	1524.61	3049.22	3055.72	6111.44	0.1	153924	3.97	5977	0.0079	1	0.010	15	0.0256
			0.5	2	1531.11	3062.22			0.1					1		15	
	4	2	0.5	1	1574.54	3149.08	3109.14	6218.28	0.1	153924	4.04	6084	0.0081	1	0.010	15	0.0261
			0.5	2	1534.6	3069.2			0.1					1		15	
Negative Control	1	2	0.5	1	4062.86	8125.72	8158.54	16317.08	0.1	153924	10.60	16183	0.0215	1	0.010	15	0.0693
			0.5	2	4095.68	8191.36			0.1					1		15	
	2	2	0.5	1	3959.37	7918.74	7931.93	15863.86	0.1	153924	10.31	15730	0.0209	1	0.010	15	0.0674
			0.5	2	3972.56	7945.12			0.1					1		15	
	3	2	0.5	1	3478.66	6957.32	6966.52	13933.04	0.1	153924	9.05	13799	0.0183	1	0.010	15	0.0591
			0.5	2	3487.86	6975.72			0.1					1		15	
	4	2	0.5	1	3360.19	6720.38	6710.79	13421.58	0.1	153924	8.72	13287	0.0176	1	0.010	15	0.0569
			0.5	2	3350.6	6701.2			0.1					1		15	
RC 8	1-1	2	0.5	1	801.77	1603.54	1614.26	3228.52	0.1	153924	2.10	3094	0.0041	1	0.010	15	0.0133
			0.5	2	812.49	1624.98			0.1					1		15	
	1-2	2	0.5	1	427.39	854.78	832.4	1664.8	0.1	153924	1.08	1531	0.0020	1	0.010	15	0.0066
			0.5	2	405.01	810.02			0.1					1		15	
	1-3	2	0.5	1	730.77	1461.54	1443.81	2887.62	0.1	153924	1.88	2753	0.0037	1	0.010	15	0.0118
			0.5	2	713.04	1426.08			0.1					1		15	
	2-1	2	0.5	1	1095.1	2190.2	2169.58	4339.16	0.1	153924	2.82	4205	0.0056	1	0.010	15	0.0180
			0.5	2	1074.48	2148.96			0.1					1		15	
	2-2	2	0.5	1	1112.73	2225.46	2227.16	4454.32	0.1	153924	2.89	4320	0.0057	1	0.010	15	0.0185
			0.5	2	1114.43	2228.86			0.1					1		15	
	2-3	2	0.5	1	1094.38	2188.76	2218.72	4437.44	0.1	153924	2.88	4303	0.0057	1	0.010	15	0.0184
			0.5	2	1124.34	2248.68			0.1					1		15	
	3-1	2	0.5	1	1673.45	3346.9	3337.52	6675.04	0.1	153924	4.34	6541	0.0087	1	0.010	15	0.0280
			0.5	2	1664.07	3328.14			0.1					1		15	
	3-2	2	0.5	1	1609.71	3219.42	3122.25	6244.5	0.1	153924	4.06	6110	0.0081	1	0.010	15	0.0262
			0.5	2	1512.54	3025.08			0.1					1		15	
	3-3	2	0.5	1	1656.8	3313.6	3351.68	6703.36	0.1	153924	4.35	6569	0.0087	1	0.010	15	0.0281
			0.5	2	1694.88	3389.76			0.1					1		15	
	4-1	2	0.5	1	2124.61	4249.62	4267.66	8535.32	0.1	153924	5.55	8401	0.0111	1	0.010	15	0.0360
			0.5	2	2142.85	4285.7			0.1					1		15	
	4-2	2	0.5	1	2240.45	4480.9	4464.56	8929.12	0.1	153924	5.80	8795	0.0117	1	0.010	15	0.0377
			0.5	2	2224.11	4448.22			0.1					1		15	
	4-3	2	0.5	1	2232.46	4464.92	4447.1	8894.2	0.1	153924	5.78	8760	0.0116	1	0.010	15	0.0375
			0.5	2	2214.64	4429.28			0.1					1		15	
	5-1	2	0.5	1	2980.97	5961.94	5997.83	11995.66	0.1	153924	7.79	11861	0.0157	1	0.010	15	0.0508
			0.5	2	3016.86	6033.72			0.1					1		15	
	5-2	2	0.5	1	2920.89	5841.98	5848.62	11697.24	0.1	153924	7.60	11563	0.0153	1	0.010	15	0.0495
			0.5	2	2927.53	5855.26			0.1					1		15	
	5-3	2	0.5	1	2851.17	5702.34	5694.25	11396.5	0.1	153924	7.40	11254	0.0149	1	0.010	15	0.0482
			0.5	2	2843.08	5686.16			0.1					1		15	
	6-1	2	0.5	1	3716.69	7433.38	7398.51	14797.02	0.1	153924	9.61	14663	0.0195	1	0.010	15	0.0628
			0.5	2	3681.82	7363.64			0.1					1		15	
	6-2	2	0.5	1	3826.55	7653.1	7636.99	15273.98	0.1	153924	9.92	15140	0.0201	1	0.010	15	0.0648
			0.5	2	3810.44	7620.88			0.1					1		15	
	6-3	2	0.5	1	3741.92	7483.84	7504.7	15009.4	0.1	153924	9.75	14875	0.0197	1	0.010	15	0.0637
			0.5	2	3762.78	7525.56			0.1					1		15	
	7-1	2	0.5	1	3307.46	6614.92	6639.14	13278.28	0.1	153924	8.63	13144	0.0174	1	0.010	15	0.0563
			0.5	2	3331.68	6663.36			0.1					1		15	
	7-2	2	0.5	1	3223.8	6447.6	6413.5	12827	0.1	153924	8.33	12693	0.0168	1	0.010	15	0.0544
			0.5	2	3189.7	6379.4			0.1					1		15	
	7-3	2	0.5	1	3252.82	6505.64	6463.98	12927.96	0.1	153924	8.40	12794	0.0170	1	0.010	15	0.0548
			0.5	2	3211.16	6422.32			0.1					1		15	
	8-1	2	0.5	1	2600.03	5200.06	5189.81	10379.62	0.1	153924	6.74	10245	0.0136	1	0.010	15	0.0439
			0.5	2	2589.78	5179.56			0.1					1		15	
	8-2	2	0.5	1	2649.83	5299.66	5302.86	10605.72	0.1	153924	6.89	10471	0.0139	1	0.010	15	0.0448
			0.5	2	2653.03	5306.06			0.1					1		15	
	8-3	2	0.5	1	2657.9	5315.8	5328.59	10657.18	0.1	153924	6.92	10523	0.0140	1	0.010	15	0.0451
			0.5	2	2670.69	5341.38			0.1					1		15	

Assay Date	8/22/2005	ID	RC 8	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0704	0.0013
Full activity	End	0.0616	0.0030
Full activity	Overall	0.0660	0.0054
Background	Beginning	0.0000	1.02348E-05
Background	End	0.0000	3.99095E-05
Background	Overall	0.0000	2.79556E-05
Positive	Beginning	0.0291	0.0004
Positive	End	0.0258	0.0003
Positive	Overall	0.0275	0.0019
Negative	Beginning	0.0683	0.0014
Negative	End	0.0580	0.0015
Negative	Overall	0.0632	0.0061

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC 8	1	1	1.00E-04	-4.00	0.0133
RC 8	1	2	1.00E-04	-4.00	0.0066
RC 8	1	3	1.00E-04	-4.00	0.0118
RC 8	2	1	1.00E-05	-5.00	0.0180
RC 8	2	2	1.00E-05	-5.00	0.0185
RC 8	2	3	1.00E-05	-5.00	0.0184
RC 8	3	1	5.00E-06	-5.30	0.0280
RC 8	3	2	5.00E-06	-5.30	0.0262
RC 8	3	3	5.00E-06	-5.30	0.0281
RC 8	4	1	2.50E-06	-5.60	0.0360
RC 8	4	2	2.50E-06	-5.60	0.0377
RC 8	4	3	2.50E-06	-5.60	0.0375
RC 8	5	1	1.00E-06	-6.00	0.0508
RC 8	5	2	1.00E-06	-6.00	0.0495
RC 8	5	3	1.00E-06	-6.00	0.0482
RC 8	6	1	1.00E-07	-7.00	0.0628
RC 8	6	2	1.00E-07	-7.00	0.0648
RC 8	6	3	1.00E-07	-7.00	0.0637
RC 8	7	1	1.00E-08	-8.00	0.0563
RC 8	7	2	1.00E-08	-8.00	0.0544
RC 8	7	3	1.00E-08	-8.00	0.0548
RC 8	8	1	1.00E-10	-10.00	0.0439
RC 8	8	2	1.00E-10	-10.00	0.0448
RC 8	8	3	1.00E-10	-10.00	0.0451

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-4.00	20.08	9.93	17.87
2	-5.00	27.29	28.04	27.93
3	-5.30	42.45	39.66	42.63
4	-5.60	54.52	57.08	56.85
5	-6.00	76.98	75.04	73.04
6	-7.00	95.16	98.26	96.54
7	-8.00	85.30	82.38	83.03
8	-10.00	66.49	67.96	68.29

Aromatase Assay Spreadsheet

Assay Date	8/30/2005	Test Chemical ID	RC8	# Concentrations tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	IVT

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	32135	1598756	
2	0.0204	32624.61	1599246	
3	0.0201	32156.54	1599828	
4	0.0202	33028.02	1635050	
5	0.0201	33280.02	1655722	
			Average DPM/g soln	1617721
			SD	26294
			CV	1.63
			µCi/g soln	0.729

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

ASDN solution	mg ASDN added	(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	8.2141 g
Mass of dilution B used in substrate prep	4.6134 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.578493 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00825 µg/g soln.
µg/g soln.

a. µCi/g soln 0.729
b. Specific activity of [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.

= 0.578493 + 0.00825
= 0.586742 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.242 µCi/µg ASDN

789640 dpm/nmol

Test

Assay Date 8/30/2005 Chemical ID RC8 tested 8

ID JG Replicate # Rep 3 Microsome type Placental Microsome ID IVT

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.575	0.347	0.173	0.106	0.056	0.045	0.039	25	100	
	0.583	0.341	0.162	0.103	0.074	0.047	0.045			
	0.567	0.341	0.170	0.110	0.060	0.045	0.039			

Samples:	10	100	microsomes
	0.064	0.301	0.105
	0.067	0.302	0.101
			0.107

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.575	0.534	0.0486
0.125	100	200	0.00013	200	0.0250	0.343	0.302	0.0271
0.05	200	1000	0.00005	200	0.0100	0.168	0.128	0.0110
0.025	100	1000	0.00003	200	0.0050	0.106	0.066	0.0053
0.01	40	1000	0.00001	200	0.0020	0.063	0.023	0.0013
0.005	20	1000	0.00001	200	0.0010	0.046	0.005	-0.0003

Blank 0.041

r^2= 0.995
m= 0.092
b= -0.001

Variables	Regression results	
m, b	0.092	-0.001
se_m, se_b	0.003	0.001
r , se_y	0.995	0.002
F, df	731	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	µL microsome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.064	0.023	0.001	200	1	1	0.000	0.000	0.008
10	0.067	0.026	0.002	200	1	1	0.000		
10					1	1			
100	0.301	0.260	0.023	200	1	1	0.000	0.000	0.117
100	0.302	0.261	0.023	200	1	1	0.000		
100									
	0.105	0.064	0.005	200	218	70018	0.008	0.008	8.202
	0.101	0.060	0.005	200	218	70018	0.008		
	0.107	0.066	0.005	200	218	70018	0.009		

Assay Date 8/30/2005 Test Chemical ID RC8 tested 8

ID JG Replicate # Rep 3 Microsome type Placental Microsome ID IVT

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.575	0.347	0.173	0.106	0.056	0.045	0.039	25	100
	0.583	0.341	0.162	0.103	0.074	0.047	0.045		
	0.567	0.341	0.170	0.110	0.060	0.045	0.039		

Protein stock ID

Samples:	10	100	microsomes
	0.064	0.301	0.105
	0.067	0.302	0.101
			0.107

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.575	0.534	0.0437
0.125	100	200	0.00013	200	0.0250	0.343	0.302	0.0247
0.05	200	1000	0.00005	200	0.0100	0.168	0.128	0.0105
0.025	100	1000	0.00003	200	0.0050	0.106	0.066	0.0054
0.01	40	1000	0.00001	200	0.0020	0.063	0.023	0.0019
0.005	20	1000	0.00001	200	0.0010	0.046	0.005	0.0005

Blank 0.041

r^2= 0.998
m= 0.082
b= 0.000

Variables	Regression results	
m, b	0.082	0.000
se_m, se_b	0.002	0.000
r , se_y	0.998	0.001
F, df	1472	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. usomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.064	0.023	0.002	200	1	1	0.000	0.000	0.010
10	0.067	0.026	0.002	200	1	1	0.000		
10					1	1			
100	0.301	0.260	0.021	200	1	1	0.000	0.000	0.107
100	0.302	0.261	0.021	200	1	1	0.000		
100									
	0.105	0.064	0.005	200	218	70018	0.009	0.008	8.444
	0.101	0.060	0.005	200	218	70018	0.008		
	0.107	0.066	0.005	200	218	70018	0.009		

Assay Date	8/30/2005	Test Chemical ID	RC8	tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	IVT

								Protein stock (mg/10 mL)	Protein stock ID
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk		
Samples:									

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/30/2005	Chemical ID	RC8	# Concentrations tested	8	Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate # Rep 3

Microsome Dilution Details

Dilution A	0.218	mL microsome Stock used
	70.018	mL total volume
	321.1835	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	321.1835	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.444
Protein Concentration (dilution added to assay, mg/mL):	0.02629

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-10

Assay Date	8/30/2005	Test Chemical ID	RC8	# Concentrations tested		# Microsome type	Placental	Microsome ID	IVT	Technician ID	JG	Replicate #	Rep 3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	3574.7	7149.4	7135.45	14270.9	0.1	161772	8.82	14115	0.0179	1	0.013	15	0.0453
			0.5	2	3560.75	7121.5			0.1					1		15	
	2	2	0.5	1	3921.21	7842.42	7824.47	15648.94	0.1	161772	9.67	15493	0.0196	1	0.013	15	0.0498
			0.5	2	3903.26	7806.52			0.1					1		15	
	3	2	0.5	1	3578.33	7156.66	7247.14	14494.28	0.1	161772	8.96	14339	0.0182	1	0.013	15	0.0460
			0.5	2	3668.81	7337.62			0.1					1		15	
	4	2	0.5	1	3633.26	7266.52	7309.5	14619	0.1	161772	9.04	14463	0.0183	1	0.013	15	0.0464
			0.5	2	3676.24	7352.48			0.1					1		15	
Background control	1	2	0.5	1	42.46	84.92	79.21	158.42	0.1	161772	0.10	3	0.0000	1	0.013	15	0.0000
			0.5	2	36.75	73.5			0.1					1		15	
	2	2	0.5	1	32.99	65.98	63.04	126.08	0.1	161772	0.08	-30	0.0000	1	0.013	15	-0.0001
			0.5	2	30.05	60.1			0.1					1		15	
	3	2	0.5	1	32.73	65.46	82.05	164.1	0.1	161772	0.10	8	0.0000	1	0.013	15	0.0000
			0.5	2	49.32	98.64			0.1					1		15	
	4	2	0.5	1	44.39	88.78	86.98	173.96	0.1	161772	0.11	18	0.0000	1	0.013	15	0.0001
			0.5	2	42.59	85.18			0.1					1		15	
Positive control	1	2	0.5	1	1763.36	3526.72	3471.27	6942.54	0.1	161772	4.29	6787	0.0086	1	0.013	15	0.0218
			0.5	2	1707.91	3415.82			0.1					1		15	
	2	2	0.5	1	1730.63	3461.26	3430.65	6861.3	0.1	161772	4.24	6706	0.0085	1	0.013	15	0.0215
			0.5	2	1700.02	3400.04			0.1					1		15	
	3	2	0.5	1	1572.92	3145.84	3109.7	6219.4	0.1	161772	3.84	6064	0.0077	1	0.013	15	0.0195
			0.5	2	1536.78	3073.56			0.1					1		15	
	4	2	0.5	1	1597.78	3195.56	3161.25	6362.5	0.1	161772	3.93	6207	0.0079	1	0.013	15	0.0199
			0.5	2	1583.47	3166.94			0.1					1		15	
Negative Control	1	2	0.5	1	3850.73	7701.46	7716.67	15433.34	0.1	161772	9.54	15276	0.0193	1	0.013	15	0.0491
			0.5	2	3865.94	7731.88			0.1					1		15	
	2	2	0.5	1	3870.25	7740.5	7678.15	15356.3	0.1	161772	9.49	15201	0.0193	1	0.013	15	0.0488
			0.5	2	3807.9	7615.8			0.1					1		15	
	3	2	0.5	1	3431.12	6862.24	6826.43	13652.86	0.1	161772	8.44	13497	0.0171	1	0.013	15	0.0433
			0.5	2	3395.31	6790.62			0.1					1		15	
	4	2	0.5	1	3161.13	6322.26	6209.47	12418.94	0.1	161772	7.68	12283	0.0155	1	0.013	15	0.0394
			0.5	2	3048.34	6096.68			0.1					1		15	
RC8	1-1	2	0.5	1	521.32	1042.64	1030.73	2061.46	0.1	161772	1.27	1906	0.0024	1	0.013	15	0.0061
			0.5	2	509.41	1018.82			0.1					1		15	
	1-2	2	0.5	1	572.55	1145.1	1145.55	2291.1	0.1	161772	1.42	2135	0.0027	1	0.013	15	0.0069
			0.5	2	573	1146			0.1					1		15	
	1-3	2	0.5	1	576.15	1152.3	1142.97	2285.94	0.1	161772	1.41	2130	0.0027	1	0.013	15	0.0068
			0.5	2	566.82	1133.64			0.1					1		15	
	2-1	2	0.5	1	1015.61	2031.22	2015.55	4031.1	0.1	161772	2.49	3875	0.0049	1	0.013	15	0.0124
			0.5	2	999.94	1999.88			0.1					1		15	
	2-2	2	0.5	1	1054.07	2108.14	2092.08	4184.16	0.1	161772	2.59	4029	0.0051	1	0.013	15	0.0129
			0.5	2	1038.01	2076.02			0.1					1		15	
	2-3	2	0.5	1	1025.65	2051.3	2090.05	4180.1	0.1	161772	2.58	4024	0.0051	1	0.013	15	0.0129
			0.5	2	1064.4	2128.8			0.1					1		15	
	3-1	2	0.5	1	1488.43	2976.86	3006.69	6013.38	0.1	161772	3.72	5858	0.0074	1	0.013	15	0.0188
			0.5	2	1518.26	3036.52			0.1					1		15	
	3-2	2	0.5	1	1599.75	3199.5	3113.39	6226.78	0.1	161772	3.85	6071	0.0077	1	0.013	15	0.0195
			0.5	2	1513.64	3027.28			0.1					1		15	
	3-3	2	0.5	1	1499.09	2998.18	3002.11	6004.22	0.1	161772	3.71	5849	0.0074	1	0.013	15	0.0188
			0.5	2	1503.02	3006.04			0.1					1		15	
	4-1	2	0.5	1	2186.73	4373.46	4377.87	8755.74	0.1	161772	5.41	8600	0.0109	1	0.013	15	0.0276
			0.5	2	2191.14	4382.28			0.1					1		15	
	4-2	2	0.5	1	2120.25	4240.5	4255.06	8510.12	0.1	161772	5.26	8354	0.0106	1	0.013	15	0.0268
			0.5	2	2134.81	4269.62			0.1					1		15	
	4-3	2	0.5	1	2080.37	4160.74	4142.61	8285.22	0.1	161772	5.12	8130	0.0103	1	0.013	15	0.0261
			0.5	2	2062.24	4124.48			0.1					1		15	
	5-1	2	0.5	1	2810.7	5621.4	5599.9	11199.8	0.1	161772	6.92	11044	0.0140	1	0.013	15	0.0355
			0.5	2	2789.2	5578.4			0.1					1		15	
	5-2	2	0.5	1	2869.72	5739.44	5687.72	11375.44	0.1	161772	7.03	11220	0.0142	1	0.013	15	0.0360
			0.5	2	2818	5636			0.1					1		15	
	5-3	2	0.5	1	2835.95	5671.9	5662.97	11325.94	0.1	161772	7.00	11170	0.0141	1	0.013	15	0.0359
			0.5	2	2827.02	5654.04			0.1					1		15	
	6-1	2	0.5	1	3637.42	7274.84	7209.89	14419.78	0.1	161772	8.91	14264	0.0181	1	0.013	15	0.0458
			0.5	2	3572.47	7144.94			0.1					1		15	
	6-2	2	0.5	1	3574.05	7148.1	7081.71	14163.42	0.1	161772	8.76	14008	0.0177	1	0.013	15	0.0450
			0.5	2	3507.66	7015.32			0.1					1		15	
	6-3	2	0.5	1	3496.49	6992.98	6962.74	13925.48	0.1	161772	8.61	13770	0.0174	1	0.013	15	0.0442
			0.5	2	3466.25	6932.5			0.1					1		15	
	7-1	2	0.5	1	3301.45	6602.9	6652.41	13304.82	0.1	161772	8.22	13149	0.0167	1	0.013	15	0.0422
			0.5	2	3350.96	6701.92			0.1					1		15	
	7-2	2	0.5	1	3337.34	6674.68	6612.69	13225.38	0.1	161772	8.18	13070	0.0166	1	0.013	15	0.0420
			0.5	2	3275.35	6550.7			0.1					1		15	
	7-3	2	0.5	1	3309.2	6618.4	6600.11	13200.22	0.1	161772	8.16	13045	0.0165	1	0.013	15	0.0419
			0.5	2	3290.91	6581.82			0.1					1		15	
	8-1	2	0.5	1	3082.6	6165.2	6161.98	12323.96	0.1	161772	7.62	12168	0.0154	1	0.013	15	0.0391
			0.5	2	3079.38	6158.76			0.1					1		15	
	8-2	2	0.5	1	2922.18	5844.36	5823.39	11646.78	0.1	161772	7.20	11491	0.0146	1	0.013	15	0.0369
			0.5	2	2901.21	5802.42			0.1					1		15	
	8-3	2	0.5	1	3191.28	6382.52	6186.38	12372.76	0.1	161772	7.65	12217	0.0155	1	0.013	15	0.0392
			0.5	2	3055.12	6110.24			0.1					1		15	

Assay Date	8/30/2005	ID	RC8	# Concentrations tested	Microsome 8 type	Placental	Microsome ID IVT	Technician ID JG	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0475	0.0031
Full activity	End	0.0462	0.0003
Full activity	Overall	0.0469	0.0020
Background	Beginning	0.0000	7.34361E-05
Background	End	0.0000	2.23896E-05
Background	Overall	0.0000	6.65584E-05
Positive	Beginning	0.0217	0.0002
Positive	End	0.0197	0.0003
Positive	Overall	0.0207	0.0012
Negative	Beginning	0.0489	0.0002
Negative	End	0.0414	0.0028
Negative	Overall	0.0452	0.0047

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC8	1	1	1.00E-04	-4.00	0.0061
RC8	1	2	1.00E-04	-4.00	0.0069
RC8	1	3	1.00E-04	-4.00	0.0068
RC8	2	1	1.00E-05	-5.00	0.0124
RC8	2	2	1.00E-05	-5.00	0.0129
RC8	2	3	1.00E-05	-5.00	0.0129
RC8	3	1	5.00E-06	-5.30	0.0188
RC8	3	2	5.00E-06	-5.30	0.0195
RC8	3	3	5.00E-06	-5.30	0.0188
RC8	4	1	2.50E-06	-5.60	0.0276
RC8	4	2	2.50E-06	-5.60	0.0268
RC8	4	3	2.50E-06	-5.60	0.0261
RC8	5	1	1.00E-06	-6.00	0.0355
RC8	5	2	1.00E-06	-6.00	0.0360
RC8	5	3	1.00E-06	-6.00	0.0359
RC8	6	1	1.00E-07	-7.00	0.0458
RC8	6	2	1.00E-07	-7.00	0.0450
RC8	6	3	1.00E-07	-7.00	0.0442
RC8	7	1	1.00E-08	-8.00	0.0422
RC8	7	2	1.00E-08	-8.00	0.0420
RC8	7	3	1.00E-08	-8.00	0.0419
RC8	8	1	1.00E-10	-10.00	0.0391
RC8	8	2	1.00E-10	-10.00	0.0369
RC8	8	3	1.00E-10	-10.00	0.0392

Percent of control values				
	Log[test	Replicate		
_evel	bstance]	1	2	3
1	-4.00	13.05	14.62	14.59
2	-5.00	26.54	27.59	27.56
3	-5.30	40.11	41.58	40.05
4	-5.60	58.89	57.21	55.67
5	-6.00	75.63	76.83	76.50
6	-7.00	97.68	95.93	94.30
7	-8.00	90.05	89.50	89.33
8	-10.00	83.33	78.69	83.66

Aromatase Assay Spreadsheet

Assay Date	9/1/2005	Test Chemical ID	RC9	# Concentrations tested	8	
ID	TNB	Replicate #	Rep 1	Microsome type	Placental	Microsome ID Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	39616.25	1970958	
2	0.0202	40379.66	1998993	
3	0.0202	40547.64	2007309	
4	0.0201	40768.21	2028269	
5	0.0201	40960.95	2037858	
			Average DPM/g soln	2008677
			SD	26238
			CV	1.31
			µCi/g soln	0.905

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

ASDN solution	mg ASDN added	(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	8.1716 g
Mass of dilution B used in substrate prep	4.6284 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.577729 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01024 µg/g soln.
µg/g soln.

a. µCi/g soln	0.905
b. Specific activity of [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.

= 0.577729 + 0.01024
= 0.587971 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.539 µCi/µg ASDN

978424 dpm/nmol

Assay Date 9/1/2005 Test Chemical ID RC9 tested 8

ID TNB Replicate # Rep 1 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.603	0.360	0.156	0.097	0.055	0.049	0.039	25	100	
	0.610	0.373	0.161	0.101	0.058	0.047	0.037			
	0.587	0.391	0.160	0.105	0.060	0.049	0.036			
Samples:	10	100	microsomes							
	0.074	0.294	0.106							
	0.067	0.303	0.106							
			0.106							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
0.25	200	200	0.00025	200	0.0500	0.600	0.563	0.0479	m, b	0.086	-0.001
0.125	100	200	0.00013	200	0.0250	0.375	0.337	0.0285	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.159	0.122	0.0099	r , se_y	0.990	0.002
0.025	100	1000	0.00003	200	0.0050	0.101	0.064	0.0049	F, df	408	4
0.01	40	1000	0.00001	200	0.0020	0.058	0.020	0.0012	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.048	0.011	0.0004			

Regression results are calculated using the function LINEST

Blank 0.037

r^2= 0.990
m= 0.086
b= -0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µL usomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.074	0.037	0.003	200	1	1	0.000	0.000	0.012
10	0.067	0.030	0.002	200	1	1	0.000		
10					1	1			
100	0.294	0.257	0.022	200	1	1	0.000	0.000	0.110
100	0.303	0.266	0.022	200	1	1	0.000		
100									
	0.106	0.068	0.005	200	83	69983	0.023	0.023	22.682
	0.106	0.069	0.005	200	83	69983	0.023		
	0.106	0.069	0.005	200	83	69983	0.023		

Assay Date 9/1/2005 Test Chemical ID RC9 tested 8

ID TNB Replicate # Rep 1 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.603	0.360	0.156	0.097	0.055	0.049	0.039	25		
	0.610	0.373	0.161	0.101	0.058	0.047	0.037			
	0.587	0.391	0.160	0.105	0.060	0.049	0.036			
Samples:	10	100	microsomes							
	0.074	0.294	0.106							
	0.067	0.303	0.106							
			0.106							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.600	0.563	0.0417
0.125	100	200	0.00013	200	0.0250	0.375	0.337	0.0252
0.05	200	1000	0.00005	200	0.0100	0.159	0.122	0.0094
0.025	100	1000	0.00003	200	0.0050	0.101	0.064	0.0051
0.01	40	1000	0.00001	200	0.0020	0.058	0.020	0.0020
0.005	20	1000	0.00001	200	0.0010	0.048	0.011	0.0013

Blank 0.037

r^2= 0.999
m= 0.073
b= 0.000

Variables	Regression results	
m, b	0.073	0.000
se_m, se_b	0.002	0.000
r , se_y	0.999	0.000
F, df	2259	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol. usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.074	0.037	0.003	200	1	1	0.000	0.000	0.015
10	0.067	0.030	0.003	200	1	1	0.000		
10					1	1			
100	0.294	0.257	0.019	200	1	1	0.000	0.000	0.098
100	0.303	0.266	0.020	200	1	1	0.000		
100									
	0.106	0.068	0.005	200	83	69983	0.023	0.023	23.249
	0.106	0.069	0.006	200	83	69983	0.023		
	0.106	0.069	0.006	200	83	69983	0.023		

Assay Date	9/1/2005	Test Chemical ID	RC9	tested	8			
ID	TNB	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	Battelle	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)
Samples:								Protein stock ID

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					
Blank		r^2=					Regression results are calculated using the function LINEST
		m=					
		b=					

A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/1/2005	Chemical ID	RC9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.1687	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.1687	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	23.249
Protein Concentration (dilution added to assay, mg/mL):	0.027573

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	9/1/2005	Test Chemical ID	RC9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	6477.06	12954.12	12631.65	25663.3	0.1	200868	12.78	25196	0.0256	1	0.014	15	0.0623
			0.5	2	6354.59	12709.18			0.1					1		15	
	2	2	0.5	1	6523.72	13047.44	13071.66	26143.32	0.1	200868	13.02	25676	0.0262	1	0.014	15	0.0634
			0.5	2	6547.94	13095.88			0.1					1		15	
	3	2	0.5	1	6186.98	12373.96	12382.7	24765.4	0.1	200868	12.33	24298	0.0248	1	0.014	15	0.0600
			0.5	2	6195.72	12391.44			0.1					1		15	
	4	2	0.5	1	6403.67	12807.14	12786.35	25572.7	0.1	200868	12.73	25106	0.0257	1	0.014	15	0.0620
			0.5	2	6382.78	12765.56			0.1					1		15	
Background control	1	2	0.5	1	114.71	229.42	235.02	470.04	0.1	200868	0.23	3	0.0000	1	0.014	15	0.0000
			0.5	2	120.31	240.62			0.1					1		15	
	2	2	0.5	1	96.86	193.32	181.44	362.88	0.1	200868	0.18	-104	-0.0001	1	0.014	15	-0.0003
			0.5	2	84.78	169.56			0.1					1		15	
	3	2	0.5	1	93.09	186.18	193.35	386.7	0.1	200868	0.19	-80	-0.0001	1	0.014	15	-0.0002
			0.5	2	100.26	200.52			0.1					1		15	
	4	2	0.5	1	155.29	310.58	324.23	648.46	0.1	200868	0.32	181	0.0002	1	0.014	15	0.0004
			0.5	2	168.94	337.88			0.1					1		15	
Positive control	1	2	0.5	1	2663.2	5326.4	5280.4	10560.8	0.1	200868	5.26	10094	0.0103	1	0.014	15	0.0249
			0.5	2	2617.2	5234.4			0.1					1		15	
	2	2	0.5	1	3349.55	6699.1	6749.92	13499.84	0.1	200868	6.72	13033	0.0133	1	0.014	15	0.0322
			0.5	2	3400.37	6800.74			0.1					1		15	
	3	2	0.5	1	2532.43	5064.86	5241.05	10482.1	0.1	200868	5.22	10015	0.0102	1	0.014	15	0.0247
			0.5	2	2708.62	5417.24			0.1					1		15	
	4	2	0.5	1	2634.98	5269.96	5231.36	10462.72	0.1	200868	5.21	9996	0.0102	1	0.014	15	0.0247
			0.5	2	2596.38	5192.76			0.1					1		15	
Negative Control	1	2	0.5	1	6492.55	12985.1	12944.79	25889.58	0.1	200868	12.89	25423	0.0260	1	0.014	15	0.0628
			0.5	2	6452.24	12904.48			0.1					1		15	
	2	2	0.5	1	6406.61	12813.22	12662.39	25324.78	0.1	200868	12.61	24858	0.0254	1	0.014	15	0.0614
			0.5	2	6255.78	12511.56			0.1					1		15	
	3	2	0.5	1	5884.66	11769.32	11800.23	23600.46	0.1	200868	11.75	23133	0.0236	1	0.014	15	0.0572
			0.5	2	5915.57	11831.14			0.1					1		15	
	4	2	0.5	1	5889.11	11778.22	11762.63	23525.26	0.1	200868	11.71	23058	0.0236	1	0.014	15	0.0570
			0.5	2	5873.52	11747.04			0.1					1		15	
RC9	1-1	2	0.5	1	442.76	885.52	909.09	1818.18	0.1	200868	0.91	1351	0.0014	1	0.014	15	0.0033
			0.5	2	466.33	932.66			0.1					1		15	
	1-2	2	0.5	1	267.37	534.74	530.74	1061.48	0.1	200868	0.53	594	0.0006	1	0.014	15	0.0015
			0.5	2	263.37	526.74			0.1					1		15	
	1-3	2	0.5	1	329.39	658.78	651.91	1303.82	0.1	200868	0.65	837	0.0009	1	0.014	15	0.0021
			0.5	2	322.52	645.04			0.1					1		15	
	2-1	2	0.5	1	3285.57	6571.14	6742.91	13485.82	0.1	200868	6.71	13019	0.0133	1	0.014	15	0.0322
			0.5	2	3457.34	6914.68			0.1					1		15	
	2-2	2	0.5	1	1914.86	3829.72	3793.88	7587.76	0.1	200868	3.78	7121	0.0073	1	0.014	15	0.0176
			0.5	2	1879.02	3758.04			0.1					1		15	
	2-3	2	0.5	1	1967.07	3934.14	3924.07	7848.14	0.1	200868	3.91	7381	0.0075	1	0.014	15	0.0182
			0.5	2	1957	3914			0.1					1		15	
	3-1	2	0.5	1	4058.31	8116.62	7997.12	15994.24	0.1	200868	7.96	15527	0.0159	1	0.014	15	0.0384
			0.5	2	3938.81	7877.62			0.1					1		15	
	3-2	2	0.5	1	4002.31	8004.62	8050.83	16101.66	0.1	200868	8.02	15635	0.0160	1	0.014	15	0.0386
			0.5	2	4048.52	8097.04			0.1					1		15	
	3-3	2	0.5	1	4297.49	8594.98	8473.97	16947.94	0.1	200868	8.44	16481	0.0168	1	0.014	15	0.0407
			0.5	2	4176.48	8352.96			0.1					1		15	
	4-1	2	0.5	1	5882.54	11765.08	11774.13	23548.26	0.1	200868	11.72	23081	0.0236	1	0.014	15	0.0570
			0.5	2	5891.59	11783.18			0.1					1		15	
	4-2	2	0.5	1	6136.68	12273.36	12302.24	24604.48	0.1	200868	12.25	24137	0.0247	1	0.014	15	0.0596
			0.5	2	6165.56	12331.12			0.1					1		15	
	4-3	2	0.5	1	5901.48	11802.96	11894.04	23788.08	0.1	200868	11.84	23321	0.0238	1	0.014	15	0.0576
			0.5	2	5992.56	11985.12			0.1					1		15	
	5-1	2	0.5	1	6549.65	13099.3	13106.76	26213.56	0.1	200868	13.05	25747	0.0263	1	0.014	15	0.0636
			0.5	2	6557.13	13114.26			0.1					1		15	
	5-2	2	0.5	1	6586.91	13173.82	13199.1	26398.2	0.1	200868	13.14	25931	0.0265	1	0.014	15	0.0641
			0.5	2	6612.19	13224.38			0.1					1		15	
	5-3	2	0.5	1	5767.46	11534.92	11749.2	23498.4	0.1	200868	11.70	23031	0.0235	1	0.014	15	0.0569
			0.5	2	5981.74	11963.48			0.1					1		15	
	6-1	2	0.5	1	6646.56	13293.12	13251.04	26502.08	0.1	200868	13.19	26035	0.0266	1	0.014	15	0.0643
			0.5	2	6604.48	13208.96			0.1					1		15	
	6-2	2	0.5	1	6309.8	12619.6	12504.72	25009.44	0.1	200868	12.45	24542	0.0251	1	0.014	15	0.0606
			0.5	2	6194.92	12389.84			0.1					1		15	
	6-3	2	0.5	1	6379.27	12758.54	12646.08	25292.16	0.1	200868	12.59	24825	0.0254	1	0.014	15	0.0613
			0.5	2	6266.81	12533.62			0.1					1		15	
	7-1	2	0.5	1	6387.59	12775.18	12801.45	25602.9	0.1	200868	12.75	25136	0.0257	1	0.014	15	0.0621
			0.5	2	6413.86	12827.72			0.1					1		15	
	7-2	2	0.5	1	6510.62	13021.24	13041.27	26082.54	0.1	200868	12.98	25616	0.0262	1	0.014	15	0.0633
			0.5	2	6530.65	13061.3			0.1					1		15	
	7-3	2	0.5	1	6469.6	12939.2	12918.57	25837.14	0.1	200868	12.86	25370	0.0259	1	0.014	15	0.0627
			0.5	2	6448.97	12897.94			0.1					1		15	
	8-1	2	0.5	1	6503.29	13006.58	13042.2	26084.4	0.1	200868	12.99	25617	0.0262	1	0.014	15	0.0633
			0.5	2	6538.91	13077.82			0.1					1		15	
	8-2	2	0.5	1	6495.24	12990.48	12889.86	25779.72	0.1	200868	12.83	25313	0.0259	1	0.014	15	0.0626
			0.5	2	6394.62	12789.24			0.1					1		15	
	8-3	2	0.5	1	5869.24	11738.48	11949	23898	0.1	200868	11.90	23431	0.0239	1	0.014	15	0.0579
			0.5	2	6080.76	12161.52			0.1					1		15	

Assay Date	9/1/2005	ID	RC9	# Concentrations tested	Microsome 8 type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0629	0.0008
Full activity	End	0.0610	0.0014
Full activity	Overall	0.0619	0.0014
Background	Beginning	-0.0001	0.000187245
Background	End	0.0001	0.000457383
Background	Overall	0.0000	0.000319739
Positive	Beginning	0.0286	0.0051
Positive	End	0.0247	0.0000
Positive	Overall	0.0266	0.0037
Negative	Beginning	0.0621	0.0010
Negative	End	0.0571	0.0001
Negative	Overall	0.0596	0.0030

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC9	1	1	1.00E-03	-3.00	0.0033
RC9	1	2	1.00E-03	-3.00	0.0015
RC9	1	3	1.00E-03	-3.00	0.0021
RC9	2	1	1.00E-04	-4.00	0.0322
RC9	2	2	1.00E-04	-4.00	0.0176
RC9	2	3	1.00E-04	-4.00	0.0182
RC9	3	1	1.00E-05	-5.00	0.0384
RC9	3	2	1.00E-05	-5.00	0.0386
RC9	3	3	1.00E-05	-5.00	0.0407
RC9	4	1	1.00E-06	-6.00	0.0570
RC9	4	2	1.00E-06	-6.00	0.0596
RC9	4	3	1.00E-06	-6.00	0.0576
RC9	5	1	1.00E-07	-7.00	0.0636
RC9	5	2	1.00E-07	-7.00	0.0641
RC9	5	3	1.00E-07	-7.00	0.0569
RC9	6	1	1.00E-08	-8.00	0.0643
RC9	6	2	1.00E-08	-8.00	0.0606
RC9	6	3	1.00E-08	-8.00	0.0613
RC9	7	1	1.00E-09	-9.00	0.0621
RC9	7	2	1.00E-09	-9.00	0.0633
RC9	7	3	1.00E-09	-9.00	0.0627
RC9	8	1	1.00E-10	-10.00	0.0633
RC9	8	2	1.00E-10	-10.00	0.0626
RC9	8	3	1.00E-10	-10.00	0.0579

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-3.00	5.39	2.37	3.34
2	-4.00	51.93	28.40	29.44
3	-5.00	61.94	62.37	65.74
4	-6.00	92.07	96.28	93.03
5	-7.00	102.70	103.44	91.87
6	-8.00	103.85	97.90	99.03
7	-9.00	100.27	102.18	101.20
8	-10.00	102.19	100.97	93.47

Aromatase Assay Spreadsheet

Assay Date	9/6/2005	Test Chemical ID	RC9	# Concentrations tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	33233.57	1678463	
2	0.0199	33635.76	1690239	
3	0.0198	32307.83	1631709	
4	0.0198	32741.24	1653598	
5	0.0197	31609.21	1604528	
			Average DPM/g soln	1651707
			SD	34749
			CV	2.10
			µCi/g soln	0.744

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

ASDN solution	mg ASDN added	(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	8.1384 g
Mass of dilution B used in substrate prep	4.5841 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563268 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00842 µg/g soln.
µg/g soln.

a. µCi/g soln 0.744
b. Specific activity of [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.

= 0.563268 + 0.00842
= 0.571690 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.301 µCi/µg ASDN

827457 dpm/nmol

Assay Date 9/6/2005 Test Chemical ID RC9 tested 8

ID TNB Replicate # Rep 2 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.545	0.336	0.157	0.103	0.065	0.051	0.043	25		
	0.560	0.344	0.158	0.100	0.062	0.051	0.044			
	0.553	0.337	0.155	0.093	0.063	0.048	0.036			
Samples:	10	100	microsomes							
	0.065	0.291	0.108							
	0.063	0.295	0.107							
			0.108							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.553	0.512	0.0483
0.125	100	200	0.00013	200	0.0250	0.339	0.298	0.0279
0.05	200	1000	0.00005	200	0.0100	0.157	0.116	0.0104
0.025	100	1000	0.00003	200	0.0050	0.099	0.058	0.0048
0.01	40	1000	0.00001	200	0.0020	0.063	0.022	0.0014
0.005	20	1000	0.00001	200	0.0010	0.050	0.009	0.0002

Blank 0.041

r^2= 0.993
m= 0.096
b= -0.001

Variables	Regression results	
m, b	0.096	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	592	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	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.065	0.024	0.002	200	1	1	0.000	0.000	0.008
10	0.063	0.022	0.001	200	1	1	0.000		
10					1	1			
100	0.291	0.250	0.023	200	1	1	0.000	0.000	0.117
100	0.295	0.254	0.024	200	1	1	0.000		
100									
	0.108	0.067	0.006	200	83	69983	0.024	0.024	24.126
	0.107	0.066	0.006	200	83	69983	0.024		
	0.108	0.067	0.006	200	83	69983	0.024		

Assay Date 9/6/2005 Test Chemical ID RC9 tested 8

ID TNB Replicate # Rep 2 Microsome type Placental Microsome ID Battelle

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.545	0.336	0.157	0.103	0.065	0.051	0.043	25	100
	0.560	0.344	0.158	0.100	0.062	0.051	0.044		
	0.553	0.337	0.155	0.093	0.063	0.048	0.036		

Protein stock ID

Samples:	10	100
	0.065	0.291
	0.063	0.295

microsomes
0.108
0.107
0.108

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.553	0.512	0.0428
0.125	100	200	0.00013	200	0.0250	0.339	0.298	0.0250
0.05	200	1000	0.00005	200	0.0100	0.157	0.116	0.0099
0.025	100	1000	0.00003	200	0.0050	0.099	0.058	0.0050
0.01	40	1000	0.00001	200	0.0020	0.063	0.022	0.0021
0.005	20	1000	0.00001	200	0.0010	0.050	0.009	0.0010

Blank 0.041

r^2= 1.000
m= 0.083
b= 0.000

Variables	Regression results	
m, b	0.083	0.000
se_m, se_b	0.000	0.000
r , se_y	1.000	0.000
F, df	43160	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	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.065	0.024	0.002	200	1	1	0.000	0.000	0.011
10	0.063	0.022	0.002	200	1	1	0.000		
10					1	1			
100	0.291	0.250	0.021	200	1	1	0.000	0.000	0.106
100	0.295	0.254	0.021	200	1	1	0.000		
100									
	0.108	0.067	0.006	200	83	69983	0.025	0.024	24.471
	0.107	0.066	0.006	200	83	69983	0.024		
	0.108	0.067	0.006	200	83	69983	0.025		

Assay Date	9/6/2005	Test Chemical ID	RC9	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

A_{raw}	A_{adj}	mg protein measured	µ µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/6/2005	Chemical ID	RC9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 2

Microsome Dilution Details

Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.16867	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.16867	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	24.471
Protein Concentration (dilution added to assay, mg/mL):	0.029023

Test Chemical Concentrations

Level	Final Concentration (M)
1	1.00E-03
2	3.00E-04
3	1.00E-04
4	3.00E-05
5	1.00E-05
6	3.00E-06
7	1.00E-06
8	1.00E-08

Assay Date	9/6/2005	Test Chemical ID	RC9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4328.01	8656.02	8722.71	17445.42	0.1	165171	10.56	17295	0.0209	1	0.015	15	0.0480
			0.5	2	4394.7	8789.4			0.1					1		15	
	2	2	0.5	1	4667.24	9334.48	9420.75	18841.5	0.1	165171	11.41	18691	0.0226	1	0.015	15	0.0519
			0.5	2	4753.51	9507.02			0.1					1		15	
	3	2	0.5	1	4221.1	8442.2	8472.3	16944.6	0.1	165171	10.26	16794	0.0203	1	0.015	15	0.0466
			0.5	2	4251.2	8502.4			0.1					1		15	
	4	2	0.5	1	4362.39	8724.78	8743.92	17487.84	0.1	165171	10.59	17338	0.0210	1	0.015	15	0.0481
			0.5	2	4381.53	8763.06			0.1					1		15	
Background control	1	2	0.5	1	43.48	86.96	83.64	167.28	0.1	165171	0.10	17	0.0000	1	0.015	15	0.0000
			0.5	2	40.16	80.32			0.1					1		15	
	2	2	0.5	1	36.61	73.22	63.2	126.4	0.1	165171	0.08	-24	0.0000	1	0.015	15	-0.0001
			0.5	2	26.59	53.18			0.1					1		15	
	3	2	0.5	1	43.33	86.66	84.93	169.86	0.1	165171	0.10	20	0.0000	1	0.015	15	0.0001
			0.5	2	41.6	83.2			0.1					1		15	
	4	2	0.5	1	34.77	69.54	68.44	136.98	0.1	165171	0.08	-13	0.0000	1	0.015	15	0.0000
			0.5	2	33.67	67.34			0.1					1		15	
Positive control	1	2	0.5	1	2441.47	4882.94	4942	9884	0.1	165171	5.98	9734	0.0118	1	0.015	15	0.0270
			0.5	2	2500.53	5001.06			0.1					1		15	
	2	2	0.5	1	2506.09	5012.18	4961.63	9923.26	0.1	165171	6.01	9773	0.0118	1	0.015	15	0.0271
			0.5	2	2455.54	4911.08			0.1					1		15	
	3	2	0.5	1	2379.05	4758.1	4783.86	9567.72	0.1	165171	5.79	9418	0.0114	1	0.015	15	0.0261
			0.5	2	2404.81	4809.62			0.1					1		15	
	4	2	0.5	1	2384.7	4769.4	4799.78	9599.56	0.1	165171	5.81	9449	0.0114	1	0.015	15	0.0262
			0.5	2	2415.08	4830.16			0.1					1		15	
Negative Control	1	2	0.5	1	4923.12	9846.24	9907.53	19815.06	0.1	165171	12.00	19665	0.0238	1	0.015	15	0.0546
			0.5	2	4984.41	9968.82			0.1					1		15	
	2	2	0.5	1	4485.94	8971.88	8944.77	17889.54	0.1	165171	10.83	17739	0.0214	1	0.015	15	0.0492
			0.5	2	4458.83	8917.66			0.1					1		15	
	3	2	0.5	1	4225.14	8450.28	8483.02	16966.04	0.1	165171	10.27	16816	0.0203	1	0.015	15	0.0467
			0.5	2	4257.88	8515.76			0.1					1		15	
	4	2	0.5	1	4001.26	8002.52	8075.26	16150.52	0.1	165171	9.78	16000	0.0193	1	0.015	15	0.0444
			0.5	2	4074	8148			0.1					1		15	
RC9	1-1	2	0.5	1	316.87	633.74	643.56	1287.12	0.1	165171	0.78	1137	0.0014	1	0.015	15	0.0032
			0.5	2	326.69	653.38			0.1					1		15	
	1-2	2	0.5	1	289.09	578.18	575.38	1150.76	0.1	165171	0.70	1001	0.0012	1	0.015	15	0.0028
			0.5	2	286.29	572.58			0.1					1		15	
	1-3	2	0.5	1	225.57	451.14	462.11	924.22	0.1	165171	0.56	774	0.0009	1	0.015	15	0.0021
			0.5	2	236.54	473.08			0.1					1		15	
	2-1	2	0.5	1	747.55	1495.1	1506.91	3013.82	0.1	165171	1.82	2864	0.0035	1	0.015	15	0.0079
			0.5	2	759.36	1518.72			0.1					1		15	
	2-2	2	0.5	1	921.8	1843.6	1904.64	3809.28	0.1	165171	2.31	3659	0.0044	1	0.015	15	0.0102
			0.5	2	982.84	1965.68			0.1					1		15	
	2-3	2	0.5	1	636.82	1273.64	1257.38	2514.76	0.1	165171	1.52	2365	0.0029	1	0.015	15	0.0066
			0.5	2	620.56	1241.12			0.1					1		15	
	3-1	2	0.5	1	1641.95	3283.9	3253.74	6507.48	0.1	165171	3.94	6357	0.0077	1	0.015	15	0.0176
			0.5	2	1611.79	3223.58			0.1					1		15	
	3-2	2	0.5	1	1650.05	3300.1	3306.83	6613.66	0.1	165171	4.00	6464	0.0078	1	0.015	15	0.0179
			0.5	2	1656.78	3313.56			0.1					1		15	
	3-3	2	0.5	1	1789.37	3578.74	3540.6	7081.2	0.1	165171	4.29	6931	0.0084	1	0.015	15	0.0192
			0.5	2	1751.23	3502.46			0.1					1		15	
	4-1	2	0.5	1	2911.45	5822.9	5800	11600	0.1	165171	7.02	11450	0.0138	1	0.015	15	0.0318
			0.5	2	2888.55	5777.1			0.1					1		15	
	4-2	2	0.5	1	3072.47	6144.94	6155.09	12310.18	0.1	165171	7.45	12160	0.0147	1	0.015	15	0.0338
			0.5	2	3082.62	6165.24			0.1					1		15	
	4-3	2	0.5	1	3033.6	6067.2	6021.64	12043.28	0.1	165171	7.29	11893	0.0144	1	0.015	15	0.0330
			0.5	2	2988.04	5976.08			0.1					1		15	
	5-1	2	0.5	1	3587.27	7174.54	7135.97	14271.94	0.1	165171	8.64	14122	0.0171	1	0.015	15	0.0392
			0.5	2	3548.7	7097.4			0.1					1		15	
	5-2	2	0.5	1	3294.5	6589	6553.72	13107.44	0.1	165171	7.94	12957	0.0157	1	0.015	15	0.0360
			0.5	2	3259.22	6518.44			0.1					1		15	
	5-3	2	0.5	1	3013.64	6027.28	5997.32	11994.64	0.1	165171	7.26	11845	0.0143	1	0.015	15	0.0329
			0.5	2	2983.68	5967.36			0.1					1		15	
	6-1	2	0.5	1	3742.01	7484.02	7578.01	15156.02	0.1	165171	9.18	15006	0.0181	1	0.015	15	0.0417
			0.5	2	3836	7672			0.1					1		15	
	6-2	2	0.5	1	3891.6	7783.2	7779.08	15558.16	0.1	165171	9.42	15408	0.0186	1	0.015	15	0.0428
			0.5	2	3887.48	7774.96			0.1					1		15	
	6-3	2	0.5	1	3723.7	7447.4	7497.73	14995.46	0.1	165171	9.08	14845	0.0179	1	0.015	15	0.0412
			0.5	2	3774.03	7548.06			0.1					1		15	
	7-1	2	0.5	1	4232.1	8464.2	8502.69	17005.38	0.1	165171	10.30	16855	0.0204	1	0.015	15	0.0468
			0.5	2	4270.59	8541.18			0.1					1		15	
	7-2	2	0.5	1	4317.49	8634.98	8574.1	17148.2	0.1	165171	10.38	16998	0.0205	1	0.015	15	0.0472
			0.5	2	4256.61	8513.22			0.1					1		15	
	7-3	2	0.5	1	4148.8	8297.6	8366.13	16732.26	0.1	165171	10.13	16582	0.0200	1	0.015	15	0.0460
			0.5	2	4217.33	8434.66			0.1					1		15	
	8-1	2	0.5	1	4504.78	9009.56	8929.37	17858.74	0.1	165171	10.81	17709	0.0214	1	0.015	15	0.0492
			0.5	2	4424.59	8849.18			0.1					1		15	
	8-2	2	0.5	1	4538.97	9077.94	9013.29	18026.58	0.1	165171	10.91	17876	0.0216	1	0.015	15	0.0496
			0.5	2	4474.32	8948.64			0.1					1		15	
	8-3	2	0.5	1	4691.77	9383.54	9349.59	18699.18	0.1	165171	11.32	18549	0.0224	1	0.015	15	0.0515
			0.5	2	4657.82	9315.64			0.1					1		15	

Assay Date	9/6/2005	ID	RC9	# Concentrations tested	Microsome 8 type	Placental	Microsome ID Battelle	Technician ID TNB	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0500	0.0027
Full activity	End	0.0474	0.0011
Full activity	Overall	0.0487	0.0023
Background	Beginning	0.0000	8.02458E-05
Background	End	0.0000	6.47384E-05
Background	Overall	0.0000	6.04402E-05
Positive	Beginning	0.0271	0.0001
Positive	End	0.0262	0.0001
Positive	Overall	0.0266	0.0005
Negative	Beginning	0.0519	0.0038
Negative	End	0.0455	0.0016
Negative	Overall	0.0487	0.0044

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC9	1	1	1.00E-03	-3.00	0.0032
RC9	1	2	1.00E-03	-3.00	0.0028
RC9	1	3	1.00E-03	-3.00	0.0021
RC9	2	1	3.00E-04	-3.52	0.0079
RC9	2	2	3.00E-04	-3.52	0.0102
RC9	2	3	3.00E-04	-3.52	0.0066
RC9	3	1	1.00E-04	-4.00	0.0176
RC9	3	2	1.00E-04	-4.00	0.0179
RC9	3	3	1.00E-04	-4.00	0.0192
RC9	4	1	3.00E-05	-4.52	0.0318
RC9	4	2	3.00E-05	-4.52	0.0338
RC9	4	3	3.00E-05	-4.52	0.0330
RC9	5	1	1.00E-05	-5.00	0.0392
RC9	5	2	1.00E-05	-5.00	0.0360
RC9	5	3	1.00E-05	-5.00	0.0329
RC9	6	1	3.00E-06	-5.52	0.0417
RC9	6	2	3.00E-06	-5.52	0.0428
RC9	6	3	3.00E-06	-5.52	0.0412
RC9	7	1	1.00E-06	-6.00	0.0468
RC9	7	2	1.00E-06	-6.00	0.0472
RC9	7	3	1.00E-06	-6.00	0.0460
RC9	8	1	1.00E-08	-8.00	0.0492
RC9	8	2	1.00E-08	-8.00	0.0496
RC9	8	3	1.00E-08	-8.00	0.0515

Percent of control values				
	Log[test	Replicate		
_evel	bstance]	1	2	3
1	-3.00	6.49	5.71	4.42
2	-3.52	16.34	20.87	13.49
3	-4.00	36.27	36.87	39.54
4	-4.52	65.32	69.37	67.85
5	-5.00	80.56	73.92	67.57
6	-5.52	85.60	87.90	84.69
7	-6.00	96.15	96.97	94.59
8	-8.00	101.02	101.98	105.81

Aromatase Assay Spreadsheet

Assay Date	9/8/2005	Test Chemical ID	RC9	# Concentrations tested	8	
ID	TNB	Replicate #	Rep 3	Microsome type	Placental	Microsome ID Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0200	34738.93	1736947		
2	0.0204	35752.88	1752592		
3	0.0201	35930.01	1787563		
4	0.0202	35142.39	1739722		
5	0.0205	35842.44	1748412		
			Average DPM/g soln		1753047
			SD		20309
			CV		1.16
			µCi/g soln		0.790

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

ASDN solution	mg ASDN added	(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	8.0602 g
Mass of dilution B used in substrate prep	4.5954 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.575836 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00894 µg/g soln.
µg/g soln.

a. µCi/g soln	0.790
b. Specific activity of [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.

= 0.575836 + 0.00894
= 0.584775 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.350 µCi/µg ASDN

858574 dpm/nmol

Test

Assay Date 9/8/2005 Chemical ID RC9 tested 8

ID TNB Replicate # Rep 3 Microsome type Placental Microsome ID Battelle

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.572	0.339	0.151	0.087	0.056	0.040	0.031	25	100	
	0.575	0.357	0.156	0.090	0.051	0.039	0.033			
	0.580	0.355	0.153	0.093	0.053	0.040	0.033			
Samples:	10	100	microsomes							
	0.053	0.285	0.109							
	0.057	0.290	0.111							
			0.110							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.576	0.543	0.0482
0.125	100	200	0.00013	200	0.0250	0.350	0.318	0.0280
0.05	200	1000	0.00005	200	0.0100	0.153	0.121	0.0104
0.025	100	1000	0.00003	200	0.0050	0.090	0.058	0.0047
0.01	40	1000	0.00001	200	0.0020	0.053	0.021	0.0014
0.005	20	1000	0.00001	200	0.0010	0.040	0.007	0.0002

Blank 0.032

r^2= 0.993
m= 0.090
b= 0.000

Variables	Regression results	
m, b	0.090	0.000
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	531	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	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.053	0.021	0.001	200	1	1	0.000	0.000	0.008
10	0.057	0.025	0.002	200	1	1	0.000		
10					1	1			
100	0.285	0.253	0.022	200	1	1	0.000	0.000	0.112
100	0.290	0.258	0.023	200	1	1	0.000		
100									
	0.109	0.077	0.006	200	83	69983	0.027	0.027	27.456
	0.111	0.079	0.007	200	83	69983	0.028		
	0.110	0.078	0.007	200	83	69983	0.027		

Test

Assay Date 9/8/2005 Chemical ID RC9 tested 8

ID TNB Replicate # Rep 3 Microsome type Placental Microsome ID Battelle

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.572	0.339	0.151	0.087	0.056	0.040	0.031	25	100	
	0.575	0.357	0.156	0.090	0.051	0.039	0.033			
	0.580	0.355	0.153	0.093	0.053	0.040	0.033			

Samples:	10	100	microsomes
	0.053	0.285	0.109
	0.057	0.290	0.111
			0.110

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
0.25	200	200	0.00025	200	0.0500	0.576	0.543	0.0425	m, b	0.077	0.000
0.125	100	200	0.00013	200	0.0250	0.350	0.318	0.0251	se_m, se_b	0.000	0.000
0.05	200	1000	0.00005	200	0.0100	0.153	0.121	0.0098	r , se_y	1.000	0.000
0.025	100	1000	0.00003	200	0.0050	0.090	0.058	0.0049	F, df	23945	3
0.01	40	1000	0.00001	200	0.0020	0.053	0.021	0.0021	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.040	0.007	0.0011			

Blank 0.032

r^2= 1.000
m= 0.077
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µL microsome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.053	0.021	0.002	200	1	1	0.000	0.000	0.011
10	0.057	0.025	0.002	200	1	1	0.000		
10					1	1			
100	0.285	0.253	0.020	200	1	1	0.000	0.000	0.101
100	0.290	0.258	0.020	200	1	1	0.000		
100									
	0.109	0.077	0.006	200	83	69983	0.027	0.027	27.349
	0.111	0.079	0.007	200	83	69983	0.028		
	0.110	0.078	0.006	200	83	69983	0.027		

Assay Date	9/8/2005	Test Chemical ID	RC9	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	Battelle

								Protein stock (mg/10 mL)	Protein stock ID
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk		
Samples:									

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/8/2005	Chemical ID	RC9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 3

Microsome Dilution Details

Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.1687	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.1687	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	27.349
Protein Concentration (dilution added to assay, mg/mL):	0.032436

Test Chemical Concentrations

Level	Final Concentration (M)
1	1.00E-03
2	3.00E-04
3	1.00E-04
4	3.00E-05
5	1.00E-05
6	3.00E-06
7	1.00E-06
8	1.00E-08

Assay Date	9/8/2005	Test Chemical ID	RC9	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	7405.83	14811.66	14782.15	29564.3	0.1	175305	16.86	28493	0.0332	1	0.016	15	0.0682
			0.5	2	7376.32	14752.64			0.1					1		15	
	2	2	0.5	1	6775.29	13550.58	13603.31	27206.62	0.1	175305	15.52	26135	0.0304	1	0.016	15	0.0626
			0.5	2	6828.02	13656.04			0.1					1		15	
	3	2	0.5	1	6345.43	12690.86	12626.03	25252.06	0.1	175305	14.40	24180	0.0282	1	0.016	15	0.0579
			0.5	2	6280.6	12561.2			0.1					1		15	
	4	2	0.5	1	6350.22	12700.44	12593.19	25186.38	0.1	175305	14.37	24115	0.0281	1	0.016	15	0.0577
			0.5	2	6242.97	12485.94			0.1					1		15	
Background control	1	2	0.5	1	32.52	65.04	73.21	146.42	0.1	175305	0.08	-925	-0.0011	1	0.016	15	-0.0022
			0.5	2	40.69	81.38			0.1					1		15	
	2	2	0.5	1	82.15	164.3	148.14	296.28	0.1	175305	0.17	-775	-0.0009	1	0.016	15	-0.0019
			0.5	2	65.99	131.98			0.1					1		15	
	3	2	0.5	1	183.38	366.76	384.45	768.9	0.1	175305	0.44	-303	-0.0004	1	0.016	15	-0.0007
			0.5	2	201.07	402.14			0.1					1		15	
	4	2	0.5	1	773.31	1546.62	1537.64	3075.28	0.1	175305	1.75	2004	0.0023	1	0.016	15	0.0048
			0.5	2	764.33	1528.66			0.1					1		15	
Positive control	1	2	0.5	1	2952.98	5905.96	5869.36	11738.72	0.1	175305	6.70	10667	0.0124	1	0.016	15	0.0255
			0.5	2	2916.38	5832.76			0.1					1		15	
	2	2	0.5	1	2840.56	5681.12	5697.15	11394.3	0.1	175305	6.50	10323	0.0120	1	0.016	15	0.0247
			0.5	2	2856.59	5713.18			0.1					1		15	
	3	2	0.5	1	2761.35	5522.7	5548.3	11096.6	0.1	175305	6.33	10025	0.0117	1	0.016	15	0.0240
			0.5	2	2786.95	5573.9			0.1					1		15	
	4	2	0.5	1	2708.23	5416.46	5481.86	10963.72	0.1	175305	6.25	9892	0.0115	1	0.016	15	0.0237
			0.5	2	2773.63	5547.26			0.1					1		15	
Negative Control	1	2	0.5	1	6732.06	13464.12	13446.64	26893.28	0.1	175305	15.34	25822	0.0301	1	0.016	15	0.0618
			0.5	2	6714.58	13429.16			0.1					1		15	
	2	2	0.5	1	3877.12	7754.24	7694.49	15388.98	0.1	175305	8.78	14317	0.0167	1	0.016	15	0.0343
			0.5	2	3817.37	7634.74			0.1					1		15	
	3	2	0.5	1	6079.51	12159.02	12104.14	24208.28	0.1	175305	13.81	23137	0.0269	1	0.016	15	0.0554
			0.5	2	6024.63	12049.26			0.1					1		15	
	4	2	0.5	1	6609.23	13218.46	13302.42	26604.84	0.1	175305	15.18	25533	0.0297	1	0.016	15	0.0611
			0.5	2	6693.19	13386.38			0.1					1		15	
RC9	1-1	2	0.5	1	247.13	494.26	498.29	996.58	0.1	175305	0.57	-75	-0.0001	1	0.016	15	-0.0002
			0.5	2	251.16	502.32			0.1					1		15	
	1-2	2	0.5	1	330.89	661.78	658.31	1316.62	0.1	175305	0.75	245	0.0003	1	0.016	15	0.0006
			0.5	2	327.42	654.84			0.1					1		15	
	1-3	2	0.5	1	257.99	515.98	516.5	1033	0.1	175305	0.59	-39	0.0000	1	0.016	15	-0.0001
			0.5	2	258.51	517.02			0.1					1		15	
	2-1	2	0.5	1	1215.36	2430.72	2398.35	4796.7	0.1	175305	2.74	3725	0.0043	1	0.016	15	0.0089
			0.5	2	1182.99	2365.98			0.1					1		15	
	2-2	2	0.5	1	759.31	1518.62	1519.22	3038.44	0.1	175305	1.73	1967	0.0023	1	0.016	15	0.0047
			0.5	2	759.91	1519.82			0.1					1		15	
	2-3	2	0.5	1	809.25	1618.5	1641.81	3283.62	0.1	175305	1.87	2212	0.0026	1	0.016	15	0.0053
			0.5	2	832.56	1665.12			0.1					1		15	
	3-1	2	0.5	1	2307.1	4614.2	4582.31	9164.62	0.1	175305	5.23	8093	0.0094	1	0.016	15	0.0194
			0.5	2	2275.21	4550.42			0.1					1		15	
	3-2	2	0.5	1	1920.98	3841.96	3847.69	7695.38	0.1	175305	4.39	6624	0.0077	1	0.016	15	0.0159
			0.5	2	1926.71	3853.42			0.1					1		15	
	3-3	2	0.5	1	2208.5	4417	4372.57	8745.14	0.1	175305	4.99	7673	0.0089	1	0.016	15	0.0184
			0.5	2	2164.07	4328.14			0.1					1		15	
	4-1	2	0.5	1	3521.48	7042.96	6990.34	13980.68	0.1	175305	7.98	12909	0.0150	1	0.016	15	0.0309
			0.5	2	3468.86	6937.72			0.1					1		15	
	4-2	2	0.5	1	3536.4	7072.8	7156.96	14313.92	0.1	175305	8.17	13242	0.0154	1	0.016	15	0.0317
			0.5	2	3620.56	7241.12			0.1					1		15	
	4-3	2	0.5	1	4134.67	8269.34	8195.79	16371.58	0.1	175305	9.34	15300	0.0178	1	0.016	15	0.0366
			0.5	2	4051.12	8102.24			0.1					1		15	
	5-1	2	0.5	1	4607.8	9215.6	9094.33	18188.66	0.1	175305	10.38	17117	0.0199	1	0.016	15	0.0410
			0.5	2	4486.53	8973.06			0.1					1		15	
	5-2	2	0.5	1	3823.57	7647.14	7744.7	15489.4	0.1	175305	8.84	14418	0.0168	1	0.016	15	0.0345
			0.5	2	3921.13	7842.26			0.1					1		15	
	5-3	2	0.5	1	4278.4	8556.8	8548.36	17096.72	0.1	175305	9.75	16025	0.0187	1	0.016	15	0.0384
			0.5	2	4269.96	8539.92			0.1					1		15	
	6-1	2	0.5	1	5588.93	11177.86	11269.25	22538.5	0.1	175305	12.86	21467	0.0250	1	0.016	15	0.0514
			0.5	2	5680.32	11360.64			0.1					1		15	
	6-2	2	0.5	1	5695.22	11390.44	11567.49	23134.98	0.1	175305	13.20	22063	0.0257	1	0.016	15	0.0528
			0.5	2	5872.27	11744.54			0.1					1		15	
	6-3	2	0.5	1	5447.83	10895.66	10909.9	21819.8	0.1	175305	12.45	20748	0.0242	1	0.016	15	0.0497
			0.5	2	5462.07	10924.14			0.1					1		15	
	7-1	2	0.5	1	6093.45	12186.9	12179.91	24359.82	0.1	175305	13.90	23288	0.0271	1	0.016	15	0.0557
			0.5	2	6086.46	12172.92			0.1					1		15	
	7-2	2	0.5	1	5785.47	11570.94	11732.75	23465.5	0.1	175305	13.39	22394	0.0261	1	0.016	15	0.0536
			0.5	2	5947.28	11894.56			0.1					1		15	
	7-3	2	0.5	1	6126.82	12253.64	12243.1	24496.2	0.1	175305	13.97	23414	0.0273	1	0.016	15	0.0561
			0.5	2	6116.28	12232.56			0.1					1		15	
	8-1	2	0.5	1	6430.75	12861.5	13011.04	26022.08	0.1	175305	14.84	24950	0.0291	1	0.016	15	0.0597
			0.5	2	6580.29	13160.58			0.1					1		15	
	8-2	2	0.5	1	6562.09	13124.18	13234.83	26469.66	0.1	175305	15.10	25398	0.0296	1	0.016	15	0.0608
			0.5	2	6672.74	13345.48			0.1					1		15	
	8-3	2	0.5	1	6654.29	13308.58	13263.11	26526.22	0.1	175305	15.13	25455	0.0296	1	0.016	15	0.0609
			0.5	2	6608.82	13217.64			0.1					1		15	

Assay Date	9/8/2005	ID	RC9	# Concentrations tested	Microsome 8 type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0654	0.0040
Full activity	End	0.0578	0.0001
Full activity	Overall	0.0616	0.0049
Background	Beginning	-0.0020	0.000253673
Background	End	0.0020	0.003904091
Background	Overall	0.0000	0.003259982
Positive	Beginning	0.0251	0.0006
Positive	End	0.0238	0.0002
Positive	Overall	0.0245	0.0008
Negative	Beginning	0.0480	0.0195
Negative	End	0.0583	0.0041
Negative	Overall	0.0531	0.0129

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC9	1	1	1.00E-03	-3.00	-0.0002
RC9	1	2	1.00E-03	-3.00	0.0006
RC9	1	3	1.00E-03	-3.00	-0.0001
RC9	2	1	3.00E-04	-3.52	0.0089
RC9	2	2	3.00E-04	-3.52	0.0047
RC9	2	3	3.00E-04	-3.52	0.0053
RC9	3	1	1.00E-04	-4.00	0.0194
RC9	3	2	1.00E-04	-4.00	0.0159
RC9	3	3	1.00E-04	-4.00	0.0184
RC9	4	1	3.00E-05	-4.52	0.0309
RC9	4	2	3.00E-05	-4.52	0.0317
RC9	4	3	3.00E-05	-4.52	0.0366
RC9	5	1	1.00E-05	-5.00	0.0410
RC9	5	2	1.00E-05	-5.00	0.0345
RC9	5	3	1.00E-05	-5.00	0.0384
RC9	6	1	3.00E-06	-5.52	0.0514
RC9	6	2	3.00E-06	-5.52	0.0528
RC9	6	3	3.00E-06	-5.52	0.0497
RC9	7	1	1.00E-06	-6.00	0.0557
RC9	7	2	1.00E-06	-6.00	0.0536
RC9	7	3	1.00E-06	-6.00	0.0561
RC9	8	1	1.00E-08	-8.00	0.0597
RC9	8	2	1.00E-08	-8.00	0.0608
RC9	8	3	1.00E-08	-8.00	0.0609

Percent of control values				
	Log[test	Replicate		
_evel	bstance]	1	2	3
1	-3.00	-0.29	0.95	-0.15
2	-3.52	14.48	7.64	8.60
3	-4.00	31.45	25.74	29.82
4	-4.52	50.17	51.46	59.46
5	-5.00	66.52	56.03	62.28
6	-5.52	83.43	85.75	80.64
7	-6.00	90.51	87.03	91.00
8	-8.00	96.97	98.71	98.93

Aromatase Assay Spreadsheet

Assay Date	9/1/2005	Test Chemical ID	RC10	# Concentrations tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	39616.25	1970958	
2	0.0202	40379.66	1998993	
3	0.0202	40547.64	2007309	
4	0.0201	40768.21	2028269	
5	0.0201	40960.95	2037858	
			Average DPM/g soln	2008677
			SD	26238
			CV	1.31
			µCi/g soln	0.905

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

ASDN solution	mg ASDN added	(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	8.1716 g
Mass of dilution B used in substrate prep	4.6284 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.577729 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01024 µg/g soln.
µg/g soln.

a. µCi/g soln	0.905
b. Specific activity of [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.

= 0.577729 + 0.01024
= 0.587971 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.539 µCi/µg ASDN

978424 dpm/nmol

Assay Date 9/1/2005 Test Chemical ID RC10 tested 8

ID TNB Replicate # Rep 1 Microsome type Placental Microsome ID Battelle

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.603	0.360	0.156	0.097	0.055	0.049	0.039	25	100	
	0.610	0.373	0.161	0.101	0.058	0.047	0.037			
	0.587	0.391	0.160	0.105	0.060	0.049	0.036			

Samples:	10	100	microsomes
	0.074	0.294	0.106
	0.067	0.303	0.106
			0.106

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.600	0.563	0.0479
0.125	100	200	0.00013	200	0.0250	0.375	0.337	0.0285
0.05	200	1000	0.00005	200	0.0100	0.159	0.122	0.0099
0.025	100	1000	0.00003	200	0.0050	0.101	0.064	0.0049
0.01	40	1000	0.00001	200	0.0020	0.058	0.020	0.0012
0.005	20	1000	0.00001	200	0.0010	0.048	0.011	0.0004

Blank 0.037

r^2= 0.990
m= 0.086
b= -0.001

Variables	Regression results	
m, b	0.086	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.990	0.002
F, df	408	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol. usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.074	0.037	0.003	200	1	1	0.000	0.000	0.012
10	0.067	0.030	0.002	200	1	1	0.000		
10					1	1			
100	0.294	0.257	0.022	200	1	1	0.000	0.000	0.110
100	0.303	0.266	0.022	200	1	1	0.000		
100									
	0.106	0.068	0.005	200	83	69983	0.023	0.023	22.682
	0.106	0.069	0.005	200	83	69983	0.023		
	0.106	0.069	0.005	200	83	69983	0.023		

Test

Assay Date 9/1/2005 Chemical ID RC10 tested 8

ID TNB Replicate # Rep 1 Microsome type Placental Microsome ID Battelle

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.603	0.360	0.156	0.097	0.055	0.049	0.039	25		
	0.610	0.373	0.161	0.101	0.058	0.047	0.037			
	0.587	0.391	0.160	0.105	0.060	0.049	0.036			

Samples:	10	100	microsomes
	0.074	0.294	0.106
	0.067	0.303	0.106
			0.106

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.600	0.563	0.0417
0.125	100	200	0.00013	200	0.0250	0.375	0.337	0.0252
0.05	200	1000	0.00005	200	0.0100	0.159	0.122	0.0094
0.025	100	1000	0.00003	200	0.0050	0.101	0.064	0.0051
0.01	40	1000	0.00001	200	0.0020	0.058	0.020	0.0020
0.005	20	1000	0.00001	200	0.0010	0.048	0.011	0.0013

Blank 0.037

r^2= 0.999
m= 0.073
b= 0.000

Variables	Regression results	
m, b	0.073	0.000
se_m, se_b	0.002	0.000
r , se_y	0.999	0.000
F, df	2259	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	µL microsomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.074	0.037	0.003	200	1	1	0.000	0.000	0.015
10	0.067	0.030	0.003	200	1	1	0.000		
10					1	1			
100	0.294	0.257	0.019	200	1	1	0.000	0.000	0.098
100	0.303	0.266	0.020	200	1	1	0.000		
100									
	0.106	0.068	0.005	200	83	69983	0.023	0.023	23.249
	0.106	0.069	0.006	200	83	69983	0.023		
	0.106	0.069	0.006	200	83	69983	0.023		

Assay Date	9/1/2005	Test Chemical ID	RC10	tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Placental	Microsome ID	Battelle

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/1/2005	Chemical ID	RC10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Microsome Dilution Details

Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.1687	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.1687	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	23.249
Protein Concentration (dilution added to assay, mg/mL):	0.027573

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	9/1/2005	Test Chemical ID	RC10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	6477.06	12954.12	12831.65	25663.3	0.1	200868	12.78	25196	0.0258	1	0.014	15	0.0623
			0.5	2	6354.59	12709.18			0.1					1		15	
	2	2	0.5	1	6523.72	13047.44	13071.66	26143.32	0.1	200868	13.02	25676	0.0262	1	0.014	15	0.0634
			0.5	2	6547.94	13095.88			0.1					1		15	
	3	2	0.5	1	6186.98	12373.96	12382.7	24765.4	0.1	200868	12.33	24296	0.0248	1	0.014	15	0.0600
			0.5	2	6195.72	12391.44			0.1					1		15	
	4	2	0.5	1	6403.57	12807.14	12786.35	25572.7	0.1	200868	12.73	25106	0.0257	1	0.014	15	0.0620
			0.5	2	6392.78	12765.56			0.1					1		15	
Background control	1	2	0.5	1	114.71	229.42	235.02	470.04	0.1	200868	0.23	3	0.0000	1	0.014	15	0.0000
			0.5	2	120.31	240.62			0.1					1		15	
	2	2	0.5	1	96.66	193.32	181.44	362.88	0.1	200868	0.18	-104	-0.0001	1	0.014	15	-0.0003
			0.5	2	84.78	169.56			0.1					1		15	
	3	2	0.5	1	93.09	186.18	193.35	386.7	0.1	200868	0.19	-80	-0.0001	1	0.014	15	-0.0002
			0.5	2	100.26	200.52			0.1					1		15	
	4	2	0.5	1	155.29	310.58	324.23	648.46	0.1	200868	0.32	181	0.0002	1	0.014	15	0.0004
			0.5	2	168.94	337.88			0.1					1		15	
Positive control	1	2	0.5	1	2663.2	5326.4	5280.4	10560.8	0.1	200868	5.26	10094	0.0103	1	0.014	15	0.0249
			0.5	2	2617.2	5234.4			0.1					1		15	
	2	2	0.5	1	3349.55	6699.1	6749.92	13499.84	0.1	200868	6.72	13033	0.0133	1	0.014	15	0.0322
			0.5	2	3400.37	6800.74			0.1					1		15	
	3	2	0.5	1	2532.43	5064.86	5241.05	10482.1	0.1	200868	5.22	10015	0.0102	1	0.014	15	0.0247
			0.5	2	2708.62	5417.24			0.1					1		15	
	4	2	0.5	1	2634.98	5269.96	5231.36	10462.72	0.1	200868	5.21	9996	0.0102	1	0.014	15	0.0247
			0.5	2	2596.38	5192.76			0.1					1		15	
Negative Control	1	2	0.5	1	6492.55	12985.1	12944.79	25889.58	0.1	200868	12.89	25423	0.0260	1	0.014	15	0.0628
			0.5	2	6452.24	12904.48			0.1					1		15	
	2	2	0.5	1	6406.61	12813.22	12662.39	25324.78	0.1	200868	12.61	24858	0.0254	1	0.014	15	0.0614
			0.5	2	6255.78	12511.56			0.1					1		15	
	3	2	0.5	1	5884.66	11768.32	11800.23	23600.46	0.1	200868	11.75	23133	0.0236	1	0.014	15	0.0572
			0.5	2	5915.57	11831.14			0.1					1		15	
	4	2	0.5	1	5889.11	11778.22	11762.63	23525.26	0.1	200868	11.71	23058	0.0236	1	0.014	15	0.0570
			0.5	2	5873.52	11747.04			0.1					1		15	
RC10	1-1	2	0.5	1	4619.86	9239.72	9199.86	18399.72	0.1	200868	9.16	17933	0.0183	1	0.014	15	0.0443
			0.5	2	4580	9160			0.1					1		15	
	1-2	2	0.5	1	5199.16	10398.32	10584.15	21168.3	0.1	200868	10.54	20701	0.0212	1	0.014	15	0.0512
			0.5	2	5384.99	10769.98			0.1					1		15	
	1-3	2	0.5	1	5021.2	10042.4	10044.35	20088.7	0.1	200868	10.00	19622	0.0201	1	0.014	15	0.0485
			0.5	2	5023.15	10046.3			0.1					1		15	
	2-1	2	0.5	1	6074.75	12149.5	12070.42	24140.84	0.1	200868	12.02	23674	0.0242	1	0.014	15	0.0585
			0.5	2	5995.67	11991.34			0.1					1		15	
	2-2	2	0.5	1	6240.81	12481.62	12418.34	24836.68	0.1	200868	12.36	24370	0.0249	1	0.014	15	0.0602
			0.5	2	6177.53	12355.06			0.1					1		15	
	2-3	2	0.5	1	6079.88	12159.76	12190.43	24380.86	0.1	200868	12.14	23914	0.0244	1	0.014	15	0.0591
			0.5	2	6110.55	12221.1			0.1					1		15	
	3-1	2	0.5	1	6463.68	12927.36	12913.35	25826.7	0.1	200868	12.86	25360	0.0259	1	0.014	15	0.0627
			0.5	2	6449.67	12899.34			0.1					1		15	
	3-2	2	0.5	1	6243.16	12486.32	12439.54	24879.08	0.1	200868	12.39	24412	0.0250	1	0.014	15	0.0603
			0.5	2	6196.38	12392.76			0.1					1		15	
	3-3	2	0.5	1	6228.25	12456.5	12492.27	24984.54	0.1	200868	12.44	24518	0.0251	1	0.014	15	0.0606
			0.5	2	6264.02	12528.04			0.1					1		15	
	4-1	2	0.5	1	6516.67	13033.34	13012.66	26025.32	0.1	200868	12.96	25558	0.0261	1	0.014	15	0.0632
			0.5	2	6495.99	12991.98			0.1					1		15	
	4-2	2	0.5	1	6385.76	12771.52	12715.18	25430.36	0.1	200868	12.66	24963	0.0255	1	0.014	15	0.0617
			0.5	2	6329.42	12658.84			0.1					1		15	
	4-3	2	0.5	1	6585.32	13170.64	13335.3	26670.6	0.1	200868	13.28	26204	0.0268	1	0.014	15	0.0648
			0.5	2	6749.98	13499.96			0.1					1		15	
	5-1	2	0.5	1	6241.76	12483.52	12684.22	25368.44	0.1	200868	12.63	24901	0.0255	1	0.014	15	0.0615
			0.5	2	6442.46	12884.92			0.1					1		15	
	5-2	2	0.5	1	6159.52	12319.04	12368.58	24737.16	0.1	200868	12.32	24270	0.0248	1	0.014	15	0.0600
			0.5	2	6209.06	12418.12			0.1					1		15	
	5-3	2	0.5	1	6208.18	12416.36	12607.53	25215.06	0.1	200868	12.55	24748	0.0253	1	0.014	15	0.0612
			0.5	2	6399.35	12798.7			0.1					1		15	
	6-1	2	0.5	1	6591.76	13183.52	13211.26	26422.52	0.1	200868	13.15	25956	0.0265	1	0.014	15	0.0641
			0.5	2	6619.5	13239			0.1					1		15	
	6-2	2	0.5	1	6857.2	13714.4	13697.96	27395.92	0.1	200868	13.64	26929	0.0275	1	0.014	15	0.0665
			0.5	2	6840.76	13681.52			0.1					1		15	
	6-3	2	0.5	1	6413.32	12826.64	12654.86	25309.72	0.1	200868	12.60	24843	0.0254	1	0.014	15	0.0614
			0.5	2	6241.54	12483.08			0.1					1		15	
	7-1	2	0.5	1	6339.89	12679.78	12599.84	25199.68	0.1	200868	12.55	24733	0.0253	1	0.014	15	0.0611
			0.5	2	6259.95	12519.9			0.1					1		15	
	7-2	2	0.5	1	6553.2	13106.4	12878.79	25757.58	0.1	200868	12.82	25291	0.0258	1	0.014	15	0.0625
			0.5	2	6325.59	12651.18			0.1					1		15	
	7-3	2	0.5	1	6372.9	12745.8	12639.14	25278.28	0.1	200868	12.58	24811	0.0254	1	0.014	15	0.0613
			0.5	2	6266.24	12532.48			0.1					1		15	
	8-1	2	0.5	1	6400.5	12801	12887.96	25775.92	0.1	200868	12.83	25309	0.0258	1	0.014	15	0.0625
			0.5	2	6487.46	12974.92			0.1					1		15	
	8-2	2	0.5	1	6251.89	12503.78	12531.99	25063.98	0.1	200868	12.48	24597	0.0251	1	0.014	15	0.0608
			0.5	2	6280.1	12560.2			0.1					1		15	
	8-3	2	0.5	1	6194.19	12388.38	12478.91	24957.82	0.1	200868	12.43	24491	0.0250	1	0.014	15	0.0605
			0.5	2	6284.72	12569.44			0.1					1		15	

Assay Date	9/1/2005	ID	RC10	# Concentrations tested	Microsome 8 type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.0629	0.0008
Full activity	End	0.0610	0.0014
Full activity	Overall	0.0619	0.0014
Background	Beginning	-0.0001	0.000187245
Background	End	0.0001	0.000457383
Background	Overall	0.0000	0.000319739
Positive	Beginning	0.0286	0.0051
Positive	End	0.0247	0.0000
Positive	Overall	0.0266	0.0037
Negative	Beginning	0.0621	0.0010
Negative	End	0.0571	0.0001
Negative	Overall	0.0596	0.0030

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC10	1	1	1.00E-03	-3.00	0.0443
RC10	1	2	1.00E-03	-3.00	0.0512
RC10	1	3	1.00E-03	-3.00	0.0485
RC10	2	1	1.00E-04	-4.00	0.0585
RC10	2	2	1.00E-04	-4.00	0.0602
RC10	2	3	1.00E-04	-4.00	0.0591
RC10	3	1	1.00E-05	-5.00	0.0627
RC10	3	2	1.00E-05	-5.00	0.0603
RC10	3	3	1.00E-05	-5.00	0.0606
RC10	4	1	1.00E-06	-6.00	0.0632
RC10	4	2	1.00E-06	-6.00	0.0617
RC10	4	3	1.00E-06	-6.00	0.0648
RC10	5	1	1.00E-07	-7.00	0.0615
RC10	5	2	1.00E-07	-7.00	0.0600
RC10	5	3	1.00E-07	-7.00	0.0612
RC10	6	1	1.00E-08	-8.00	0.0641
RC10	6	2	1.00E-08	-8.00	0.0665
RC10	6	3	1.00E-08	-8.00	0.0614
RC10	7	1	1.00E-09	-9.00	0.0611
RC10	7	2	1.00E-09	-9.00	0.0625
RC10	7	3	1.00E-09	-9.00	0.0613
RC10	8	1	1.00E-10	-10.00	0.0625
RC10	8	2	1.00E-10	-10.00	0.0608
RC10	8	3	1.00E-10	-10.00	0.0605

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-3.00	71.53	82.58	78.27
2	-4.00	94.43	97.21	95.39
3	-5.00	101.16	97.38	97.80
4	-6.00	101.95	99.58	104.53
5	-7.00	99.33	96.81	98.72
6	-8.00	103.54	107.42	99.10
7	-9.00	98.66	100.88	98.97
8	-10.00	100.96	98.12	97.69

Aromatase Assay Spreadsheet

Assay Date	9/6/2005	Test Chemical ID	RC10	# Concentrations tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	33233.57	1678463	
2	0.0199	33635.76	1690239	
3	0.0198	32307.83	1631709	
4	0.0198	32741.24	1653598	
5	0.0197	31609.21	1604528	
			Average DPM/g soln	1651707
			SD	34749
			CV	2.10
			µCi/g soln	0.744

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

ASDN solution	mg ASDN added	(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	8.1384 g
Mass of dilution B used in substrate prep	4.5841 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563268 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00842 µg/g soln.
µg/g soln.

a. µCi/g soln	0.744
b. Specific activity of [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.

= 0.563268 + 0.00842
= 0.571690 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.301 µCi/µg ASDN

827457 dpm/nmol

Assay Date	9/6/2005	Test Chemical ID	RC10	tested	8				
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle		
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.545	0.336	0.157	0.103	0.065	0.051	0.043	25	100
	0.560	0.344	0.158	0.100	0.062	0.051	0.044		
	0.553	0.337	0.155	0.093	0.063	0.048	0.036		
Samples:	10	100	microsomes						
	0.065	0.291	0.108						
	0.063	0.295	0.107						
			0.108						

Protein stock ID

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.553	0.512	0.0483
0.125	100	200	0.00013	200	0.0250	0.339	0.298	0.0279
0.05	200	1000	0.00005	200	0.0100	0.157	0.116	0.0104
0.025	100	1000	0.00003	200	0.0050	0.099	0.058	0.0048
0.01	40	1000	0.00001	200	0.0020	0.063	0.022	0.0014
0.005	20	1000	0.00001	200	0.0010	0.050	0.009	0.0002

Variables	Regression results	
m, b	0.096	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	592	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank 0.041

r^2= 0.993
m= 0.096
b= -0.001

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol. usomes prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.065	0.024	0.002	200	1	1	0.000	0.000	0.008
10	0.063	0.022	0.001	200	1	1	0.000		
10					1	1			
100	0.291	0.250	0.023	200	1	1	0.000	0.000	0.117
100	0.295	0.254	0.024	200	1	1	0.000		
100									
	0.108	0.067	0.006	200	83	69983	0.024	0.024	24.126
	0.107	0.066	0.006	200	83	69983	0.024		
	0.108	0.067	0.006	200	83	69983	0.024		

Assay Date 9/6/2005 Test Chemical ID RC10 tested 8

ID TNB Replicate # Rep 2 Microsome type Placental Microsome ID Battelle

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.545	0.336	0.157	0.103	0.065	0.051	0.043	25	100
	0.560	0.344	0.158	0.100	0.062	0.051	0.044		
	0.553	0.337	0.155	0.093	0.063	0.048	0.036		

Protein stock ID

Samples:	10	100	microsomes
	0.065	0.291	0.108
	0.063	0.295	0.107
			0.108

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.553	0.512	0.0428
0.125	100	200	0.00013	200	0.0250	0.339	0.298	0.0250
0.05	200	1000	0.00005	200	0.0100	0.157	0.116	0.0099
0.025	100	1000	0.00003	200	0.0050	0.099	0.058	0.0050
0.01	40	1000	0.00001	200	0.0020	0.063	0.022	0.0021
0.005	20	1000	0.00001	200	0.0010	0.050	0.009	0.0010

Blank 0.041

r^2= 1.000
m= 0.083
b= 0.000

Variables	Regression results	
m, b	0.083	0.000
se_m, se_b	0.000	0.000
r , se_y	1.000	0.000
F, df	43160	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	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.065	0.024	0.002	200	1	1	0.000	0.000	0.011
10	0.063	0.022	0.002	200	1	1	0.000		
10					1	1			
100	0.291	0.250	0.021	200	1	1	0.000	0.000	0.106
100	0.295	0.254	0.021	200	1	1	0.000		
100									
	0.108	0.067	0.006	200	83	69983	0.025	0.024	24.471
	0.107	0.066	0.006	200	83	69983	0.024		
	0.108	0.067	0.006	200	83	69983	0.025		

Assay Date	9/6/2005	Test Chemical ID	RC10	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Placental	Microsome ID	Battelle

								Protein stock (mg/10 mL)	Protein stock ID
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk		

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					
Blank		r^2=					Regression results are calculated using the function LINEST
		m=					
		b=					

A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/6/2005	Chemical ID	RC10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.1687	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.1687	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	24.471
Protein Concentration (dilution added to assay, mg/mL):	0.029023

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	RC10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 2

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	4326.01	8656.02	8722.71	17445.42	0.1	165171	10.56	17295	0.0209	1	0.015	15	0.0480
			0.5	2	4394.7	8789.4			0.1					1		15	
	2	2	0.5	1	4667.24	9334.48	9420.75	18841.5	0.1	165171	11.41	18691	0.0226	1	0.015	15	0.0519
			0.5	2	4753.51	9507.02			0.1					1		15	
	3	2	0.5	1	4231.1	8462.2	8472.3	16944.6	0.1	165171	10.26	16794	0.0203	1	0.015	15	0.0466
			0.5	2	4251.2	8502.4			0.1					1		15	
	4	2	0.5	1	4382.39	8724.78	8743.92	17487.84	0.1	165171	10.59	17338	0.0210	1	0.015	15	0.0481
			0.5	2	4381.53	8763.06			0.1					1		15	
Background control	1	2	0.5	1	43.48	86.96	83.64	167.28	0.1	165171	0.10	17	0.0000	1	0.015	15	0.0000
			0.5	2	40.16	80.32			0.1					1		15	
	2	2	0.5	1	36.61	73.22	63.2	126.4	0.1	165171	0.08	-24	0.0000	1	0.015	15	-0.0001
			0.5	2	26.59	53.18			0.1					1		15	
	3	2	0.5	1	43.33	86.66	84.93	169.86	0.1	165171	0.10	20	0.0000	1	0.015	15	0.0001
			0.5	2	41.6	83.2			0.1					1		15	
	4	2	0.5	1	34.77	69.54	68.44	136.88	0.1	165171	0.08	-13	0.0000	1	0.015	15	0.0000
			0.5	2	33.67	67.34			0.1					1		15	
Positive control	1	2	0.5	1	2441.47	4882.94	4942	9884	0.1	165171	5.98	9734	0.0118	1	0.015	15	0.0270
			0.5	2	2500.53	5001.06			0.1					1		15	
	2	2	0.5	1	2506.09	5012.18	4961.63	9923.26	0.1	165171	6.01	9773	0.0118	1	0.015	15	0.0271
			0.5	2	2455.54	4911.08			0.1					1		15	
	3	2	0.5	1	2379.05	4758.1	4783.86	9567.72	0.1	165171	5.79	9418	0.0114	1	0.015	15	0.0261
			0.5	2	2404.81	4809.62			0.1					1		15	
	4	2	0.5	1	2384.7	4769.4	4799.78	9599.56	0.1	165171	5.81	9449	0.0114	1	0.015	15	0.0262
			0.5	2	2415.08	4830.16			0.1					1		15	
Negative Control	1	2	0.5	1	4923.12	9846.24	9907.53	19815.06	0.1	165171	12.00	19665	0.0238	1	0.015	15	0.0546
			0.5	2	4984.41	9968.82			0.1					1		15	
	2	2	0.5	1	4485.94	8971.88	8944.77	17889.54	0.1	165171	10.83	17739	0.0214	1	0.015	15	0.0492
			0.5	2	4458.63	8917.66			0.1					1		15	
	3	2	0.5	1	4225.14	8450.28	8483.02	16966.04	0.1	165171	10.27	16816	0.0203	1	0.015	15	0.0467
			0.5	2	4257.88	8515.76			0.1					1		15	
	4	2	0.5	1	4001.26	8002.52	8075.26	16150.52	0.1	165171	9.78	16000	0.0193	1	0.015	15	0.0444
			0.5	2	4074	8148			0.1					1		15	
RC10	1-1	2	0.5	1	2630.18	5260.36	5288.9	10577.8	0.1	165171	6.40	10428	0.0126	1	0.015	15	0.0289
			0.5	2	2658.72	5317.44			0.1					1		15	
	1-2	2	0.5	1	3971.44	7942.88	7880.98	15761.96	0.1	165171	9.54	15612	0.0189	1	0.015	15	0.0433
			0.5	2	3909.54	7819.08			0.1					1		15	
	1-3	2	0.5	1	3907.54	7815.08	7914.15	15828.3	0.1	165171	9.58	15678	0.0189	1	0.015	15	0.0435
			0.5	2	4006.61	8013.22			0.1					1		15	
	2-1	2	0.5	1	4161.37	8322.74	8404.14	16808.28	0.1	165171	10.18	16658	0.0201	1	0.015	15	0.0462
			0.5	2	4242.77	8485.54			0.1					1		15	
	2-2	2	0.5	1	4185.19	8370.38	8443.97	16887.94	0.1	165171	10.22	16738	0.0202	1	0.015	15	0.0465
			0.5	2	4256.78	8517.36			0.1					1		15	
	2-3	2	0.5	1	4297.71	8595.42	8518.95	17037.9	0.1	165171	10.32	16888	0.0204	1	0.015	15	0.0469
			0.5	2	4221.24	8442.48			0.1					1		15	
	3-1	2	0.5	1	4526.8	9053.6	9017.85	18035.7	0.1	165171	10.92	17886	0.0216	1	0.015	15	0.0497
			0.5	2	4491.05	8982.1			0.1					1		15	
	3-2	2	0.5	1	4480.87	8961.74	9065.62	18131.24	0.1	165171	10.98	17981	0.0217	1	0.015	15	0.0499
			0.5	2	4584.75	9169.5			0.1					1		15	
	3-3	2	0.5	1	4472.64	8945.28	8991.51	17983.02	0.1	165171	10.89	17833	0.0216	1	0.015	15	0.0495
			0.5	2	4518.87	9037.74			0.1					1		15	
	4-1	2	0.5	1	4362.49	8724.98	8772.24	17544.48	0.1	165171	10.62	17394	0.0210	1	0.015	15	0.0483
			0.5	2	4409.75	8819.5			0.1					1		15	
	4-2	2	0.5	1	4249.29	8498.58	8497.48	16994.96	0.1	165171	10.29	16845	0.0204	1	0.015	15	0.0468
			0.5	2	4248.19	8496.38			0.1					1		15	
	4-3	2	0.5	1	4371.23	8742.46	8727.12	17454.24	0.1	165171	10.57	17304	0.0209	1	0.015	15	0.0480
			0.5	2	4355.89	8711.78			0.1					1		15	
	5-1	2	0.5	1	4298.13	8596.26	8753.52	17507.04	0.1	165171	10.60	17357	0.0210	1	0.015	15	0.0482
			0.5	2	4455.39	8910.78			0.1					1		15	
	5-2	2	0.5	1	4394.17	8788.34	8719.89	17439.78	0.1	165171	10.56	17290	0.0209	1	0.015	15	0.0480
			0.5	2	4325.72	8651.44			0.1					1		15	
	5-3	2	0.5	1	4349.56	8699.12	8503.1	17006.2	0.1	165171	10.30	16856	0.0204	1	0.015	15	0.0468
			0.5	2	4153.54	8307.08			0.1					1		15	
	6-1	2	0.5	1	4508.54	9017.08	9054.4	18108.8	0.1	165171	10.96	17959	0.0217	1	0.015	15	0.0499
			0.5	2	4545.86	9091.72			0.1					1		15	
	6-2	2	0.5	1	4380.53	8761.06	8694.21	17388.42	0.1	165171	10.53	17238	0.0208	1	0.015	15	0.0479
			0.5	2	4313.68	8627.36			0.1					1		15	
	6-3	2	0.5	1	4226.84	8453.68	8741.22	17482.44	0.1	165171	10.58	17332	0.0209	1	0.015	15	0.0481
			0.5	2	4514.38	9028.76			0.1					1		15	
	7-1	2	0.5	1	4201.54	8403.08	8544.62	17089.24	0.1	165171	10.35	16939	0.0205	1	0.015	15	0.0470
			0.5	2	4343.08	8686.16			0.1					1		15	
	7-2	2	0.5	1	4460.69	8921.38	8893.25	17786.5	0.1	165171	10.77	17636	0.0213	1	0.015	15	0.0490
			0.5	2	4432.56	8865.12			0.1					1		15	
	7-3	2	0.5	1	4463.33	8926.66	8829.97	17659.94	0.1	165171	10.69	17510	0.0212	1	0.015	15	0.0486
			0.5	2	4366.64	8733.28			0.1					1		15	
	8-1	2	0.5	1	4256.01	8512.02	8594.11	17188.22	0.1	165171	10.41	17038	0.0206	1	0.015	15	0.0473
			0.5	2	4338.1	8676.2			0.1					1		15	
	8-2	2	0.5	1	4330.31	8660.62	8720.29	17440.58	0.1	165171	10.56	17290	0.0209	1	0.015	15	0.0480
			0.5	2	4389.98	8779.96			0.1					1		15	
	8-3	2	0.5	1	4267.93	8535.86	8611.65	17223.3	0.1	165171	10.43	17073	0.0206	1	0.015	15	0.0474
			0.5	2	4343.72	8687.44			0.1					1		15	

Assay Date	9/6/2005	ID	RC10	# Concentrations tested	Microsome 8 type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0500	0.0027
Full activity	End	0.0474	0.0011
Full activity	Overall	0.0487	0.0023
Background	Beginning	0.0000	8.02458E-05
Background	End	0.0000	6.47384E-05
Background	Overall	0.0000	6.04402E-05
Positive	Beginning	0.0271	0.0001
Positive	End	0.0262	0.0001
Positive	Overall	0.0266	0.0005
Negative	Beginning	0.0519	0.0038
Negative	End	0.0455	0.0016
Negative	Overall	0.0487	0.0044

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC10	1	1	1.00E-03	-3.00	0.0289
RC10	1	2	1.00E-03	-3.00	0.0433
RC10	1	3	1.00E-03	-3.00	0.0435
RC10	2	1	1.00E-04	-4.00	0.0462
RC10	2	2	1.00E-04	-4.00	0.0465
RC10	2	3	1.00E-04	-4.00	0.0469
RC10	3	1	1.00E-05	-5.00	0.0497
RC10	3	2	1.00E-05	-5.00	0.0499
RC10	3	3	1.00E-05	-5.00	0.0495
RC10	4	1	1.00E-06	-6.00	0.0483
RC10	4	2	1.00E-06	-6.00	0.0468
RC10	4	3	1.00E-06	-6.00	0.0480
RC10	5	1	1.00E-07	-7.00	0.0482
RC10	5	2	1.00E-07	-7.00	0.0480
RC10	5	3	1.00E-07	-7.00	0.0468
RC10	6	1	1.00E-08	-8.00	0.0499
RC10	6	2	1.00E-08	-8.00	0.0479
RC10	6	3	1.00E-08	-8.00	0.0481
RC10	7	1	1.00E-09	-9.00	0.0470
RC10	7	2	1.00E-09	-9.00	0.0490
RC10	7	3	1.00E-09	-9.00	0.0486
RC10	8	1	1.00E-10	-10.00	0.0473
RC10	8	2	1.00E-10	-10.00	0.0480
RC10	8	3	1.00E-10	-10.00	0.0474

Percent of control values				
	Log[test	Replicate		
.evel	bstance]	1	2	3
1	-3.00	59.49	89.06	89.44
2	-4.00	95.03	95.48	96.34
3	-5.00	102.03	102.58	101.73
4	-6.00	99.23	96.09	98.71
5	-7.00	99.01	98.63	96.16
6	-8.00	102.45	98.34	98.87
7	-9.00	96.63	100.61	99.89
8	-10.00	97.20	98.64	97.40

Aromatase Assay Spreadsheet

Assay Date	9/8/2005	Test Chemical ID	RC10	# Concentrations tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	Battelle

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	34738.93	1736947	
2	0.0204	35752.88	1752592	
3	0.0201	35930.01	1787563	
4	0.0202	35142.39	1739722	
5	0.0205	35842.44	1748412	
			Average DPM/g soln	1753047
			SD	20309
			CV	1.16
			µCi/g soln	0.790

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

ASDN solution	mg ASDN added	(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	8.0602 g
Mass of dilution B used in substrate prep	4.5954 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.575836 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00894 µg/g soln.
µg/g soln.

a. µCi/g soln	0.790
b. Specific activity of [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.

= 0.575836 + 0.00894
= 0.584775 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.350 µCi/µg ASDN

858574 dpm/nmol

Assay Date	9/8/2005	Test Chemical ID	RC10	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	Battelle

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.572	0.339	0.151	0.087	0.056	0.040	0.031	25	100	
	0.575	0.357	0.156	0.090	0.051	0.039	0.033			
	0.580	0.355	0.153	0.093	0.053	0.040	0.033			
Samples:	10	100	microsomes							
	0.053	0.285	0.109							
	0.057	0.290	0.111							
			0.110							

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results	
0.25	200	200	0.00025	200	0.0500	0.576	0.543	0.0482	m, b	0.090	0.000
0.125	100	200	0.00013	200	0.0250	0.350	0.318	0.0280	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.153	0.121	0.0104	r , se_y	0.993	0.002
0.025	100	1000	0.00003	200	0.0050	0.090	0.058	0.0047	F, df	531	4
0.01	40	1000	0.00001	200	0.0020	0.053	0.021	0.0014	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.040	0.007	0.0002			

Regression results are calculated using the function LINEST

Blank 0.032

r^2= 0.993
m= 0.090
b= 0.000

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	µSOMES prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.053	0.021	0.001	200	1	1	0.000	0.000	0.008
10	0.057	0.025	0.002	200	1	1	0.000		
10					1	1			
100	0.285	0.253	0.022	200	1	1	0.000	0.000	0.112
100	0.290	0.258	0.023	200	1	1	0.000		
100									
	0.109	0.077	0.006	200	83	69983	0.027	0.027	27.456
	0.111	0.079	0.007	200	83	69983	0.028		
	0.110	0.078	0.007	200	83	69983	0.027		

Assay Date 9/8/2005 Test Chemical ID RC10 tested 8

ID TNB Replicate # Rep 3 Microsome type Placental Microsome ID Battelle

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.572	0.339	0.151	0.087	0.056	0.040	0.031	25	100	
	0.575	0.357	0.156	0.090	0.051	0.039	0.033			
	0.580	0.355	0.153	0.093	0.053	0.040	0.033			

Samples:	10	100	microsomes
	0.053	0.285	0.109
	0.057	0.290	0.111
			0.110

concentration (mg/mL)	Volume of stock used	Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
0.25	200	200	0.00025	200	0.0500	0.576	0.543	0.0425
0.125	100	200	0.00013	200	0.0250	0.350	0.318	0.0251
0.05	200	1000	0.00005	200	0.0100	0.153	0.121	0.0098
0.025	100	1000	0.00003	200	0.0050	0.090	0.058	0.0049
0.01	40	1000	0.00001	200	0.0020	0.053	0.021	0.0021
0.005	20	1000	0.00001	200	0.0010	0.040	0.007	0.0011

Blank 0.032

r^2= 1.000
m= 0.077
b= 0.000

Variables	Regression results	
m, b	0.077	0.000
se_m, se_b	0.000	0.000
r , se_y	1.000	0.000
F, df	23945	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

	A_{raw}	A_{adj}	mg protein measured	µL diluted µSOMES	Vol. usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.053	0.021	0.002	200	1	1	0.000	0.000	0.011
10	0.057	0.025	0.002	200	1	1	0.000		
10					1	1			
100	0.285	0.253	0.020	200	1	1	0.000	0.000	0.101
100	0.290	0.258	0.020	200	1	1	0.000		
100									
	0.109	0.077	0.006	200	83	69983	0.027	0.027	27.349
	0.111	0.079	0.007	200	83	69983	0.028		
	0.110	0.078	0.006	200	83	69983	0.027		

Assay Date	9/8/2005	Test Chemical ID	RC10	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Placental	Microsome ID	Battelle

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID

Samples:

mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

Blank

r^2=
m=
b=

Regression results are calculated using the function LINEST

		mg protein	µL diluted		Final vol. Diluted usomes		mg protein/µL		
A_{raw}	$A_{adj.}$	measured	µSOMES	prep. (µL)	(µL)		Prep.	average mg/µL	mg/mL

Assay Date	9/8/2005	Chemical ID	RC10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 3

Microsome Dilution Details

Dilution A	0.083	mL microsome Stock used
	69.983	mL total volume
	843.1687	dilution factor
Dilution B	1	mL microsome Dilution A used
	1	mL total volume
	1	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	843.1687	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	27.349
Protein Concentration (dilution added to assay, mg/mL):	0.032436

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	RC10	# Concentrations tested	8	Microsome type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 3

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol H_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 (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	microsomes used in assay tube (mL)	assay (mg/mL)	Incubation time (min)	estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	7405.83	14811.66	14782.15	29564.3	0.1	175305	16.86	28493	0.0332	1	0.016	15	0.0682
			0.5	2	7376.32	14752.64			0.1					1		15	
	2	2	0.5	1	6775.29	13550.58	13803.31	27606.62	0.1	175305	15.52	26135	0.0304	1	0.016	15	0.0626
			0.5	2	6828.02	13656.04			0.1					1		15	
	3	2	0.5	1	6345.43	12690.86	12626.03	25252.06	0.1	175305	14.40	24180	0.0282	1	0.016	15	0.0579
			0.5	2	6280.6	12561.2			0.1					1		15	
	4	2	0.5	1	6350.22	12700.44	12593.19	25186.38	0.1	175305	14.37	24115	0.0281	1	0.016	15	0.0577
			0.5	2	6242.97	12485.94			0.1					1		15	
Background control	1	2	0.5	1	32.52	65.04	73.21	146.42	0.1	175305	0.08	-925	-0.0011	1	0.016	15	-0.0022
			0.5	2	40.69	81.38			0.1					1		15	
	2	2	0.5	1	82.15	164.3	148.14	296.28	0.1	175305	0.17	-775	-0.0009	1	0.016	15	-0.0019
			0.5	2	65.99	131.98			0.1					1		15	
	3	2	0.5	1	183.38	366.76	384.45	768.9	0.1	175305	0.44	-303	-0.0004	1	0.016	15	-0.0007
			0.5	2	201.07	402.14			0.1					1		15	
	4	2	0.5	1	773.31	1546.62	1537.64	3075.28	0.1	175305	1.75	2004	0.0023	1	0.016	15	0.0048
			0.5	2	764.33	1528.66			0.1					1		15	
Positive control	1	2	0.5	1	2952.96	5905.96	5869.36	11738.72	0.1	175305	6.70	10667	0.0124	1	0.016	15	0.0255
			0.5	2	2916.38	5832.76			0.1					1		15	
	2	2	0.5	1	2840.56	5681.12	5697.15	11394.3	0.1	175305	6.50	10323	0.0120	1	0.016	15	0.0247
			0.5	2	2856.59	5713.18			0.1					1		15	
	3	2	0.5	1	2761.35	5522.7	5548.3	11096.6	0.1	175305	6.33	10025	0.0117	1	0.016	15	0.0240
			0.5	2	2786.95	5573.9			0.1					1		15	
	4	2	0.5	1	2708.23	5416.46	5481.86	10963.72	0.1	175305	6.25	9882	0.0115	1	0.016	15	0.0237
			0.5	2	2773.63	5547.26			0.1					1		15	
Negative Control	1	2	0.5	1	6732.06	13464.12	13446.64	26893.28	0.1	175305	15.34	25822	0.0301	1	0.016	15	0.0618
			0.5	2	6714.58	13429.16			0.1					1		15	
	2	2	0.5	1	3877.12	7754.24	7694.49	15388.98	0.1	175305	8.78	14317	0.0167	1	0.016	15	0.0343
			0.5	2	3817.37	7634.74			0.1					1		15	
	3	2	0.5	1	6079.51	12159.02	12104.14	24208.28	0.1	175305	13.81	23137	0.0269	1	0.016	15	0.0554
			0.5	2	6024.63	12049.26			0.1					1		15	
	4	2	0.5	1	6609.23	13218.46	13302.42	26604.84	0.1	175305	15.18	25533	0.0297	1	0.016	15	0.0611
			0.5	2	6693.19	13386.38			0.1					1		15	
RC10	1-1	2	0.5	1	5371.27	10742.54	10702.9	21405.8	0.1	175305	12.21	20334	0.0237	1	0.016	15	0.0487
			0.5	2	5331.63	10663.26			0.1					1		15	
	1-2	2	0.5	1	5573.03	11146.06	11160.87	22321.74	0.1	175305	12.73	21250	0.0248	1	0.016	15	0.0509
			0.5	2	5587.84	11175.68			0.1					1		15	
	1-3	2	0.5	1	5511.29	11022.58	10900.14	21800.28	0.1	175305	12.44	20729	0.0241	1	0.016	15	0.0496
			0.5	2	5388.85	10777.7			0.1					1		15	
	2-1	2	0.5	1	6190.85	12381.7	12373.72	24747.44	0.1	175305	14.12	23676	0.0276	1	0.016	15	0.0567
			0.5	2	6182.87	12365.74			0.1					1		15	
	2-2	2	0.5	1	5985.1	11970.2	12133.23	24266.46	0.1	175305	13.84	23195	0.0270	1	0.016	15	0.0555
			0.5	2	6148.13	12296.26			0.1					1		15	
	2-3	2	0.5	1	6067.15	12134.3	12310.79	24621.58	0.1	175305	14.05	23550	0.0274	1	0.016	15	0.0564
			0.5	2	6243.64	12487.28			0.1					1		15	
	3-1	2	0.5	1	6547.95	13095.9	12936.8	25873.6	0.1	175305	14.76	24802	0.0289	1	0.016	15	0.0594
			0.5	2	6388.85	12777.7			0.1					1		15	
	3-2	2	0.5	1	6418.02	12836.04	12887.12	25774.24	0.1	175305	14.70	24703	0.0288	1	0.016	15	0.0591
			0.5	2	6469.1	12938.2			0.1					1		15	
	3-3	2	0.5	1	6917.1	13034.2	13083.16	26166.32	0.1	175305	14.93	25095	0.0292	1	0.016	15	0.0601
			0.5	2	6566.06	13132.12			0.1					1		15	
	4-1	2	0.5	1	6475.35	12950.7	12975.44	25950.88	0.1	175305	14.80	24879	0.0290	1	0.016	15	0.0596
			0.5	2	6500.09	13000.18			0.1					1		15	
	4-2	2	0.5	1	6375.61	12751.22	12771.66	25543.32	0.1	175305	14.57	24472	0.0285	1	0.016	15	0.0586
			0.5	2	6396.05	12792.1			0.1					1		15	
	4-3	2	0.5	1	6341.12	12682.24	12629.74	25259.48	0.1	175305	14.41	24188	0.0282	1	0.016	15	0.0579
			0.5	2	6288.62	12577.24			0.1					1		15	
	5-1	2	0.5	1	6989.83	13979.66	13918.6	27837.2	0.1	175305	15.88	26765	0.0312	1	0.016	15	0.0641
			0.5	2	6928.77	13857.54			0.1					1		15	
	5-2	2	0.5	1	6337.45	12674.9	12757.14	25514.28	0.1	175305	14.55	24443	0.0285	1	0.016	15	0.0585
			0.5	2	6419.69	12839.38			0.1					1		15	
	5-3	2	0.5	1	6421.28	12842.56	12775.22	25550.44	0.1	175305	14.57	24479	0.0285	1	0.016	15	0.0586
			0.5	2	6353.94	12707.88			0.1					1		15	
	6-1	2	0.5	1	6590.46	13180.92	13064.31	26128.62	0.1	175305	14.90	25057	0.0292	1	0.016	15	0.0600
			0.5	2	6473.85	12947.7			0.1					1		15	
	6-2	2	0.5	1	6289.29	12578.58	12680.72	25361.44	0.1	175305	14.47	24290	0.0283	1	0.016	15	0.0581
			0.5	2	6391.43	12782.86			0.1					1		15	
	6-3	2	0.5	1	6382.34	12764.68	12854.06	25708.12	0.1	175305	14.66	24636	0.0287	1	0.016	15	0.0590
			0.5	2	6471.72	12943.44			0.1					1		15	
	7-1	2	0.5	1	6379.95	12759.9	12935.68	25871.36	0.1	175305	14.76	24800	0.0289	1	0.016	15	0.0594
			0.5	2	6555.73	13111.46			0.1					1		15	
	7-2	2	0.5	1	6379.62	12759.24	12722.37	25444.74	0.1	175305	14.51	24373	0.0284	1	0.016	15	0.0583
			0.5	2	6342.75	12685.5			0.1					1		15	
	7-3	2	0.5	1	6232.81	12465.62	12704.09	25408.18	0.1	175305	14.49	24336	0.0283	1	0.016	15	0.0583
			0.5	2	6471.28	12942.56			0.1					1		15	
	8-1	2	0.5	1	6475.02	12950.04	13045.22	26090.44	0.1	175305	14.88	25019	0.0291	1	0.016	15	0.0599
			0.5	2	6570.2	13140.4			0.1					1		15	
	8-2	2	0.5	1	11140.62	22281.24	22375.5	44751	0.1	175305	25.53	43679	0.0509	1	0.016	15	0.1046
			0.5	2	11234.88	22469.76			0.1					1		15	
	8-3	2	0.5	1	6451.24	12902.48	12796.1	25592.2	0.1	175305	14.60	24520	0.0286	1	0.016	15	0.0587
			0.5	2	6344.86	12689.72			0.1					1		15	

Assay Date	9/8/2005	ID	RC10	# Concentrations tested	Microsome 8 type	Placental	Microsome ID	Battelle	Technician ID	TNB	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.0654	0.0040
Full activity	End	0.0578	0.0001
Full activity	Overall	0.0616	0.0049
Background	Beginning	-0.0020	0.000253673
Background	End	0.0020	0.003904091
Background	Overall	0.0000	0.003259982
Positive	Beginning	0.0251	0.0006
Positive	End	0.0238	0.0002
Positive	Overall	0.0245	0.0008
Negative	Beginning	0.0480	0.0195
Negative	End	0.0583	0.0041
Negative	Overall	0.0531	0.0129

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC10	1	1	1.00E-03	-3.00	0.0487
RC10	1	2	1.00E-03	-3.00	0.0509
RC10	1	3	1.00E-03	-3.00	0.0496
RC10	2	1	1.00E-04	-4.00	0.0567
RC10	2	2	1.00E-04	-4.00	0.0555
RC10	2	3	1.00E-04	-4.00	0.0564
RC10	3	1	1.00E-05	-5.00	0.0594
RC10	3	2	1.00E-05	-5.00	0.0591
RC10	3	3	1.00E-05	-5.00	0.0601
RC10	4	1	1.00E-06	-6.00	0.0596
RC10	4	2	1.00E-06	-6.00	0.0586
RC10	4	3	1.00E-06	-6.00	0.0579
RC10	5	1	1.00E-07	-7.00	0.0641
RC10	5	2	1.00E-07	-7.00	0.0585
RC10	5	3	1.00E-07	-7.00	0.0586
RC10	6	1	1.00E-08	-8.00	0.0600
RC10	6	2	1.00E-08	-8.00	0.0581
RC10	6	3	1.00E-08	-8.00	0.0590
RC10	7	1	1.00E-09	-9.00	0.0594
RC10	7	2	1.00E-09	-9.00	0.0583
RC10	7	3	1.00E-09	-9.00	0.0583
RC10	8	1	1.00E-10	-10.00	0.0599
RC10	8	2	1.00E-10	-10.00	0.1046
RC10	8	3	1.00E-10	-10.00	0.0587

Percent of control values				
	Log[test	Replicate		
Level	bstance]	1	2	3
1	-3.00	79.03	82.59	80.56
2	-4.00	92.01	90.14	91.52
3	-5.00	96.39	96.00	97.53
4	-6.00	96.69	95.11	94.00
5	-7.00	104.02	94.99	95.13
6	-8.00	97.38	94.40	95.75
7	-9.00	96.38	94.72	94.58
8	-10.00	97.23	169.76	95.30

Appendix E

Statistical Analysis Summary (BioSTAT Consultants, Inc.)

Statistical Analysis Summary
WIL Research Laboratories, LLC
Study Protocol – 431009
Version 1.1

1 Statistical Analysis
1.1 Analysis of Variance Comparisons Among Concentration Response Curve Fits
1.2 Full Enzyme Activity and Background Activity Percent of Control Values Across Replicates

BioSTAT Consultants, Inc.
Statement of Quality Control

This report was quality checked in accordance with BioSTAT Procedural Guideline 2.1 (Quality Control Process for Tables and Reports). The statistical methodology and results of inferential statistics were verified by an independent quality control statistician. Based on these documented quality control activities, it is concluded that the statistical results incorporated in this report accurately reflect the statistical analysis of data received by BioSTAT.

1 Statistical Analysis

1.1 Analysis of Variance Comparisons Among Concentration Response Curve Fits

Top (T), bottom (B), slope (β) and log10IC50 (μ) were subjected to a random effects analysis of variance. Response variable were T, B, β or μ and replicates were treated as random effects.

The SAS procedure Proc Mixed was used for analysis using the following statements:

```
proc mixed covtest;
class replicate;
model mean = /solution cl;
random replicate;
repeated/group=replicate;
parms (1) (STD1^2) (STD2^2) (STD3^2)/hold=2,3,4;
run;
```

where $STD1^2$, $STD2^2$ and $STD3^2$ were the squares of the standard errors for each replicate.

T, B, β and μ were estimated, separately within each reference chemical and replicate, and presented along with the average across replicates (LSMean) and associated 95% confidence interval across replicates.

Reference Chemical	Parameter	Statistic	Replicate 1	2	3	Overall
1	Bottom	Prism Best Fit Value (S.E.)	-6.198 (2.991)	2.121 (2.972)	0.113 (1.764)	--
		Prism 95% CI (lower, upper)	(-12.440, 0.042)	(-4.078, 8.320)	(-3.567, 3.793)	--
		LSM (LSM s.e.)	--	--	--	-1.108 (2.224)
		95% CI (lower, upper)	--	--	--	(-10.679, 8.462)
	Slope (β)	Prism Best Fit Value (S.E.)	-0.861 (0.057)	-1.080 (0.105)	-0.999 (0.053)	--
		Prism 95% CI (lower, upper)	(-0.981, -0.742)	(-1.299, -0.861)	(-1.110, -0.888)	--
		LSM (LSM s.e.)	--	--	--	-0.963 (0.060)
		95% CI (lower, upper)	--	--	--	(-1.223, -0.703)
	Top	Prism Best Fit Value (S.E.)	103.720 (0.700)	103.480 (1.170)	102.470 (0.680)	--
		Prism 95% CI (lower, upper)	(102.260, 105.180)	(101.030, 105.930)	(101.050, 103.890)	--
		LSM (LSM s.e.)	--	--	--	103.143 (0.479)
		95% CI (lower, upper)	--	--	--	(101.080, 105.206)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-4.196 (0.043)	-4.169 (0.037)	-4.202 (0.023)	--
		Prism 95% CI (lower, upper)	(-4.286, -4.105)	(-4.245, -4.093)	(-4.249, -4.155)	--
		LSM (LSM s.e.)	--	--	--	-4.193 (0.018)
		95% CI (lower, upper)	--	--	--	(-4.270, -4.117)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
2	Bottom	Prism Best Fit Value (S.E.)	-0.322 (0.514)	0.091 (1.047)	1.503 (1.001)	--
		Prism 95% CI (lower, upper)	(-1.394, 0.750)	(-2.092, 2.274)	(-0.585, 3.590)	--
		LSM (LSM s.e.)	--	--	--	0.187 (0.539)
		95% CI (lower, upper)	--	--	--	(-2.132, 2.506)
	Slope (ß)	Prism Best Fit Value (S.E.)	-0.974 (0.023)	-0.992 (0.046)	-1.140 (0.056)	--
		Prism 95% CI (lower, upper)	(-1.021, -0.927)	(-1.088, -0.897)	(-1.258, -1.023)	--
		LSM (LSM s.e.)	--	--	--	-1.026 (0.049)
		95% CI (lower, upper)	--	--	--	(-1.236, -0.816)
	Top	Prism Best Fit Value (S.E.)	97.250 (0.390)	100.280 (0.770)	101.050 (0.830)	--
		Prism 95% CI (lower, upper)	(96.430, 98.070)	(98.670, 101.890)	(99.330, 102.780)	--
		LSM (LSM s.e.)	--	--	--	99.435 (1.197)
		95% CI (lower, upper)	--	--	--	(94.285, 104.585)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-6.410 (0.014)	-6.332 (0.026)	-6.422 (0.027)	--
		Prism 95% CI (lower, upper)	(-6.438, -6.381)	(-6.386, -6.279)	(-6.478, -6.366)	--
		LSM (LSM s.e.)	--	--	--	-6.390 (0.027)
		95% CI (lower, upper)	--	--	--	(-6.505, -6.274)

Reference Chemical	Parameter	Statistic	Replicate 1	2	3	Overall
3	Bottom	Prism Best Fit Value (S.E.)	-0.161 (1.199)	-20.490 (3.159)	-0.303 (1.147)	--
		Prism 95% CI (lower, upper)	(-2.661, 2.339)	(-27.080, -13.900)	(-2.695, 2.090)	--
		LSM (LSM s.e.)	--	--	--	-6.695 (6.603)
		95% CI (lower, upper)	--	--	--	(-35.107, 21.717)
	Slope (S)	Prism Best Fit Value (S.E.)	-0.984 (0.076)	-1.062 (0.128)	-0.952 (0.049)	--
		Prism 95% CI (lower, upper)	(-1.143, -0.826)	(-1.330, -0.794)	(-1.053, -0.851)	--
		LSM (LSM s.e.)	--	--	--	-0.971 (0.039)
		95% CI (lower, upper)	--	--	--	(-1.138, -0.803)
	Top	Prism Best Fit Value (S.E.)	108.570 (1.930)	100.780 (3.180)	101.630 (1.230)	--
		Prism 95% CI (lower, upper)	(104.550, 112.600)	(94.150, 107.410)	(99.070, 104.200)	--
		LSM (LSM s.e.)	--	--	--	103.824 (2.494)
		95% CI (lower, upper)	--	--	--	(93.093, 114.554)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-7.620 (0.043)	-7.546 (0.053)	-7.600 (0.025)	--
		Prism 95% CI (lower, upper)	(-7.710, -7.530)	(-7.657, -7.436)	(-7.651, -7.549)	--
		LSM (LSM s.e.)	--	--	--	-7.597 (0.020)
		95% CI (lower, upper)	--	--	--	(-7.683, -7.511)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
4	Bottom	Prism Best Fit Value (S.E.)	0.278 (0.546)	0.626 (1.441)	0.563 (0.505)	--
		Prism 95% CI (lower, upper)	(-0.861, 1.417)	(-2.379, 3.631)	(-0.490, 1.615)	--
		LSM (LSM s.e.)	--	--	--	0.444 (0.359)
		95% CI (lower, upper)	--	--	--	(-1.101, 1.989)
	Slope (ß)	Prism Best Fit Value (S.E.)	-1.858 (0.049)	-1.974 (0.113)	-2.031 (0.043)	--
		Prism 95% CI (lower, upper)	(-1.960, -1.756)	(-2.209, -1.739)	(-2.120, -1.941)	--
		LSM (LSM s.e.)	--	--	--	-1.952 (0.062)
		95% CI (lower, upper)	--	--	--	(-2.219, -1.685)
	Top	Prism Best Fit Value (S.E.)	104.540 (0.240)	100.510 (0.790)	98.580 (0.280)	--
		Prism 95% CI (lower, upper)	(104.050, 105.040)	(98.850, 102.170)	(98.000, 99.160)	--
		LSM (LSM s.e.)	--	--	--	101.226 (1.787)
		95% CI (lower, upper)	--	--	--	(93.536, 108.915)
	log10IC50 (µ)	Prism Best Fit Value (S.E.)	-4.602 (0.012)	-4.578 (0.022)	-4.561 (0.008)	--
		Prism 95% CI (lower, upper)	(-4.627, -4.576)	(-4.623, -4.533)	(-4.577, -4.545)	--
		LSM (LSM s.e.)	--	--	--	-4.579 (0.014)
		95% CI (lower, upper)	--	--	--	(-4.640, -4.519)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
6	Bottom	Prism Best Fit Value (S.E.)	0.078 (2.128)	-20.199 (2.116)	0.605 (1.610)	--
		Prism 95% CI (lower, upper)	(-4.361, 4.518)	(-24.629, -15.770)	(-2.754, 3.964)	--
		LSM (LSM s.e.)	--	--	--	-6.474 (6.835)
		95% CI (lower, upper)	--	--	--	(-35.881, 22.933)
	Slope (ß)	Prism Best Fit Value (S.E.)	-1.025 (0.102)	-1.022 (0.081)	-1.047 (0.072)	--
		Prism 95% CI (lower, upper)	(-1.238, -0.812)	(-1.193, -0.852)	(-1.197, -0.896)	--
		LSM (LSM s.e.)	--	--	--	-1.034 (0.048)
		95% CI (lower, upper)	--	--	--	(-1.239, -0.829)
	Top	Prism Best Fit Value (S.E.)	101.660 (1.240)	81.040 (1.850)	87.830 (1.260)	--
		Prism 95% CI (lower, upper)	(99.080, 104.250)	(77.170, 84.900)	(85.210, 90.450)	--
		LSM (LSM s.e.)	--	--	--	90.229 (6.063)
		95% CI (lower, upper)	--	--	--	(64.141, 116.317)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-5.197 (0.046)	-5.149 (0.039)	-5.113 (0.033)	--
		Prism 95% CI (lower, upper)	(-5.293, -5.102)	(-5.231, -5.067)	(-5.182, -5.043)	--
		LSM (LSM s.e.)	--	--	--	-5.145 (0.023)
		95% CI (lower, upper)	--	--	--	(-5.245, -5.045)

Reference Chemical	Parameter	Statistic	Replicate 1	2	3	Overall
7	Bottom	Prism Best Fit Value (S.E.)	0.057 (0.144)	0.742 (0.395)	0.529 (0.455)	--
		Prism 95% CI (lower, upper)	(-0.245, 0.358)	(-0.082, 1.565)	(-0.419, 1.478)	--
		LSM (LSM s.e.)	--	--	--	0.315 (0.238)
		95% CI (lower, upper)	--	--	--	(-0.710, 1.340)
	Slope (S)	Prism Best Fit Value (S.E.)	-1.095 (0.017)	-1.070 (0.029)	-1.134 (0.038)	--
		Prism 95% CI (lower, upper)	(-1.129, -1.060)	(-1.130, -1.010)	(-1.212, -1.056)	--
		LSM (LSM s.e.)	--	--	--	-1.094 (0.013)
		95% CI (lower, upper)	--	--	--	(-1.152, -1.037)
	Top	Prism Best Fit Value (S.E.)	99.780 (0.460)	104.730 (0.970)	97.090 (1.050)	--
		Prism 95% CI (lower, upper)	(98.810, 100.740)	(102.710, 106.750)	(94.910, 99.280)	--
		LSM (LSM s.e.)	--	--	--	100.533 (2.200)
		95% CI (lower, upper)	--	--	--	(91.066, 110.000)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-8.794 (0.007)	-8.765 (0.013)	-8.761 (0.015)	--
		Prism 95% CI (lower, upper)	(-8.809, -8.780)	(-8.791, -8.738)	(-8.791, -8.731)	--
		LSM (LSM s.e.)	--	--	--	-8.776 (0.011)
		95% CI (lower, upper)	--	--	--	(-8.825, -8.727)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
8	Bottom	Prism Best Fit Value (S.E.)	8.333 (5.093)	16.119 (4.975)	13.826 (2.595)	--
		Prism 95% CI (lower, upper)	(-2.290, 18.957)	(5.742, 26.497)	(8.412, 19.241)	--
		LSM (LSM s.e.)	--	--	--	13.302 (2.097)
		95% CI (lower, upper)	--	--	--	(4.281, 22.324)
	Slope (ß)	Prism Best Fit Value (S.E.)	-1.014 (0.177)	-1.549 (0.385)	-1.388 (0.157)	--
		Prism 95% CI (lower, upper)	(-1.383, -0.645)	(-2.351, -0.746)	(-1.714, -1.061)	--
		LSM (LSM s.e.)	--	--	--	-1.258 (0.157)
		95% CI (lower, upper)	--	--	--	(-1.932, -0.585)
	Top	Prism Best Fit Value (S.E.)	98.820 (2.250)	82.620 (2.790)	89.190 (1.440)	--
		Prism 95% CI (lower, upper)	(94.130, 103.520)	(76.810, 88.440)	(86.180, 92.200)	--
		LSM (LSM s.e.)	--	--	--	90.296 (4.612)
		95% CI (lower, upper)	--	--	--	(70.454, 110.139)
	log10IC50 (µ)	Prism Best Fit Value (S.E.)	-5.613 (0.089)	-5.451 (0.081)	-5.493 (0.040)	--
		Prism 95% CI (lower, upper)	(-5.798, -5.428)	(-5.620, -5.283)	(-5.576, -5.410)	--
		LSM (LSM s.e.)	--	--	--	-5.503 (0.033)
		95% CI (lower, upper)	--	--	--	(-5.646, -5.360)

Reference Chemical	Parameter	Statistic	Replicate 1	2	3	Overall
9	Bottom	Prism Best Fit Value (S.E.)	-3.409 (4.305)	-17.170 (9.803)	-17.010 (7.392)	--
		Prism 95% CI (lower, upper)	(-12.420, 5.602)	(-37.620, 3.281)	(-32.430, -1.590)	--
		LSM (LSM s.e.)	--	--	--	-10.380 (5.313)
		95% CI (lower, upper)	--	--	--	(-33.241, 12.482)
	Slope (S)	Prism Best Fit Value (S.E.)	-0.692 (0.072)	-0.624 (0.080)	-0.603 (0.069)	--
		Prism 95% CI (lower, upper)	(-0.843, -0.541)	(-0.790, -0.457)	(-0.747, -0.458)	--
		LSM (LSM s.e.)	--	--	--	-0.639 (0.042)
		95% CI (lower, upper)	--	--	--	(-0.822, -0.457)
	Top	Prism Best Fit Value (S.E.)	100.910 (1.130)	102.210 (2.490)	99.230 (2.520)	--
		Prism 95% CI (lower, upper)	(98.550, 103.270)	(97.020, 107.400)	(93.970, 104.480)	--
		LSM (LSM s.e.)	--	--	--	100.860 (0.953)
		95% CI (lower, upper)	--	--	--	(96.761, 104.959)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-4.576 (0.086)	-4.078 (0.133)	-4.307 (0.110)	--
		Prism 95% CI (lower, upper)	(-4.755, -4.397)	(-4.356, -3.800)	(-4.538, -4.077)	--
		LSM (LSM s.e.)	--	--	--	-4.334 (0.144)
		95% CI (lower, upper)	--	--	--	(-4.954, -3.713)

1.2 Full Enzyme Activity and Background Activity Percent of Control Values Across Replicates

Three factor mixed effects analysis of variance models were fit separately for the full enzyme activity controls, the background activity controls, and the positive and negative control tubes. The fixed effect factors in the analysis of variance were reference chemical (chemicals 1&2, 3&6, 4, 5, 7&8 and 9&10 – 5 degrees of freedom), portion (beginning or end – 1 degree of freedom) and the portion by reference chemical interaction (5 degrees of freedom). The random effects were replicate nested within reference chemical and portion by replicate within reference chemical interaction.

The SAS procedure Proc Mixed was used for analysis using the following statements:

```
proc mixed covtest;
class chemical portion replicate;
model value = chemical portion chemical*portion;
random replicate(chemical) portion*replicate(chemical)/group=chemical;
lsmeans chemical portion chemical*portion/cl;
run;
```

If the portion by replicate within reference chemical interaction was significant (at the 0.05 level) the nature of the effect was assessed by comparing the portion effect within each replicate within reference chemical to the portion effect averaged across replicates within the same reference chemical. Simultaneity of inference was adjusted for by Bonferroni's method.

P-values from the ANOVA were presented in the tables. In addition, estimates for the LSMeans, standard errors and 95% confidence intervals were presented.

Activity	Portion	Statistic	Chemical							
			1 & 2	3 & 6	4	5	7 & 8	9 & 10	Overall
Background	Beginning	LSM	-0.067	1.139	-0.006	0.041	-0.098	-1.175	-0.028
		LSM s.e.	0.366	1.349	0.366	0.366	0.366	1.066	0.311
		95% CI - lower	-0.864	-1.799	-0.804	-0.756	-0.895	-3.498	-0.706
		95% CI - upper	0.731	4.078	0.791	0.839	0.700	1.148	0.651
	End	LSM	0.067	-1.139	0.006	-0.041	0.098	1.175	0.028
		LSM s.e.	0.366	1.349	0.366	0.366	0.366	1.066	0.311
		95% CI - lower	-0.731	-4.078	-0.791	-0.839	-0.700	-1.148	-0.651
		95% CI - upper	0.864	1.800	0.804	0.756	0.895	3.498	0.706
	Overall	LSM	-0.000	0.000	0.000	-0.000	0.000	0.000	
		LSM s.e.	0.259	0.954	0.259	0.259	0.259	0.754	
		95% CI - lower	-0.564	-2.078	-0.564	-0.564	-0.564	-1.643	
		95% CI - upper	0.564	2.078	0.564	0.564	0.564	1.643	
	P-Values	Chemical = 1.0000							
		Portion = 0.9024							
		Chemical*Portion = 0.5741							
		Replicate(Chemical) 1 & 2 = .							
		Replicate(Chemical) 3 & 6 = .							
		Replicate(Chemical) 4 = .							
		Replicate(Chemical) 5 = .							
		Replicate(Chemical) 7 & 8 = .							
		Replicate(Chemical) 9 & 10 = .							
		Portion*Replicate(Chemical) 1 & 2 = .							
		Portion*Replicate(Chemical) 3 & 6 = 0.0951							
		Portion*Replicate(Chemical) 4 = .							
		Portion*Replicate(Chemical) 5 = .							
		Portion*Replicate(Chemical) 7 & 8 = .							
		Portion*Replicate(Chemical) 9 & 10 = 0.1062							

Activity	Portion	Statistic	Chemical							
			1 & 2	3 & 6	4	5	7 & 8	9 & 10	Overall
Full Activity	Beginning	LSM	105.076	108.236	102.985	95.122	102.940	103.421	102.963
		LSM s.e.	2.027	4.612	2.027	2.220	2.027	2.027	1.088
		95% CI - lower	100.660	98.188	98.569	90.285	98.524	99.005	100.592
		95% CI - upper	109.491	118.285	107.400	99.960	107.356	107.836	105.334
	End	LSM	94.924	91.764	97.016	104.064	97.060	96.580	96.901
		LSM s.e.	2.027	4.612	2.027	2.027	2.027	2.027	1.078
		95% CI - lower	90.509	81.715	92.600	99.649	92.644	92.164	94.553
		95% CI - upper	99.340	101.812	101.431	108.480	101.475	100.995	99.249
	Overall	LSM	100.000	100.000	100.000	99.593	100.000	100.000	
		LSM s.e.	1.433	3.261	1.433	1.503	1.433	1.433	
		95% CI - lower	96.878	92.895	96.878	96.319	96.878	96.878	
		95% CI - upper	103.122	107.105	103.122	102.868	103.122	103.122	

P-Values

Chemical = 0.9999
Portion = 0.0019
Chemical*Portion = 0.0065
Replicate(Chemical) 1 & 2 = .
Replicate(Chemical) 3 & 6 = .
Replicate(Chemical) 4 = .
Replicate(Chemical) 5 = .
Replicate(Chemical) 7 & 8 = .
Replicate(Chemical) 9 & 10 = .
Portion*Replicate(Chemical) 1 & 2 = .
Portion*Replicate(Chemical) 3 & 6 = 0.1272
Portion*Replicate(Chemical) 4 = .
Portion*Replicate(Chemical) 5 = .
Portion*Replicate(Chemical) 7 & 8 = .
Portion*Replicate(Chemical) 9 & 10 = .

Activity	Portion	Statistic	Chemical						
			1 & 2	3 & 6	4	5	7 & 8	9 & 10	Overall
Negative	Beginning	LSM	103.114	105.521	103.224	106.764	104.360	94.990	102.995
		LSM s.e.	2.716	4.764	2.716	6.123	2.716	6.169	1.829
		95% CI - lower	97.196	95.141	97.306	93.423	98.442	81.549	99.011
		95% CI - upper	109.032	115.900	109.142	120.105	110.278	108.431	106.980
	End	LSM	92.648	88.461	95.707	96.954	90.795	93.435	93.000
		LSM s.e.	2.716	4.764	2.716	6.123	2.716	6.169	1.829
		95% CI - lower	86.730	78.081	89.789	83.613	84.877	79.995	89.016
		95% CI - upper	98.566	98.840	101.625	110.294	96.713	106.876	96.984
	Overall	LSM	97.881	96.991	99.466	101.859	97.577	94.213	
		LSM s.e.	1.921	3.368	1.921	5.484	1.921	4.362	
		95% CI - lower	93.696	89.651	95.281	89.911	93.393	84.709	
		95% CI - upper	102.065	104.330	103.650	113.807	101.762	103.716	
	P-Values	Chemical = 0.8443							
		Portion = 0.0011							
		Chemical*Portion = 0.6666							
		Replicate(Chemical) 1 & 2 = .							
		Replicate(Chemical) 3 & 6 = .							
		Replicate(Chemical) 4 = .							
		Replicate(Chemical) 5 = 0.2323							
		Replicate(Chemical) 7 & 8 = .							
		Replicate(Chemical) 9 & 10 = .							
		Portion*Replicate(Chemical) 1 & 2 = .							
		Portion*Replicate(Chemical) 3 & 6 = 0.1710							
		Portion*Replicate(Chemical) 4 = .							
		Portion*Replicate(Chemical) 5 = 0.3084							
		Portion*Replicate(Chemical) 7 & 8 = .							
		Portion*Replicate(Chemical) 9 & 10 = 0.1275							

Activity	Portion	Statistic	Chemical 1 & 2	3 & 6	4	5	7 & 8	9 & 10	Overall
Positive	Beginning	LSM	49.414	40.903	43.934	40.498	43.645	47.518	44.318
		LSM s.e.	2.197	3.803	1.458	2.369	1.871	4.661	1.205
		95% CI - lower	44.627	32.617	40.756	35.336	39.568	37.361	41.693
		95% CI - upper	54.200	49.188	47.111	45.660	47.723	57.674	46.944
	End	LSM	45.251	30.269	40.969	43.532	39.303	44.143	40.578
		LSM s.e.	2.197	3.803	1.458	2.369	1.871	4.661	1.205
		95% CI - lower	40.464	21.983	37.792	38.370	35.226	33.987	37.953
		95% CI - upper	50.037	38.555	44.147	48.694	43.381	54.299	43.203
	Overall	LSM	47.332	35.586	42.452	42.015	41.474	45.830	
		LSM s.e.	1.940	2.689	1.031	1.675	1.562	4.546	
		95% CI - lower	43.105	29.727	40.205	38.365	38.072	35.926	
		95% CI - upper	51.559	41.445	44.698	45.665	44.877	55.735	

P-Values

```
Chemical = 0.0687
Portion = 0.0117
Chemical*Portion = 0.3755
Replicate(Chemical) 1 & 2  = 0.2369
Replicate(Chemical) 3 & 6  = .
Replicate(Chemical) 4      = .
Replicate(Chemical) 5      = .
Replicate(Chemical) 7 & 8  = 0.2872
Replicate(Chemical) 9 & 10 = 0.1712
Portion*Replicate(Chemical) 1 & 2  = .
Portion*Replicate(Chemical) 3 & 6  = 0.1141
Portion*Replicate(Chemical) 4      = .
Portion*Replicate(Chemical) 5      = 0.1913
Portion*Replicate(Chemical) 7 & 8  = .
Portion*Replicate(Chemical) 9 & 10 = .
```

APPENDIX E

BATTELLE INTERLABORATORY STISTICAL ANALYSIS REPORT

DRAFT REPORT

INTERLABORATORY STATISTICAL ANALYSIS

on

PLACENTAL AROMATASE VALIDATION STUDY

CONDUCT MULTIPLE CHEMICAL STUDIES WITH MICROSOMES PREPARED IN PARTICIPATING LABORATORIES

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-16, TASK 7

June 8, 2006

Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
ENDOCRINE DISRUPTOR SCREENING PROGRAM
WASHINGTON, D.C.

Prepared by

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Columbus, Ohio 43201

Placental Aromatase Validation Study

Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories

Interlaboratory Statistical Analysis

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-16, TASK 7

Zhenxu J. Ma, Author _____ Date _____

Paul I. Feder, Reviewer _____ Date _____

Quality Assurance Statement

Study Number: WA 4-16 Task 7

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	6/8/2006	6/8/2006
Audit interlaboratory statistics draft report	6/8/2006	6/8/2006

Quality Assurance Unit **Date**

_____ _____

Terri L. Pollock

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This report discusses the methods and results of the interlaboratory statistical analysis to compare and combine the intralaboratory results obtained at Battelle, In Vitro Technologies, Research Triangle Institute, and WIL Laboratories for the multiple chemical study with the placental aromatase assay under Work Assignment 4-16, Task 7, Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories.

Summary and Conclusions

Human placental microsomes prepared by Battelle and In Vitro Technologies were distributed to the four participating laboratories. They were utilized to conduct placental aromatase assays for ten test substances: aminoglutethimide (AG), ketoconazole (KCZ), prochloraz (PCZ), 4-nonylphenol (NYP), dibenz[a,h]anthracene (DBA), fenarimol (FRM), econazole (ECZ), chrysin (CYN), dicofol (DCF), and atrazine (ATZ) (coded: chemicals I, II,...., X). Within each laboratory five of the ten test substances were tested with microsomes prepared by Battelle and five were tested with microsomes prepared by In Vitro Technologies. Placental assay aromatase activity levels were determined for various graded concentrations of each of the substances. The intralaboratory analyses were performed on the "percent of control" responses for the placental aromatase assays.

The intralaboratory analyses included concentration response curve fits for individual chemicals and analysis of variance comparisons of the background activity, full enzyme activity, positive, and negative control responses combined across the chemicals. The interlaboratory analysis compared across the four laboratories the $\log_{10}IC_{50}$, the slope, the top and the bottom parameters of the concentration response curve fits determined in the intralaboratory analyses for each chemical. The interlaboratory analysis also compared across the four laboratories the background activity, full enzyme activity, the positive and the negative control responses at the beginnings and the ends of the replicates. It determined whether and how the differences in control activity between the beginning and the end of each replicate within laboratories varied across laboratories. In addition, the interlaboratory analysis compared across laboratories the percent of control between the microsomes prepared by Battelle and those prepared by In Vitro Technologies.

The principal results of the interlaboratory analysis are summarized below.

Concentration Response Relations

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

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

differences. For slope there were no significant differences. For the top threshold, for ketoconazole the Battelle microsomes were on average lower than the In Vitro microsomes. Otherwise there were no significant differences between microsome sources. For the bottom threshold, there were no significant differences between microsome sources.

2. Differences among laboratories for $\log_{10}IC_{50}$. The estimates for the $\log_{10}IC_{50}$ were similar among the four laboratories for most chemicals except for econazole. For econazole, the estimated $\log_{10}IC_{50}$ for In Vitro was more negative than the other three laboratories, and the associated 95 percent confidence interval for In Vitro did not overlap with those for the other three laboratories. The CVs for the IC_{50} (note that CVs were calculated for IC_{50} rather than $\log_{10}IC_{50}$) were 22.5 percent for econazole, 22.6 percent for chrysin, 47.4 percent for dicofol, and less than 15 percent for the other five chemicals. The ratios of the among laboratory variances to the pooled within laboratory variance ratios were 6.8 for econazole, 4.6 for chrysin, 2.3 for prochloraz, and less than 0.05 for the remaining five chemicals. The relatively high CVs for econazole and for chrysin might be explained by their relatively large among laboratory variances. However the relatively large CV for dicofol was due to the relatively large within laboratory variances, which were for the most part an order of magnitude larger than those for the other test substances. The pooled within laboratory variances were 0.045 for dicofol and less than or equal to 0.002 for the other test substances.

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

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

Control Activity

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

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

a. For the background activity controls there were no significant differences between the beginnings and the ends of replicates, for each laboratory and combined across laboratories.
b. For the full enzyme activity controls the ends of replicates had lower activity than the beginnings, for each laboratory and combined across laboratories.
c. For the negative controls the ends of replicates had lower activity than the beginnings for RTI, Battelle, and WIL. The ends had greater activity than the beginnings for In Vitro. The confidence interval at In Vitro did not overlap with those from any of the other laboratories. The results at In Vitro were inconsistent with those at the other laboratories. Combined across laboratories, there was not a significant difference between beginnings and ends of replicates. This was due to the very large among laboratories variance.
d. The results for the positive controls were similar to those for the negative controls, but not as extreme. For the positive controls the ends of replicates had lower activity than the beginnings for RTI, Battelle, and WIL. The ends had nearly significantly greater activity than the beginnings for In Vitro. The confidence interval at In Vitro did not overlap with those from any of the other laboratories. The results at In Vitro were inconsistent with those at the other laboratories. Combined across laboratories, there was not a significant difference between beginnings and ends of replicates. This was due to the relatively large among laboratories variance.

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

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

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

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

7. Summary and Conclusions

   a. In Vitro differed from the other laboratories with respect to $\log_{10}IC_{50}$ and slope for econazole, for slope for 4-nonylphenol, for differences between end and beginning portions for negative and positive controls, and for the difference between Battelle and In Vitro for negative controls. It is not understood why the activity for the negative and positive controls at In Vitro increased between the beginning and end portions of replicates.
   b. In future analyses comparisons between sources of microsomes should be based on unnormalized aromatase activity endpoints rather than percent of control. Normalizing by the sum of the full enzyme activity control values within each replicate completely masks differences between sources in aromatase activity of full enzyme activity controls and likely at least partially masks differences between sources in aromatase activity of positive and negative controls.

Introduction and Background

Task 7 of Work Assignment 4-16, the Placental Aromatase Validation Study, involved four laboratories (RTI, Battelle, WIL and In Vitro) independently carrying out the placental aromatase assay with ten reference chemicals on the aromatase activity of human placental microsomes according to a common protocol. Intralaboratory statistical analyses were carried out based on each laboratory's test data according to a common statistical analysis plan. The interlaboratory statistical analysis discussed in this report combines summary values developed in each intralaboratory analysis and assesses the relationships among them, the extent of interlaboratory variation, and overall consensus estimates. This report discusses the methods used and the results obtained from combining the intralaboratory statistical analysis results.

The intralaboratory analyses were performed on the "percent of control" responses for the placental aromatase assays. The intralaboratory analyses included concentration response curve fits for individual chemicals and analysis of variance comparisons of the background activity, full enzyme activity, positive, and negative control responses combined across the chemicals. The interlaboratory analysis compared across the four laboratories the $\log_{10}IC_{50}$, the slope, the top and the bottom parameters of the concentration response curve fits determined in the intralaboratory analyses for each chemical. The interlaboratory analysis also compared across the four laboratories the background activity, full enzyme activity, the positive and the negative control responses at the beginnings and the ends of the replicates. It determined whether and how the differences in control activity between the beginning and the end of each replicate within laboratories varied across laboratories. In addition, the interlaboratory analysis compared across laboratories the percent of control between the microsomes prepared by Battelle and those prepared by In Vitro Technologies.

Test Organization

Human placental microsomes prepared by Battelle and In Vitro Technologies were distributed to the four participating laboratories. They were utilized in the assays for the ten chemicals as summarized in Table M-D-T. Within each laboratory five of the ten chemicals were tested with microsomes prepared by Battelle and five were tested with microsomes prepared by In Vitro Technologies. Placental assay aromatase activity levels were determined for various graded concentrations of each of the ten reference chemicals: aminoglutethimide (AG), ketoconazole (KCZ), prochloraz (PCZ), 4-nonylphenol (NYP), dibenz[a,h]anthracene (DBA), fenarimol (FRM), econazole (ECZ), chrysin (CYN), dicofol (DCF), and atrazine (ATZ). Three replicates of the inhibitor assay were carried out at each laboratory for each chemical using either Battelle microsomes or In Vitro microsomes, as shown in Table M-D-T. Within each replicate three repetitions were run at each of the chemical concentrations. In addition, for each chemical, two repeat tubes of the full enzyme activity and background activity controls were run prior to the chemical runs and two repeat tubes of the full enzyme activity and background activity controls were run following the chemical runs.

Table M-D-T. Microsome Distribution and Testing Matrix

Chemical	Analysis Laboratory			
	Laboratory 1 RTI	**Laboratory 2 Battelle**	**Laboratory 3 WIL**	**Laboratory 4 In Vitro Technologies**
Chemical 1 (AG)	M_2	M_2	M_4	M_4
Chemical 2 (KCZ)	M_2	M_2	M_4	M_4
Chemical 3 (PCZ)	M_4	M_4	M_2	M_2
Chemical 4 (NYP)	M_2	M_4	M_2	M_4
Chemical 5 (DBA)	M_4	M_2	M_4	M_2
Chemical 6 (FRM)	M_4	M_4	M_2	M_2
Chemical 7 (ECZ)	M_2	M_4	M_4	M_2
Chemical 8 (CYN)	M_2	M_4	M_4	M_2
Chemical 9 (DCF)	M_4	M_2	M_2	M_4
Chemical 10 (ATZ)	M_4	M_2	M_2	M_4

* M2 and M4 denote Battelle and In Vitro microsomes respectively.

Intralaboratory 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 average background adjusted 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 chemical concentrations and approximately 100 percent near the low chemical concentrations, but this may vary with the inhibitor. Thus four parameter concentration response relations were fitted to the inhibition data, with variable top and bottom threshold parameters.

Intralaboratory statistical analyses were performed based on a common analysis plan. The following results were reported in each intralaboratory analysis.

1. A concentration response curve (four parameter) fit within each replicate to describe the trend in the percent of control activity across varying concentrations of the test chemical.

2. Estimates of the $\log_{10}IC_{50}$ concentration, the slope, the top and bottom thresholds, and associated standard errors within each replicate for each chemical.

3. Average values for the $\log_{10}IC_{50}$ concentration, the slope, the top and the bottom, and associated standard errors across replicates for each chemical.

4. Results of analysis of variance applied to the data for the full enzyme activity, background activity, positive and negative controls obtained at the beginning and those obtained at the end of each replicate.

The control activity results were reported differently by the various laboratories (Appendix B). RTI reported least squares means and associated standard errors averaged across the end portions and the beginning portions, separately for each chemical. WIL reported least squares means averaged across the end portions and across chemicals and averaged across the beginning portions and across chemicals. WIL also reported least squares means and associated standard errors for the positive and negative controls averaged across then beginning and end portions, separately for each set of chemicals for which independent controls were run. Battelle and In Vitro reported least squares means differences between the beginning and the end portions averaged across chemicals, and associated standard errors. In addition, Battelle and In Vitro reported results differences in averages across replicates between the microsomes prepared by Battelle and the microsomes prepared by In Vitro for the negative and the positive controls. Note that for the full enzyme activity controls and the background activity controls the difference in responses between the Battelle microsomes and the In Vitro microsomes are constrained to be zero, since the averages across replicates are constrained to be 100 percent (full activity controls) and 0 percent (background activity controls) respectively.

The interlaboratory statistical analysis combined summary values developed in each of the intralaboratory analyses to assess relationships among the results at each laboratory, the extent of laboratory-to-laboratory variation, and overall consensus estimates among the laboratories with associated variability estimates (incorporating laboratory-to-laboratory variability). The interlaboratory analysis compared the average values for the $\log_{10}IC_{50}$, the slope, the top and bottom threshold parameters of the concentration response curve fits determined by each of the participating laboratories, as reported in the intralaboratory analyses. It also compared among laboratories the background activity control results, the full enzyme activity control results, the negative activity control results, and the positive activity control results obtained at the end of each replicate with those obtained at the beginning. In addition, the interlaboratory analysis compared the intralaboratory results averaged across replicates between the Battelle microsomes and the In Vitro microsomes for the negative and the positive controls.

The objectives of the interlaboratory statistical analysis were to:

- Determine the average values and the variabilities among laboratories for each of the parameters mentioned above.
- Determine the interlaboratory coefficients of variation among laboratories for the $\log_{10}IC_{50}$, the slope, and the top threshold parameters.
- Estimate the ratio of the among laboratory variation to the average within laboratory variation for the parameters mentioned above.
- Compare the percent activity of the Battelle and the In Vitro microsomes for the $\log_{10}IC_{50}$, the slope, the top and bottom threshold parameters of the concentration response concentration relations, and for the negative and positive controls.

Statistical Analysis Methods

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

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

both laboratory-to-laboratory variation and within laboratory variation. The degrees of freedom associated with the overall weighted average were calculated as

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

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

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

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

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

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

2. For WIL, the differences between the beginning and the end portion values (averaged across chemicals) were calculated. The associated standard errors for the differences were calculated as |LS Mean|/(F-value)$^{1/2}$, where F-values were corresponding to the p-values for the portion main effect, as reported in the WIL Laboratories intralaboratory analysis report (Table B-3a).

3. Results for Battelle and In Vitro were used as reported in the intralaboratory analysis reports (averaged across chemicals with associated standard errors) (Tables B-2, B-4).

Within each of the laboratories the intralaboratory analyses of the control responses combined across chemicals were based on mixed effects analysis of variance models that assumed random replicate effects and independent responses within replicates. The models did not account for the constraints on the full enzyme activity control responses (sum to 100 percent) or the background activity control responses (sum to 0 percent) within each replicate. This affects the standard errors that are presented in the tables in Appendix B. As an example, for the RTI (Table B-1a) aminoglutethimide background activity control the replicate-to-replicate variability is zero and so the standard error of the end minus beginning portions is given as 0.305 $\equiv 0.216\sqrt{2}$. These standard errors given in Appendix B do not account for the constraints on the sums of the background activity control and the full enzyme activity control values within replicates.

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

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

1. For RTI, for each chemical averages were calculated averages across the beginning and the end portions. For each chemical, the associated standard errors for the averages were calculated as $(1/2)(2(S_e^2+S_b^2)-S_d^2)^{1/2}$, where S_e, S_b, and S_d were the standard errors associated with the end, the beginning, and the difference between the end and the beginning respectively. S_e and S_b were reported in the intralaboratory analysis, and S_d was calculated as discussed above (see above item 1). This expression accounts for possible correlation between the beginning and the end portions within each replicate due to replicate-to-replicate variation. The difference between the two microsome sources was then calculated as the difference between the averages over the five chemicals per source, $(1/5)(\sum M_{1i}-\sum M_{2i})$, with associated standard errors $[(\sum s_{1i}^2+\sum s_{2i}^2)/5]^{1/2}$, where M_{1i} and s_{1i} are the average and associated standard error for the i^{th} chemical within the Battelle microsomes and M_{2i} and s_{2i} are the average and associated standard error for the i^{th} chemical within the In Vitro microsomes.

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

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

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

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

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

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

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

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

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

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

$$CV=|S/M|$$

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

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

where $t_{df}(NC)$ is noncentral t distribution with noncentrality parameter NC ($= \mu/(\sigma/\sqrt{4})$) and degrees of freedom df is the estimated degree of freedom among the four laboratories, shown above. The solution to the first equation gives the lower bound of the CI, and the solution for the

second equation gives the upper bound of the CI. A Newton-type algorithm is used to solve for the noncentrality parameters in these equations (Lehmann, 1986).

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

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

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

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

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

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

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

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

Round off

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

Figure Symbols

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

Statistical Analysis Results

As discussed in the method section, the interlaboratory comparisons for the $\log_{10}IC_{50}$, the slope, the top, and the bottom parameters were performed separately for each chemical. The interlaboratory comparisons for the background activity, full enzyme activity, negative and positive control data were performed combined across chemicals.

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

Convergence problems prevented successful concentration response curve fits for the two non-inhibitor chemicals dibenz[a,h]anthracene (DBA) and atrazine (ATZ) in all four intralaboratory analyses of the individual laboratory data. Therefore the interlaboratory analyses of the concentration response curve parameters were performed only for the other eight chemicals. The mixed model analysis of variance comparisons among the laboratories accounted for potential systematic differences between the two sources of microsomes.

Table 1 displays the estimated $\log_{10}IC_{50}$ and slope within each laboratory and the associated 95 percent confidence intervals about the $\log_{10}IC_{50}$ and slope for each chemical[1]. Table 1 also displays the mean difference between the two sources of microsomes and the overall mean values across laboratories and their associated 95 percent confidence intervals, incorporating among laboratory variation based on the random effects analysis of variance and heterogeneous within laboratory variation. These means and confidence intervals are displayed in Figures 1 through 16. 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 1.

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

Table 2 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 2 also displays the among laboratory component of random variation, the p-values associated with the tests that the among laboratory variation is zero, and the squares of the standard errors of the overall mean values, as well as their associated degrees of freedom. The ratios of the among laboratory variances to the unweighted average within laboratory variances are also displayed, with their associated 95 percent confidence intervals.

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

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

The estimates for $\log_{10}IC_{50}$ (Table 1) were similar among the four laboratories for most chemicals except for econazole (ECZ). For econazole, the estimated $\log_{10}IC_{50}$ for In Vitro was smaller than for any of the other three laboratories and the associated 95 percent confidence interval for In Vitro did not overlap with those for the other three laboratories (Figure 11). The CVs for IC_{50} exceeded 22.5 percent for econazole and for chrysin and 47.3 percent for dicofol. They were less than 14.7 percent for the IC_{50}s for the other chemicals (Table 1). The ratios of the among laboratory variance to the average within laboratory variance were 6.8 for econazole, 4.6 for chrysin, 2.3 for prochloraz, and less than 0.05 for the remaining five chemicals (Table 2). The large CV for dicofol (47.4 percent, Table 1) was caused by the relatively large within laboratory variance. The pooled within laboratory variance was 0.045 for dicofol, compared to less than or equal to 0.002 for the other seven chemicals. For chrysin, the estimated $\log_{10}IC_{50}$ for RTI was smaller than those for the other three laboratories (Figure 13), which was the principal reason for the relatively large CV and among laboratory variance for this chemical.

The estimates for slope (Table 1) were similar among the four laboratories for most chemicals except for econazole (ECZ). For econazole, the estimated slope for In Vitro was less negative than the other three laboratories and the associated 95 percent confidence interval did not overlap with those for the other three laboratories (Figure 12). For 4-nonylphenol the In Vitro slope was more negative than those at the other laboratories (Figure 8). The ratios of the

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

among laboratory variances to the pooled within laboratory variance were 9.0 for econazole and zero for the seven other chemicals (Table 2). The CVs for the slope were 10.2 percent for chrysin, 15.2 percent for dicofol, and less than or equal to 8.8 percent for the other chemicals (Table 1). The relatively large CVs for chrysin and for dicofol were due to the relatively large within laboratory variances for these chemicals. The pooled within laboratory variances were 0.024 for dicofol, 0.021 for chrysin, 0.013 for 4-nonylphenol, and less than or equal to 0.004 for the other chemicals. Note that for 4-nonylphenol (NYP), the estimated slope for In Vitro was more negative than for the other three laboratories (-2.48 compared to the closest one at -1.95) (Table 1 and Figure 8). However for 4-nonylphenol (NYP), the within-laboratory variance for In Vitro was at least 3 times of the other three laboratories, and therefore less weight was given to In Vitro results when combining the intralaboratory results (Table 2).

The estimates for the top were similar for the four laboratories. The CVs for the top were less than 3 percent for each of the chemicals (Table A-1). The among laboratory variances to the pooled within laboratory variance ratios were 2.3 for 4-nonylphenol, 0.7 for dicofol, and zero for the rest (Table A-2). Some laboratories had very large within laboratory variances relatively to the others for some chemicals, however there were no consistent patterns for any laboratory.

The estimated bottom thresholds for In Vitro were higher than those for the other three laboratories for 4-nonylphenol and for dicofol (Table A-1, Figure A-8, Figure A-16). The associated 95 percent 4-nonylphenol confidence interval for In Vitro did not overlap with those for the other three laboratories (Figure A-8). The ratios of the among laboratory variances to the pooled within laboratory variance ratios were 12.6 for 4-nonylphenol and 5.6 for chrysin (Table A-2), and zero for the other chemicals. Some laboratories had very large within laboratory variances relatively to the others for some chemicals, however there were no consistent patterns for any laboratory (Table A-2).

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

Table 3 displays the estimated parameter values and the associated within laboratory 95 percent confidence intervals for the differences between the beginning and the end, and for the differences between the two sources of microsomes[3]. It also displays the overall mean differences across laboratories and their associated 95 percent confidence intervals, incorporating among laboratory variation based on the random effects analysis of variance. The mean differences and confidence intervals are displayed in Figures 17 to 22. Each figure includes a reference line corresponding to the overall average. Figures 17 through 20 display the results for the differences between the beginning and the end, one for each control endpoint. Figures 21 and 22 display the results for the differences between the two microsome sources for the negative and positive controls respectively.

Table 4 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 4 also displays the laboratory to laboratory random

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

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

The following results were observed:

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

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

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

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

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

References

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Table 1. Parameter Estimates and the 95 percent Confidence Intervals for $Log_{10}IC_{50}$ and Slope Parameter of Concentration Response Curves for Placental Assay. Estimated by Chemical

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

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

Table 2. Variance Components and Ratio of Between and Within Laboratories Variances for $Log_{10}IC_{50}$ and Slope Parameter of Concentration Response Curves for Placental Assay. Estimated by Chemical

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

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

Table 3. Parameter Estimate and the 95 percent Confidence Interval for Differences Between Beginning and End (End Minus Beginning) and Between the Two Sources of Microsomes (Battelle minus In Vitro) for the Percent of Control Responses in the Placental Aromatase Assay.

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

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

Table 4. Variance Components and Ratio of among and Within Laboratories Variances for Differences between Beginning and End and between the Two Sources of Microsomes by Battelle and In Vitro for the Percent of Control Responses for Placental Aromatase Assay

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

1. The within laboratory variance for each laboratory is the square of the standard error associated with the parameter estimate, as reported in the intralaboratory analyses for the four participating laboratories (Appendix B).
2. Pooled average for the within laboratory variances is the unweighted average of the within laboratory variances among the four laboratories. Associated degrees of freedom were based on Satterthwaite's approximation
3. Among laboratories variance is based on a one-way random effects analysis of variance model with heterogeneous within laboratory variances, equal to the squares of the within laboratory standard errors.
4. Mean variance is the square of the standard error of the pooled weighted mean value. It includes both within and among laboratory variation.
5. Degrees of freedom for the (mean) overall effect variance were estimated as $2*((1/K)*\sum(S_L^2+S_i^2))^2/(var(S_L^2)+(2/K^2)*\sum(S_i^4/df_i))$, where S_L^2 is the among laboratory variance, S_i^2 and df_i are the reported variance and degrees of freedom for laboratory i, $var(S_L^2)$ is the variance of S_L^2, and K is the number of laboratories (Hartung and Makambi, 2001).
6. Ratio of the among-laboratory variance and the pooled average within laboratory variance.
7. Microsomes were prepared by Battelle and In Vitro laboratories and distributed to the four laboratories as shown in Table M-D-T. Results for the difference between the two sources of microsomes within each laboratory were listed in Appendix B.

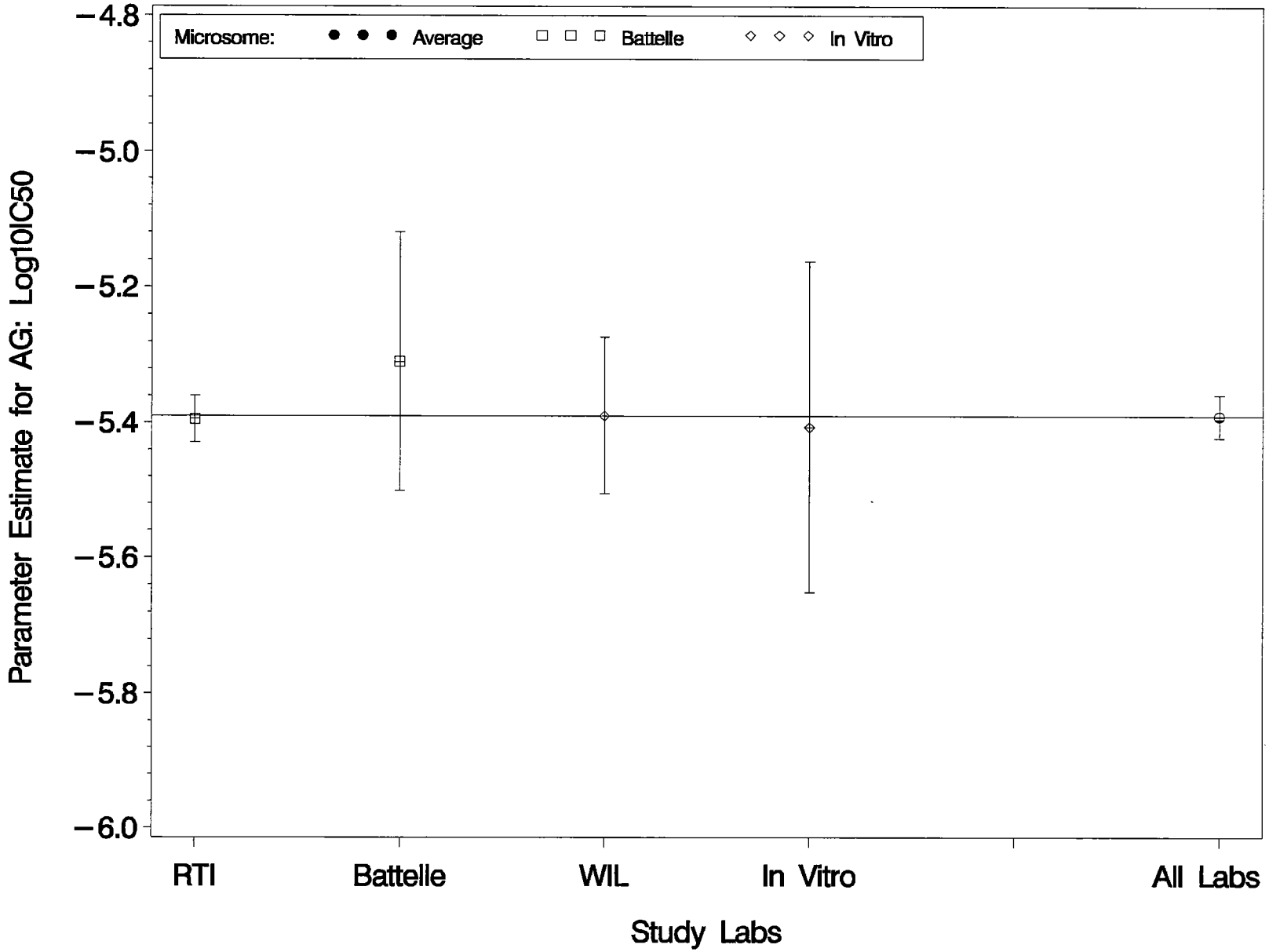


Figure 1. **<u>Aminoglutethimide</u>: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

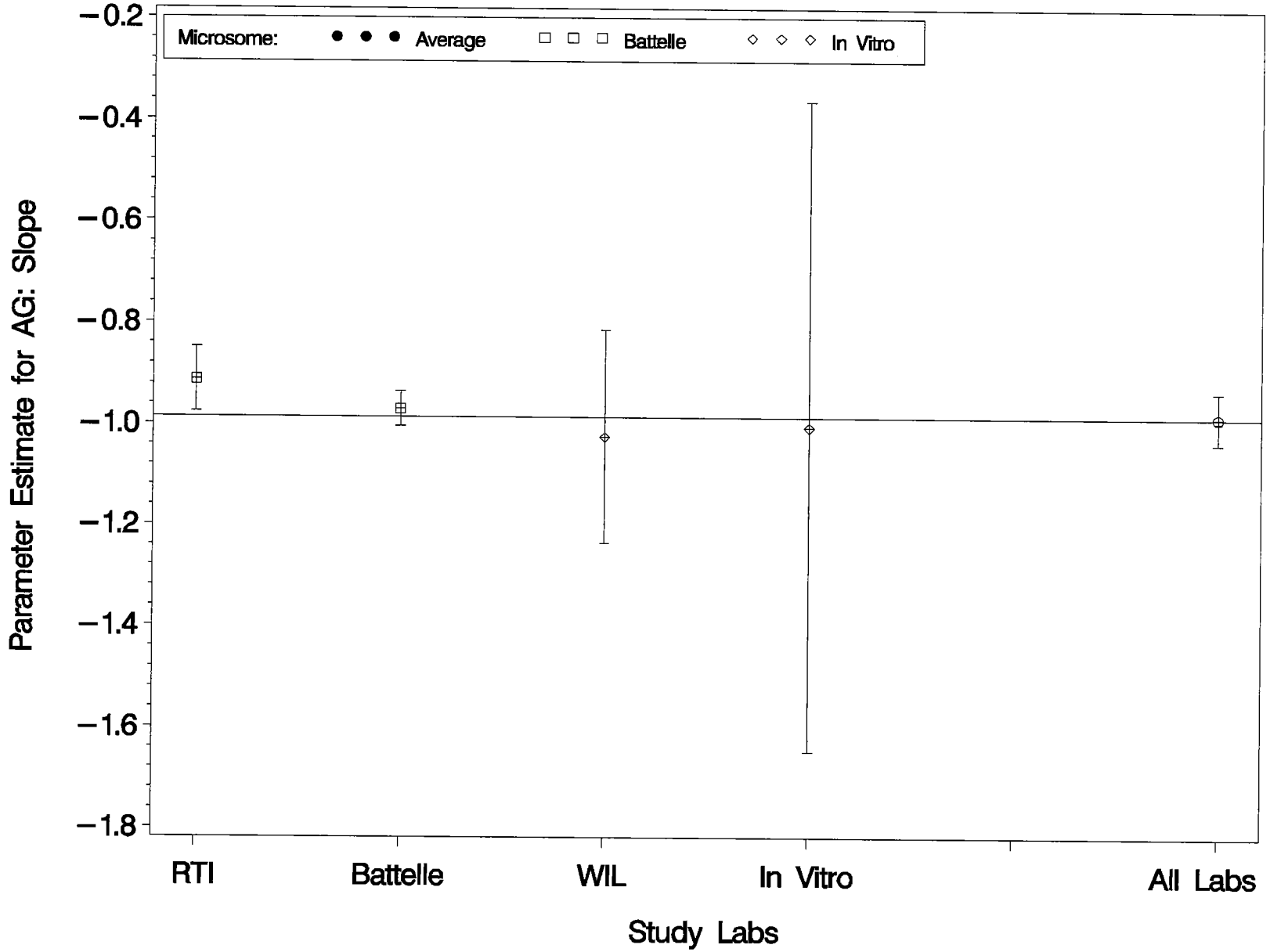


Figure 2. **Aminoglutethimide: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

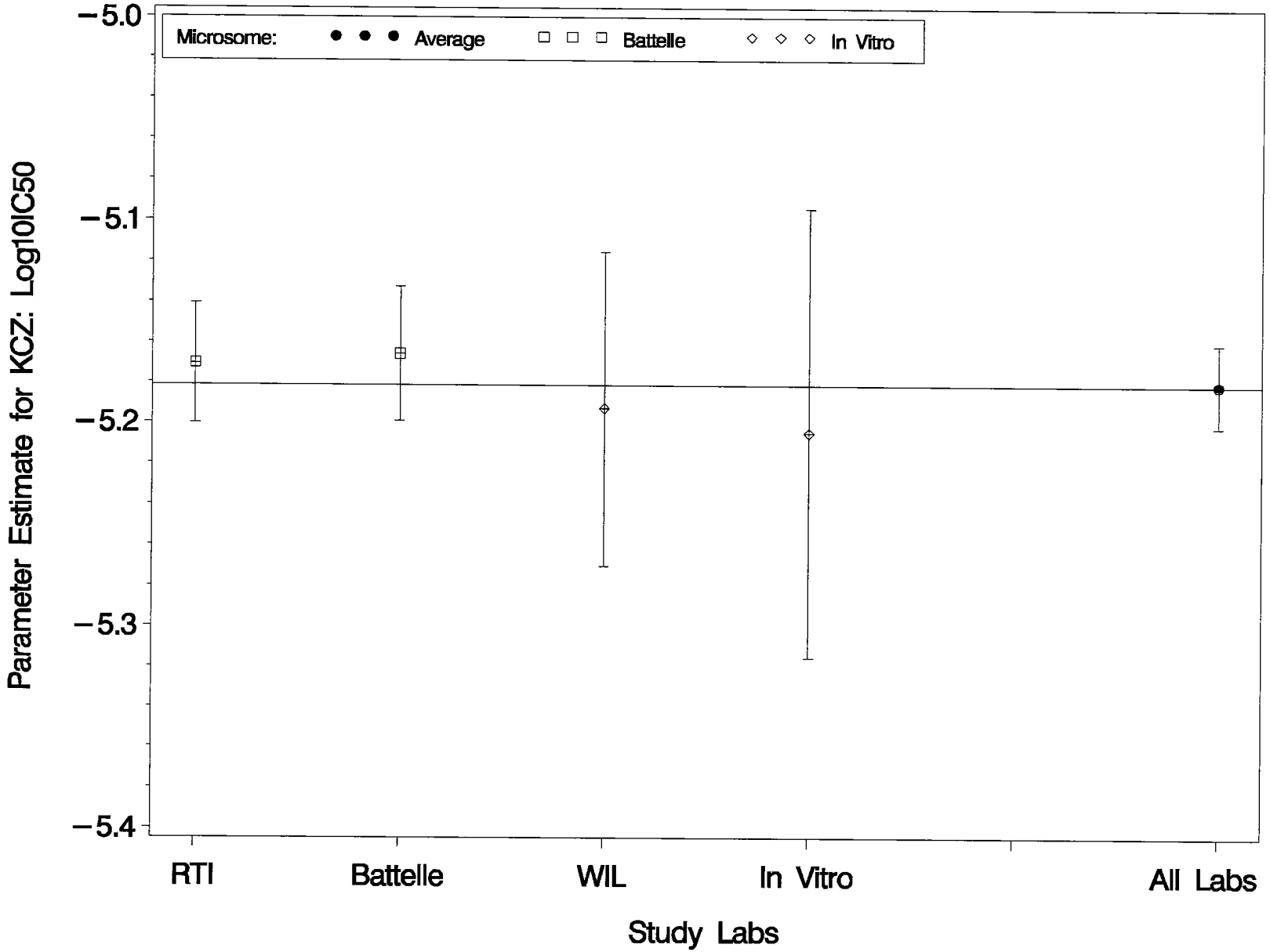


Figure 3. **Ketoconazole: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

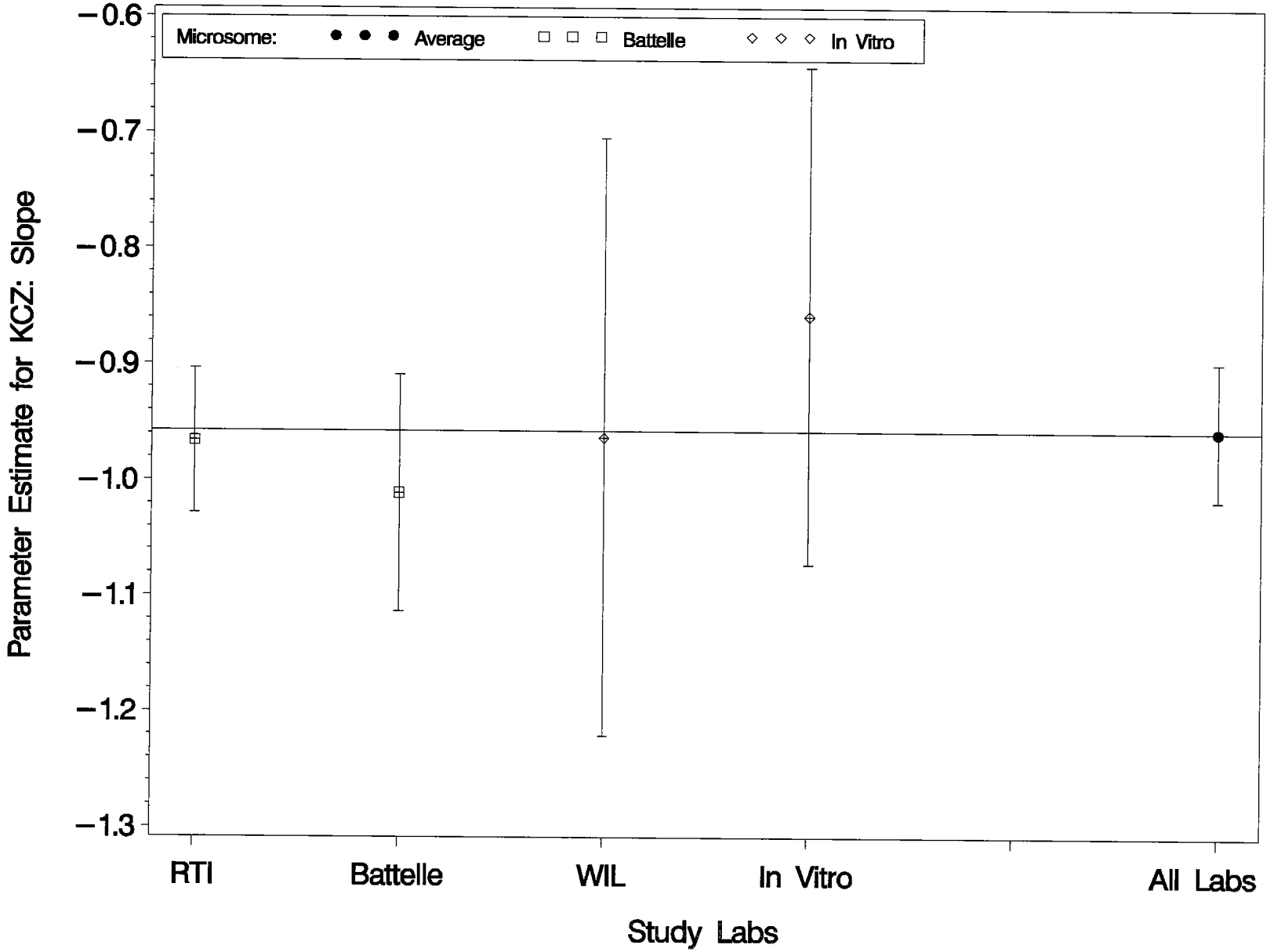


Figure 4. **Ketoconazole: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

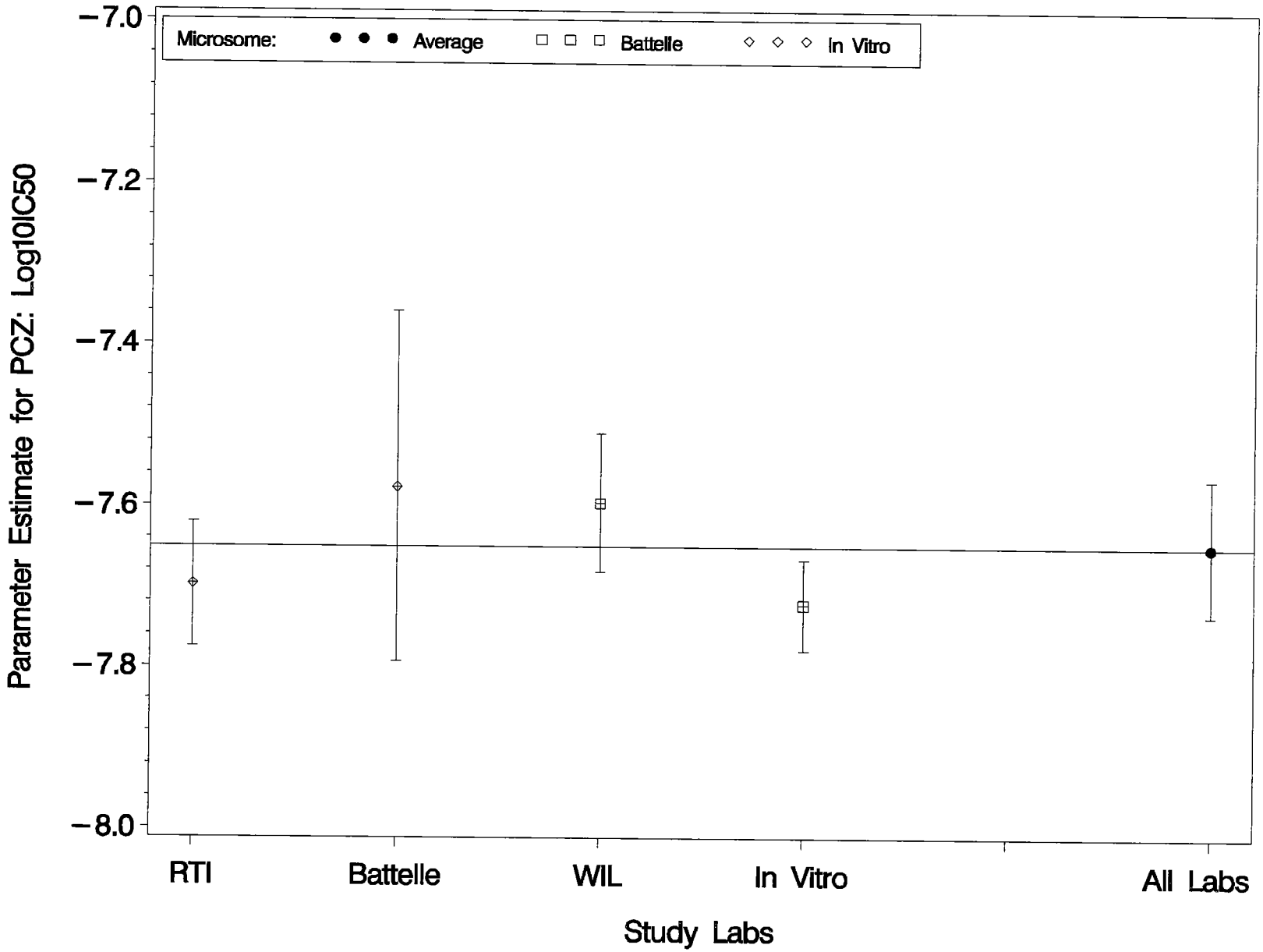


Figure 5. **<u>Prochloraz:</u> Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

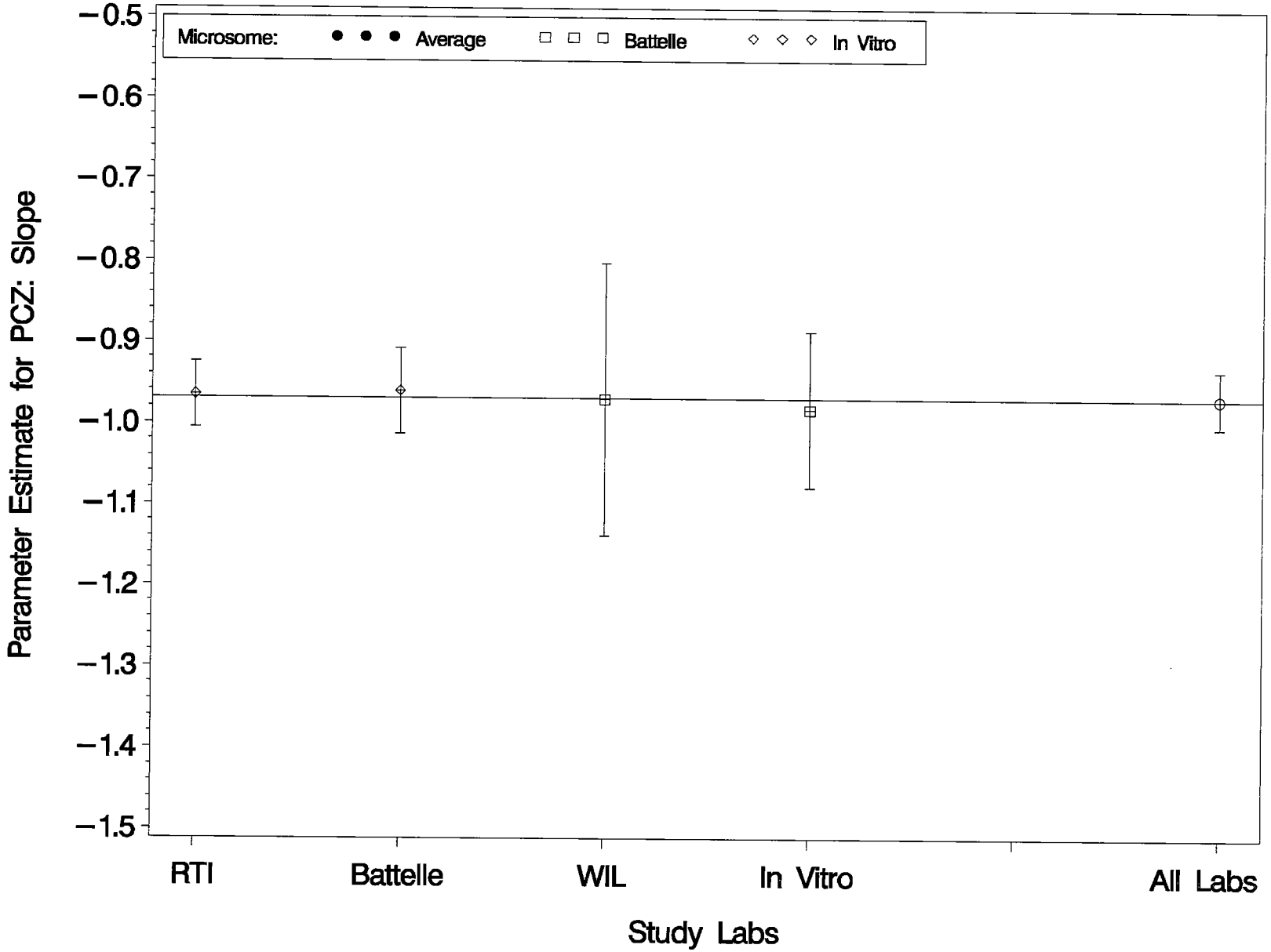


Figure 6. **Prochloraz: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

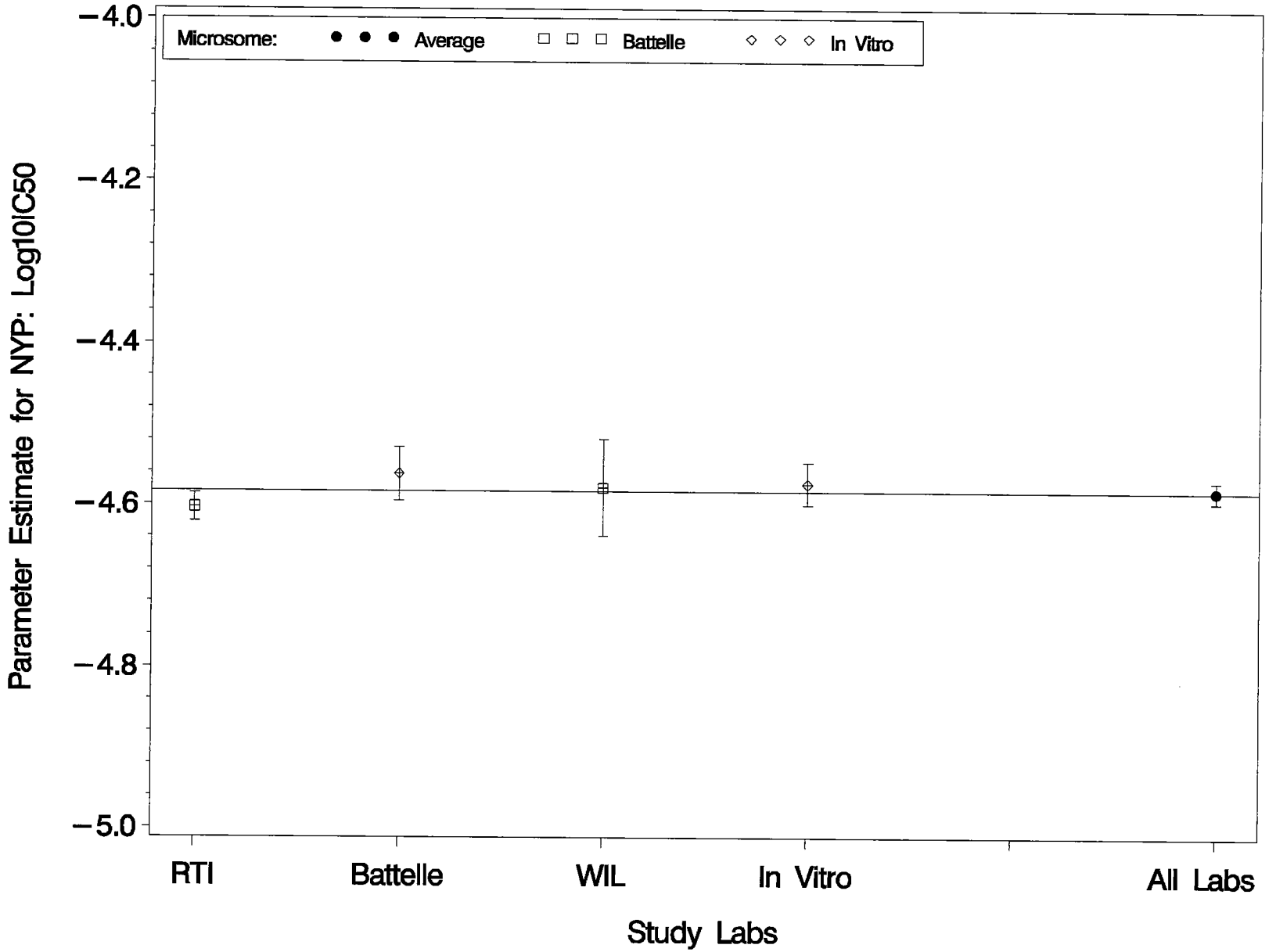


Figure 7. **4-Nonylphenol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

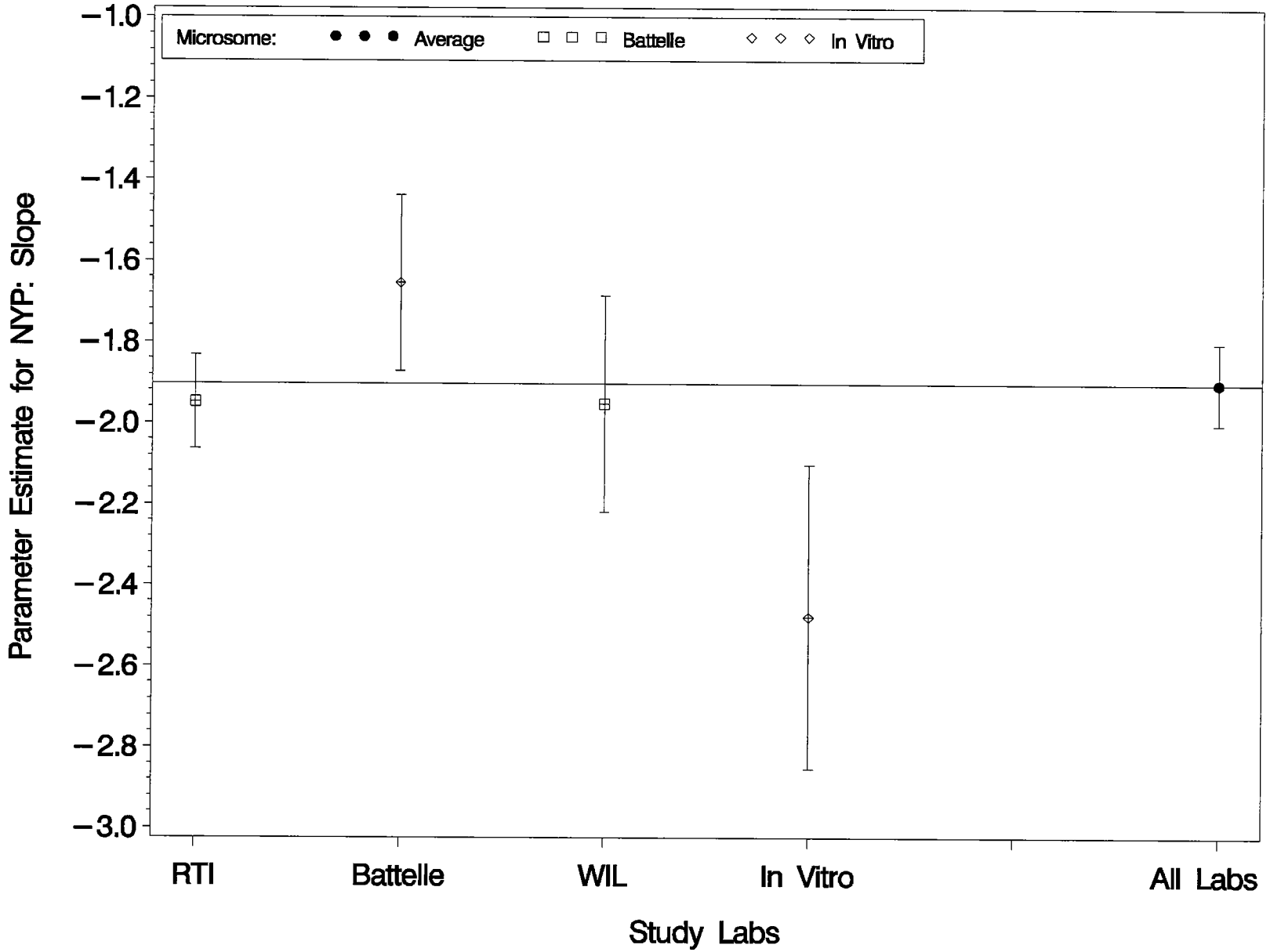


Figure 8. **4-Nonylphenol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

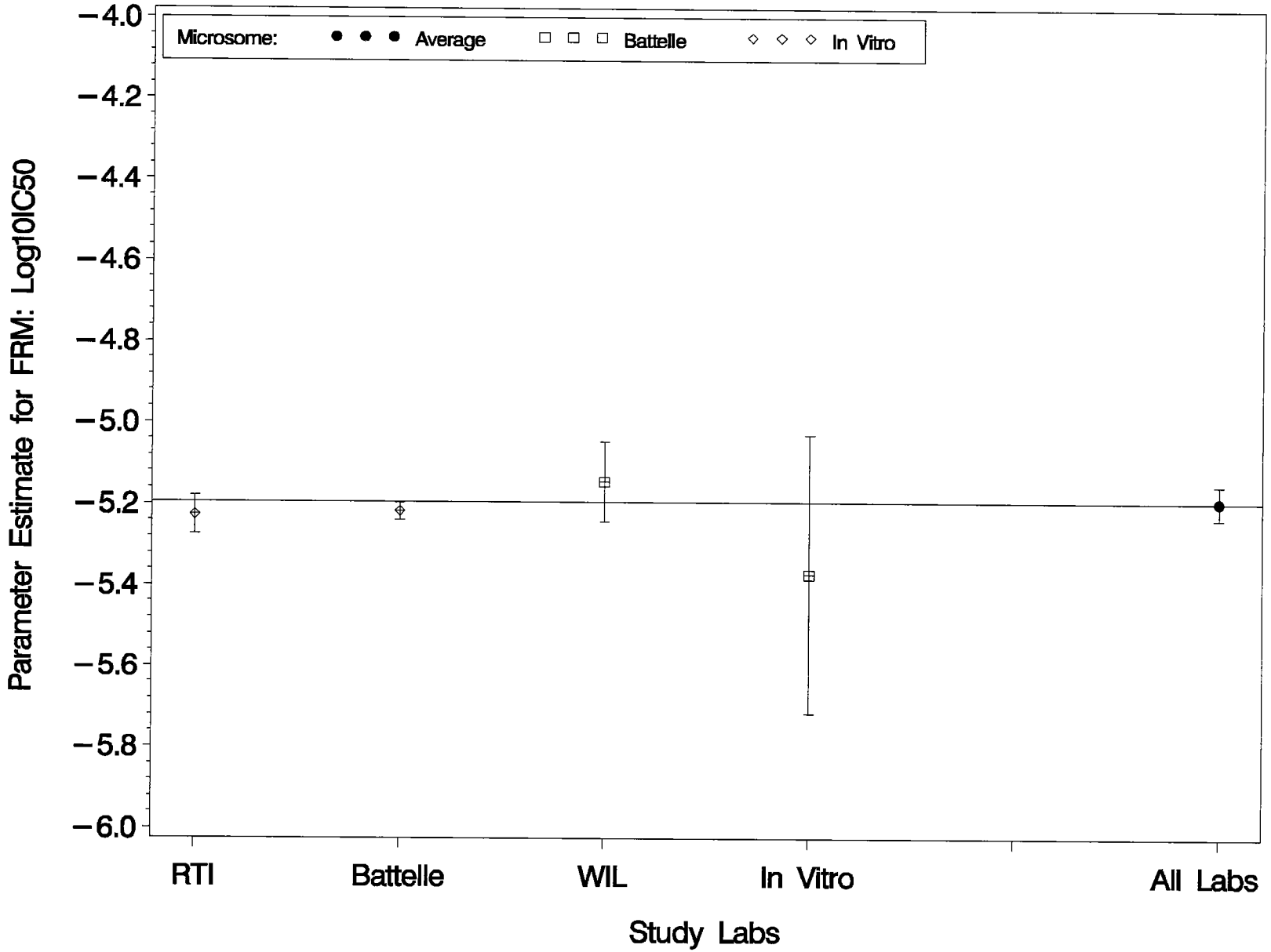


Figure 9. **Fenarimol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

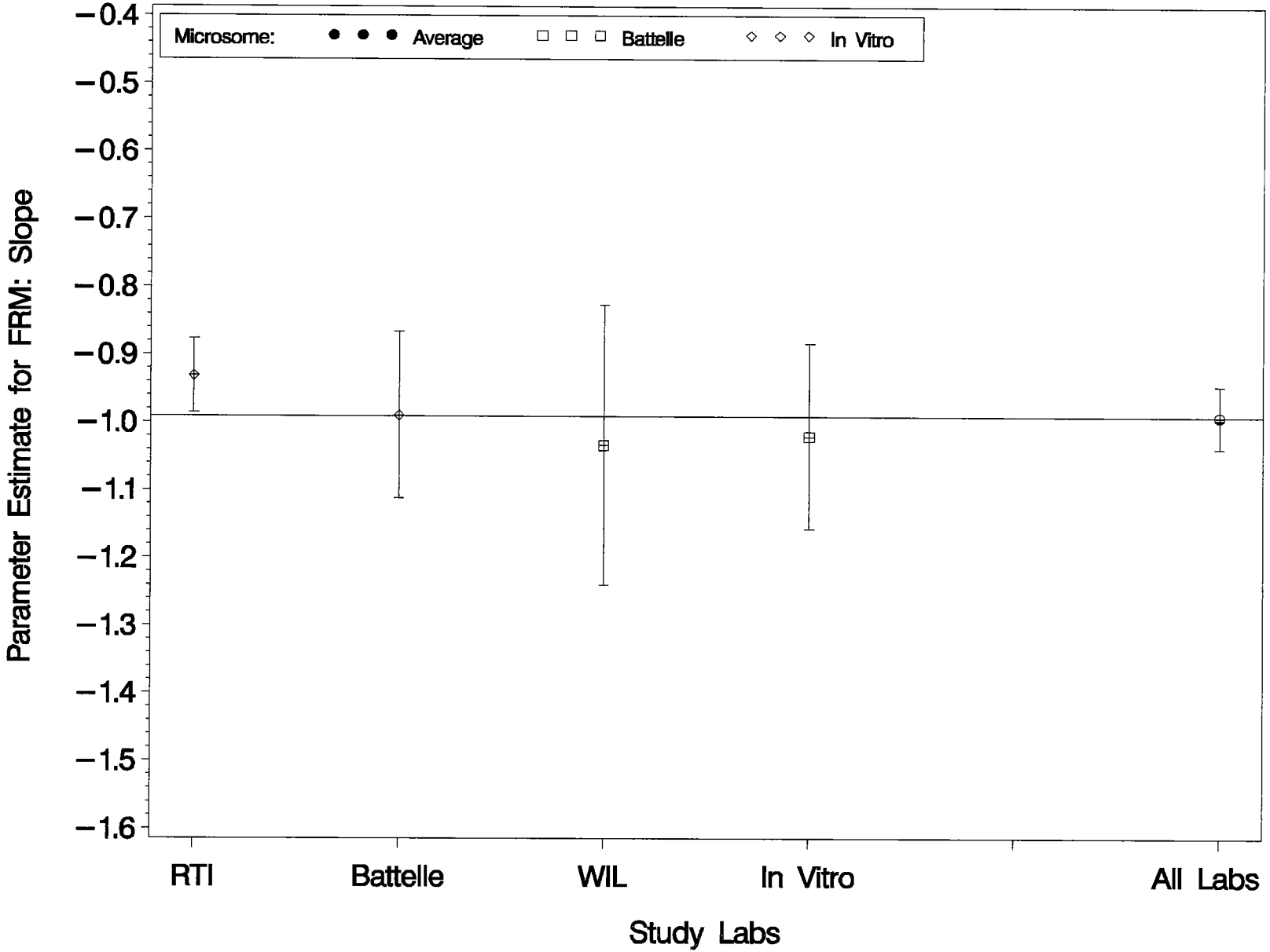


Figure 10. **Fenarimol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

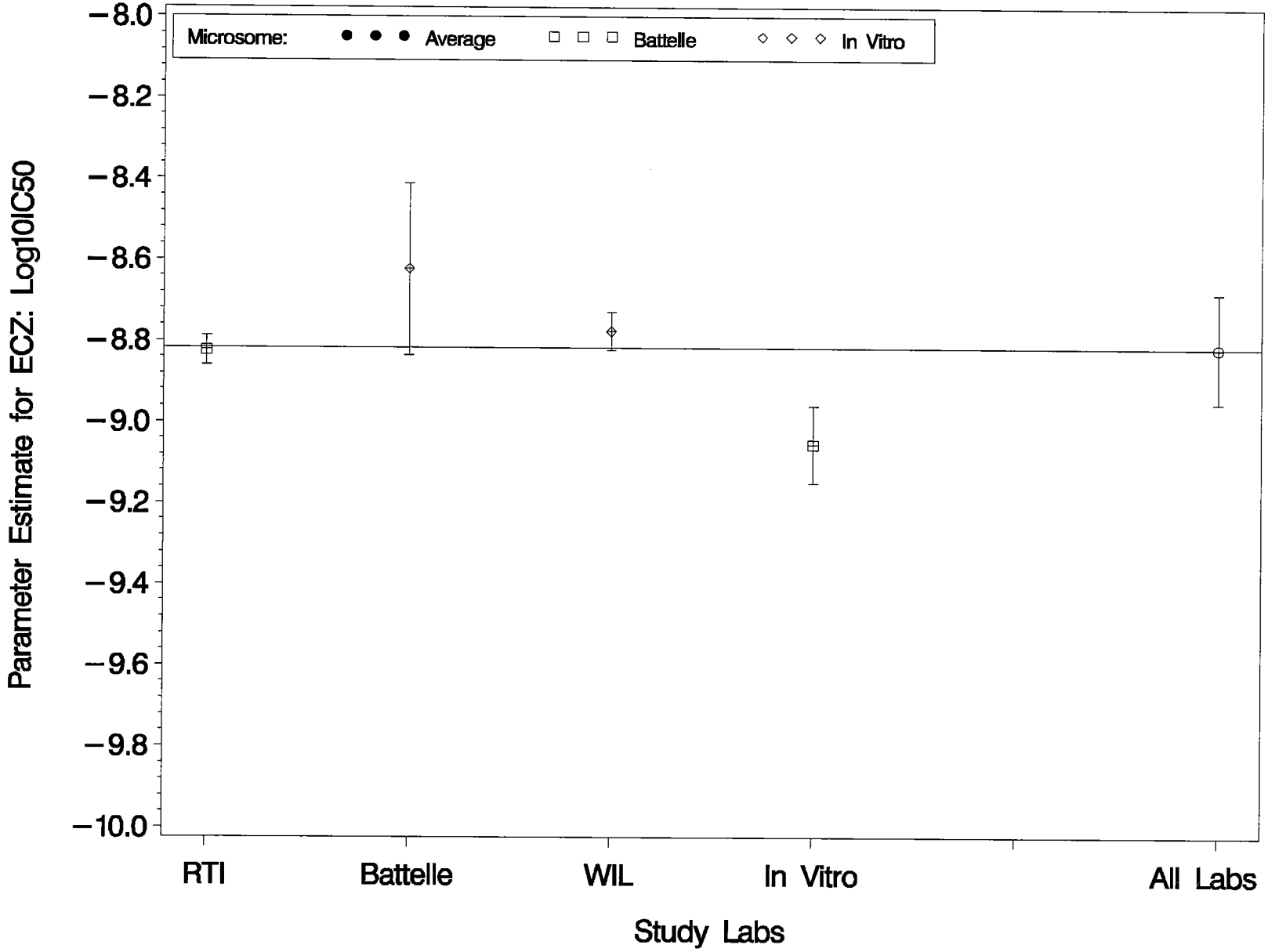


Figure 11. **<u>Econazole:</u> Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

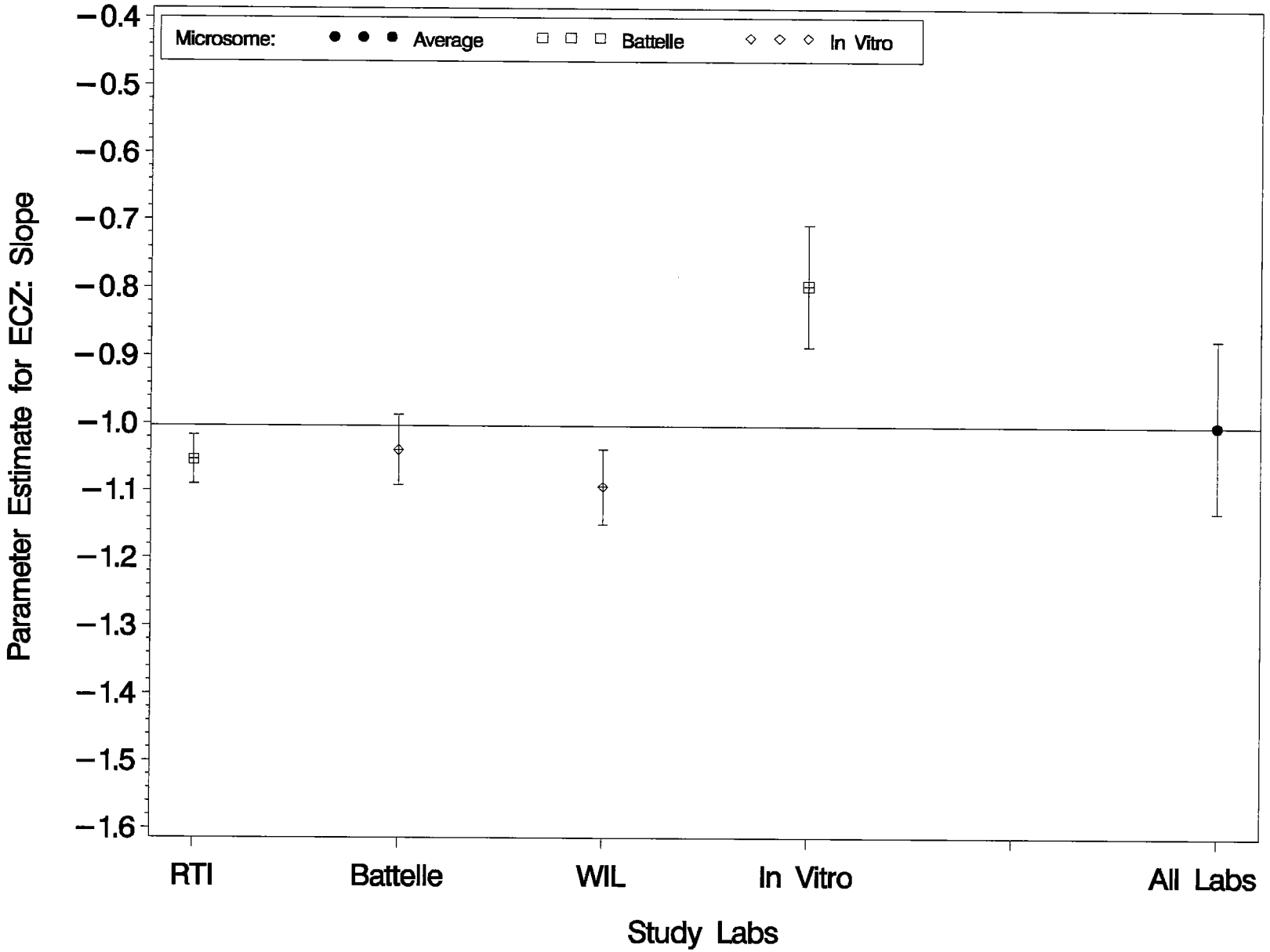


Figure 12. **<u>Econazole:</u> Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

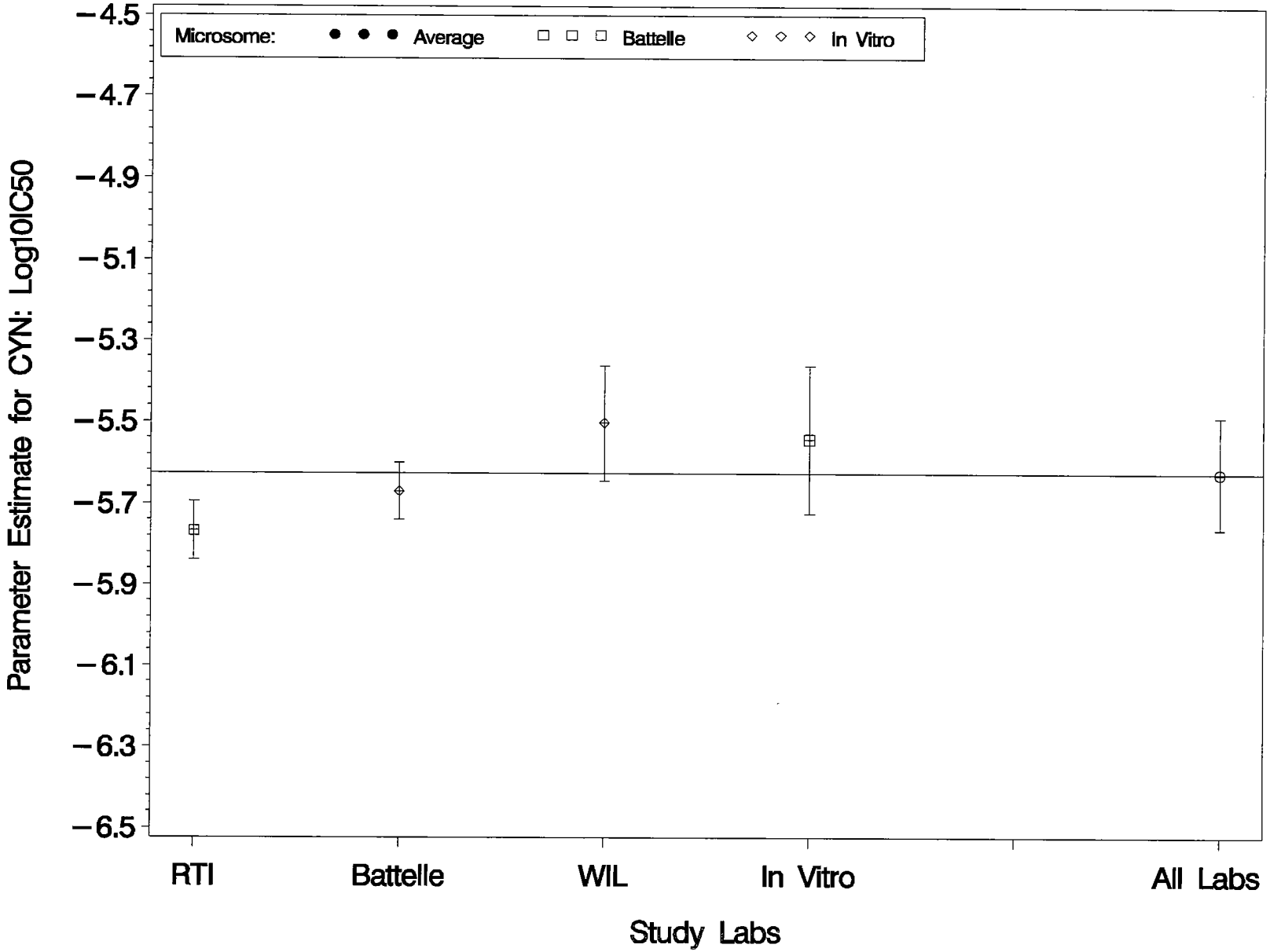


Figure 13. **Chrysin: Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

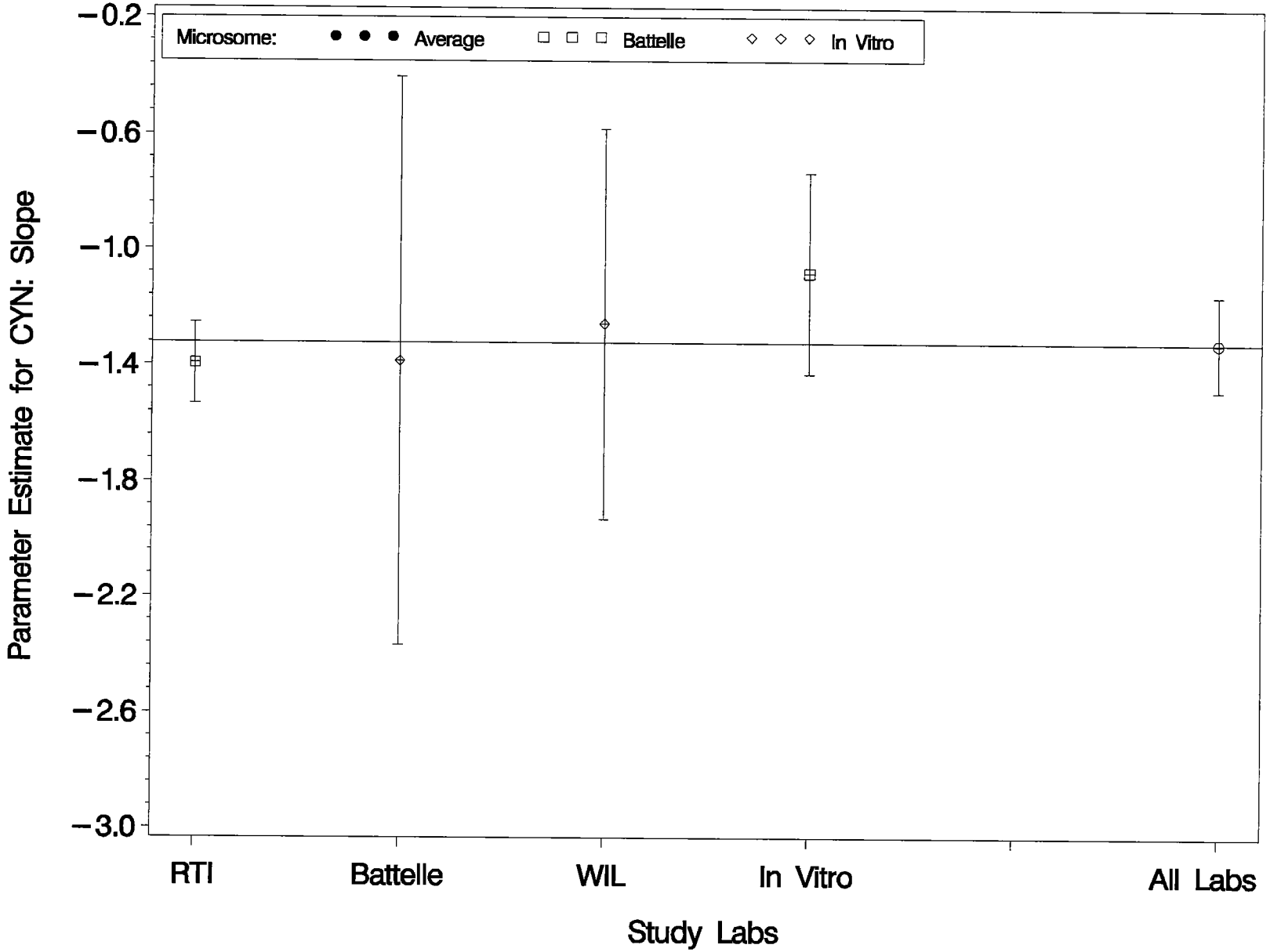


Figure 14. **Chrysin: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

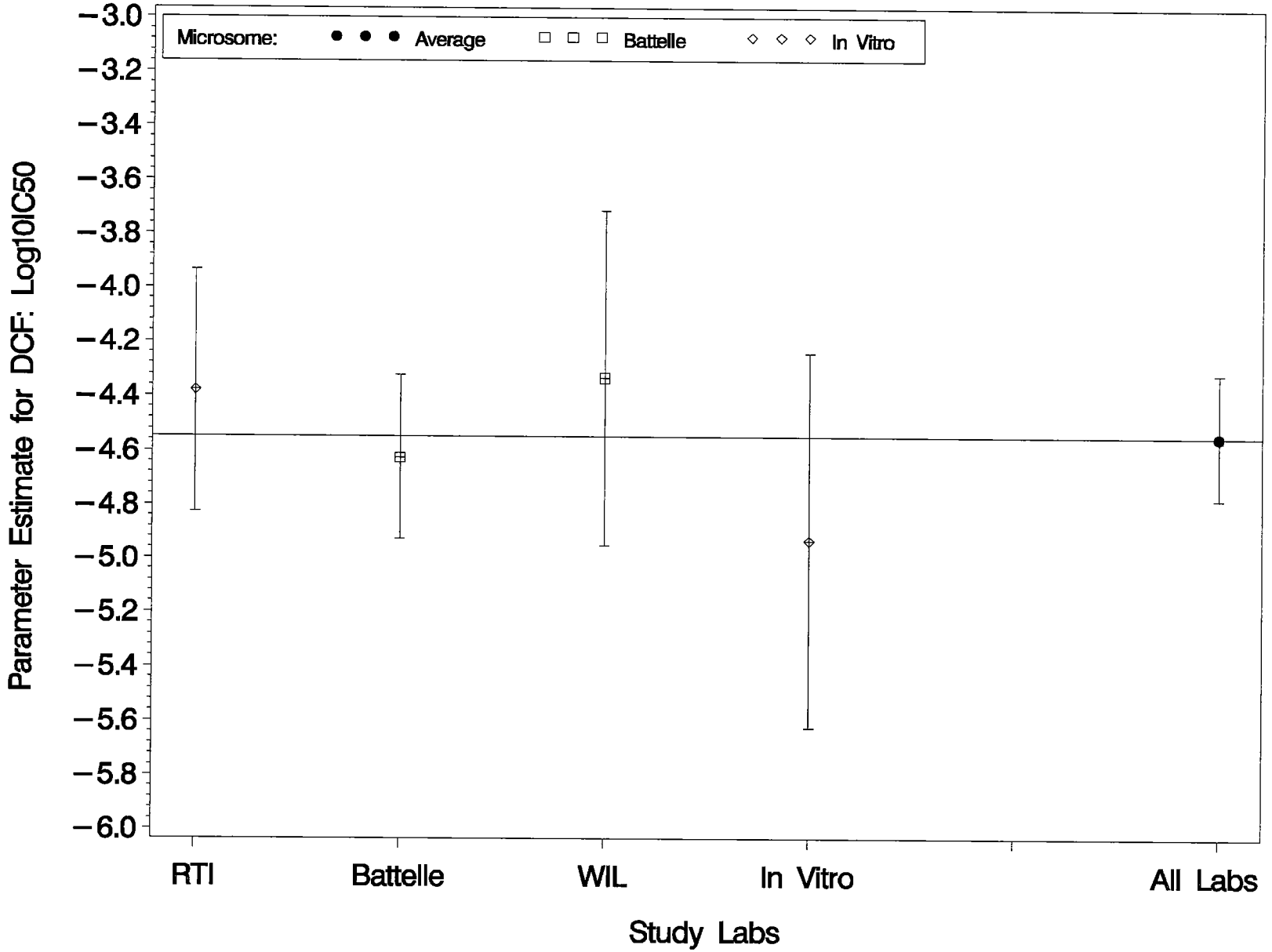


Figure 15. **<u>Dicofol:</u> Parameter Estimates and Their Associated 95 percent Confidence Intervals for $Log_{10}IC_{50}$ in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

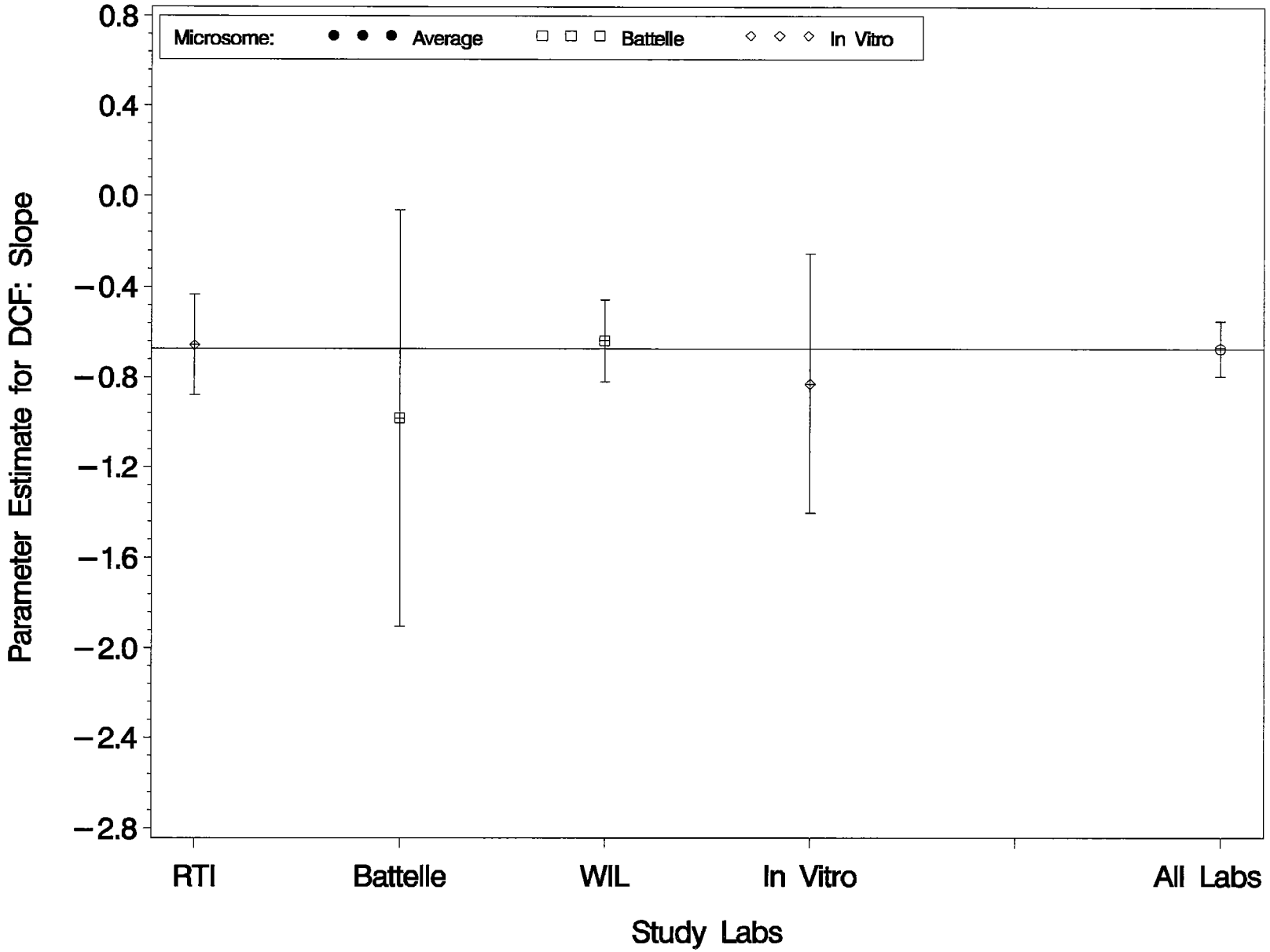


Figure 16. **Dicofol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Slope in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

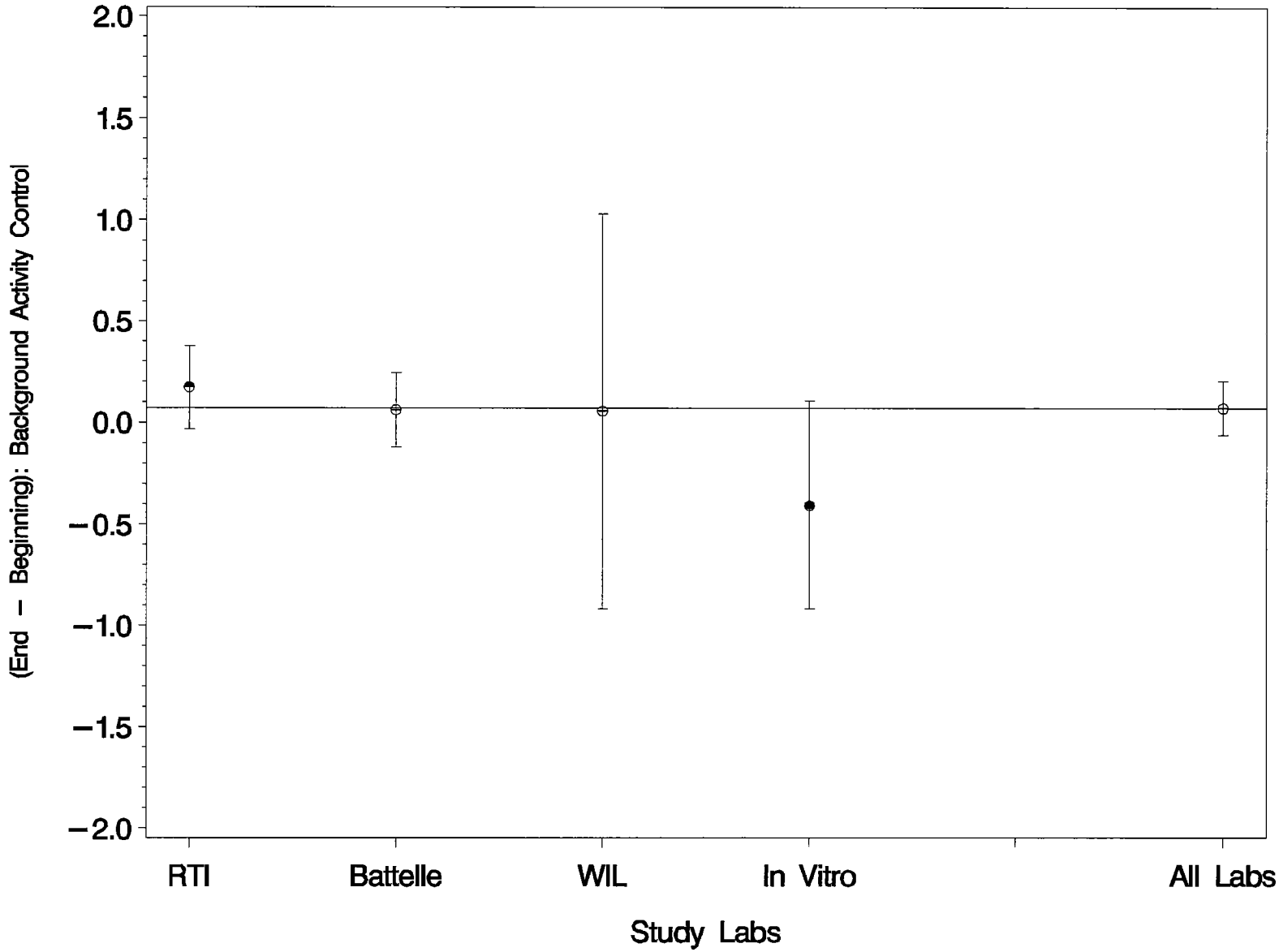


Figure 17. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference (End Minus Beginning) for Background Activity Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

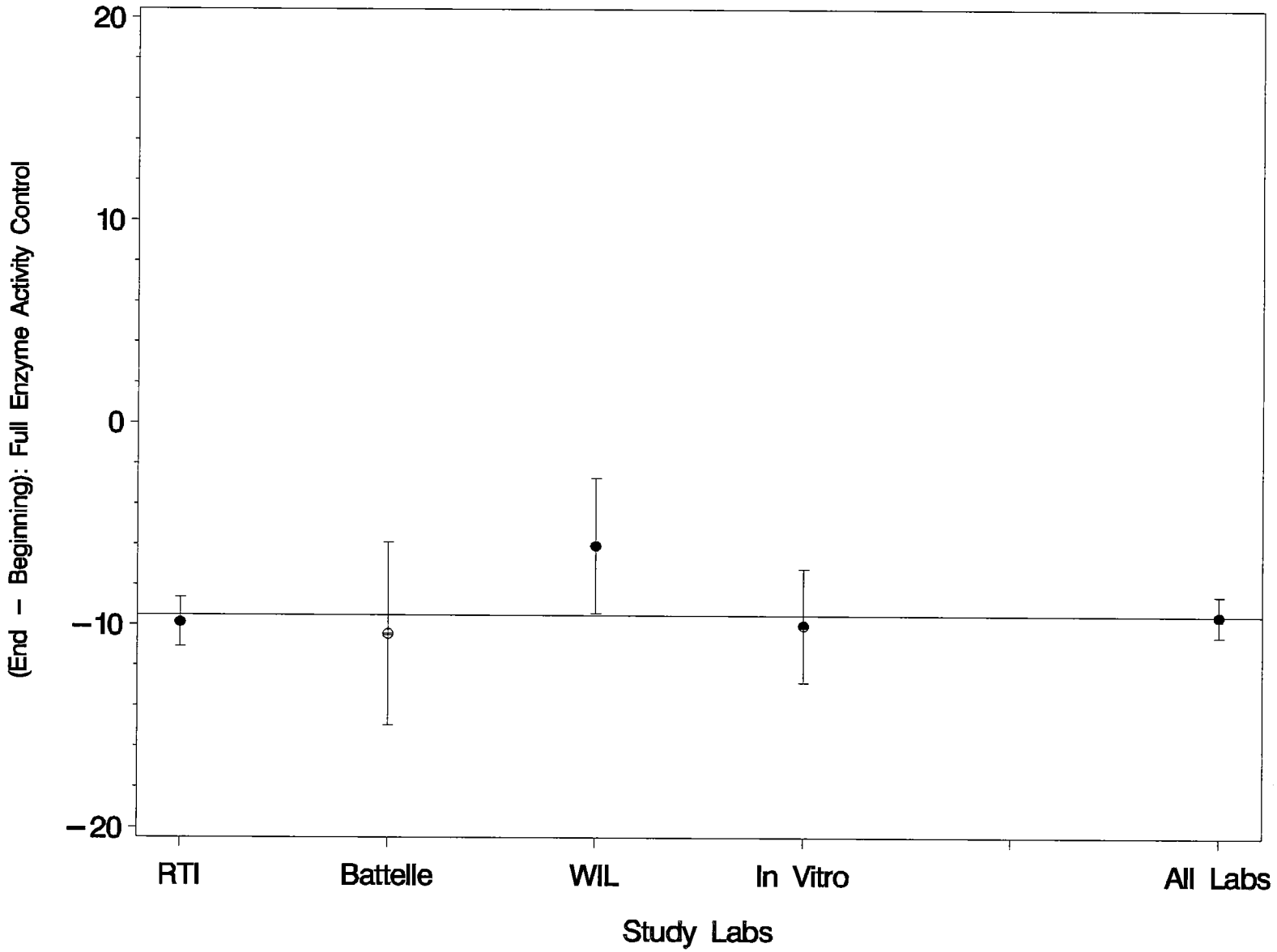


Figure 18. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference (End Minus Beginning) for Full Enzyme Activity Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

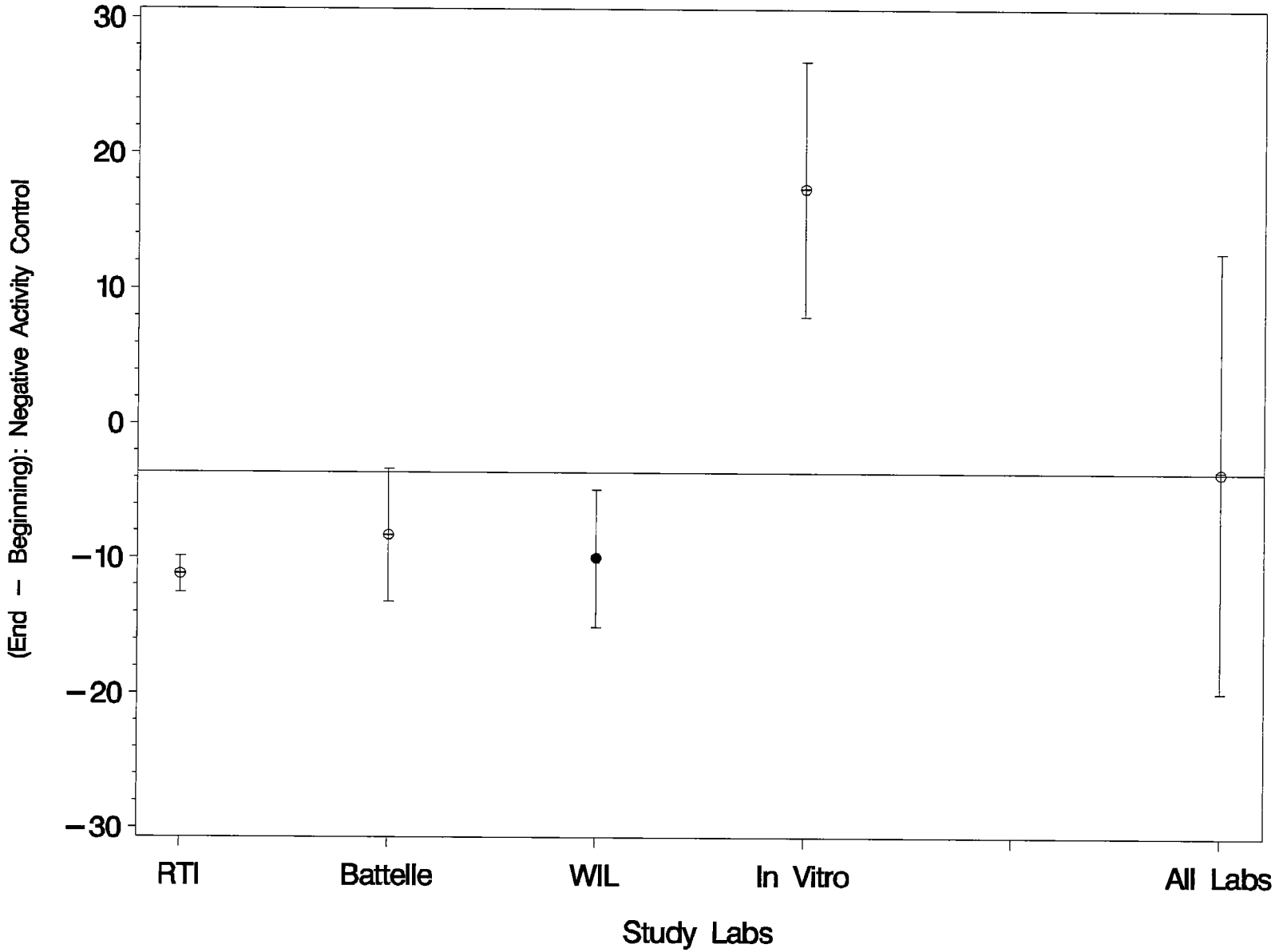


Figure 19. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference (End Minus Beginning) for Negative Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

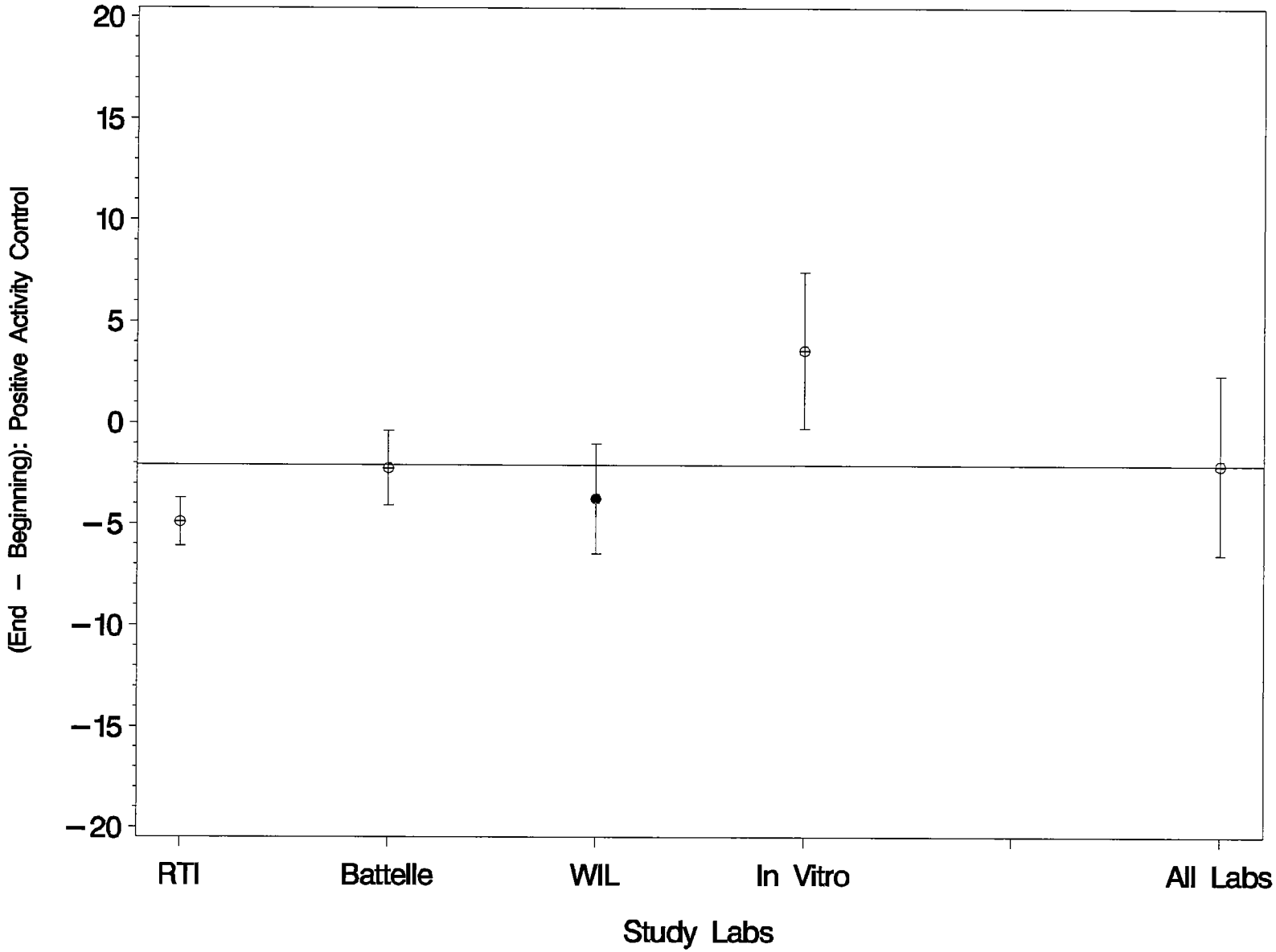


Figure 20. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference (End Minus Beginning) for Positive Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

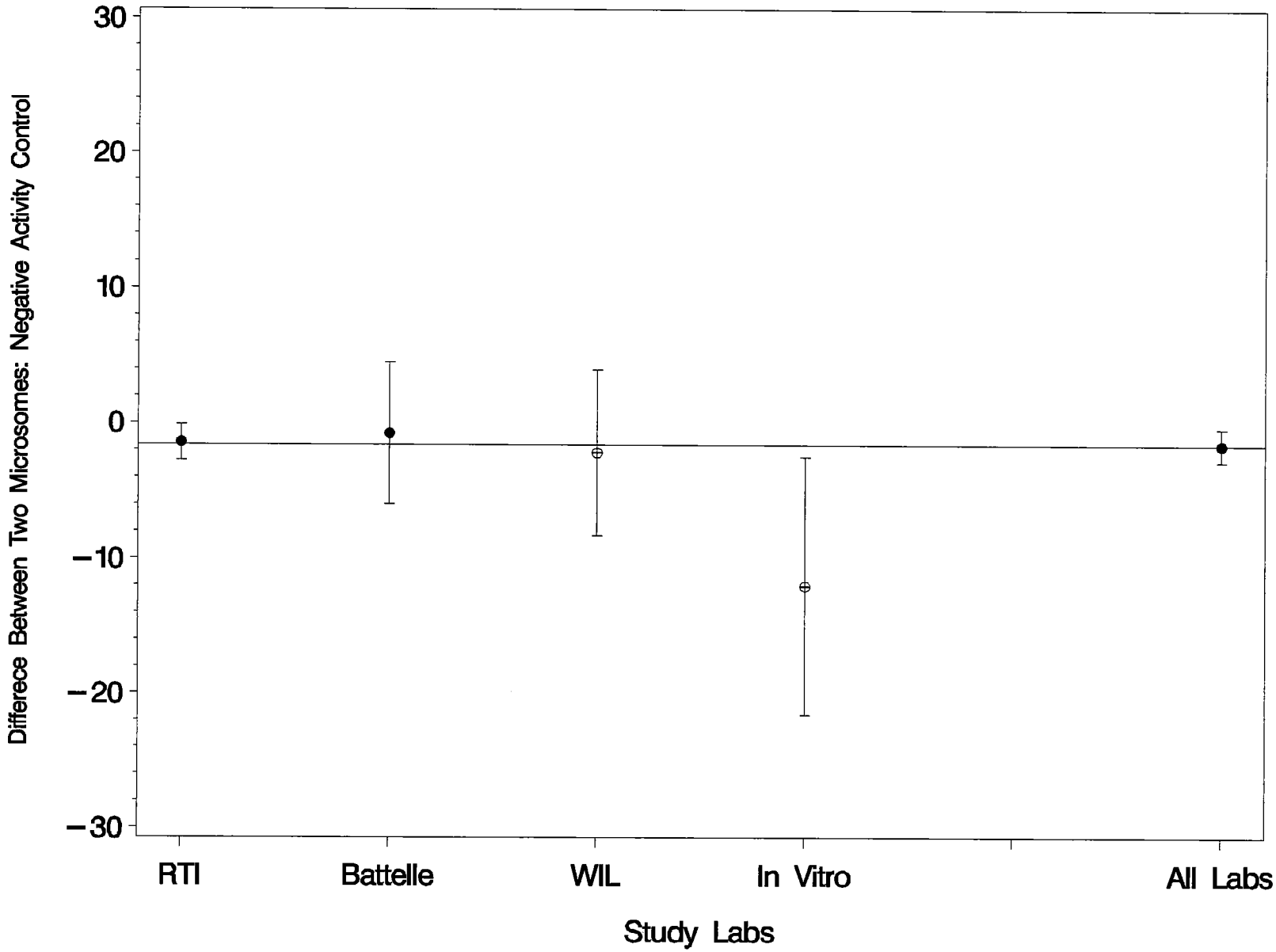


Figure 21. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference Between Sources (Battelle Minus In Vitro) for Negative Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

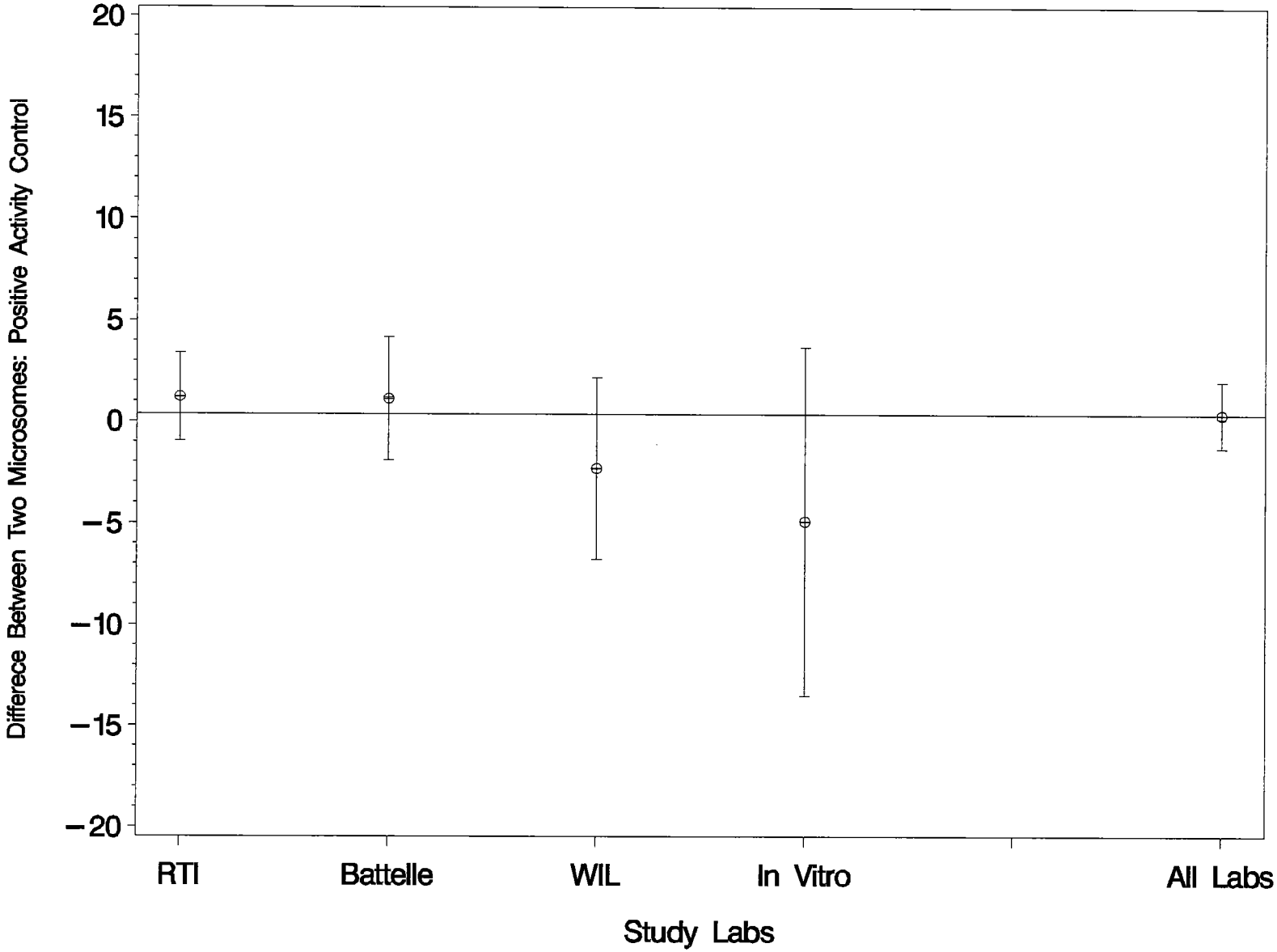


Figure 22. **Parameter Estimates and Their Associated 95 percent Confidence Intervals for Difference Between Sources (Battelle Minus In Vitro) for Positive Controls in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

Appendix A. Results of Interlaboratory Analysis for Top and Bottom Parameters of Concentration Response Curves.

- **Table A-1. Parameter Estimates and the 95 percent Confidence Intervals for Top and Bottom Parameters of Concentration Response Curves for Placental Assay. Estimated by Chemical.**

- **Table A-2. Variance Components and Ratio of Between and Within Laboratories Variances for Top and Bottom Parameters of Concentration Response Curves for Placental Assay. Estimated by Chemical.**

- **Figures A-1 through A-16. Parameter Estimates and the 95 percent Confidence Intervals for Top and Bottom Parameters of Concentration Response Curves for Placental Assay. Estimated by Chemical.**

Table A-1. Parameter Estimates and the 95 percent Confidence Intervals for Top and Bottom Parameters of Concentration Response Curves for Placental Assay. Estimated by Chemical.

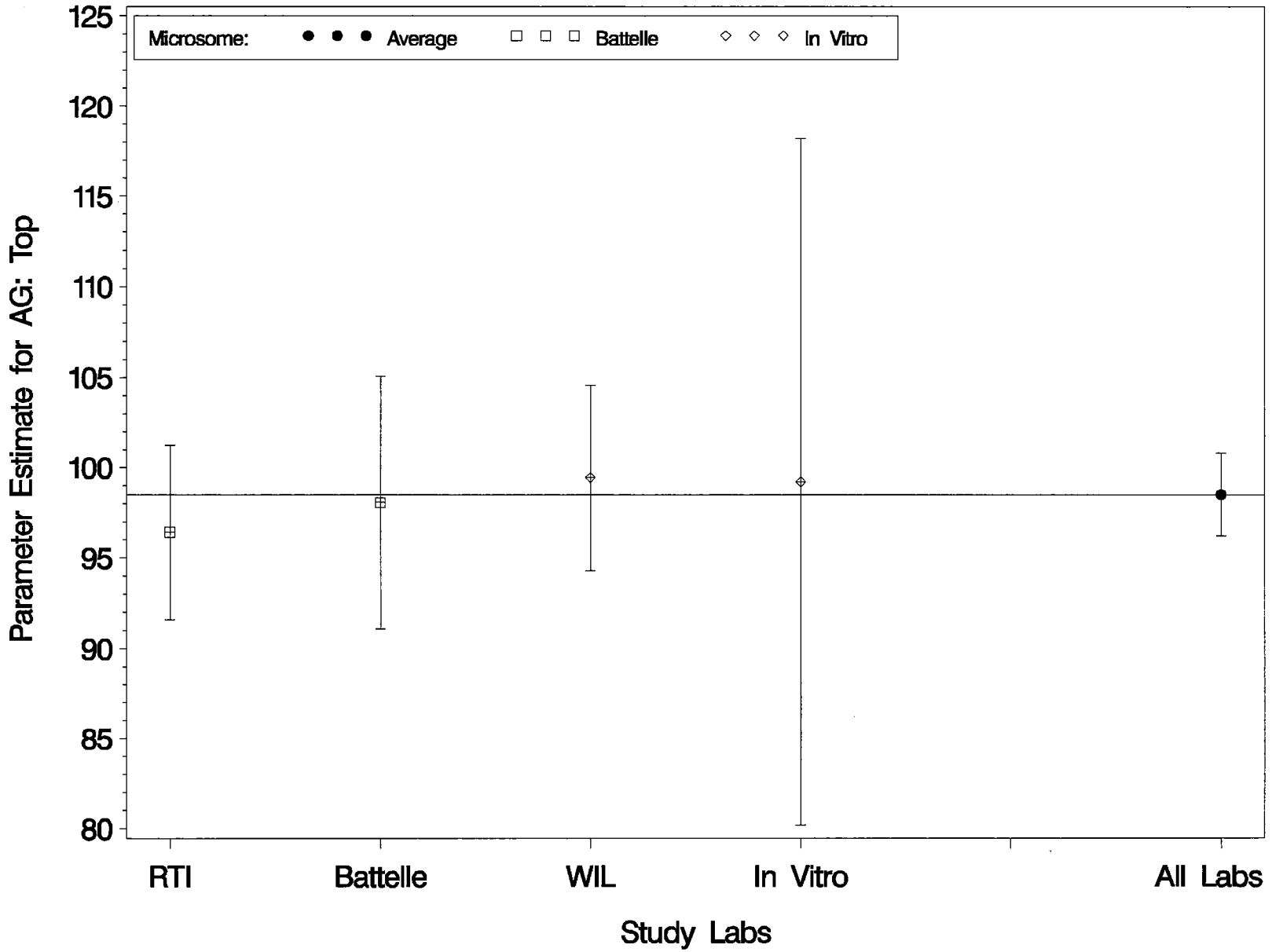
Chemical[5]	Param	Estimate and 95 percent Confidence Interval						CV(percent) and 95 percent CI[4]
		RTI[1,2]	Battelle[1,2]	WIL[1,2]	In Vitro[1,2]	Difference Between Two Microsome Sources (Battelle – In Vitro)[3]	Overall	
AG	Top	96.41(91.58,101.25)	98.07(91.09,105.05)	99.44(94.28,104.59)	99.20(80.16,118.24)	-1.82(-6.45,2.80)	98.51(96.19,100.82)	1.70(1.02,4.83)
AG	Bottom	-1.04(-2.79,0.72)	-0.46(-1.27,0.35)	0.19(-2.13,2.51)	0.10(-1.47,1.68)	-0.73(-1.89,0.43)	-0.21(-0.78,0.37)	
KCZ	Top	99.12(95.78,102.47)	96.77(90.12,103.43)	103.14(101.08,105.20)	102.77(100.67,104.87)	-5.16(-7.75,-2.58)	100.50(99.20,101.79)	1.18(0.84,1.96)
KCZ	Bottom	-1.86(-4.27,0.55)	-0.45(-3.47,2.58)	-1.11(-10.68,8.46)	13.67(-68.80,96.13)	-0.41(-10.33,9.50)	-1.11(-6.07,3.84)	
PCZ	Top	101.47(94.27,108.67)	98.02(95.37,100.66)	103.82(93.09,114.55)	91.80(58.66,124.93)	4.53(-2.84,11.89)	100.40(96.72,104.09)	2.45(1.42,8.18)
PCZ	Bottom	0.06(-0.74,0.85)	-0.18(-0.76,0.40)	-6.70(-35.11,21.72)	0.61(-0.94,2.15)	0.60(-2.70,3.90)	0.21(-1.44,1.86)	
NYP	Top	98.97(97.43,100.51)	96.42(89.85,102.99)	101.23(93.54,108.91)	88.97(81.47,96.47)	7.29(-0.43,15.00)	96.23(92.37,100.09)	2.72(1.59,8.81)
NYP	Bottom	0.49(-0.85,1.84)	1.56(-0.97,4.09)	0.44(-1.10,1.99)	12.62(9.82,15.42)	-6.55(-17.34,4.23)	3.75(-1.65,9.14)	
FRM	Top	93.06(82.02,104.11)	98.53(95.81,101.26)	90.23(64.14,116.32)	95.69(85.90,105.48)	-3.57(-9.31,2.17)	96.67(93.80,99.54)	2.49(1.64,5.17)
FRM	Bottom	0.26(-1.10,1.62)	0.55(-0.54,1.64)	-6.47(-35.88,22.93)	5.36(-17.88,28.60)	1.12(-10.30,12.54)	0.99(-4.72,6.70)	
ECZ	Top	98.83(96.41,101.24)	83.52(24.92,142.11)	100.53(91.07,110.00)	107.85(100.56,115.14)	-0.47(-9.31,8.38)	99.87(95.45,104.29)	2.43(1.32,11.56)
ECZ	Bottom	0.10(-0.38,0.57)	-0.26(-0.76,0.25)	0.32(-0.71,1.34)	-0.62(-1.76,0.51)	-0.05(-0.59,0.49)	0.02(-0.25,0.29)	
CYN	Top	93.33(89.59,97.08)	91.38(86.78,95.99)	90.30(70.45,110.14)	90.70(61.33,120.06)	1.87(-4.13,7.86)	92.23(89.23,95.22)	2.35(1.41,6.71)
CYN	Bottom	20.63(18.96,22.31)	20.76(18.07,23.44)	13.30(4.28,22.32)	5.59(-8.75,19.93)	-2.75(-19.07,13.58)	15.82(7.65,23.98)	
DCF	Top	99.71(95.40,104.03)	97.60(96.39,98.81)	100.86(96.76,104.96)	94.65(84.85,104.45)	1.67(-3.66,7.00)	98.23(95.56,100.89)	2.18(1.38,5.01)
DCF	Bottom	-4.86(-8.64,-1.08)	0.64(-2.41,3.69)	-10.38(-33.24,12.48)	37.20(1.82,72.57)	4.20(-0.56,8.96)	-2.30(-4.68,0.08)	

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 chemical.
2. The overall estimates and confidence intervals in this table were estimated using a mixed effects analysis of variance, with microsome source as a fixed effect and laboratory as a random effect, and with heterogeneous variances among the four laboratories. The variances for each laboratory were specified as the squares of the within laboratory standard errors.
3. Microsomes were prepared by Battelle and In Vitro laboratories and distributed to the four laboratories, as shown in Table M-D-T. The differences in average responses between the two sources of microsomes were estimated based on the model fit, accounting for among laboratory variation and heterogeneous within laboratory variation. The degrees of freedom are same as those for the overall means.
4. CV was calculated for the top based on the average results.
5. Concentration response relations were not fitted for dibenz [a,h]anthracene and for atrazine, since they resulted in no aromatase inhibition.

Table A-2 . Variance Components and Ratio of Between and Within Laboratories Variances for Top and Bottom Parameters of Concentration Response Curves for Placental Assay. Estimated by Chemical.

Chemical[7]	Param	Within Laboratory Variance[1]					Among Laboratory Variance[3] and (p-value) (df=3)	Mean Variance[4,5]	Ratio and 95 percent CI[6]
		RTI[1,2]	Battelle[1,2]	WIL[1,2]	In Vitro[1,2]	Pooled Results			
AG	Top	5.193/df=16.0	2.079/df=1.8	1.433/df=2.0	18.775/df=2.0	6.870/df=4.1	0.000 (1)	0.704/df=4.1	0.000 (0.000, 0.000)
AG	Bottom	0.684/df=16.0	0.159/df=35.9	0.291/df=2.0	0.601/df=35.3	0.434/df=36.5	0.000 (1)	0.081/df=36.5	0.000 (0.000, 0.000)
KCZ	Top	2.489/df=16.0	2.347/df=2.0	0.229/df=2.0	1.051/df=27.0	1.529/df=11.6	0.000 (1)	0.349/df=11.6	0.000 (0.000, 0.000)
KCZ	Bottom	1.290/df=16.0	2.100/df=20.1	4.946/df=2.0	360.278/df=2.0	92.153/df=2.1	0.000 (.)	1.420/df=2.1	0.000 (0.000, 0.000)
PCZ	Top	11.541/df=16.0	0.430/df=2.2	6.220/df=2.0	58.400/df=2.0	19.148/df=3.4	0.000 (1)	1.509/df=3.4	0.000 (0.000, 0.000)
PCZ	Bottom	0.142/df=16.0	0.083/df=48.0	43.600/df=2.0	0.585/df=39.5	11.102/df=2.1	0.000 (1)	0.157/df=2.1	0.000 (0.000, 0.000)
NYP	Top	0.527/df=16.0	2.586/df=2.1	3.193/df=2.0	2.170/df=1.7	2.119/df=6.5	4.865 (0.17311)	1.712/df=3.5	2.296 (0.370, 33.688)
NYP	Bottom	0.405/df=16.0	1.578/df=47.6	0.129/df=2.0	1.927/df=44.1	1.009/df=105.3	12.709 (0.10772)	3.429/df=3.6	12.590 (3.883, 175.678)
FRM	Top	27.147/df=16.0	0.401/df=2.0	36.760/df=2.0	6.345/df=2.2	17.663/df=6.7	0.000 (1)	1.452/df=6.7	0.000 (0.000, 0.000)
FRM	Bottom	0.411/df=16.0	0.297/df=59.1	46.717/df=2.0	22.194/df=1.8	17.405/df=3.5	0.000 (1)	3.805/df=3.5	0.000 (0.000, 0.000)
ECZ	Top	1.293/df=16.0	185.641/df=2.0	4.840/df=2.0	13.155/df=47.9	51.232/df=2.4	0.000 (1)	1.474/df=2.4	0.000 (0.000, 0.000)
ECZ	Bottom	0.050/df=16.0	0.064/df=55.1	0.057/df=2.0	0.320/df=53.7	0.123/df=64.3	0.000 (1)	0.018/df=64.3	0.000 (0.000, 0.000)
CYN	Top	3.121/df=16.0	1.946/df=2.8	21.271/df=2.0	45.051/df=2.0	17.847/df=4.0	0.000 (1)	1.175/df=4.0	0.000 (0.000, 0.000)
CYN	Bottom	0.625/df=16.0	1.756/df=37.3	4.397/df=2.0	12.795/df=2.2	4.893/df=4.5	27.637 (0.12359)	7.991/df=3.6	5.648 (0.648, 84.647)
DCF	Top	4.141/df=16.0	0.365/df=55.4	0.908/df=2.0	5.230/df=2.0	2.661/df=7.5	1.944 (0.20873)	1.142/df=5.6	0.730 (0.130, 10.649)
DCF	Bottom	3.175/df=16.0	2.326/df=57.8	28.228/df=2.0	287.981/df=20.2	80.427/df=23.0	0.000 (1)	1.322/df=23.0	0.000 (0.000, 0.000)

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



8.

Figure A-1. **Aminoglutethimide: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Top in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

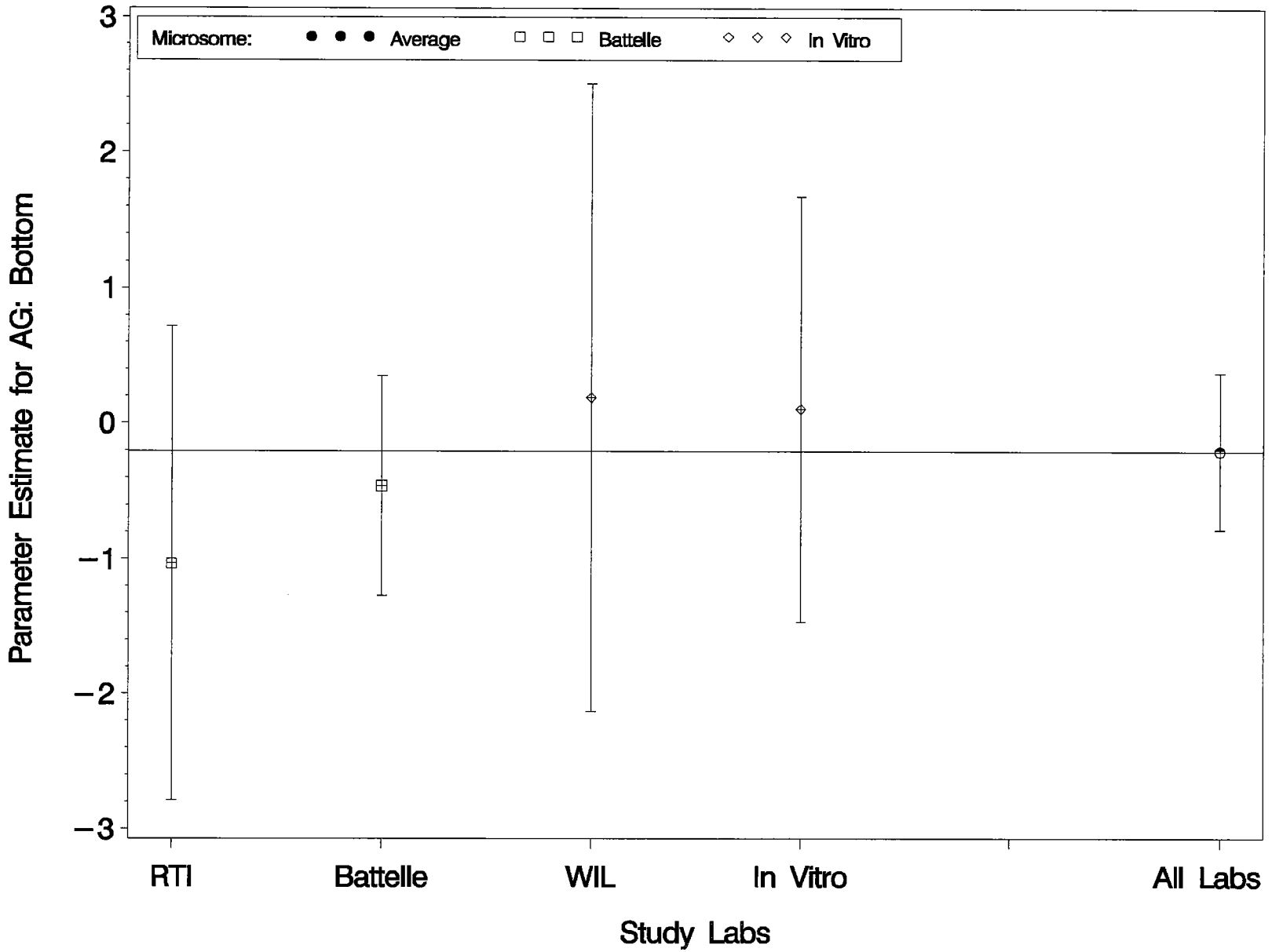


Figure A-2. **Aminoglutethimide: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Bottom in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

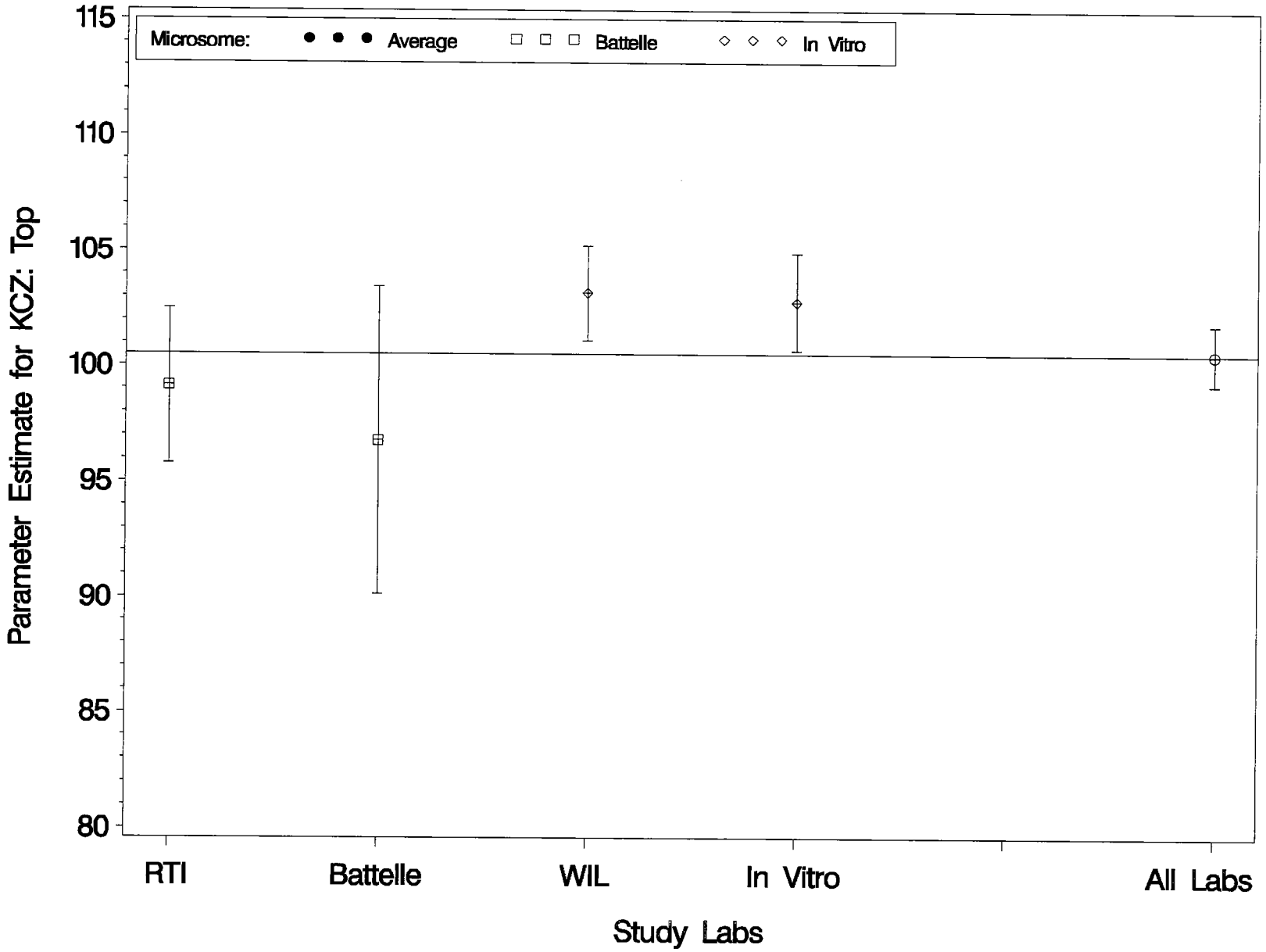


Figure A-3. **Ketoconazole: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Top in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

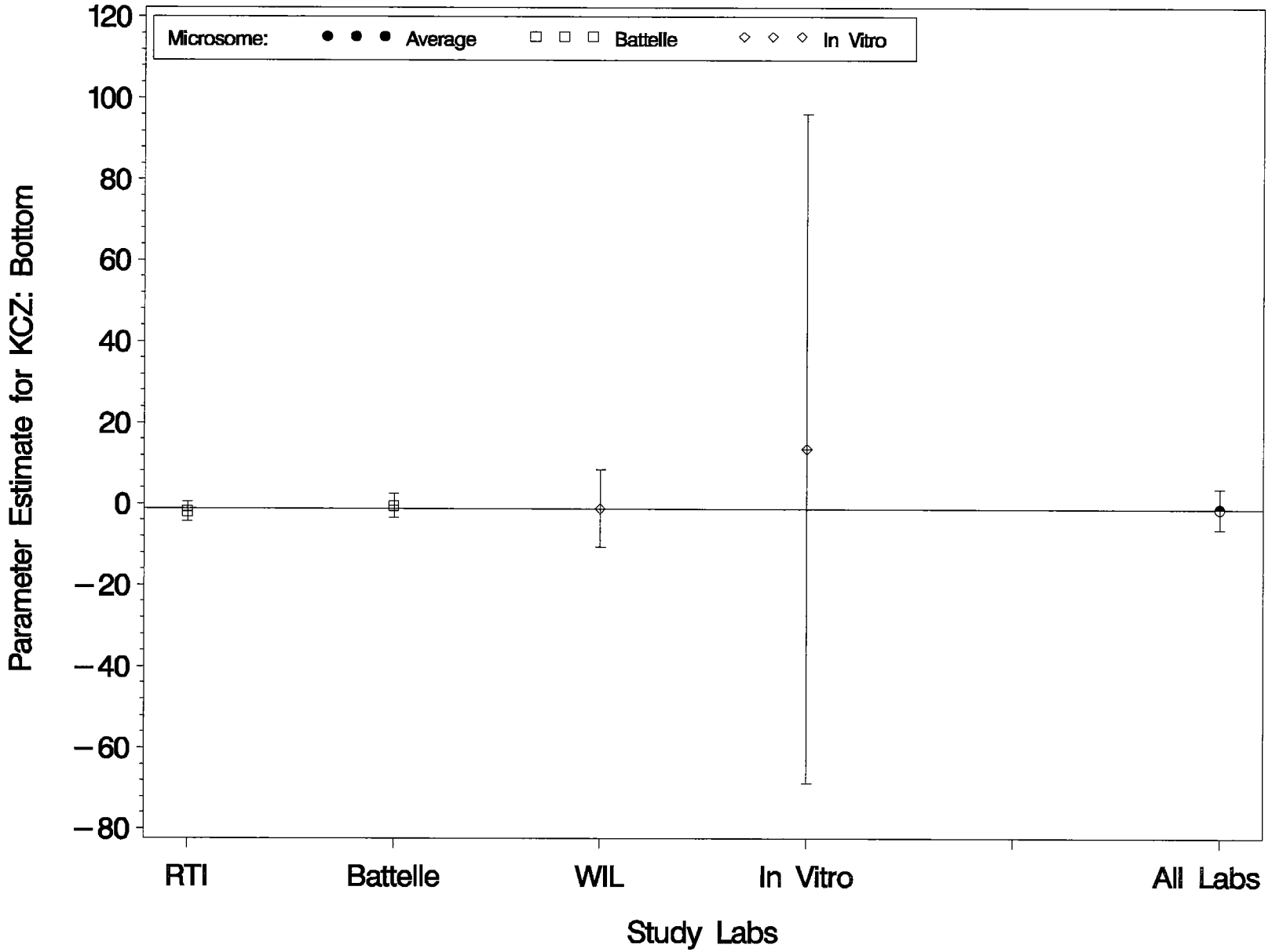


Figure A-4. **Ketoconazole: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Bottom in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

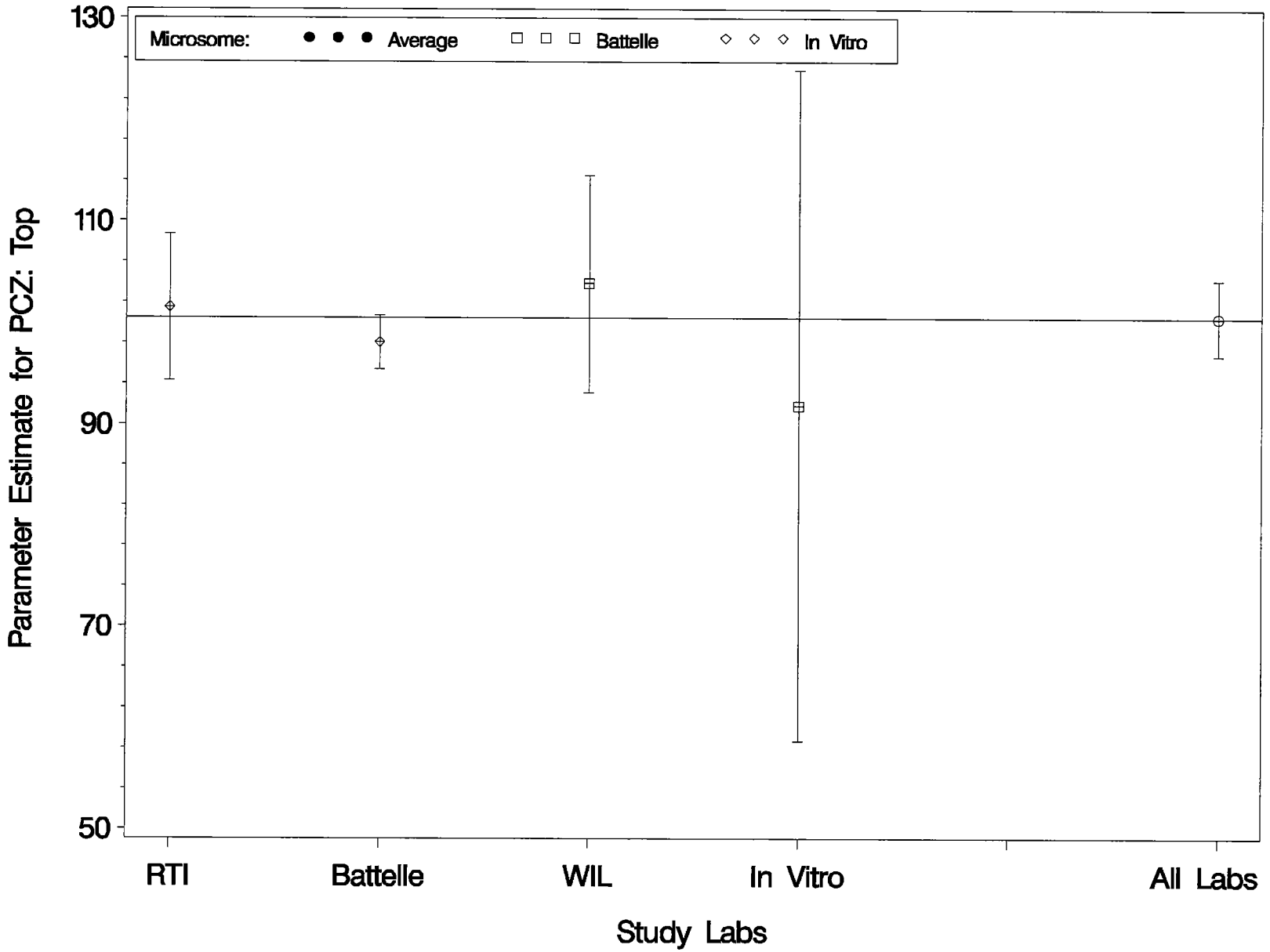


Figure A-5. **<u>Prochloraz:</u> Parameter Estimates and Their Associated 95 percent Confidence Intervals for Top in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

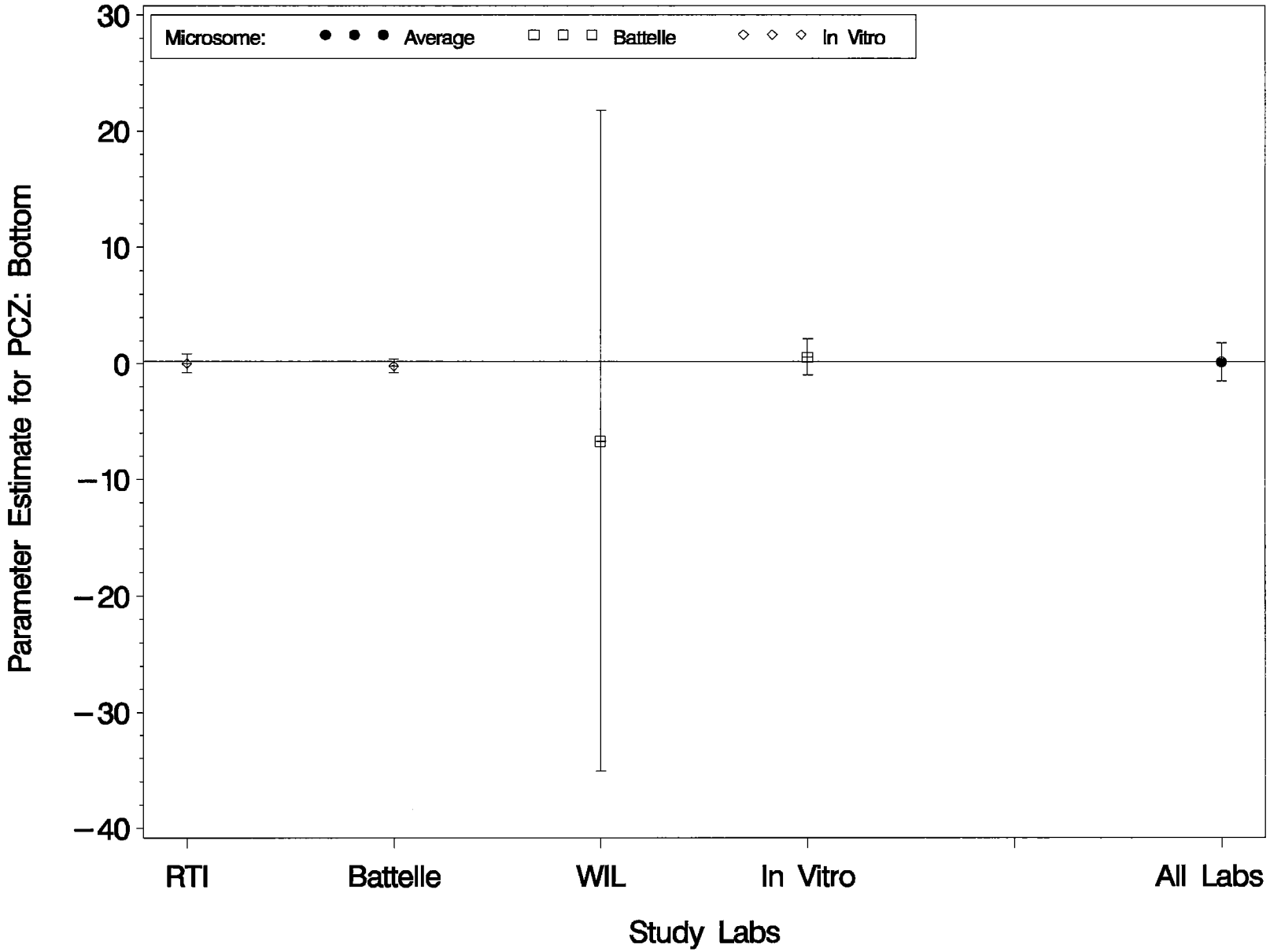


Figure A-6. **<u>Prochloraz:</u> Parameter Estimates and Their Associated 95 percent Confidence Intervals for Bottom in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

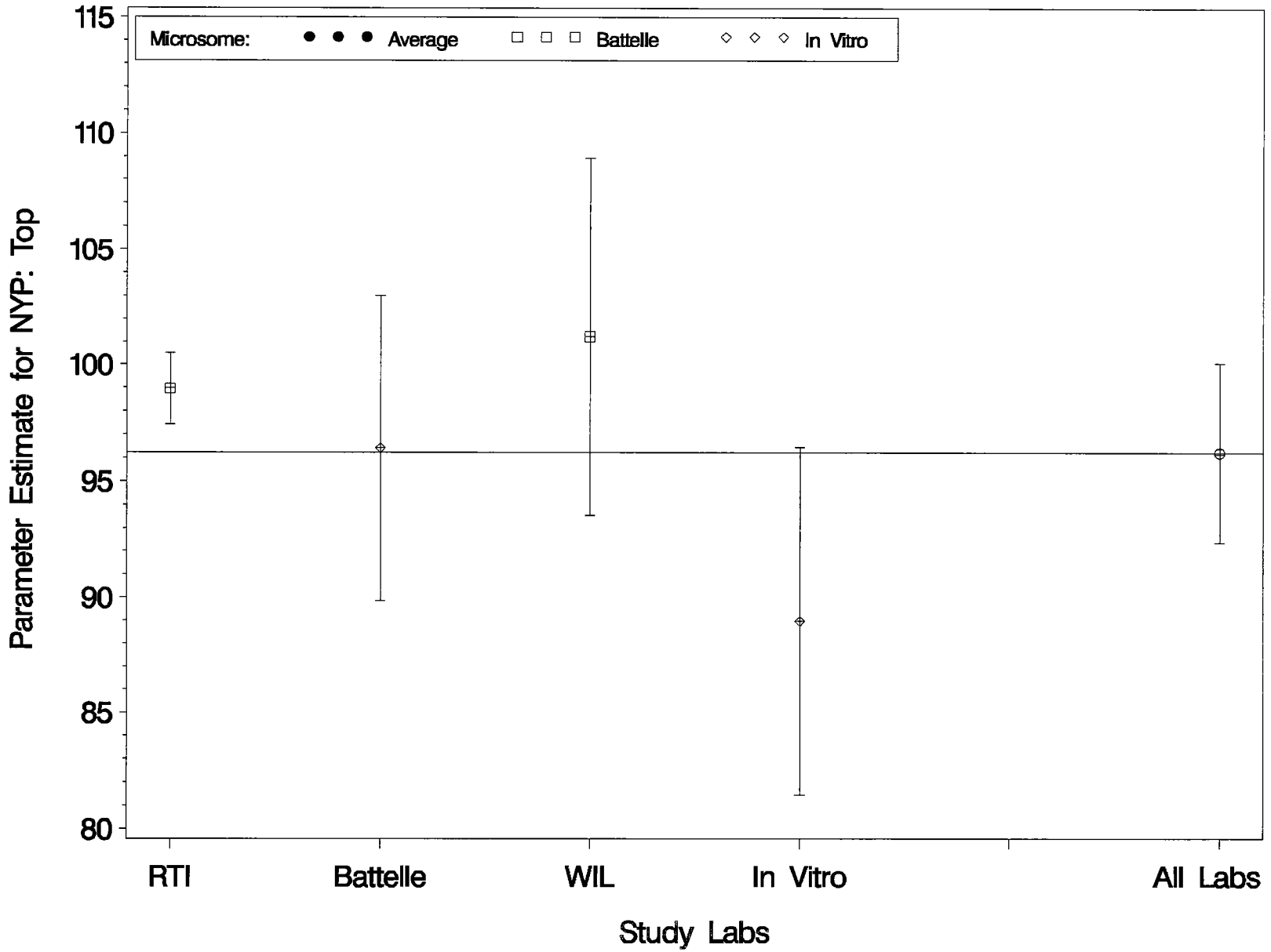


Figure A-7. **4-Nonylphenol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Top in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

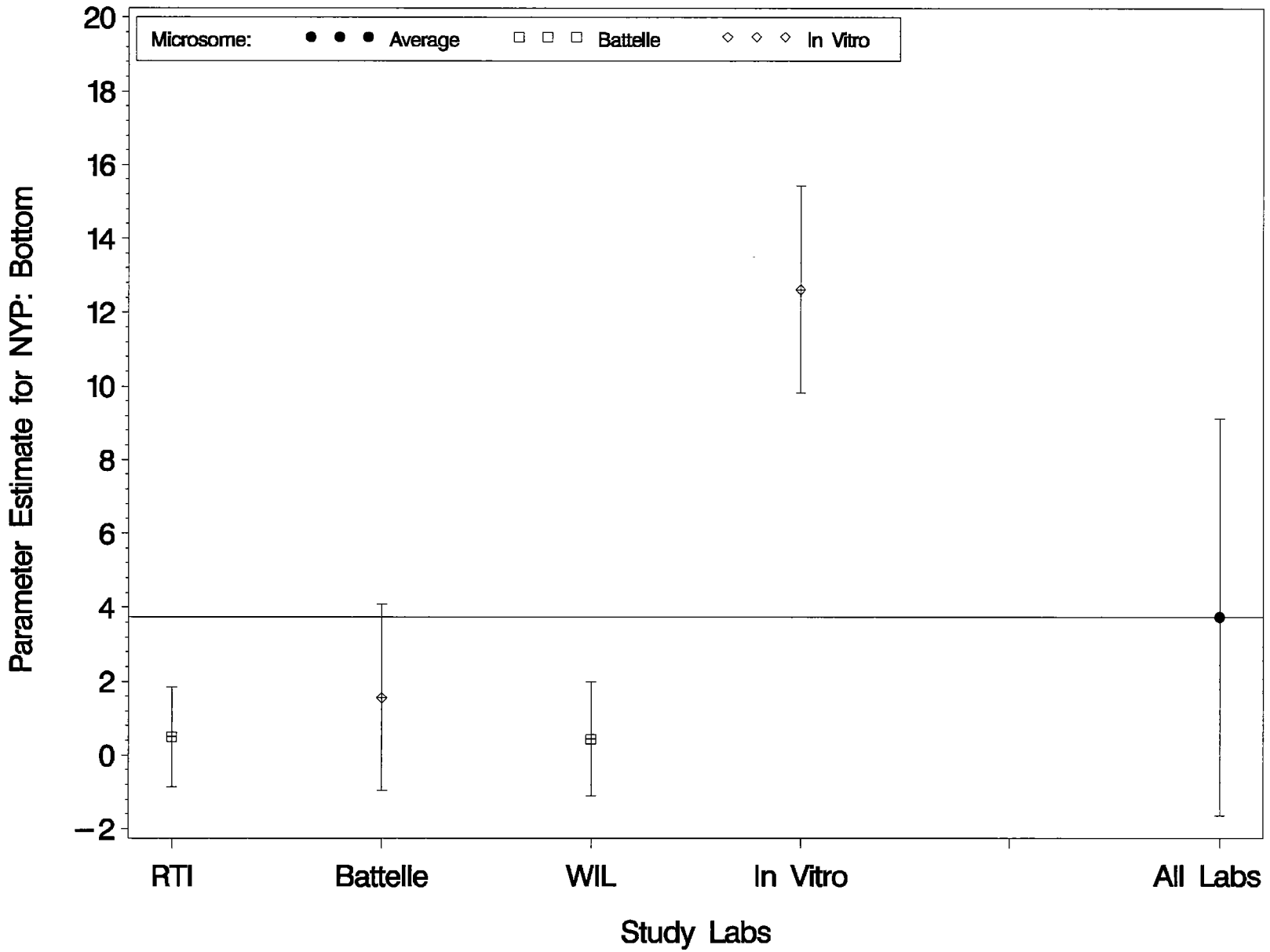


Figure A-8. **4-Nonylphenol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Bottom in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

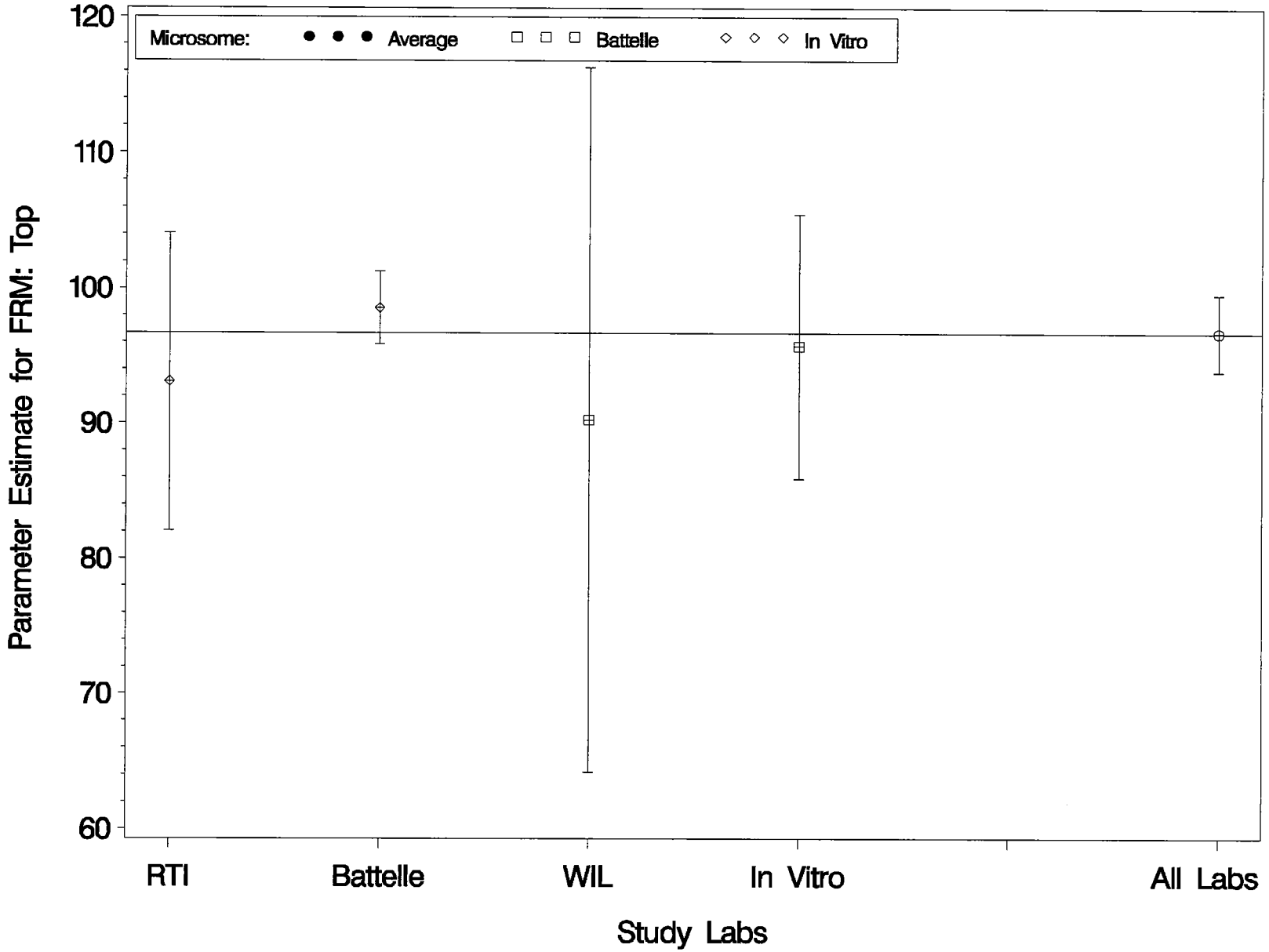


Figure A-9. **Fenarimol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Top in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

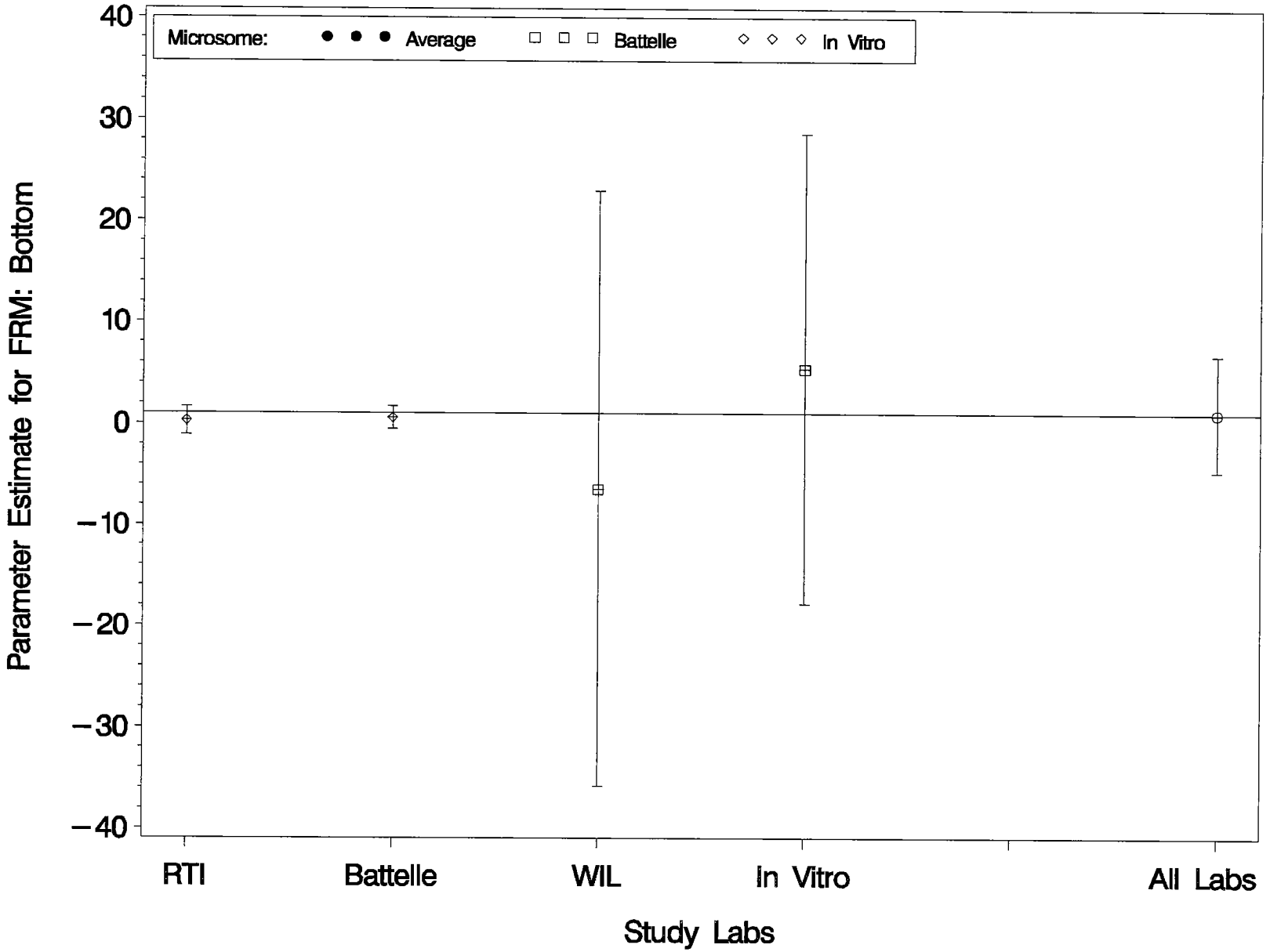


Figure A-10. **Fenarimol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Bottom in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

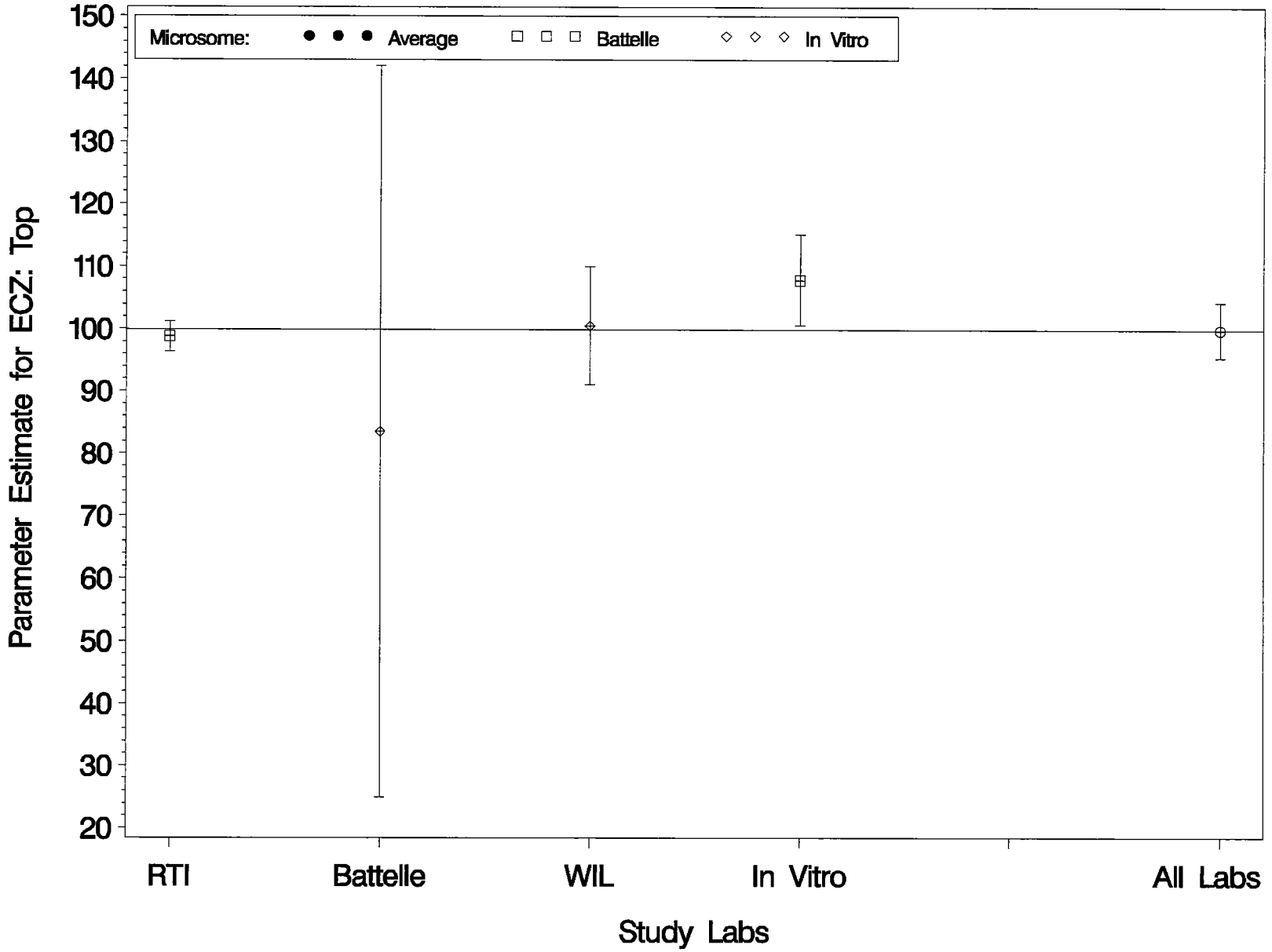


Figure A-11. **<u>Econazole:</u> Parameter Estimates and Their Associated 95 percent Confidence Intervals for Top in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

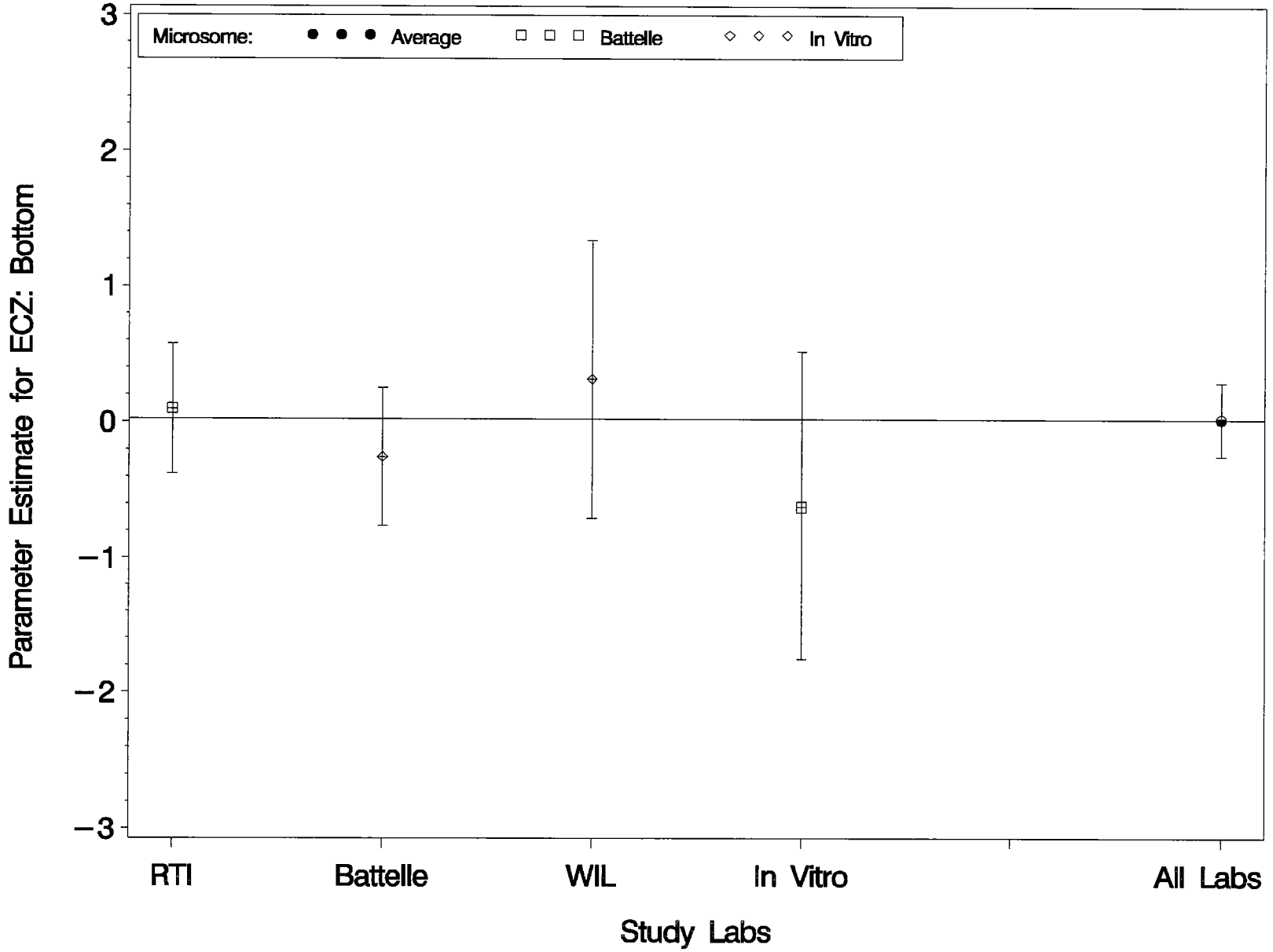


Figure A-12. **Econazole: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Bottom in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

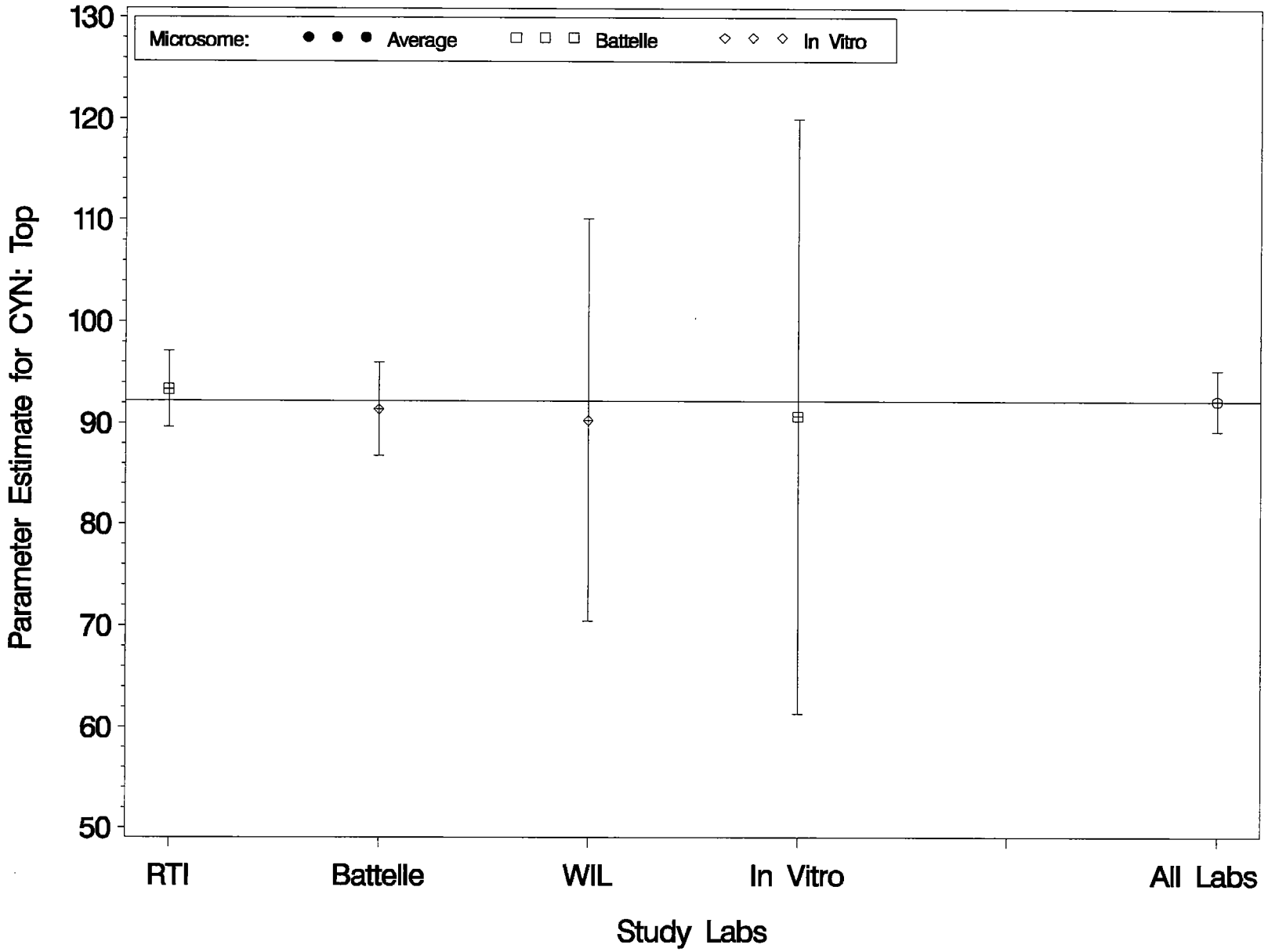


Figure A-13. **Chrysin: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Top in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

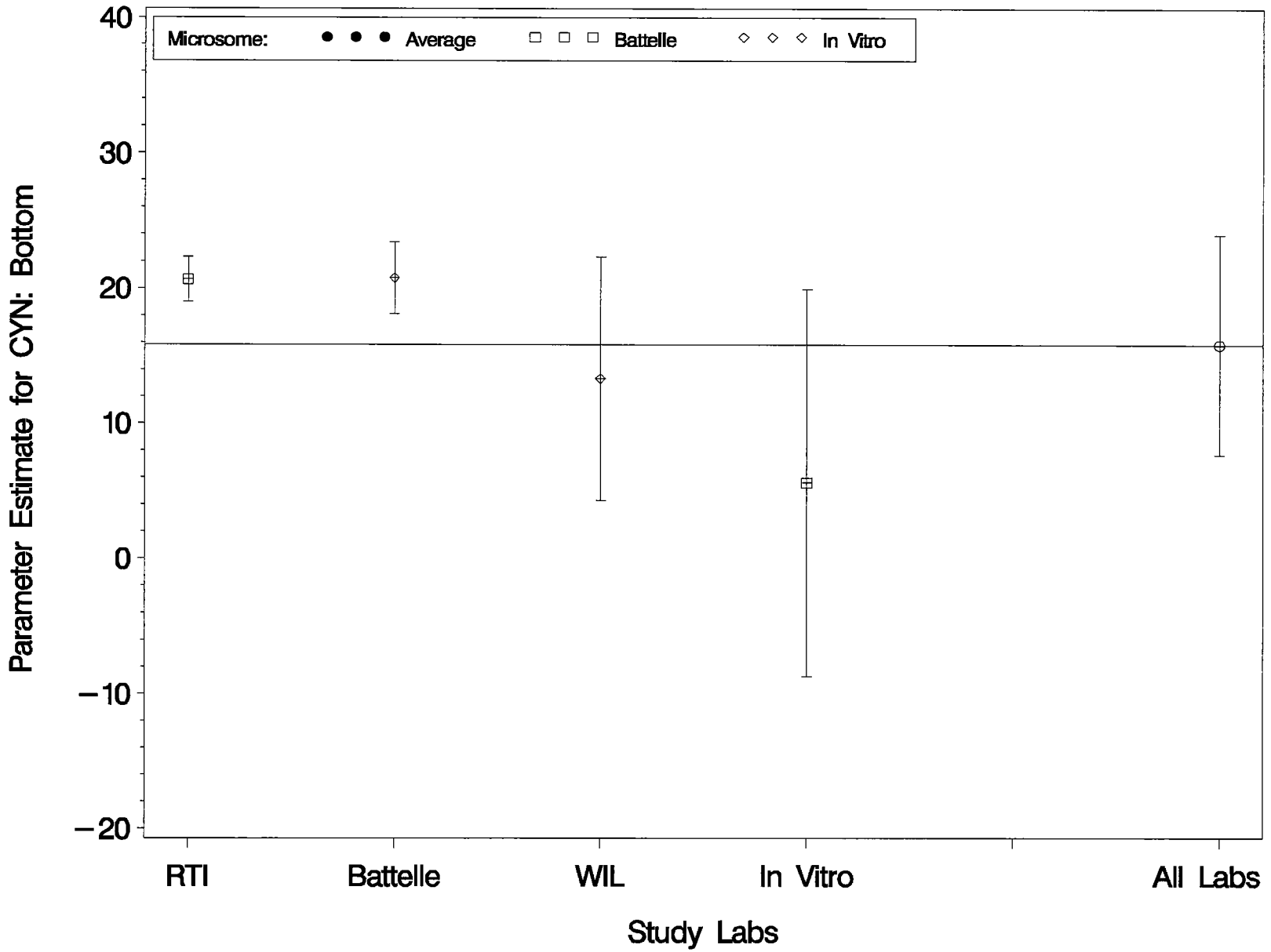


Figure A-14. **Chrysin: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Bottom in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

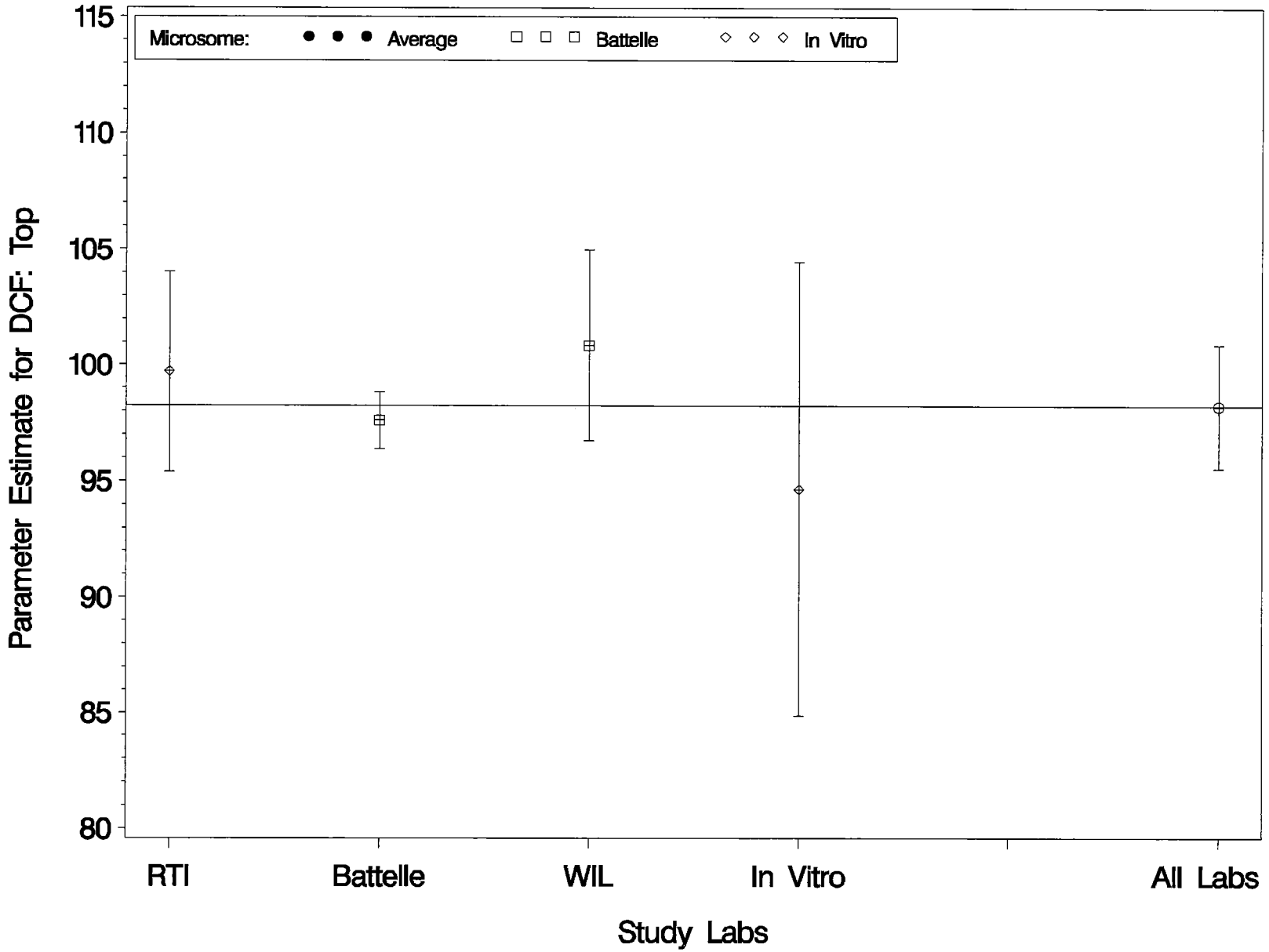


Figure A-15. **Dicofol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Top in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

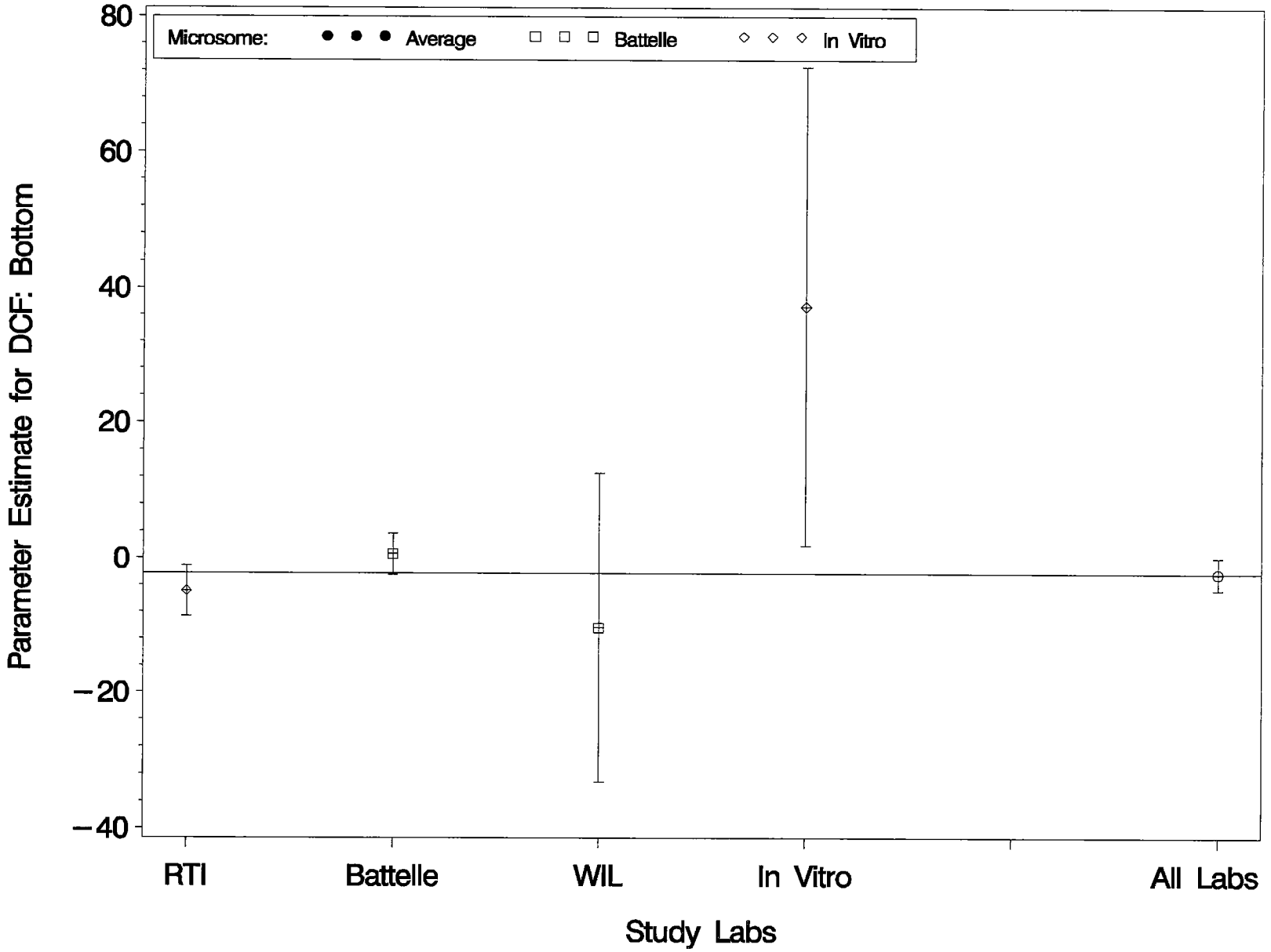


Figure A-16. **Dicofol: Parameter Estimates and Their Associated 95 percent Confidence Intervals for Bottom in the Placental Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

Appendix B. Results of Intralaboratory Analyses of Control Data at Each of the Four Participating Laboratories.

- **Tables B-1a, B-1b. Results for RTI.**
- **Table B-2. Results for Battelle.**
- **Tables B-3a, B-3b. Results for WIL.**
- **Table B-4. Results for In Vitro.**

Table B-1a. Least Squares Means and Associated Standard Errors for the Beginning and the End. F-Test Results for the Portion Effect for Each Chemical, as Reported by RTI. Derived Results for the Differences Between the Beginning and the End, and Averages Across the Beginning and the End.

Control Parameter[4,5]	Chemical	LSM (Std Error)		Degree of Freedom	F-Value (P-Value)	End-Beginning (Std Error)[1]	Average (Std Error)[2,3]
		Beginning	End				
BAC	AG	-0.522 (0.216)	0.522 (0.216)	20	11.710 (0.0027)	1.045 (0.305)	0.000 (0.153)
	ATZ	0.062 (0.216)	-0.062 (0.216)	20	0.160 (0.6902)	-0.123 (0.309)	0.000 (0.151)
	CYN	-0.016 (0.216)	0.016 (0.216)	20	0.010 (0.9151)	0.033 (0.330)	0.000 (0.139)
	DBA	0.066 (0.216)	-0.066 (0.216)	20	0.190 (0.6704)	-0.132 (0.303)	0.000 (0.154)
	DCF	0.062 (0.216)	-0.062 (0.216)	20	0.160 (0.6902)	-0.123 (0.309)	0.000 (0.151)
	ECZ	-0.016 (0.216)	0.016 (0.216)	20	0.010 (0.9151)	0.033 (0.330)	0.000 (0.139)
	FRM	0.040 (0.216)	-0.040 (0.216)	20	0.070 (0.7966)	-0.080 (0.301)	0.000 (0.155)
	KCZ	-0.550 (0.216)	0.550 (0.216)	20	12.970 (0.0018)	1.100 (0.305)	0.000 (0.153)
	NYP	-0.023 (0.216)	0.023 (0.216)	20	0.020 (0.8810)	0.046 (0.327)	0.000 (0.141)
	PCZ	0.040 (0.216)	-0.040 (0.216)	20	0.070 (0.7966)	-0.080 (0.301)	0.000 (0.155)
FEAC	AG	105.360 (1.308)	94.644 (1.308)	20	33.560 (<.0001)	-10.716 (1.850)	100.002 (0.924)
	ATZ	105.340 (1.308)	94.658 (1.308)	20	33.390 (<.0001)	-10.682 (1.849)	99.999 (0.925)
	CYN	106.380 (1.308)	93.617 (1.308)	20	47.670 (<.0001)	-12.763 (1.849)	99.998 (0.925)
	DBA	99.257 (1.308)	100.740 (1.308)	20	0.650 (0.4310)	1.483 (1.840)	99.998 (0.929)
	DCF	105.340 (1.308)	94.658 (1.308)	20	33.390 (<.0001)	-10.682 (1.849)	99.999 (0.925)
	ECZ	106.380 (1.308)	93.617 (1.308)	20	47.670 (<.0001)	-12.763 (1.849)	99.998 (0.925)
	FRM	106.450 (1.308)	93.547 (1.308)	20	48.710 (<.0001)	-12.903 (1.849)	99.999 (0.925)
	KCZ	105.340 (1.308)	94.662 (1.308)	20	33.340 (<.0001)	-10.679 (1.849)	100.001 (0.924)
	NYP	103.060 (1.308)	96.936 (1.308)	20	10.980 (0.0035)	-6.124 (1.848)	99.998 (0.925)
	PCZ	106.450 (1.308)	93.547 (1.308)	20	48.710 (<.0001)	-12.903 (1.849)	99.999 (0.925)
Negative	AG	104.100 (1.512)	91.079 (1.409)	20	39.690 (<.0001)	-13.021 (2.067)	97.590 (1.033)
	ATZ	101.740 (1.409)	91.236 (1.409)	20	27.820 (<.0001)	-10.504 (1.992)	96.488 (0.996)
	CYN	102.040 (1.409)	88.528 (1.409)	20	45.980 (<.0001)	-13.512 (1.993)	95.284 (0.996)
	DBA	103.730 (1.409)	99.239 (1.409)	20	5.080 (0.0356)	-4.491 (1.993)	101.485 (0.996)
	DCF	101.740 (1.409)	91.236 (1.409)	20	27.820 (<.0001)	-10.504 (1.992)	96.488 (0.996)
	ECZ	102.040 (1.409)	88.528 (1.409)	20	45.980 (<.0001)	-13.512 (1.993)	95.284 (0.996)
	FRM	105.300 (1.409)	91.654 (1.512)	20	43.580 (<.0001)	-13.646 (2.067)	98.477 (1.033)
	KCZ	103.090 (1.409)	90.263 (1.409)	20	41.480 (<.0001)	-12.827 (1.992)	96.677 (0.996)
	NYP	102.580 (1.409)	96.124 (1.409)	20	10.490 (0.0041)	-6.456 (1.993)	99.352 (0.995)
	PCZ	105.300 (1.409)	91.654 (1.512)	20	43.580 (<.0001)	-13.646 (2.067)	98.477 (1.033)
Positive	AG	51.936 (1.895)	46.313 (1.895)	20	9.410 (0.0061)	-5.623 (1.833)	49.125 (1.659)
	ATZ	49.596 (1.895)	44.964 (1.895)	20	6.390 (0.0200)	-4.632 (1.833)	47.280 (1.659)
	CYN	52.547 (1.895)	45.769 (1.895)	20	13.670 (0.0014)	-6.778 (1.833)	49.158 (1.659)
	DBA	47.205 (1.895)	46.429 (1.895)	20	0.180 (0.6767)	-0.776 (1.829)	46.817 (1.660)

Control Parameter[4,5]	Chemical	LSM (Std Error)		Degree of Freedom	F-Value (P-Value)	End-Beginning (Std Error)[1]	Average (Std Error)[2,3]
		Beginning	End				
	DCF	49.596 (1.895)	44.964 (1.895)	20	6.390 (0.0200)	-4.632 (1.833)	47.280 (1.659)
	ECZ	52.547 (1.895)	45.769 (1.895)	20	13.670 (0.0014)	-6.778 (1.833)	49.158 (1.659)
	FRM	50.584 (1.895)	45.720 (1.895)	20	7.040 (0.0152)	-4.864 (1.833)	48.152 (1.659)
	KCZ	52.172 (1.895)	45.925 (1.895)	20	11.610 (0.0028)	-6.246 (1.833)	49.049 (1.659)
	NYP	49.212 (1.895)	45.275 (1.895)	20	4.610 (0.0442)	-3.937 (1.834)	47.243 (1.659)
	PCZ	50.584 (1.895)	45.720 (1.895)	20	7.040 (0.0152)	-4.864 (1.833)	48.152 (1.659)

1. For each chemical, the associated standard errors for the differences between the end and beginning were calculated as |LS Mean Diff|/(F-value)$^{1/2}$, where F-values were test results for portion effect for a given chemical, as provided in the intralaboratory report.
2. For each chemical, the associated standard errors for the averages over the beginning and the end were calculated as $(1/2)(2(S_e^2+S_b^2)-S_d^2)^{1/2}$, where S_e, S_b, and S_d were the standard error associated with the end, the beginning, and the differences between the end and the beginning respectively.
3. The standard error calculations do not account for the constraints on the full enzyme activity and the background activity control values within replicates.
4. Control values are given corresponding to each of ten chemicals.
5. For RTI, the chemicals AG, KCZ, NYP, ECZ and CYN were analyzed with Battelle microsomes and chemicals PCZ, FRM, DBA, DCF and ATZ were analyzed with In Vitro microsomes.

Table B-1b. Least Squares Means and Associated Standard Error Over the Beginning and the End, as Reported by RTI, and the Calculated Results for the Differences between the Battelle Microsome and the In Vitro Microsome[1,2].

Endpoint	Control Parameter	LS Mean	Standard Error[3]	Degree of Freedom
End-Beginning	BAC	0.1718	0.09874	20.00
	FEAC	-9.8733	0.58436	20.00
	Negative	-11.2118	0.63719	20.00
	Positive	-4.9131	0.57953	20.00
Battelle-IVT	Negative	-1.4456	0.63706	20.00
	Positive	1.2102	1.04929	20.00

1. The differences between the beginning and the end are averages across all chemicals. The associated standard errors were calculated as $(1/10)(\sum s_i^2)^{1/2}$, where s_i is the standard error associated with the difference for the i^{th} chemical (Table B-1a).
2. The differences between Battelle microsomes and the In Vitro microsomes were calculated as $(1/5)(\sum m_{1i} - \sum m_{2i})$, and the associated standard errors as $(1/5)(\sum S_{1i}^2 + \sum S_{2i}^2)^{1/2}$, where m_{1i} and S_{1i} are the LS mean and standard error for i^{th} chemical with Battelle microsomes, and m_{2i} and S_{2i} are the LS mean and standard error for i^{th} chemical with In Vitro microsomes (Table B-1a).
3. The standard error calculations do not account for the constraints on the full enzyme activity and the background activity control values within replicates.

Table B-2. Least Squares Means and Associated Standard Errors, as Reported By Battelle.

Endpoint[2]	Control Parameter	LS Mean	Standard Error[1]	Degree of Freedom
End-Beginning	BAC	0.0620	0.0900	61.80
	FEAC	-10.4440	1.8370	5.81
	Negative	-8.2940	2.0880	7.04
	Positive	-2.2330	0.7650	6.07
Battelle-IVT	Negative	-0.7818	2.3082	8.70
	Positive	1.1094	1.4075	12.30

1. The standard error calculations do not account for the constraints on the full enzyme activity and the background activity control values within replicates
2. For Battelle, the chemicals AG, KCZ, DBA, DCF, and ATZ were analyzed with Battelle microsomes, and chemicals PCZ, NYP, FRM, ECZ, and CYN were analyzed with In Vitro microsomes.

Table B-3a. Least Squares Means for the Beginning and the End, and the P-value for the Portion Effect, as Reported by WIL, and the Calculated Results for the Differences between the Beginning and the End.

Control Parameter	LSM		P-Value	Degree of Freedom	End-Beginning	
	Beginning	End			LSM	Std Error[1,2]
BAC	-0.028	0.028	0.9024	12	0.056	0.44711
FEAC	102.963	96.901	0.0019	12	-6.062	1.53156
Negative	102.995	93.000	0.0011	12	-9.995	2.34413
Positive	44.318	40.578	0.0117	12	-3.740	1.25924

1. The associated standard errors for the differences between beginning and end (end minus beginning) were calculated as |LS Mean|/(F-value)$^{1/2}$, where F-value is the critical value corresponding to the p-value.
2. The standard error calculations do not account for the constraints on the full enzyme activity and the background activity control values within replicates

Table B-3b. Least Squares Means and Associated Standard Error Over the Beginning and the End, as Reported by WIL, and the Calculated Results for the Differences between the Battelle Microsomes and the In Vitro Microsomes[1].

Control Parameter	Statistics	Chemical[1]						Battelle-In Vitro[2]
		AG-KCZ	PCZ-FRM	NYP	DBA	ECZ-CYN	DCF-ATZ	
Negative	LSM	97.881	96.991	99.466	101.859	97.577	94.213	-2.21567
	Std Error	1.921	3.368	1.921	5.484	1.921	4.362	2.81889
Positive	LSM	47.332	35.586	42.452	42.015	41.474	45.830	-2.31767
	Std Error	1.940	2.689	1.031	1.675	1.562	4.546	2.05396

1. For WIL, the chemicals AG, KCZ, DBA, ECZ, and CYN were analyzed with In Vitro microsomes and chemicals PCZ, FRM, NYP, DCF, and ATZ were analyzed with Battelle microsomes. The analysis was carried out in six groups, as shown in the table.
2. The differences between the Battelle microsomes and the In Vitro microsomes were calculated as $(1/3)(\sum m_{1i} - \sum m_{2i})$, and the associated standard errors is $(1/3)(\sum S_{1i}^2 + \sum S_{2i}^2)^{1/2}$, where m_{1i}, and S_{1i} are the LS mean and standard error for i^{th} chemicals with Battelle microsomes, and m_{2i}, and S_{2i} are the LS mean and standard error for i^{th} chemicals with In Vitro microsomes.

Table B-4. Least Squares Means and Associated Standard Errors, as Reported By In Vitro.

Endpoint[2]	Control Parameter	LS Mean	Standard Error[1]	Degree of Freedom
End-Beginning	BAC	-0.4070	0.2280	9.25
	FEAC	-10.0160	1.3710	22.72
	Negative	17.3840	4.2630	10.10
	Positive	3.5810	1.6360	7.17
Battelle-IVT	Negative	-12.0629	4.3324	10.90
	Positive	-4.9048	3.1050	4.02

1. The standard error calculations do not account for the constraints on the full enzyme activity and the background activity control values within replicates
2. For In Vitro, the chemicals PCZ, DBA, FRM, ECZ, and CYN were analyzed with Battelle microsomes, and chemicals AG, KCZ, NYP, DCF, and ATZ and were analyzed with In Vitro microsomes.

APPENDIX F

CHEMISTRY REPORTS

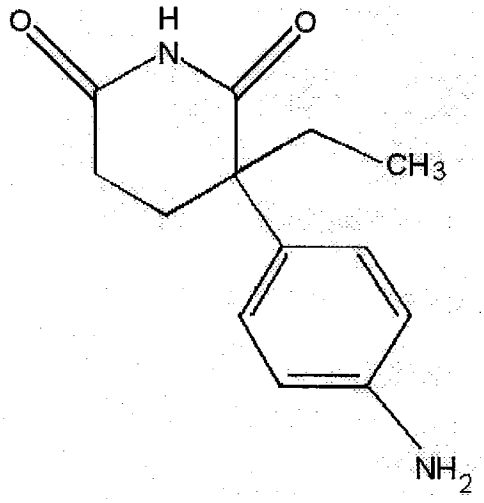
Battelle
The Business of Innovation

ANALYTICAL CHEMISTRY ACTIVITIES REPORT

AMINOGLUTETHIMIDE

CAS No.: 125-84-8	Lot No.: 043K0939 (Sigma Aldrich)
Receipt Date: 10/27/04	Amount Received: 2.40 g
Appearance: Solid	Vendor Purity: >99% by TLC
Receipt Date: 6/24/05	Lot No.: 06016JS (Sigma Aldrich)
Appearance: Solid	Amount Received: 3.0 g
Storage Conditions (@ Battelle): Room Temperature (~25°C)	Vendor Purity: 99% by TLC

STRUCTURE:



Mol. Wt.: 232.28 g/mol

Mol. Formula: $C_{13}H_{16}N_2O_2$

Prepared By:

Denise A. Contos

Denise A. Contos, M.S.

Approved By:

Steven W. Graves 10-18-05

Steven W. Graves, B.S.

Manager, Chemistry Technical Center

Battelle Study No. WA 4-16/17

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to the Study Director and Management as follows:

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Test substance receipt*	10/26/04	10/26/04
Formulation preparation*	12/2/04	12/2/04
Dispensing*	12/2/04	12/2/04
Formulation analysis*	12/2/04	12/2/04
Audit study file	7/26/05	7/26/05
Audit analytical report	7/26/05	7/26/05
Audit study file	10/5/05	10/5/05
Audit analytical report	10/5/05	10/5/05

* These inspections are serving the purpose for all reference chemicals since QA was required to see only one phase inspection of a chemical.

Hillary Gilroy 10-14-05

Quality Assurance Unit Date

EXECUTIVE SUMMARY

The title compound, aminoglutethimide (AG), was analyzed in support of the Environment Protection Agency (EPA) Placental and Recombinant Aromatase Assay Prevalidation work, Work Assignment 4-16/17.

Solubility of aminoglutethimide was determined to be acceptable in dimethylsulfoxide (DMSO) at a concentration of 23.2 mg/mL (0.1 M).

An aminoglutethimide formulation analysis method was validated on the previous EPA WA 3-10 study. This method was used without technical modification for analysis of formulation and stability samples on the current study.

Storage stability was previously determined (EPA WA 3-10 study) as 39 days when stored at approximately 5°C and protected from light at a target formulation concentration of 27.6 mg/mL in DMSO. In the current study, a formulation sample at a target concentration of 23.2 mg/mL in DMSO was stable when stored refrigerated and protected from light for 59 days.

The stock formulation prepared for shipment to the testing laboratory was analyzed and met the established acceptance criteria.

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Figure 4. Vehicle/Calibration Standard Curve 1/24/2005 Analysis 7

Figure 5. Vehicle/Calibration Standard Curve 6/30/2005 Analysis 8

1 INTRODUCTION

The purpose of this work was to provide all necessary chemistry support activities for aminoglutethimide on EPA Work Assignment 4-16/17, and consisted of:

- Determining solubility in dimethylsulfoxide (DMSO)
- Preparing and analyzing a stock formulation and a formulation stability sample.

This work was done at Battelle, 505 King Avenue, Columbus, OH 43201.

2 CHEMICAL RECEIPT AND STORAGE

One 15-mL amber glass bottle containing 2.40 grams of aminoglutethimide, Lot No. 043K0939, and one 30-mL clear glass bottle containing 3.0 grams of aminoglutethimide, Lot No. 06016JS was received on October 27, 2004 and on June 24, 2005, respectively, from the repository at Battelle's Marine Sciences Laboratory in Sequim, WA. The chemicals were received and subsequently stored at room temperature.

A copy of the manufacturer's Certificates of Analysis for these lots are shown in Figures 1 and 2. The purity of the chemicals were > 99% and 99%, respectively, based on thin layer chromatography.

SIGMA-ALDRICH

Certificate of Analysis

Product Name	DL-Aminoglutethimide
Product Number	A9657
Product Brand	SIGMA
CAS Number	125-84-8
Molecular Formula	$C_{13}H_{16}N_2O_2$
Molecular Weight	232.28

TEST	SPECIFICATION	LOT 043K0939 RESULTS
APPEARANCE	WHITE TO OFF-WHITE POWDER	WHITE POWDER
SOLUBILITY	CLEAR COLORLESS SOLUTION AT 50 MG/ML OF ACETIC ACID:METHANOL (1:1)	CONFORMS
IDENTITY	CONSISTENT WITH STRUCTURE BY IR OR NMR	IR SPECTRUM CONFORMS
CARBON	66.0 TO 68.5%	67.2% *
NITROGEN	11.8 TO 12.3%	12.1% *
PURITY BY THIN LAYER CHROMATOGRAPHY	98% MINIMUM	> 99%
		* SUPPLIER'S INFORMATION
SHELF LIFE SOP QC-12-006	7 YEARS	OCTOBER 2007
QC ACCEPTANCE DATE		APRIL 2003
PRODUCT CROSS REFERENCE INFORMATION		REPLACEMENT FOR ALDRICH #259195

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

Figure 1 – Certificate of Analysis, Lot No. 043K0939

Battelle
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ANALYTICAL CHEMISTRY ACTIVITIES REPORT

CHRYSIN

CAS No.: 480-40-0

Lot No.: 10101DC (Sigma Aldrich)

Receipt Date: 10/26/04

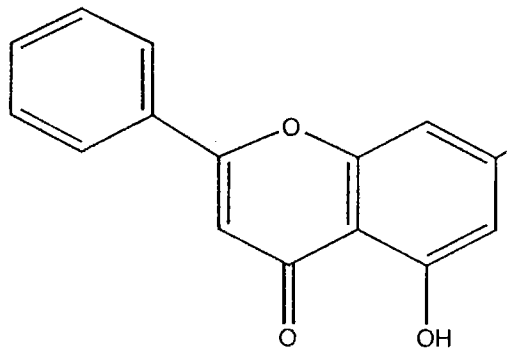
Amount Received: 25 g

Appearance: Solid

Vendor Purity: 98.20% by HPLC

Storage Conditions (@ Battelle): Room temperature (~25°C)

STRUCTURE:



Mol. Wt.: 254.24 g/mol

Mol. Formula: $C_{15}H_{10}O_4$

Prepared By:

Denise A. Contos, M.S. 12/22/05

Approved By:

Steven W. Graves, B.S.
Manager, Chemistry Technical Center

Battelle Study No. WA 4-16/17

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit (QAU) and reports were submitted to the Study Director and Management as follows:

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Test substance receipt	10/26/04	10/26/04
Dispensing*	12/2/04	12/2/04
Formulation analysis*	12/2/04	12/2/04
Formulation preparation*	12/2/04	12/2/04
Audit analytical report	8/9/05	8/9/05
Audit study file	8/9/05	8/9/05
Audit analytical report	12/14/05	12/14/05
Audit study file	12/14/05	12/14/05

* These inspections are serving the purpose for all reference chemicals since QA was required to see only one phase inspection of a chemical.

Quality Assurance Unit — Date: 12.22.05

Battelle

EXECUTIVE SUMMARY

The title compound, chrysin (CHRY), was analyzed in support of the EPA Placental and Recombinant Aromatase Assay Prevalidation work, Work Assignment 4-16/17.

The solubility of CHRY was determined to be acceptable in dimethylsulfoxide (DMSO) for preparing formulations.

A formulation analysis method was developed and validated to analyze CHRY in DMSO at a target concentration of 2.54 mg/mL (0.01 M). This method was used to analyze samples from both the formulation and formulation storage stability studies.

The storage stability study indicated that a 2.54 mg/mL formulation stored in sealed amber glass bottles and protected from light was stable for 100 days at approximately 5°C.

The stock formulations prepared for shipment to the testing laboratory was analyzed and met the established acceptance criteria.

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1 INTRODUCTION

The purpose of this work was to provide all necessary chemistry support activities for Chrysin (CHRY) on Environmental Protection Agency (EPA) Work Assignment 4-16/17, and consisted of:

- Determining solubility in dimethylsulfoxide (DMSO)
- Developing and validating a formulation analysis method
- Conducting a storage stability study
- Preparing and analyzing a stock formulation.

This work was done at Battelle, 505 King Avenue, Columbus, OH 43201.

2 CHEMICAL RECEIPT AND STORAGE

One 60-mL amber glass bottle of CHRY, 10101DC, was received from the repository at Battelle's Marine Sciences Laboratory in Sequim, WA on October 26, 2004. The label amount indicated 25 grams was sent. The chemical was received and subsequently stored at room temperature.

A copy of the manufacturer's Certificate of Analysis for this lot is shown in Figure 1, which states that purity was 98.20% based on high performance liquid chromatography (HPLC).

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Chrysin
Product Number	C8,010-5
Product Brand	ALDRICH
CAS Number	480-40-0
Molecular Formula	$C_{15}H_{10}O_4$
Molecular Weight	254.24

TEST	SPECIFICATION	LOT 10101DC RESULTS
APPEARANCE	YELLOW TO YELLOW GREEN TO TAN POWDER	YELLOW POWDER
INFRARED SPECTRUM	CONFORMS TO STRUCTURE AND STANDARD.	CONFORMS TO STRUCTURE AND STANDARD.
UV-VISIBLE SPECTRUM	C IN 0.1N NAOH	0.01G/L,0.01N NAOH
	E(348 +/- 2NM) = > 8,000	E348= 8,500
	E(282 +/- 2NM) = >22,000	E282=23,400
	E(263 +/- 2NM) = >20,000	E264=20,400
	E(224 +/- 2NM) = >27,000	E224=28,000
MISCELLANEOUS ASSAYS	97.0% - 103.0% (WITH TBAH)	101.4 % (WITH TBAH)
TITRATION	97.0% - 103.0% (WITH NAOH)	99.3 % (WITH NAOH)
HIGH PRESSURE LIQUID CHROMATOGRAPHY	CONSISTENT WITH CONTROL	98.20 %
SOLUBILITY	50 MG/ML PYRIDINE; CLEAR TO SLIGHT HAZY,	5%, PYRIDINE; CLEAR, YELLOW SOLUTION
QUALITY CONTROL ACCEPTANCE DATE		APRIL, 2004

Ronnie J. Martin, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Figure 1 – Certificate of Analysis

3 SOLUBILITY STUDIES

A solubility study was conducted to determine the solubility of CHRY in 100% DMSO, at a concentration of at least 2.54 mg/mL. CHRY (0.50848 ± 0.05085 g) was weighed into a 10-mL volumetric flask. DMSO was added until the flask was approximately 80% full. The contents were mixed until the CHRY dissolved. The contents of the flask were diluted to volume with DMSO, sealed, and mixed well. The CHRY went readily into solution. Although the solution was prepared at approximately 50 mg/mL, higher than the target concentration, CHRY was readily soluble and would therefore, be soluble at the target concentration 2.54 mg/mL. This experiment showed that DMSO was an acceptable solvent for the 2.54 mg/mL formulation (0.01M).

4 FORMULATION ANALYSIS METHOD PERFORMANCE EVALUATION (MPE)

This section describes the evaluation of a method developed to analyze formulations of CHRY in DMSO at a target concentration of 2.54 mg/mL (0.01 M) for the stability study and the results and conclusions from this evaluation.

4.1 Method Development

Method development for this chemical involved the evaluation of various chromatographic conditions. The selected method was one which produced acceptable retention time and peak shape. The detection method chosen was HPLC with ultraviolet (UV) detection with the wavelength set at the absorbance maximum above 270 nm.

4.2 Method

The HPLC parameters for CHRY are presented in Table 1.

Table 1 – HPLC System

Instrument System	Waters (Milford, MA) and Agilent (Palo Alto, CA)
Column	Supelcosil LC-ABZ, 5 µm particle size, 150 mm × 4.6 mm (ID) (Supelco, Bellefonte, PA)
Mobile Phase	70:30 (v/v) Methanol:0.15% Ammonium Acetate, Isocratic
Flow Rate	1.0 mL/minute
Injection Volume	10 µL
Detector Type	UV
Detector Wavelength	270 nm
Run Time	~15 minutes

4.3 Method Validation

Validation was accomplished using a single experiment.

Triplicate vehicle/calibration standards at the highest and lowest of four concentrations were prepared. A single standard was prepared at each intermediate concentration. The high and low concentrations were used to assess the precision of the method. The precision of the low concentration was used to calculate limits of detection (LOD) and quantitation (LOQ). Triplicate vehicle/calibration blanks with and without internal standard (IS) were used to assess the specificity of the method.

4.3.1 Preparation of Mobile Phase

A 0.15% ammonium acetate solution was prepared by weighing approximately 1.5 grams of ammonium acetate into a 1-L volumetric flask. The content of the flask was diluted to volume with Milli-Q water, sealed, and mixed well.

The mobile phase was prepared by mixing 700 mL of methanol and 300 mL of 0.15% ammonium acetate.

ANALYTICAL CHEMISTRY ACTIVITIES REPORT

ECONAZOLE

CAS No.: 24169-02-6

Lot No.: 123K1220 (Sigma Aldrich)

Receipt Date: 10/26/04 and 12/4/04

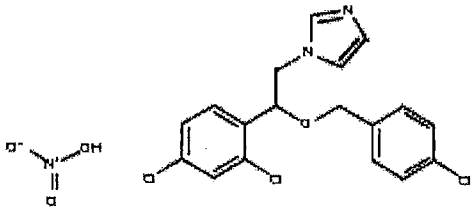
Amount Received: 10 g

Appearance: Solid/White Powder

Vendor Purity: 98% by TLC

Storage Conditions (@ Battelle): Room temperature (~25°C)

STRUCTURE:



Mol. Wt.: 444.70 g/mol

Mol. Formula: $C_{18}H_{15}Cl_3N_2O \cdot HNO_3$

Prepared By:

Denise A. Contos, M.S.

Approved By:

Steven W. Graves, B.S.
Manager, Chemistry Technical Center

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit (QAU) and reports were submitted to the Study Director and Management as follows:

Phase Inspected	Inspection Date	Date Reported to Study Director/Management
Test substance receipt	10/26/2004	10/26/2004
Formulation preparation*	12/2/2004	12/2/2004
Dispensing*	12/2/2004	12/2/2004
Formulation analysis*	12/2/2004	12/2/2004
Audit study file	1/3/2006	1/3/2006
Audit analytical report	1/3/2006	1/3/2006

* These inspections are serving the purpose for all reference chemicals since QA was required to see only one phase inspection of a chemical.

Quality Assurance Unit — 1-5-06 Date

EXECUTIVE SUMMARY

The title compound, econazole, was analyzed in support of the Environmental Protection Agency (EPA) Placental and Recombinant Aromatase Assay Prevalidation work, Work Assignment 4-16/17.

The solubility of econazole was determined to be acceptable in dimethylsulfoxide (DMSO) for preparing formulations.

A formulation analysis method was developed and validated to analyze econazole in DMSO at a concentration of 44.47 mg/mL (0.1 M). This method was used to analyze samples from both formulation and formulation storage stability studies at 44.47 mg/mL.

The storage stability study indicated that a 44.47 mg/mL formulation stored in sealed amber glass bottles and protected from light was stable for 56 days at approximately 5°C.

The stock formulations prepared for shipment to the testing laboratory were determined to meet the established acceptance criteria.

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1 INTRODUCTION

The purpose of this work was to provide all necessary chemistry support activities for econazole on the Environmental Protection Agency (EPA) Work Assignment 4-16/17, and consisted of:

- Determining solubility in dimethylsulfoxide (DMSO).
- Developing and validating a formulation analysis method.
- Conducting a storage stability study.
- Preparing and analyzing a stock formulation.

This work was done at Battelle, 505 King Avenue, Columbus, OH 43201.

2 CHEMICAL RECEIPT AND STORAGE

Two 15-mL amber glass bottles of econazole, Lot No. 123K1220, were received from the repository at Battelle's Marine Sciences Laboratory in Sequim, WA (one each on October 26, 2004 and December 4, 2004). The label amount indicated 5 grams was sent at each shipment. The chemical was received and subsequently stored at room temperature.

A copy of the manufacturer's Certificate of Analysis for this lot is shown in Figure 1. This states that purity was 98% based on thin layer chromatography.

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Econazole nitrate salt,
Product Number	E4632
Product Brand	SIGMA
CAS Number	24169-02-6
Molecular Formula	$C_{18}H_{15}Cl_3N_2O \cdot HNO_3$
Molecular Weight	444.70

TEST	SPECIFICATION	LOT 123K1220 RESULTS
APPEARANCE	WHITE TO OFF-WHITE POWDER	WHITE POWDER
SOLUBILITY	CLEAR COLORLESS TO FAINT YELLOW SOLUTION WITH SOME INSOLUBLES AT 25MG/ML IN CHLOROFORM:METHANOL (1:1)	CLEAR COLORLESS
WATER BY KARL FISCHER	NMT 0.5%	0.0%
ELEMENTAL ANALYSIS	47.6 TO 49.6% CARBON	48.6%
	9.2 TO 9.8% NITROGEN	9.4%
IR SPECTRUM	CONSISTENT WITH STRUCTURE	CONFORMS (SUPPLIER DATA)
PURITY BY THIN LAYER CHROMATOGRAPHY	NLT 98%	98%
QC ACCEPTANCE DATE		JANUARY 2004

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

Figure 1 – Certificate of Analysis

3 SOLUBILITY STUDIES

A solubility study was conducted to determine the solubility of econazole in 100% DMSO, at a concentration of at least 44.47 mg/mL (0.1 M). Econazole (0.44470 ± 0.04447 g) was weighed into a 10-mL volumetric flask. DMSO was added until the flask was approximately 80% full. The contents were mixed until the econazole dissolved. The contents of flask were diluted to volume with DMSO, sealed, and mixed well. The econazole went readily into solution. This experiment showed that DMSO was an acceptable solvent for the 44.47 mg/mL formulation.

4 FORMULATION ANALYSIS METHOD PERFORMANCE EVALUATION (MPE)

This section describes the evaluation of a method developed to analyze formulations of econazole in DMSO at a target concentration of 44.47 mg/mL for the stability study, the results and the conclusions from this evaluation.

4.1 Method Development

Method development for this chemical involved the evaluation of various chromatographic conditions. The selected method was one which produced acceptable retention time and peak shape. The detection method chosen was high pressure liquid chromatography with ultraviolet detection (HPLC/UV) with the wavelength set at the absorbance maximum of 271 nm.

4.2 Method

The HPLC parameters for econazole are shown in Table 1.

Table 1 – HPLC System

Instrument System	Agilent (Palo Alto, CA), Waters (Milford, MA)
Column	Supelcosil LC-ABZ, 150 mm × 4.6 mm (ID) (Supelco, Bellefonte, PA)
Guard Column	C-18 guard column
Mobile Phase	70:30 (v/v) Methanol:0.15% Ammonium Acetate, Isocratic
Flow Rate	1.0 mL/minute
Injection Volume	50 µL
Detector Type	UV
Detector Wavelength	271 nm
Run Time	~20 minutes

4.3 Method Validation

Validation was accomplished using a single experiment.

Triplicate vehicle/calibration standards at the highest and lowest of four concentrations were prepared. A single standard was prepared at each intermediate concentration. The high and low concentrations were used to assess the precision of the method. The precision of the low concentration was used to calculate limits of

detection (LOD) and limits of quantitation (LOQ). Triplicate vehicle/calibration blanks with and without internal standard (IS) were used to assess the specificity of the method.

4.3.1 Preparation of Mobile Phase

A 0.15% ammonium acetate solution was prepared by weighing approximately 1.5 grams of ammonium acetate into a 1-L volumetric flask. The flask was diluted to volume with Milli-Q water, sealed, and mixed well.

The mobile phase was prepared by mixing 700 mL of methanol and 300 mL of 0.15% ammonium acetate.

4.3.2 Preparation of Standards and Blanks

4.3.2.1 Internal Standard (IS)

An IS solution was prepared by weighing 100 ± 4 mg of terconazole into a 50-mL volumetric flask. The content of the flask was diluted to volume with methanol, sealed, and mixed well.

4.3.2.2 Stock Standards

Two stock standards (A and B) were prepared by accurately weighing 25 ± 1 mg of econazole each into two individual 25-mL volumetric flasks and dissolving in and diluting to volume with HPLC mobile phase. This produced stocks A and B with target concentrations of 1 mg/mL each.

4.3.2.3 Vehicle/Calibration Standards

Vehicle/calibration standards were prepared as shown in Table 2. The contents of the flasks were diluted to volume with HPLC mobile phase, sealed, and mixed well. Triplicate vehicle/calibration standards were prepared at the low and high concentrations with single vehicle/calibration standards prepared at the two intermediate concentrations.

Table 2 – Preparation of Vehicle/Calibration Standards

Vehicle/ Calibration Std	Target Final Conc (µg/mL)	Source	Source Volume (mL)	IS (mL)	DMSO (mL)	Final Volume (mL)
VS1	500	A	5	1	0.1	10
VS2	400	B	4	1	0.1	10
VS3	200	A	2	1	0.1	10
VS4	100	B	1	1	0.1	10

4.3.2.4 Blanks

Triplicate blanks without IS were prepared by pipetting 0.1 mL of DMSO into three individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with HPLC mobile phase, sealed, and mixed well.

Triplicate blanks with IS were prepared by pipetting 1 mL IS and 0.1 mL of DMSO into three individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with HPLC mobile phase, sealed, and mixed well.

4.3.3 Analysis

A portion of each vehicle/calibration standard, blank and sample were transferred to individual autoinjector vials and the vials were sealed. Single injections were made from each vial using the same chromatographic system and parameters determined during method development (Table 1).

4.3.4 Calculations

The integration of the econazole and IS peaks by the chromatography data system was evaluated to assure it was correct in all chromatograms and manually reintegrated, if necessary. A linear regression equation was calculated relating the response ratio of econazole divided by the IS (y) to the concentration of the vehicle/calibration standards (x). The concentration of each vehicle/calibration standard was calculated using its individual response ratio and the regression equation. These values were used to calculate the individual and average concentrations, percent relative errors (RE), standard deviation (s), and percent relative standard deviation (RSD) as appropriate for the vehicle/calibration standards at each concentration.

4.3.5 Results

Specificity is shown by the representative overlaid chromatograms from a high and low vehicle/calibration standard, a blank with IS, and a blank from the validation as indicated in Figure 2. The blank and blank with IS exhibited no peaks that would significantly interfere with the econazole or IS peaks. The regression analysis results from the standard curve indicate linearity and are shown in Table 3.

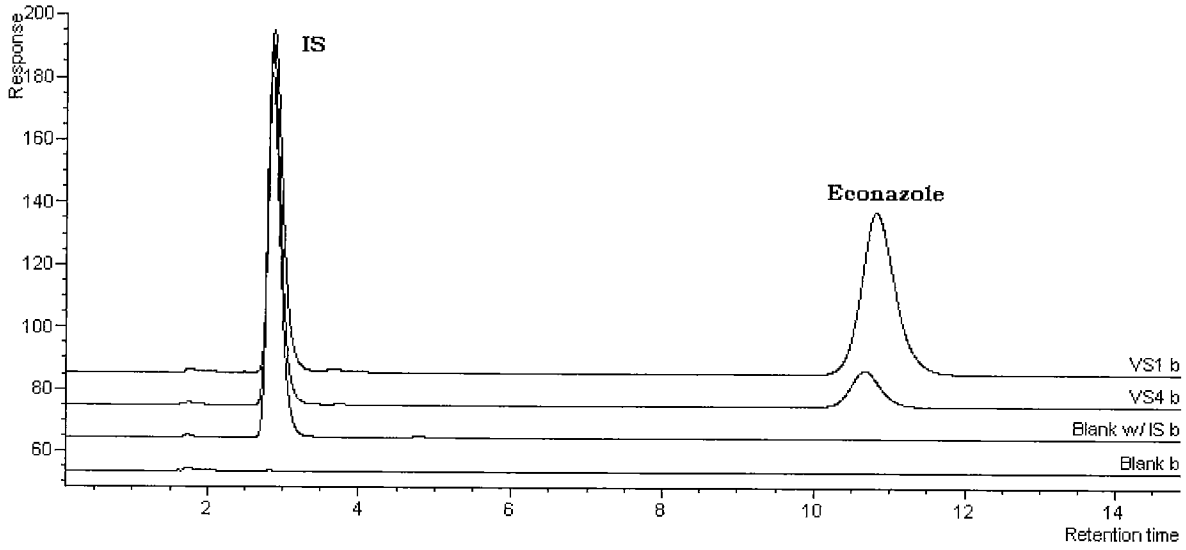


Figure 2 – Representative Overlaid Chromatograms from a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from the Validation (Shown Top to Bottom)

Table 3 – Regression Analysis Results

Slope	y-Intercept	Correlation Coefficient	Standard Error
0.0023	0.0088	1.000	0.0021

The precision and accuracy of the vehicle/calibration standard validation results are shown in Table 4.

Table 4 – Vehicle/Calibration Standard Validation Results

Nominal Std Conc (µg/mL)	Det'd Std Conc (µg/mL)	Avg Det'd Std Conc (µg/mL)	s (µg/mL)	% RSD	% RE	Avg % RE
512.6	513.2	512.5	0.8	0.1	0.1	0.0
	512.7				0.0	
	511.7				-0.2	
406.9	406.7	NA	NA	NA	0.0	NA
205.0	206.8	NA	NA	NA	0.9	NA
101.7	101.3	101.3	0.1	0.1	-0.4	-0.4
	101.2				-0.5	
	101.3				-0.4	

The method validation sensitivity was 0.1732 µg/mL, the LOD, which is defined as three times the standard deviation of the low vehicle/calibration standard. This is equivalent to a formulation concentration of 20 µg/mL when a formulation is diluted 1 to 100 for analysis. The LOQ, defined as ten times the standard deviation of the lowest standard because there was no blank response, was 0.5774

µg/mL. This is equivalent to a formulation concentration of 60 µg/mL when a formulation is diluted 1 to 100 for analysis. The estimated limit of quantitation (ELOQ), defined as the lowest vehicle/calibration standard with acceptable accuracy and precision, was 101.7 µg/mL.

4.3.6 Conclusions

The method met all acceptance criteria for specificity, linearity, precision, accuracy and sensitivity. The method was suitable for the stability study and subsequent formulation analysis for which it was used.

5 FORMULATION STABILITY STUDIES

A formulation stability study was conducted at a target concentration of 44.47 mg/mL in DMSO for 56 days (8 weeks) in sealed, amber glass bottles stored at approximately 5°C.

5.1 Study Design

A formulation sample was analyzed on the day of preparation (Day 0) and Day 14. A second formulation sample was analyzed on the day of preparation (Day 0), Week 4 and Week 8. Three aliquots were analyzed from each sample at each storage time.

5.2 Formulation Method

A formulation was prepared on November 19, 2004, Day 0 of the storage stability study at a target concentration of 44.47 mg/mL in DMSO by accurately weighing 1110 ± 10 mg into a 25-mL volumetric flask. The chemical was dissolved in and diluted to approximately three quarters of the total volume with DMSO. The flask was sealed and sonicated for approximately 5 minutes to mix the contents. The contents of the flask was diluted to volume with DMSO, sealed, and mixed well.

Approximately 6 mL of formulation was transferred into each of four 8-mL amber glass vials which were then sealed. One vial was used for the Day 0 analysis and the other three were stored at approximately 5°C until use. After 14 days of storage, a vial was removed from the refrigerator, allowed to warm to room temperature, and triplicate aliquots were prepared and analyzed.

A second formulation was prepared on February 7, 2005, Day 0, at a target concentration of 44.47 mg/mL in DMSO by accurately weighing 2.22350 ± 0.08894 g into a 50-mL volumetric flask. The flask was diluted to approximately 80% volume with DMSO, sealed and sonicated for approximately 5 minutes. The content of the flask was inverted 10 times, and diluted to volume with DMSO and shaken to mix well. Approximately 9 mL were dispensed into an amber glass bottle, sealed and stored refrigerated. A formulation sample aliquot was prepared for analysis on Day 0, Weeks 4 and 8 for storage stability determination.

5.3 Analysis Method

Vehicle/calibration standards and blanks with and without IS were prepared as described in the validation experiment (Section 4.3.2) of this report.

In triplicate, 1 mL of the formulation was pipetted into three individual 10-mL volumetric flasks, diluted to volume with HPLC mobile phase, sealed, and mixed well. One (1) mL of the diluted formulation and 1-mL of the IS were pipetted into individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with HPLC mobile phase, sealed, and mixed well. An appropriate volume of each was transferred to an autoinjector vial and the vials were sealed and analyzed using the chromatographic system in Table 1.

5.4 Results

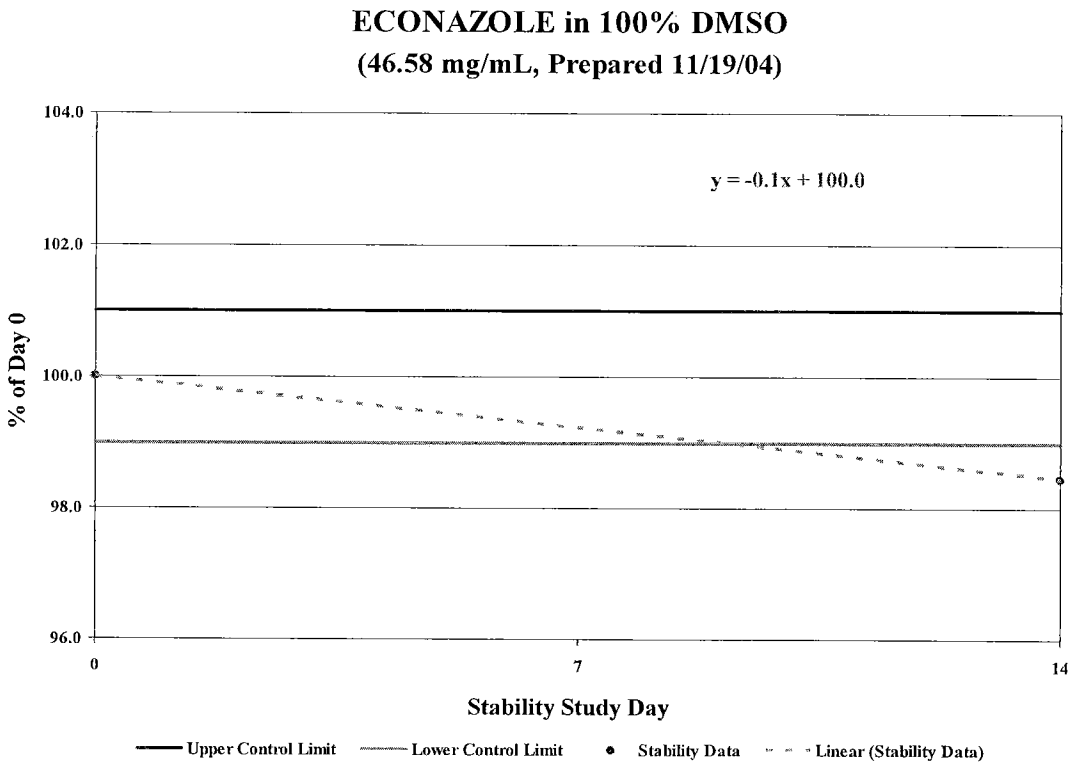
The results from the storage stability study are shown in Table 5 and presented in control chart format in Figure 3.

Table 5 – Formulation Storage Stability Results (44.47 mg/mL)

Preparation Date	Analysis Date	Day	Det'd Conc (mg/mL)			Avg Det'd Conc (mg/mL) ± s	% of Day 0 Conc ± s
11/19/04	11/19/04	0	46.91	46.37	46.47	46.58 ± 0.29	100.0 ± 0.6
11/19/04	12/3/04	14	45.91	45.88	45.80	45.86 ± 0.06	98.5 ± 0.1
2/7/05	2/7/05	0	46.24	46.08	45.89	46.07 ± 0.17	100.0 ± 0.4
2/7/05	3/7/05	28	45.03	44.74	44.66	44.81 ± 0.19	97.3 ± 0.4
2/7/05	4/4/05	56	42.95	42.92	41.94	42.60 ± 0.58	92.5 ± 1.3

For the formulation stability sample prepared on November 19, 2004, the pooled RSD of the analytical method was 0.44%. This means that there would have to be a difference of more than 1.01% from the Day 0 value for the difference to be statistically significant at a 95% confidence level.

For the formulation stability sample prepared on February 7, 2005, the pooled RSD of the analytical method was 0.79%. This means that there would have to be a difference of more than 1.80% from the Day 0 value for the difference to be statistically significant at a 95% confidence level.



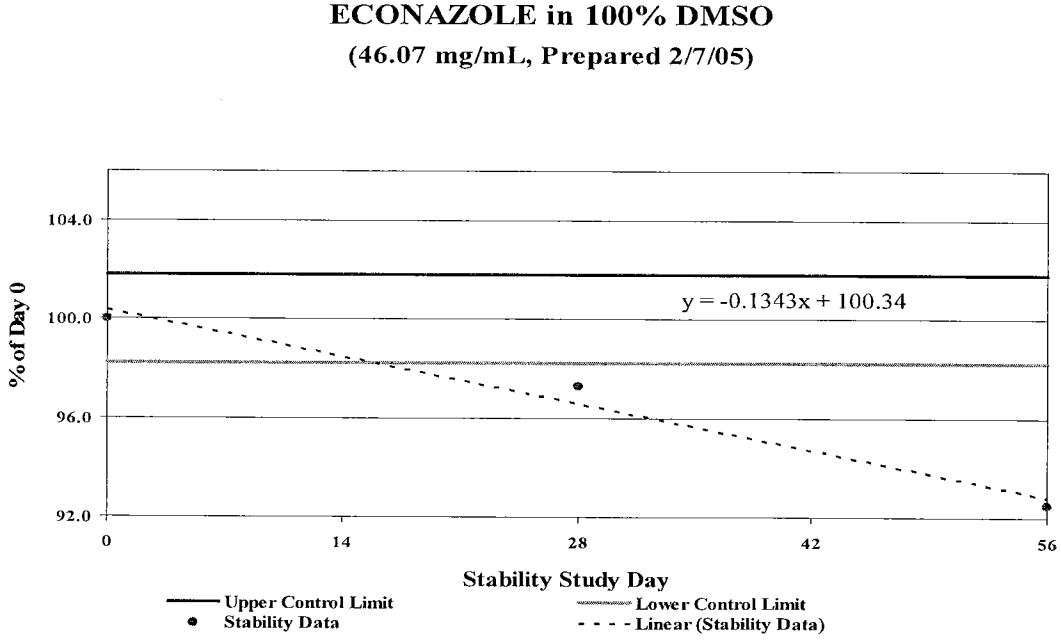


Figure 3 – Control Charts for the Storage Stability Analysis

5.5 Discussion and Conclusions

The Day 0 determined value for the November 19, 2004 formulation was approximately 4.7% above the nominal value (the calculated concentration based on the weight of the chemical). The concentration of the sample stored at approximately 5°C protected from light in amber glass vial for Day 14 was below the lower significance level due to the tight precision of the assay but was within 1.5% of the Day 0 value (November 19, 2004) and met acceptance criteria ± 10%.

The Day 0 determined value for the February 7, 2005 formulation was approximately 3.5% above the nominal value (the calculated concentration based on the weight of the chemical). The concentrations of the sample stored at approximately 5°C protected from light in an amber glass vial for Days 28 and 56 was below the lower significance level due to the tight precision of the assay but was within 2.7 and 7.5%, respectively of the Day 0 value (February 7, 2005) and met acceptance criteria ± 10%.

These data indicate the formulation was stable when protected from light at approximately 5°C for 56 days.

6 FORMULATION PREPARATION AND ANALYSIS

Formulations were prepared and analyzed on February 7, 2005 and August 8, 2005 according to SOP COMSPEC.II-031, "Standard Operating Procedure (SOP) for the Formulation and Analysis of Econazole in 100% DMSO." This section describes the method, results, and conclusions.

6.1 Preparation of Formulation

Econazole (2.22350 ± 0.08894 g) was weighed into a 50-mL volumetric flask. DMSO was added until the flask was approximately 80% full. The contents were sonicated for approximately 5 minutes until the econazole dissolved. The contents of the flask were diluted to volume with DMSO, sealed, and mixed well.

6.2 Preparation of Standards and Blanks

Standards and blanks were prepared as described for the validation (Section 4.3.2 of this report).

6.3 Preparation of Formulation Samples

One (1) mL of the formulation was pipetted into three individual 10-mL volumetric flasks, diluted to volume with HPLC mobile phase, sealed, and mixed well. One (1) mL of the diluted formulation and 1 mL of the IS were pipetted into individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with HPLC mobile phase, sealed, and mixed well.

6.4 Analysis

Autoinjector vials were filled with aliquots of each standard, blank and sample. A single injection was made from each vial using the HPLC conditions from the validation (Table 1).

6.5 Calculations

The integration of the econazole and IS peaks by the chromatography data system was evaluated to assure it was correct in all chromatograms and manually reintegrated, if necessary. A linear regression equation was calculated relating the response ratio of econazole divided by the IS (y) to the concentration of the vehicle/calibration standards (x). The concentration of each vehicle/calibration standard was calculated using its individual response ratio and the regression equation. The percent RE for each vehicle/calibration standard was calculated by subtracting the nominal value from the determined value, dividing by the nominal value, and then multiplying by 100. The percent RE for each formulation sample was calculated by subtracting the target value from the determined value, dividing by the target value, and then multiplying by 100. The average determined concentration, standard deviation (s), and percent RSD were calculated for the vehicle/calibration standards and formulation samples when applicable.

6.6 Results

Specificity is shown by the representative overlaid chromatograms of the high and low vehicle/calibration standards, blank with working IS and a blank are presented in Figure 4.

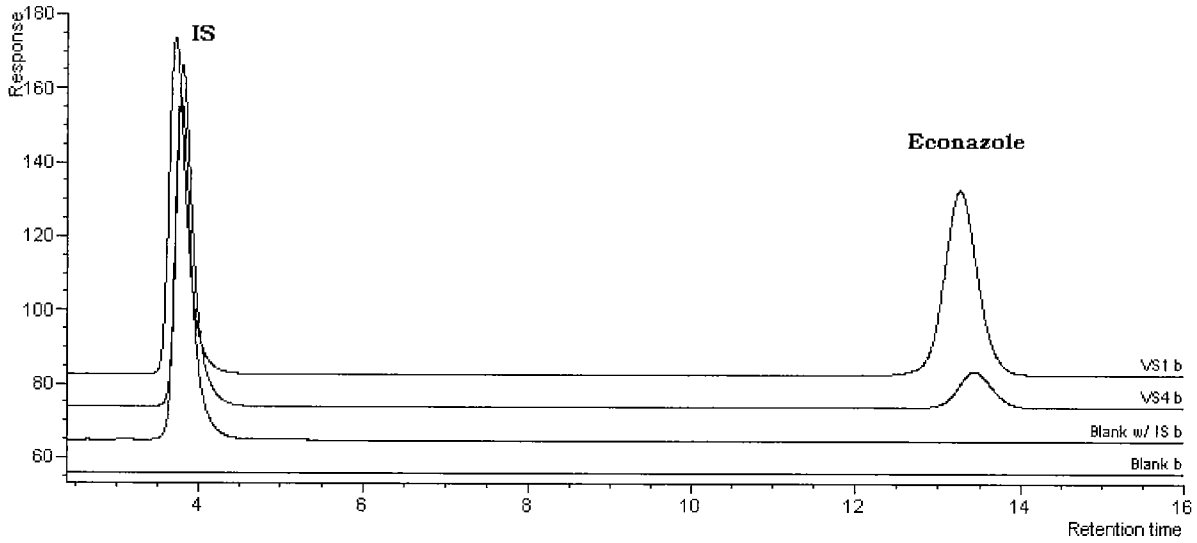


Figure 4 – Representative Overlaid Chromatograms of a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from a Formulation Analysis (Shown Top to Bottom)

The vehicle/calibration standard curve and the results of the regression analysis indicated linearity and are shown in Figure 5.

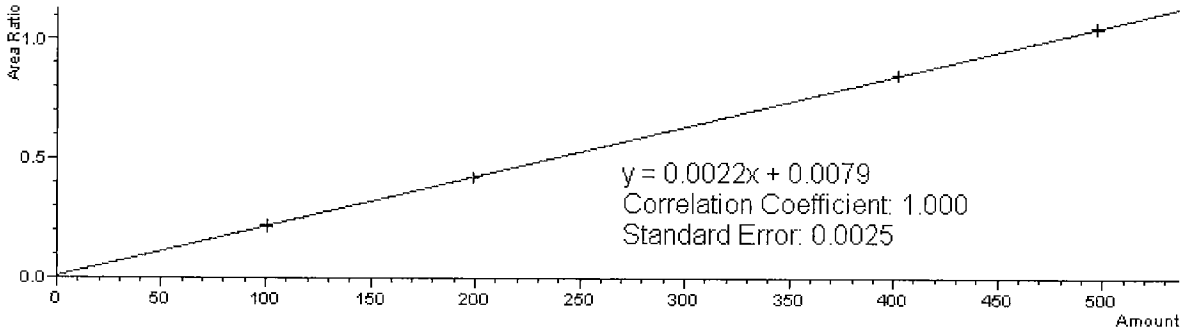


Figure 5 – Vehicle/Calibration Standard Curve and Regression Analysis Results

The results of the formulation analysis are shown in Table 6. The formulations met acceptance criteria (RE within 10% of target and RSD of $\leq$ 10%).

Table 6 – Formulation Analysis Results

Batch No.	Analysis Date	Det'd Conc (mg/mL)			Avg Det'd Conc (mg/mL)	Avg % RE	RSD (%)
1-ECON-1	2/7/05	46.24	46.08	45.89	46.07	3.5	0.4
2-ECON-1	8/8/05	44.58	44.49	44.14	44.40	-0.2	0.5

6.7 Conclusions

The average concentration of the stock formulations and its percent RSDs were within acceptance criteria. Therefore, the formulations were suitable for use.

The econazole formulation at a target concentration of 44.47 mg/mL in DMSO was stable for 56 days when stored refrigerated and protected from light.

7 ACKNOWLEDGMENTS

Analytical support for this work was provided by Christina Zielinski, Sandy Runyon, Tudor Fernando, Hans Whittenburg and Ron Haney. The report was written by Denise Contos. Review of the data and report for completeness and accuracy was performed by Maria Evascu.

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ANALYTICAL CHEMISTRY ACTIVITIES REPORT

LINDANE

CAS No.: 58-89-9

Lot No.: 14419EB (Sigma Aldrich)

Receipt Date: 1/6/05

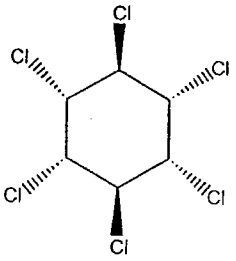
Amount Received: 10 g

Appearance: White Solid

Vendor Purity: 99.6% by GC

Storage Conditions (@ Battelle): Room temperature (~25°C)

STRUCTURE:



Mol. Wt.: 290.83 g/mol

Mol. Formula: $C_6H_6Cl_6$

Prepared By:

Denise A. Contos, M.S.

Approved By:

Steven W. Graves, B.S.
Manager, Chemistry Technical Center

Battelle Study No. WA 4-16/17

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit (QAU) and reports were submitted to the Study Director and Management as follows:

Phase Inspected	Inspection Date	Date Reported to Study Director/Management
Test substance receipt*	10/26/2004	10/26/2004
Formulation preparation*	12/2/2004	12/2/2004
Dispensing*	12/2/2004	12/2/2004
Formulation analysis*	12/2/2004	12/2/2004
Audit analytical report	12/22/2005	12/22/2005
Audit study file	12/22/2005	12/22/2005

* These inspections are serving the purpose for all reference chemicals since QA was required to see only one phase inspection of a chemical.

Kathleen E. Reed 1-6-06

Quality Assurance Unit Date

EXECUTIVE SUMMARY

The title compound, lindane, was analyzed in support of the Environmental Protection Agency (EPA) Placental and Recombinant Aromatase Assay Prevalidation Work, Work Assignment 4-16/17.

Solubility of lindane was determined to be acceptable in dimethylsulfoxide (DMSO) for preparing formulations.

A formulation analysis method was developed and validated to analyze lindane in DMSO at a concentration of 29.08 mg/mL (0.1M). This method was used to analyze samples from both formulation and formulation storage stability studies at 29.08 mg/mL.

Storage stability study indicated that a 29.08 mg/mL formulation stored in sealed amber glass bottles and protected from light was stable for 168 days at approximately 5°C.

The formulations prepared for shipment to the testing laboratory were determined and met the established acceptance criteria.

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1 INTRODUCTION

The purpose of this work was to provide all necessary chemistry support activities for lindane on Environmental Protection Agency (EPA) Work Assignment 4-16/17, and consisted of:

- Determining solubility in dimethylsulfoxide (DMSO).
- Developing and validating a formulation analysis method.
- Conducting a storage stability study.
- Preparing and analyzing a stock formulation.

This work was done at Battelle, 505 King Avenue, Columbus, OH 43201.

2 CHEMICAL RECEIPT AND STORAGE

One 20-mL amber glass bottle of lindane, 14419EB, was received from the repository at Battelle's Marine Sciences Laboratory in Sequim, WA on January 6, 2005. The label amount indicated 10 grams was sent. The chemical was received and subsequently stored at room temperature.

A copy of the manufacturer's Certificate of Analysis for this lot is shown in Figure 1. This states that purity was 99.6% based on gas chromatography (GC).

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Lindane
Product Number	23,339-0
Product Brand	ALDRICH
CAS Number	58-89-9
Molecular Formula	$C_6H_6Cl_6$
Molecular Weight	290.83

TEST	**SPECIFICATION**	**LOT 14419EB RESULTS**
APPEARANCE	WHITE TO OFF-WHITE POWDER	OFF WHITE POWDER
INFRARED SPECTRUM	CONFORMS TO STRUCTURE AND STANDARD.	CONFORMS TO STRUCTURE AND STANDARD
GAS LIQUID CHROMATOGRAPHY	96.5% (MINIMUM)	99.6%
QUALITY CONTROL ACCEPTANCE DATE		MAY, 2003

Ronnie J. Martin, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Figure 1 – Certificate of Analysis

3 SOLUBILITY STUDIES

A solubility study was conducted to determine the solubility of lindane in 100% DMSO, at a concentration of at least 29.08 mg/mL. Lindane (0.29080 ± 0.02908 g) was weighed into a 10-mL volumetric flask. DMSO was added until the flask was approximately 80% full. The contents were mixed until the lindane dissolved. The contents of the flask were diluted to volume with DMSO, sealed, and mixed well. The lindane went readily into solution. This experiment showed that DMSO was an acceptable solvent for the 29.08 mg/mL formulation.

4 FORMULATION ANALYSIS METHOD PERFORMANCE EVALUATION (MPE)

This section describes the evaluation of a method developed to analyze formulations of lindane in DMSO at a target concentration of 29.08 mg/mL for the stability study and the results and conclusions from this evaluation.

4.1 Method Development

Method development for this chemical involved the evaluation of various chromatographic conditions. The selected method was one which produced acceptable retention time for the major peak, apparent resolution of significant impurities and acceptable peak shape. The detection method chosen was gas chromatography with flame ionization detection (FID).

4.2 Method

The GC parameters for lindane are presented in Table 1.

Table 1 – GC System

GC	Agilent 6890 (Palo Alto, CA)
Column	RTX-5, 30 m × 0.25 mm (ID), 0.25 µm film thickness (Restek, Bellefonte, PA)
Carrier Gas and Flow Rate	Helium at ~2 mL/minute
Oven Temperature	150°C, hold for ~2 minutes, increase at 20°C/minute to 300°C; hold for 2 minutes
Detector Type	Flame Ionization (FID)
Detector Flow Rates	Hydrogen at ~30 mL/minute; Air at ~380 mL/minute
Detector Temperature	320°C
Injector Temperature	285°C
Injection Volume	1 µL
Injection Mode	Split 5:1
Run Time	~12 minutes

4.3 Method Validation

Validation was accomplished using a single experiment.

Triplicate vehicle/calibration standards at the highest and lowest of four concentrations were prepared. A single standard was prepared at each intermediate concentration. The high and low concentrations were used to assess the precision of the method. The precision of the low concentration was used to calculate limits of detection (LOD) and limits of quantitation (LOQ). Triplicate vehicle/calibration blanks with and without working internal standard (WIS) were used to assess the specificity of the method.

4.3.1 Preparation of Standards and Blanks

4.3.1.1 Internal Standard (IS)

Approximately 25 ± 1 mg of phenanthrene was added to a 25-mL volumetric flask. The contents of the flask was diluted to volume with methanol, sealed, and mixed well.

The IS was prepared by pipetting 10 mL of stock IS into a 25-mL volumetric flask. The contents of the flask was diluted to volume with methanol, sealed, and mixed well.

4.3.1.2 Stock Standards

Two stock standards were prepared by accurately weighing 50 ± 2 mg of lindane each into two individual 25-mL volumetric flasks and dissolving in and diluting to volume with methanol. This produced stocks A and B with target concentrations of 2000 µg/mL each.

4.3.1.3 Vehicle/Calibration Standards

Vehicle/calibration standards were prepared as shown in Table 2. The contents of the flasks were diluted to volume with methanol and mixed well. Triplicate vehicle/calibration standards were prepared at the low and high concentrations with single vehicle/calibration standards prepared at the two middle concentrations.

Table 2 – Preparation of Vehicle/Calibration Standards

Vehicle/Calibration Std	Target Final Conc (µg/mL)	Source	Source Volume (mL)	WIS (mL)	DMSO (mL)	Final Volume (mL)
VS1	800	A	4	1	0.1	10
VS2	600	B	3	1	0.1	10
VS3	400	A	2	1	0.1	10
VS4	200	B	1	1	0.1	10

4.3.1.4 Blanks

Triplicate blanks without IS were prepared by pipetting 0.1 mL of DMSO into three individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with methanol, sealed, and mixed well.

Triplicate blanks with IS were prepared by pipetting 1 mL IS and 0.1 mL of DMSO into three individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with methanol, sealed, and mixed well.

4.3.2 Analysis

A portion of each vehicle/calibration standard and blank was transferred to individual autoinjector vials and the vials were sealed. Single injections were made from each vial using the same chromatographic system and parameters determined during method development as shown in Table 1.

4.3.3 Calculations

The integration of the lindane and IS peaks by the chromatography data system was evaluated to assure it was consistent in all chromatograms and manually reintegrated, if necessary. A linear regression equation was calculated relating the response ratio of lindane divided by the IS (y) to the concentration of

the vehicle/calibration standards (x). The concentration of each vehicle/calibration standard was calculated using its individual response ratio and the regression equation. These values were used to calculate the individual and average concentrations, percent relative errors (RE), standard deviation (s), and percent relative standard deviation (RSD) as appropriate for the vehicle/calibration standards at each concentration.

4.3.4 Results

Specificity is shown by the representative overlaid chromatograms from a high and low vehicle/calibration standard, a blank with IS, and a blank from the validation as indicated in Figure 2. The blank and blank with IS exhibited no peaks that would significantly interfere with the lindane or IS peaks. The regression analysis results from the standard curve indicate the linearity and are shown in Table 3.

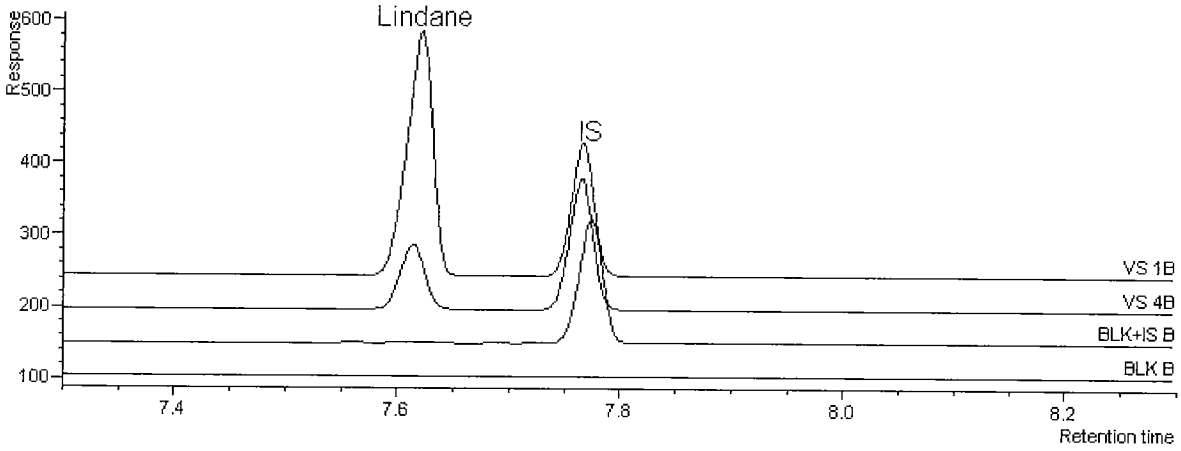


Figure 2 – Representative Overlaid Chromatograms from a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from the Validation (Shown Top to Bottom)

Table 3 – Method Validation Regression Analysis Results

Slope	y-Intercept	Correlation Coefficient	Standard Error
0.0027	-0.0351	1.000	0.0047

The precision and accuracy of the vehicle/calibration standard validation results are shown in Table 4.

Table 4 – Vehicle/Calibration Standard Validation Results

Nominal Std Conc (µg/mL)	Det'd Std Conc (µg/mL)	Avg Det'd Std Conc (µg/mL)	s (µg/mL)	%RSD	%RE	Avg %RE
776.3	777.3	776.8	1.1	0.1	0.1	0.1
	777.6				0.2	
	775.6				-0.1	
600.2	598.4	NA	NA	NA	-0.3	NA
388.2	387.0	NA	NA	NA	-0.3	NA
200.1	202.8	200.5	2.1	1.1	1.4	0.2
	200.1				0.0	
	198.6				-0.7	

The sensitivity of the method resulted in 6.4 µg/mL LOD which is defined as three times the standard deviation of the low vehicle/calibration standard. This is equivalent to a formulation concentration of 640 µg/mL when a formulation is diluted 1 to 100 for analysis. The LOQ, defined as ten times the standard deviation of the lowest standard because there was no blank response, was 21.3 µg/mL. This is equivalent to a formulation concentration of 2130 µg/mL when a formulation is diluted 1 to 100 for analysis. The estimated limit of quantitation (ELOQ), defined as the lowest standard with acceptable accuracy and precision, was 200.1 µg/mL.

4.3.5 Conclusions

The method met all acceptance criteria for specificity, linearity, precision, accuracy, and sensitivity. The method was suitable for the stability study and subsequent formulation analyses.

5 FORMULATION STABILITY STUDIES

A formulation stability study was conducted at a target concentration of 29.08 mg/mL in DMSO for 168 days (24 weeks) in sealed, amber glass bottles stored at approximately 5°C.

5.1 Study Design

A single sample was analyzed on the day of preparation (Day 0), Day 14, Weeks 4, 8 and 12. A second formulation sample was prepared and analyzed on January 24, 2005 (Day 0) and on Week 24. Three aliquots were analyzed from each sample at each storage time.

5.2 Formulation Method

A formulation was prepared on January 13, 2005, Day 0 of the storage stability study at a target concentration of 29.08 mg/mL in DMSO by accurately weighing 727 ± 7 mg of lindane into a 25-mL volumetric flask. The chemical was dissolved in and diluted to approximately three quarters of the total volume with

DMSO. The flask was sealed and manually shaken to mix the contents. The contents of the flask was diluted to volume with DMSO, sealed, and mixed well.

Approximately 6 mL of formulation was transferred into each of four, 8-mL amber glass vials which were then sealed. One vial was used for the Day 0 analysis and the other three were stored at approximately 5°C until use. After the desired storage period, a vial was removed from storage, allowed to warm to room temperature, and triplicate aliquots were prepared and analyzed.

A second formulation (Batch 1-LIN-1) was prepared on January 24, 2005 (Day 0) at a target concentration of 29.08 mg/mL in DMSO by accurately weighing 1.45400 ± 0.058 g into a 50-mL volumetric flask. The content of the flask was diluted to approximately 80% volume with DMSO, sealed and mixed well. The contents of the flask was diluted to volume with DMSO and mixed well. Approximately 9 mL were dispensed into an amber glass bottle, sealed and stored refrigerated. A formulation sample aliquot was prepared for analysis on Days 0 and 168 for storage stability determination.

5.3 Analysis Method

Vehicle/calibration standards and blanks with and without IS were prepared as described in the validation experiment (Section 4.3.1) of this report.

One (1) mL of the formulation was pipetted into three individual 10-mL volumetric flasks, diluted to volume with methanol, sealed, and mixed well. One (1) mL of the diluted formulation and 1-mL of IS were pipetted into 10-mL volumetric flasks, diluted to volume with methanol, sealed, and mixed well. An appropriate volume of each was transferred to an autoinjector vial and the vials were sealed and analyzed using the chromatographic system in Table 1.

5.4 Results

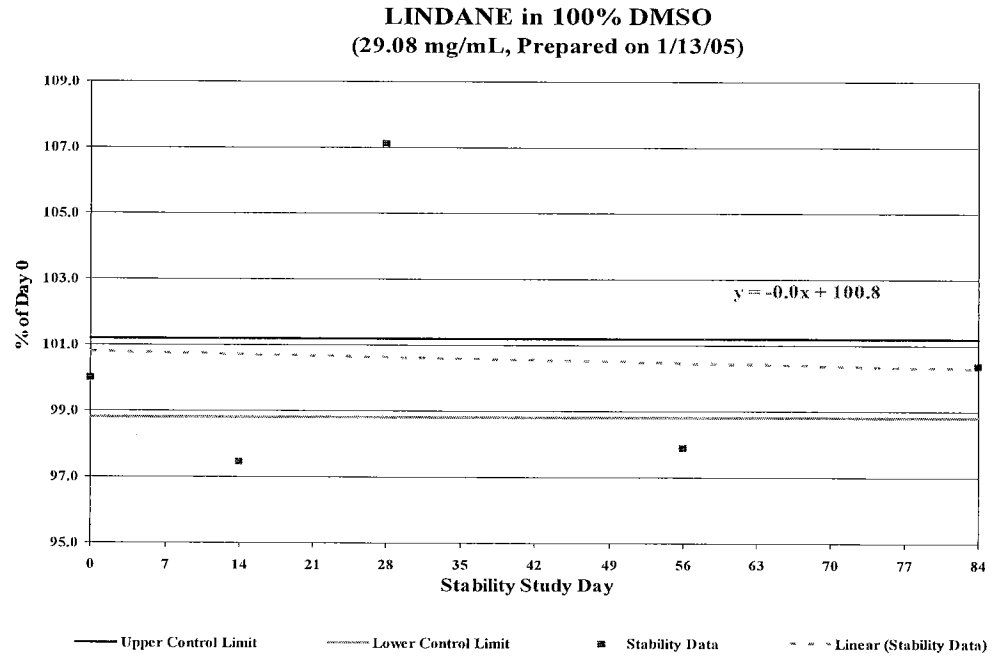
The results from the storage stability study are shown in Table 5 and presented in control chart format in Figure 3.

Table 5 – Formulation Storage Stability Results (29.08 mg/mL)

Preparation Date	Analysis Date	Day	Det'd Conc (mg/mL)			Avg Det'd Conc (mg/mL) ± s	% of Day 0 Conc ± s
1/13/05	1/13/05	0	29.38	29.48	29.18	29.35 ± 0.15	100 ± 0.5
1/13/05	1/27/05	14	28.56	28.56	28.67	28.60 ± 0.06	97.4 ± 0.2
1/13/05	2/10/05	28	31.36	31.30	31.64	31.43 ± 0.18	107 ± 0.6
1/13/05	3/10/05	56	28.77	28.76	28.65	28.73 ± 0.07	97.9 ± 0.2
1/13/05	4/7/05	84	29.22	29.67	29.47	29.45 ± 0.23	100 ± 0.8
1/24/05	1/24/05	0	30.02	29.88	29.93	29.95 ± 0.07	100 ± 0.2
1/24/05	7/11/05	168	29.64	29.72	29.95	29.77 ± 0.16	99.4 ± 0.5

For the formulation sample prepared on January 13, 2005, the pooled relative standard deviation of the analytical method was 0.5%. This means that there would have to be a difference of more than 1.2% from the Day 0 value for the difference to be statistically significant at a 95% confidence level.

For the formulation sample prepared on January 24, 2005, the pooled RSD of the analytical method was 0.6%. This means that there would have to be a difference of more than 1.3% from the Day 0 value for the difference to be statistically significant at a 95% confidence level.



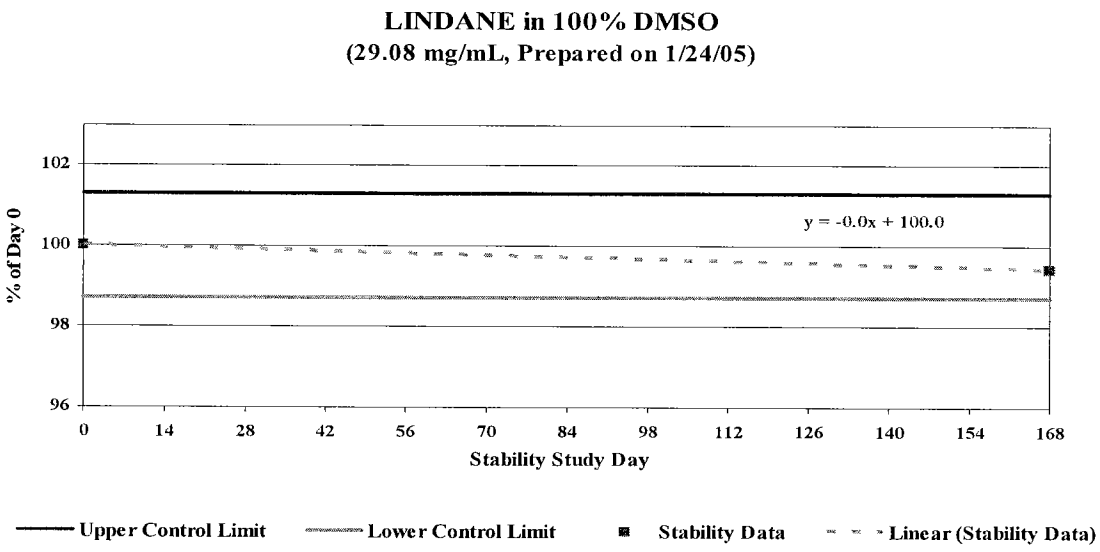


Figure 3 – Control Chart for the Storage Stability Study

5.5 Discussion and Conclusions

The Day 0 determined value for the formulation prepared on January 13, 2005 was approximately 1.0% above nominal (the calculated concentration based on the weight of the chemical). The concentrations of the samples stored at approximately 5°C protected from light in amber glass vials for Days 14 and 56 were below the lower significance level and for Day 28 it was above the upper significance level due to the tight precision of the assay. The average concentrations of the samples were within 2.6% (Day 14), 7.1% (Day 28), 2.1% (Day 56), and 0.4% (Day 84) of the Day 0 value and met acceptance criteria of ± 10%. These data indicate the formulation was stable at approximately 5°C for 84 days.

The formulation stability sample prepared on January 24, 2005 (Day 0) and analyzed on Day 0 and Day 168 (July 11, 2005) was approximately 3.0% above nominal for Day 0 (the calculated concentration based on the weight of the chemical) and for Day 168, 0.6% below the Day 0 value and met acceptance criteria of ± 10%. These data indicate the formulation was stable at approximately 5°C protected from light for 168 days.

6 FORMULATION PREPARATION AND ANALYSIS

Formulations were prepared and analyzed on January 24, 2005, March 21, 2005 and July 1, 2005, according to SOP COMSPEC.II-029, "Standard Operating Procedure (SOP) for the Formulation and Analysis of Lindane in 100% Dimethylsulfoxide (DMSO)." This section describes the method, results, and conclusions.

6.1 Preparation of Formulation

Lindane (1.45400 ± 0.058 g) was weighed into a 50-mL volumetric flask. DMSO was added until the flask was approximately 80% full. The contents were mixed until the lindane dissolved. The contents of the flask were diluted to volume with DMSO, sealed, and mixed well.

6.2 Preparation of Standards and Blanks

Standards and blanks were prepared as described for the validation (Section 4.3.1 of this report).

6.3 Preparation of Formulation Samples

One (1) mL of the formulation was pipetted into three individual 10-mL volumetric flasks, diluted to volume with methanol, sealed, and mixed well. One (1) mL of the diluted formulation and 1-mL of IS were pipetted into individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with methanol, sealed, and mixed well.

6.4 Analysis

Autosampler vials were filled with aliquots of each standard, blank and sample. A single injection was made from each vial using the GC conditions from the validation (Table 1). Representative overlaid chromatograms of the high and low vehicle/calibration standards, blank with IS, and a blank are shown in Figure 4.

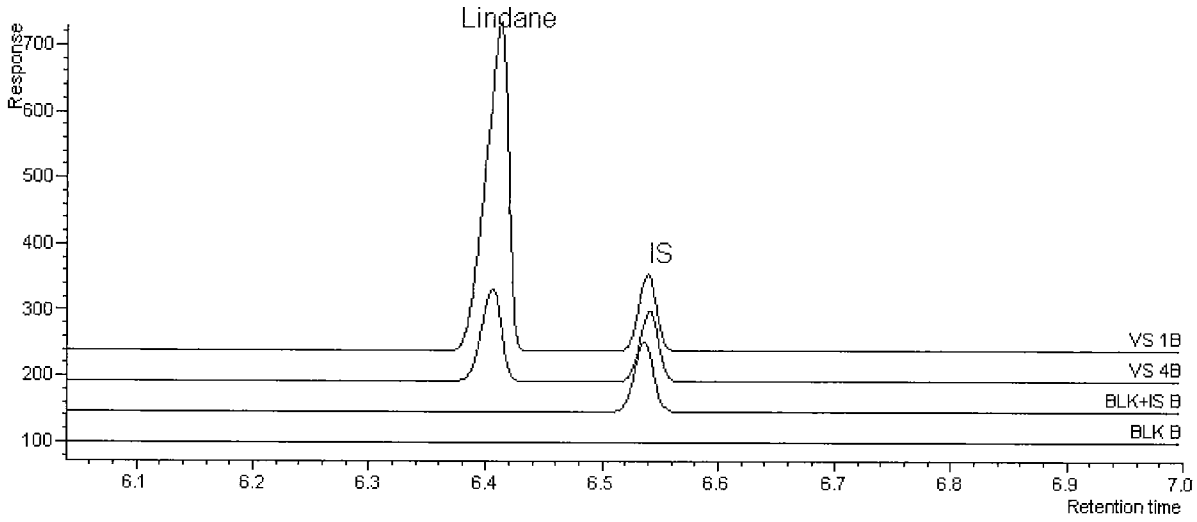


Figure 4 – Representative Overlaid Chromatograms of a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from a Formulation Analysis (Shown Top to Bottom)

6.5 Calculations

The peaks for lindane and the IS were integrated for each injection by the chromatography data system. Any peak with improper integration was manually reintegrated. A linear regression equation was calculated relating the response ratio (lindane/IS) to the concentration of the vehicle/calibration standards. This regression equation and the response ratios were used to calculate the concentration in each standard and formulation sample. The percent RE for each standard was calculated by subtracting the nominal value from the determined value, dividing by the nominal value, and then multiplying by 100. The percent RE for each formulation sample was calculated by subtracting the target value from the determined value, dividing by the target value, and then multiplying by 100. The average determined concentration, standard deviation, and percent RSD were calculated for the vehicle/calibration standards and formulation samples when applicable.

6.6 Results

The regression analysis results of the vehicle/calibration standard curves indicated linearity and are shown in Table 6.

Table 6 – Formulation Regression Analysis Results

Formulation Date	Slope	y-Intercept	Correlation Coefficient
1/24/05	6.8029	-0.0081	1.000
3/21/05	7.2898	-0.0197	1.000
7/1/05	6.8477	-0.1022	1.000

The results of the formulation analysis are shown in Table 7. Formulations met all acceptance criteria (RE within 10% of target and RSD of ≤ 10%).

Table 7 – Formulation Analysis Results

Formulation Date	Det'd Conc (mg/mL)			Avg Det'd Conc (mg/mL)	Avg %RE	%RSD
1/24/05	30.02	29.88	29.93	29.95	3.0	0.2
3/21/05	29.23	29.67	29.20	29.37	1.0	0.9
7/1/05	29.32	29.26	29.63	29.40	1.1	0.7

6.7 Conclusions

The average concentration of the formulations and its percent RSD were within acceptance criteria. Therefore the formulation was suitable for use.

7 ACKNOWLEDGMENTS

Analytical support for this work was provided by Barb Harritos, Darren Brown, John Kelly, Christina Zielinski, Jim Hoskinson, Melinda Pauff, Tudor Fernando, and Sandy Runyon. The report was written by Denise Contos. Review of the data and report for completeness and accuracy was performed by Maria Evascu.

ANALYTICAL CHEMISTRY ACTIVITIES REPORT

KETOCONAZOLE

CAS No.: 65277-42-1

Receipt Date: 10/26/04

Appearance: Solid

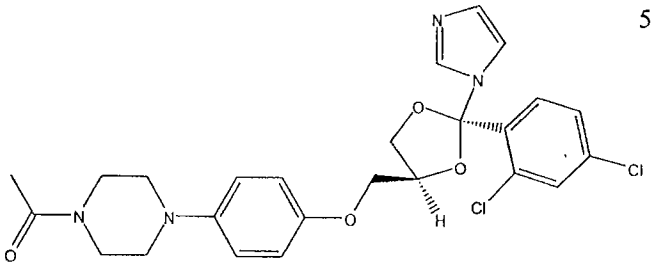
Storage Conditions (@ Battelle): Refrigerated (~5°C)

Lot No.: 121H0524 (Sigma Aldrich)

Amount Received: 2.7 g

Vendor Purity: > 99% by TLC

STRUCTURE:



Mol. Wt.: 531.43 g/mol

Mol. Formula: $C_{26}H_{28}Cl_2N_4O_4$

Prepared By:

Denise A. Contos, M.S.

Approved By:

Steven W. Graves, B.S.

Manager, Chemistry Technical Center

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit (QAU) and reports were submitted to the Study Director and Management as follows:

Phase Inspected	Inspection Date	Date Reported to Study Director/Management
Test substance receipt	10/26/2004	10/26/2004
Formulation preparation*	12/2/2004	12/2/2004
Dispensing*	12/2/2004	12/2/2004
Formulation analysis*	12/2/2004	12/2/2004
Audit study file	8/23/2005	8/23/2005
Audit analytical report	8/23/2005	8/23/2005
Audit study file	12/16/2005	12/16/2005
Audit analytical report	12/16/2005	12/16/2005

* These inspections are serving the purpose for all reference chemicals since QA was required to see only one phase inspection of a chemical.

Quality Assurance Unit — Date: 12-27-05

EXECUTIVE SUMMARY

The title compound, ketoconazole, was analyzed in support of the Environmental Protection Agency (EPA) Placental and Recombinant Aromatase Assay Prevalidation work, Work Assignment 4-16/17.

Solubility of ketoconazole was determined to be acceptable in dimethylsulfoxide (DMSO) at a concentration of 5.31 mg/mL (0.01 M).

A ketoconazole formulation analysis method was validated on the previous EPA WA 3-10 study. The method was modified by including an additional 1:10 dilution of the formulation which resulted in a 1:200 final dilution of the 5.31 mg/mL (0.01 M) formulation prior to analysis. This modified method was used to analyze both stability and formulation analysis samples.

Storage stability was previously determined (EPA WA 3-10 study, Analytical Chemistry Activities Report, Ketoconazole, 2004) as 28 days when stored at approximately 5°C and protected from light at a target formulation concentration of 0.532 mg/mL in DMSO. In the current study, a formulation sample with a target concentration of 5.31 mg/mL in DMSO was stable when stored refrigerated and protected from light for 60 days.

The stock formulations prepared for shipment to the testing laboratory were analyzed and met the established acceptance criteria.

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1 INTRODUCTION

The purpose of this work was to provide all necessary chemistry support activities for ketoconazole on the Environmental Protection Agency (EPA) Work Assignment 4-16/17, and consisted of:

- Determining solubility in dimethylsulfoxide (DMSO).
- Preparing and analyzing a stock formulation and a formulation stability sample.

This work was done at Battelle, 505 King Avenue, Columbus, OH 43201.

2 CHEMICAL RECEIPT AND STORAGE

One 15-mL amber glass bottle of ketoconazole, Lot No. 121H0524, was received from the repository at Battelle's Marine Sciences Laboratory in Sequim, WA on October 26, 2004. The label amount indicated 2.7 grams was sent. The chemical was received and subsequently stored refrigerated.

A copy of the manufacturer's Certificate of Analysis for this lot is shown in Figure 1. This states that purity was greater than 99% based on thin layer chromatography.

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Ketoconazole
Product Number	K1003
Product Brand	SIGMA
CAS Number	65277-42-1
Molecular Formula	$C_{26}H_{28}Cl_2N_4O_4$
Molecular Weight	531.43

TEST	SPECIFICATION	LOT 121H0524 RESULTS
APPEARANCE	WHITE TO YELLOW WITH A LIGHT TAN CAST POWDER	WHITE POWDER WITH A LIGHT YELLOW CAST
SOLUBILITY	CLEAR FAINT YELLOW TO YELLOW SOLUTION AT 50MG/ML IN METHANOL	CLEAR FAINT YELLOW SOLUTION AT 200 MG PLUS 4 ML OF METHANOL
ELEMENTAL ANALYSIS	57.6 TO 59.9% CARBON	58.6% CARBON 10.5% NITROGEN
SPECIFIC ROTATION	+1 TO -1 DEG (C=4 IN METHANOL AT 20DEGCENTIGRADE)	+0.08 DEG (C = 3.8 IN METHANOL AT 20 DEG CENTIGRADE)
PURITY BY THIN LAYER CHROMATOGRAPHY	NLT 98%	GREATER THAN 99%
SHELF LIFE	3 YEARS	MARCH 2005
QC ACCEPTANCE DATE		MARCH 2004

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

Figure 1 - Certificate of Analysis

3 SOLUBILITY STUDY

A solubility study was conducted to determine the solubility of ketoconazole in either 95% ethanol or 100% DMSO. Initially, both 95% ethanol and DMSO were used to prepare a 53.14 mg/mL (0.1 M) ketoconazole solution. Neither of the solvents dissolved the ketoconazole at the 0.1 M concentration. At the direction of the Task Leader, a 5.31 mg/mL ketoconazole solution (0.01 M) in 100% DMSO was prepared by weighing 0.05314 ± 0.00531 g into a

10-mL volumetric flask. DMSO was added until the flask was approximately 80% full. The flask was capped and contents were mixed. The content of the flask was diluted to volume with DMSO, sealed, mixed and sonicated. The ketoconazole went into solution with minimal shaking and sonication. This experiment showed that DMSO was an acceptable solvent for a 5.31 mg/mL (0.01 M) formulation.

4 FORMULATION PREPARATION AND ANALYSIS

Formulations were prepared and analyzed on February 4, 2005 and June 29, 2005 according to SOP COMSPEC.II-018-02, "Standard Operating Procedure (SOP) for the Formulation and Analysis of Ketoconazole in 100% DMSO." In addition, the February 4, 2005 formulation was re-analyzed to determine stability on April 5, 2005, 60 days after storage at approximately 5°C and protected from light. This section describes the method, results, and conclusions.

4.1 Preparation of Formulation

A ketoconazole formulation with a target concentration of 5.31 mg/mL (0.01 M) in DMSO was prepared on February 4, 2005 by accurately weighing 265.5 ± 5 mg of ketoconazole into a tarred 50-mL volumetric flask. DMSO was added until the flask was approximately 80% full. The flask was sealed and sonicated for approximately 10 minutes then inverted ten times. The content of the flask was diluted to volume with DMSO, sealed, and mixed well by inverting at least ten times.

4.2 Preparation of High Performance Liquid Chromatography (HPLC) Mobile Phase

An accurate amount of ammonium acetate (3.0 g) was weighed into a 2000-mL HPLC mobile phase bottle. A 600 mL volume of Milli-Q water was added to the bottle and the contents were mixed well. A final volume of 1400 mL of methanol and 2.8 mL of diethanolamine were added to the bottle and the contents were mixed well. This produced a HPLC mobile phase containing 70:30:0.14 (v:v:v) of methanol:0.5% ammonium acetate: diethanolamine.

4.3 Preparation of Standards and Blanks

4.3.1 Internal Standard (IS)

Fifteen (15) ± 2 mg terconazole was added to a 50-mL volumetric flask. The content of the flask was diluted to volume with HPLC mobile phase, sealed, and mixed well. This produced a solution with a target concentration of 300 µg/mL.

4.3.2 Stock Standards

Two stock standards were prepared by accurately weighing 30 ± 3.0 mg of ketoconazole into two individual 200-mL volumetric flasks and dissolving in and diluting to volume with HPLC mobile phase. This produced stocks A and B with target concentrations of 150 µg/mL each.

4.3.3 Vehicle/Calibration Standards

Vehicle/calibration standards were prepared as shown in Table 1. The contents of the flasks were diluted to volume with HPLC mobile phase, sealed, and mixed well. Triplicate vehicle/calibration standards were prepared at the low and high concentrations with single vehicle/calibration standards prepared at the two intermediate concentrations.

Table 1 – Preparation of Vehicle/Calibration Standards

Vehicle Std	Target Final Conc (µg/mL)	Source	Source Volume (mL)	Internal Std (mL)	DMSO (mL)	Final Volume (mL)
VS1	60	A	4	1	0.05	10
VS2	45	B	3	1	0.05	10
VS3	30	A	2	1	0.05	10
VS4	15	B	1	1	0.05	10

4.3.4 Blanks

Triplicate blanks without IS were prepared by pipetting 0.05 mL of DMSO into three individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with HPLC mobile phase, sealed, and mixed well.

Triplicate blanks with IS were prepared by pipetting 1 mL IS and 0.05 mL of DMSO into three individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with HPLC mobile phase, sealed, and mixed well.

4.4 Preparation of Formulation and Formulation Stability Samples

Triplicate 1 mL aliquots of the formulation were pipetted into three individual 10-mL volumetric flasks, diluted to volume with HPLC mobile phase, sealed, and mixed well. A 0.5 mL aliquot of the diluted formulation and 1 mL of the IS were pipetted into individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with HPLC mobile phase, sealed, and mixed well.

4.5 Analysis

A portion of each vehicle/calibration standard, blank and sample were transferred to individual autoinjector vials and the vials were sealed. Single injections were made from each vial using the HPLC parameters for ketoconazole which are presented in Table 2.

Table 2 – HPLC System

Instrument System	Agilent (Palo Alto, CA); Waters (Milford, MA)
Column	Supelcosil LC-ABZ, 5 µm particle size, 250 mm × 4.6 mm (ID) (Supelco, St. Louis, MO)
Guard	C_{18} guard cartridge
Mobile Phase	70:30:0.14 (v/v/v) Methanol:0.5% Ammonium Acetate:Diethanolamine, Isocratic
Flow Rate	1.5 mL/minute
Injection Volume	20 µL
Detector Type	Ultraviolet (UV)
Detector Wavelength	245 nm

4.6 Calculations

The integration of the ketoconazole and the IS peaks by the chromatography data system were evaluated to assure it was consistent in all chromatograms and manually reintegrated, if necessary. A linear regression equation weighted 1/x was calculated relating the response ratio of ketoconazole/IS (y) to the concentration of the concentration of the vehicle/calibration standards (x). This regression equation and the response ratios were used to calculate the concentration in each vehicle/calibration standard and formulation sample. These values were used to calculate the individual and average concentrations, percent relative errors (RE), standard deviation (s), and percent relative standard deviation (RSD) as appropriate for the vehicle/calibration standards at each concentration.

4.7 Results

Specificity is shown by the representative overlaid chromatograms from a high and low vehicle/ calibration standard, a blank with IS, and a blank as presented in Figure 2. The blank and blank with IS exhibited no peaks that would significantly interfere with the ketoconazole or IS peaks.

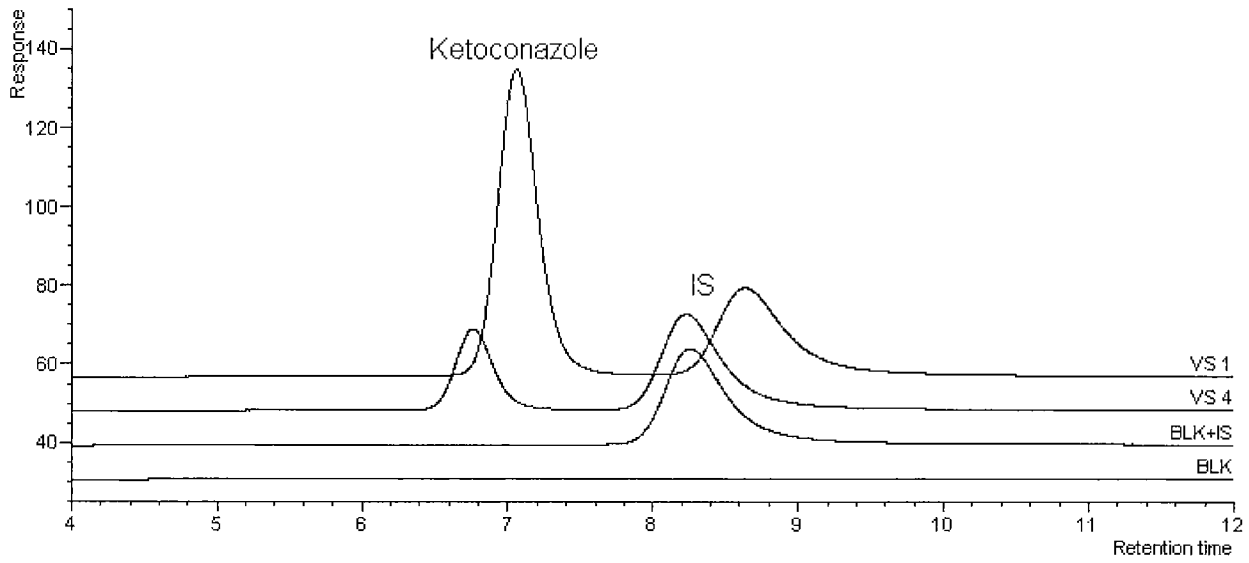

Figure 2 – Representative Overlaid Chromatograms of a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from 2/4/2005 Analysis (Shown Top to Bottom)

The regression analysis results from the standard curve for February 4, 2005 analysis indicate linearity and are shown in Figure 3.

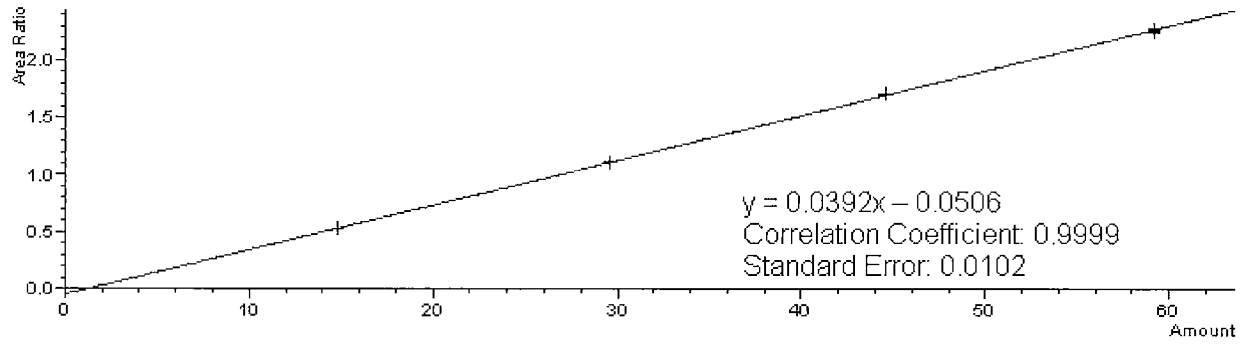

Figure 3 – Vehicle/Calibration Standard Curve for 2/4/2005 Analysis

The precision and accuracy of the vehicle/calibration standard results from February 4, 2005 analysis are shown in Table 3.

Table 3 –Vehicle/Calibration Standard Results for 2/4/2005 Analysis

Nominal Std Conc (µg/mL)	Det'd Conc (µg/mL)	Avg Det'd Conc (µg/mL)	s (µg/mL)	% RSD	% RE	Avg % RE
59.20	59.15	59.08	0.30	0.5	-0.1	-0.2
	59.33				0.2	
	58.75				-0.8	
44.60	44.98	NA	NA	NA	0.9	NA
29.60	29.64	NA	NA	NA	0.1	NA
14.87	14.69	14.85	0.14	0.9	-1.2	-0.1
	14.92				0.4	
	14.94				0.5	

The results of the formulation and formulation stability sample analysis are shown in Table 4 and 5. The formulation stability sample was the same formulation sample prepared and analyzed on February 4, 2005 that had been stored refrigerated for 60 days, protected from light in an amber glass bottle.

Table 4 – Formulation Analysis Results

Batch No.	Analysis Date	Det'd Conc (mg/mL)			Avg Det'd Conc (mg/mL)	Avg % RE	RSD (%)
1-KET-1	2/4/2005	5.136	5.122	5.134	5.131	-3.4	0.1
2-KET-1	6/29/2005	5.458	5.487	5.464	5.470	3.0	0.3

The formulations met all acceptance criteria (RE within 10% of target and RSD of ≤ 10%).

Table 5 –Formulation Stability Analysis Results

Analysis Date	Det'd Conc (mg/mL)			Avg Det'd Conc (mg/mL)	Avg % of Day 0 Conc ± s
4/5/2005	5.316	5.359	5.369	5.348	104.2 ± 0.5

The formulation stability sample analyzed on April 5, 2005 was within 4.2% of the Day 0 value (February 4, 2005 analysis value) and met acceptance criteria + 10 %.

4.8 Conclusions

The average concentration of the stock formulation and its percent relative standard deviation were within acceptance criteria. Therefore the formulations were suitable for use.

The ketoconazole formulation at a target concentration of 5.31 mg/mL in DMSO was stable for 60 days when stored refrigerated and protected from light.

5 ACKNOWLEDGMENTS

Analytical support for this work was provided by Jim Hoskinson and Tudor Fernando. The report was written by Denise Contos. Review of the data and report for completeness and accuracy was performed by Maria Evascu.

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The Business of Innovation

ANALYTICAL CHEMISTRY ACTIVITIES REPORT

4-HYDROXYANDROSTENEDIONE (4-OH ASDN)

CAS No.: 566-48-3

Lot No.: 063K4069 (Sigma Aldrich)

Receipt Date: 10/22/04

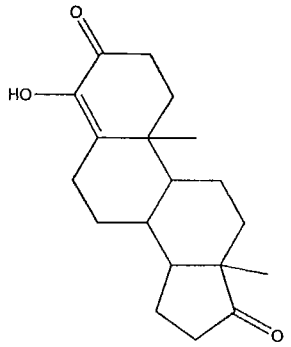
Amount Received: 3.1 g

Appearance: Solid

Vendor Purity: 99% by TLC

Storage Conditions (@ Battelle): Refrigerated (~5°C)

STRUCTURE:



Mol. Wt.: 302.41 g/mol

Mol. Formula: $C_{19}H_{26}O_3$

Prepared By:

Denise A. Contos 12/19/05

Denise A. Contos, M.S.

Approved By:

Steven W. Graves

Steven W. Graves, B.S.

Manager, Chemistry Technical Center

Battelle Study No. WA 4-16/17

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit (QAU) and reports were submitted to the Study Director and Management as follows:

Phase Inspected	Inspection Date	Date Reported to Study Director/Management
Test substance receipt*	10/26/04	10/26/04
Formulation preparation	12/2/04	12/2/04
Dispensing	12/2/04	12/2/04
Formulation analysis	12/2/04	12/2/04
Audit analytical report	10/20/05	10/20/05
Audit study file	10/20/05	10/20/05

* These inspections are serving the purpose for all reference chemicals since QA was required to see only one phase inspection of a chemical.

Quality Assurance Unit — 12-14-05 Date

EXECUTIVE SUMMARY

The title compound, 4-hydroxyandrostenedione (4-OH ASDN), was analyzed in support of the Environmental Protection Agency (EPA) Placental and Recombinant Aromatase Assay Prevalidation Work, Work Assignment 4-16/17.

The solubility of 4-hydroxyandrostenedione was determined to be acceptable in 95% ethanol for preparing formulations.

A formulation analysis method was developed and validated to analyze 4-hydroxyandrostenedione in 95% ethanol at a concentration of 3.02 mg/mL (0.01M). This method was used to analyze samples from both formulation and formulation storage stability studies at 3.02 mg/mL.

The storage stability study indicated that a 3.02 mg/mL formulation, stored in sealed amber glass bottles and protected from light, was stable for 173 days at approximately 5°C.

The stock formulation prepared for shipment to the testing laboratory was analyzed and met the established acceptance criteria.

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1 INTRODUCTION

The purpose of this work was to provide all necessary chemistry support activities for 4-hydroxyandrostenedione on Environmental Protection Agency (EPA) Work Assignment 4-16/17, and consisted of:

- Determining solubility in 95% ethanol.
- Developing and validating a formulation analysis method.
- Conducting a storage stability study.
- Preparing and analyzing a stock formulation.

This work was done at Battelle, 505 King Avenue, Columbus, OH 43201.

2 CHEMICAL RECEIPT AND STORAGE

One 15-mL amber glass bottle of 4-hydroxyandrostenedione, 063K4069, was received from the repository at Battelle's Marine Sciences Laboratory in Sequim, WA on October 22, 2004. The label amount indicated 3.1 grams was sent. The chemical was received and subsequently stored at approximately 5°C.

A copy of the manufacturer's Certificate of Analysis for this lot is shown in Figure 1. This states that purity was 99% based on thin layer chromatography (TLC).

3 SOLUBILITY STUDIES

A solubility study was conducted to determine the solubility of 4-hydroxyandrostenedione (4-OH ASDN) in 95% ethanol, at a concentration of at least 30.2 mg/mL. The 4-OH ASDN (0.30200 ± 0.03020 g) was weighed into a 10-mL volumetric flask, diluted to approximately 80% volume with 95% ethanol, sealed and shaken to mix. The flask was diluted to volume with 95% ethanol, sealed, shaken, sonicated for approximately 50 minutes and stirred. The 4-OH ASDN did not go into solution.

A second solubility study was conducted to determine the solubility of 4-OH ASDN in 95% ethanol, with a solubility of at least 3.02 mg/mL being required for acceptability. The 4-OH ASDN (0.03020 ± 0.00302 g) was weighed into a 10-mL volumetric flask, diluted to approximately 80% volume with 95% ethanol, sealed and shaken to mix. The flask was diluted to volume with 95% ethanol, sealed, shaken and sonicated for approximately 2 minutes. The 4-OH ASDN went into solution. This experiment showed that 95% ethanol was an acceptable solvent for the 3.02 mg/mL formulation (0.01M).

SIGMA-ALDRICH

Certificate of Analysis

Product Name	4-Androsten-4-ol-3,17-dione
Product Number	A5791
Product Brand	SIGMA
CAS Number	566-48-3
Molecular Formula	$C_{19}H_{26}O_3$
Molecular Weight	302.41

TEST	SPECIFICATION	LOT 063K4069 RESULTS
APPEARANCE		WHITE POWDER
SOLUBILITY		CLEAR COLORLESS SOLUTION AT 10 MG/ML OF METHANOL
ELEMENTAL ANALYSIS		75.45% CARBON
PROTON NMR SPECTRUM		CONSISTENT WITH STRUCTURE
PURITY BY THIN LAYER CHROMATOGRAPHY		99%
QC ACCEPTANCE DATE		JUNE 2003

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

Figure 1 – Certificate of Analysis

4 FORMULATION ANALYSIS METHOD PERFORMANCE EVALUATION (MPE)

This section describes the evaluation of a method developed to analyze formulations of 4-hydroxyandrostenedione in 95% ethanol at a target concentration of 3.02 mg/mL (0.01 M) for the stability study and the results and conclusions from this evaluation.

4.1 Method Development

Method development for this chemical involved the evaluation of various chromatographic columns and conditions. The selected method was one which produced acceptable retention time for the major peak, apparent resolution of significant impurities and acceptable peak shape. The detection method chosen was gas chromatography with flame ionization detection (GC/FID).

4.2 Method

The GC parameters for 4-hydroxyandrostenedione are presented in Table 1.

Table 1 – GC System

GC	Agilent 6890 (Palo Alto, CA)
Column	RTX-5 MS, 15 m × 0.25 mm (ID), 0.25 µm film thickness (Restek, Bellefonte, PA)
Carrier Gas and Flow Rate	Helium at 2 mL/minute
Oven Temperature	150°C, hold for 1 minute, increase at 15°C/minute to 320°C
Detector Type	Flame Ionization (FID)
Detector Flow Rates	Hydrogen at 30 mL/minute; Air at 380 mL/minute
Detector Temperature	320°C
Injector Temperature	250°C
Injection Volume	1 µL
Injection Mode	Split 1:10
Run Time	~12 minutes

4.3 Method Validation

Validation was accomplished using a single experiment.

Triplicate vehicle/calibration standards at the highest and lowest of four concentrations were prepared. A single standard was prepared at each intermediate concentration. The high and low concentrations were used to assess the precision of the method. The precision of the low concentration was used to calculate limits of detection (LOD) and limits of quantitation (LOQ). Triplicate vehicle blanks with and without internal standard (IS) were used to assess the specificity of the method.

4.3.1 Preparation of Standards and Blanks

4.3.1.1 Internal Standard (IS)

Fifty (50) milligrams ± 4 mg of benzophenone was added to a 25-mL volumetric flask. The content of the flask was diluted to volume with methanol, sealed, and mixed well.

4.3.1.2 Stock Standards

Two stock standards (A, B) were prepared by accurately weighing 50 ± 1 mg of 4-OH ASDN each into individual 50-mL volumetric flasks and dissolving in and diluting to volume with methanol. This produced stocks A and B with target concentrations of 1000 µg/mL each.

4.3.1.3 Vehicle/Calibration Standards

Vehicle/calibration standards were prepared as shown in Table 2. The contents of the flasks were diluted to volume with methanol, and mixed well. Triplicate vehicle/calibration standards were prepared at the low and high concentrations with single vehicle/calibration standards prepared at the two intermediate concentrations.

Table 2 – Preparation of Vehicle/Calibration Standards

Vehicle/Calibration Std	Target Final Conc (µg/mL)	Source	Source Volume (mL)	IS (mL)	95% Ethanol (mL)	Final Volume (mL)
VS1	500	A	5	1	1	10
VS2	300	B	3	1	1	10
VS3	200	A	2	1	1	10
VS4	100	B	1	1	1	10

4.3.1.4 Blanks

Triplicate blanks without IS were prepared by pipetting 1 mL of 95% ethanol into three individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with methanol, sealed, and mixed well.

Triplicate blanks with IS were prepared by pipetting 1 mL IS and 1 mL of 95% ethanol into three individual 10-mL volumetric flasks. The contents of the flasks were diluted to volume with methanol, sealed, and mixed well.

4.3.2 Analysis

A portion of each vehicle/calibration standard and blank was transferred to individual autoinjector vials and the vials were sealed. Single injections were made from each vial using the same chromatographic system and parameters determined during method development (Table 1).

4.3.3 Calculations

The integration of the 4-OH ASDN and IS peaks by the chromatography data system was evaluated to assure it was consistent in all chromatograms and manually reintegrated, if necessary. A linear regression equation weighted 1/x was calculated relating the response ratio of 4-OH ASDN divided by the IS (y) to the concentration of the vehicle/calibration standards (x). The concentration of each vehicle/calibration standard was calculated using its individual response ratio and the regression equation. These values were used to calculate the individual and average concentrations, percent relative errors (RE), standard deviation (s), and percent relative standard deviation (RSD) as appropriate for the vehicle/calibration standard at each concentration.

4.3.4 Results

Specificity is shown by representative overlaid chromatograms from low and high vehicle/calibration standards, blank with IS, and a blank from the validation data as presented in Figure 2.

The blank and blank with IS exhibited no peaks that would significantly interfere with the 4-OH ASDN or IS peaks.

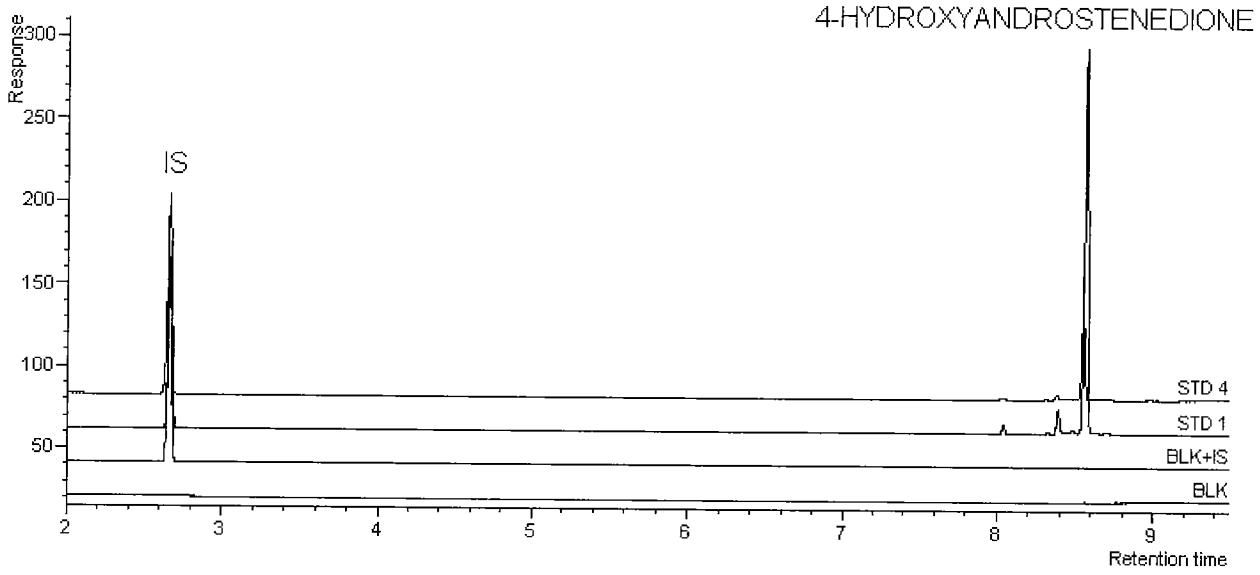

Figure 2 – Representative Overlaid Chromatograms from a Low and High Vehicle/Calibration Standard, Blank with IS, and Blank from the Validation (Shown Top to Bottom)

The regression analysis results from the validation standard curve indicate linearity and are shown in Table 3.

Table 3 – Regression Analysis Validation Results

Slope	y-Intercept	Correlation Coefficient	Standard Error
0.0038	-0.0272	0.9975	0.0565

The vehicle/calibration standard validation results are shown in Table 4.

Table 4 –Vehicle/Calibration Standard Validation Results

Nominal Std Conc (µg/mL)	Det'd Std Conc (µg/mL)	Avg Det'd Std Conc (µg/mL)	s (µg/mL)	% RSD	%RE	Avg %RE
506.4	496.8	509.6	24.2	4.7	-1.9	0.6
	494.5				-2.3	
	537.5				6.1	
298.1	289.4	NA	NA	NA	-2.9	NA
202.6	198.8	NA	NA	NA	-1.9	NA
99.38	100.7	100.4	0.4	0.4	1.3	1.0
	99.89				0.5	
	100.5				1.1	

The method validation sensitivity was 1.266 µg/mL, the LOD, which is defined as three times the standard deviation of the low vehicle/calibration standard. This is equivalent to a formulation concentration of 13 µg/mL when a formulation is diluted 1 to 10 for analysis. The LOQ was 4.219 µg/mL, defined as ten times the standard deviation of the lowest standard because there was no blank response. This is equivalent to a formulation concentration of 42 µg/mL when a formulation is diluted 1 to 10 for analysis. The estimated limit of quantitation (ELOQ), defined as the lowest standard with acceptable accuracy and precision, was 99.38 µg/mL.

4.3.5 Conclusions

The method met all acceptance criteria for precision, accuracy, linearity, sensitivity and specificity. The method was suitable for the stability study and subsequent formulation analyses for which it was used.

5 FORMULATION STABILITY STUDIES

A formulation stability study was conducted at a concentration of 3.02 mg/mL (0.01 M) in 95% ethanol for 173 days in sealed, amber glass bottles stored at approximately 5°C.

5.1 Study Design

A sample was analyzed on the day of preparation (Day 0) and Day 14. A second sample was analyzed on the day of preparation Day 0, Days 27, 54, 83 and 173. Three aliquots were analyzed from each sample at each storage time.

5.2 Formulation Method

A formulation was prepared on November 10, 2004 (Day 0) for the storage stability study at a target concentration of 3.02 mg/mL (0.01 M) in 95% ethanol by accurately weighing 75.50 ± 0.75 mg of 4-OH ASDN into a 25-mL volumetric flask. The chemical was dissolved in and diluted to approximately three quarters of the total volume with 95% ethanol. The flask was sealed, sonicated for 10 minutes and allowed to cool to room temperature. The contents of the flask was diluted to volume with 95% ethanol, sealed, and mixed well.

Approximately 6 mL of formulation was transferred into each of four, 8-mL amber glass vials which were then sealed. One vial was used for the Day 0 analysis and the other three were stored at approximately 5°C until use. After 14 days of storage, a vial was removed from the refrigerator, allowed to warm to room temperature, and triplicate aliquots were prepared and analyzed.

A second formulation was prepared on December 2, 2004 (Day 0) at a target concentration of 3.02 mg/mL (0.01 M) in 95% ethanol by accurately weighing 151.00 ± 0.50 mg into a 50-mL volumetric flask. The content of the flask was diluted to approximately 80% volume with 95% ethanol, sealed and mixed well. The contents of the flask were diluted to volume with 95% ethanol and mixed well. Approximately 18 mL were dispensed into an amber glass bottle, sealed and stored refrigerated. A formulation sample aliquot was prepared for analysis on Days 0, 27, 54, 83 and 173 for storage stability determination.

5.3 Analysis Method

Vehicle/calibration standards, blanks with and without IS were prepared as described in the validation experiment (Section 4.3.1) of this report with the exception that the standard stocks were prepared by accurately weighing 25 ± 1 mg of 4-OH ASDN into 25-mL volumetric flasks.

In triplicate, 1 mL of the formulation and 1 mL of IS were pipetted into three individual 10-mL volumetric flasks, diluted to volume with methanol, sealed and mixed well. An appropriate volume of each was transferred to an autoinjector vial and the vials were sealed and analyzed using the chromatographic system in Table 1.

5.4 Results

The results from the storage stability study are shown in Table 5 and presented in control chart format in Figure 3.

Table 5 – Formulation Storage Stability Results (3.02 mg/mL)

Preparation Date	Analysis Date	Day	Det'd Conc (mg/mL)			Avg Det'd Conc (mg/mL) ± s	% of Day 0 Conc ± s
11/10/04	11/10/04	0	2.871	2.873	2.928	2.891 ± 0.032	100.0 ± 1.1
11/10/04	11/24/04	14	3.006	3.085	3.149	3.080 ± 0.072	106.5 ± 2.5
12/2/04	12/2/04	0	3.005	3.022	3.005	3.011 ± 0.010	100.0 ± 0.3
12/2/04	12/29/04	27	3.168	3.123	3.117	3.136 ± 0.028	104.2 ± 0.9
12/2/04	1/25/05	54	3.008	3.126	3.110	3.081 ± 0.064	102.3 ± 2.1
12/2/04	2/23/05	83	3.027	3.131	3.217	3.125 ± 0.095	103.8 ± 3.2
12/2/04	5/24/05	173	3.126	3.142	3.129	3.133 ± 0.008	104.1 ± 0.3

For the sample prepared November 10, 2004, the pooled RSD of the analytical method was 1.9%. This means that there would have to be a difference of more than 4.4% from the Day 0 value for the difference to be statistically significant at a 95% confidence level.

For the sample prepared December 2, 2004, the pooled RSD of the analytical method was 1.8%. This means that there would have to be a difference of more than 4.0% from the Day 0 value for the difference to be statistically significant at a 95% confidence level.

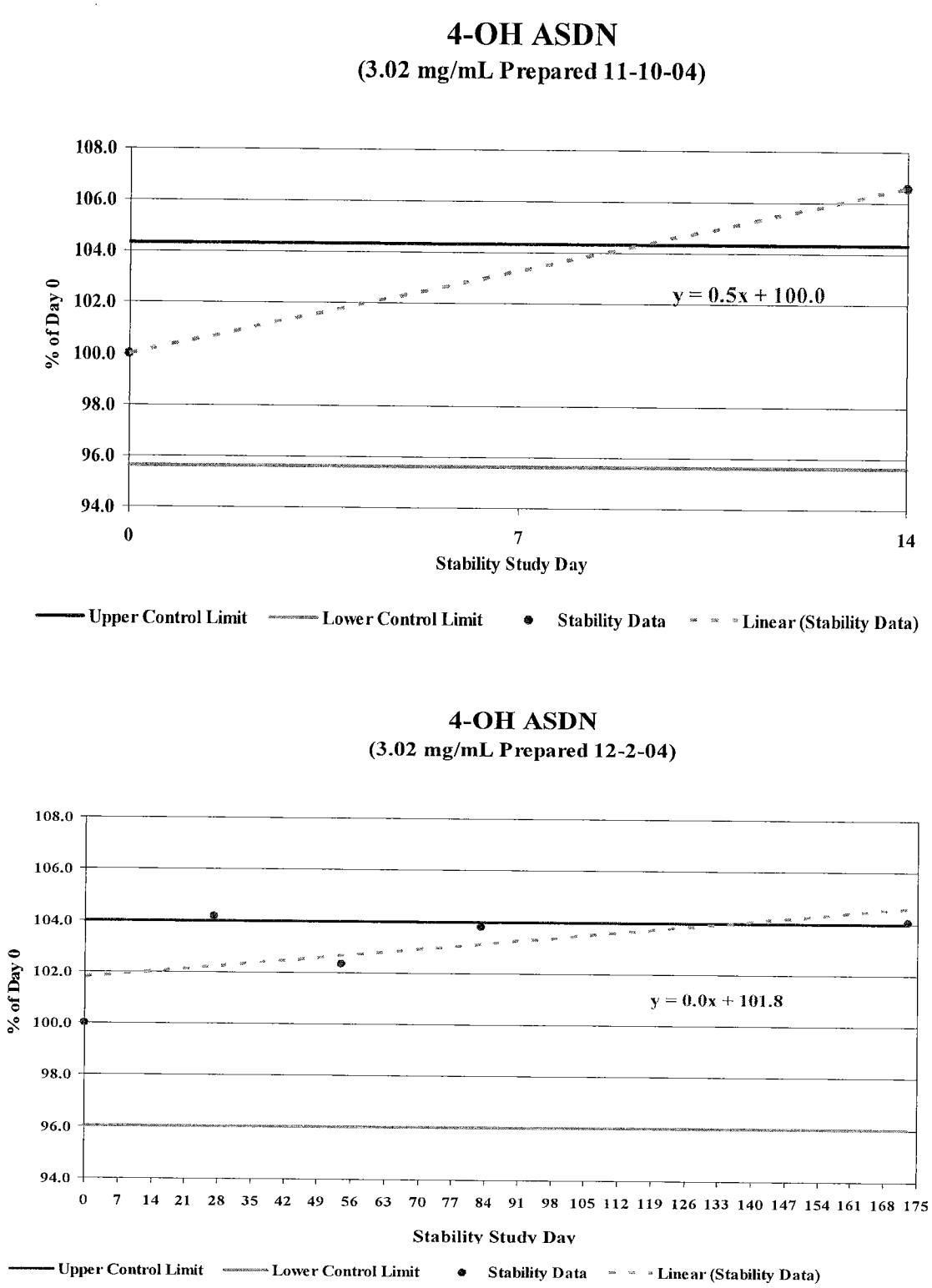

Figure 3 – Control Charts for the Storage Stability Studies

5.5 Discussion and Conclusions

The concentration of the samples stored at approximately 5°C protected from light in amber glass vials for Day 14 was above the upper significance level, but was within 6.5% of the Day 0 value (prepared November 10, 2004). Concentrations for Days 54 and 83 samples were within the upper and lower significance levels and Days 27 and 173 were just above the upper significant level. A linear trend analysis indicated there was no significant trend to changing concentration over time for the samples. These data indicate the formulation was stable when stored protected from light at approximately 5°C for 173 days.

6 FORMULATION PREPARATIONS AND ANALYSES

Formulations were prepared and analyzed on December 2, 2004, January 25, 2005, March 21, 2005, and June 27, 2005, according to SOP No. COMSPEC.II-027, "Standard Operating Procedure (SOP) for the Formulation and Analysis of 4-Hydroxyandrostenedione (4-OH ASDN) in 95% Ethanol." This section describes the method, results, and conclusions.

6.1 Preparation of Formulations

An accurate weight of 151.00 ± 0.50 mg of 4-OH ASDN was added to a 50-mL volumetric flask. The content of the flask was diluted to approximately 80% volume with 95% ethanol, sealed and mixed well. The contents of the flask were diluted to volume with 95% ethanol and mixed well. This produced a target concentration of 3.02 mg/mL (0.01 M) 4-OH ASDN in 95% ethanol.

6.2 Preparation of Standards and Blanks

Standards and blanks were prepared as described for the method validation, Section 4.3.1 of this report.

6.3 Preparation of Formulation Samples

One (1) mL of the formulation and 1-mL of IS were pipetted into three individual 10-mL volumetric flasks, diluted to volume with methanol, sealed, and mixed well.

6.4 Analysis

Autoinjector vials were filled with aliquots of each standard, blank and sample. A single injection was made from each vial using the conditions from the method validation (Table 1).

6.5 Calculations

The peaks for 4-hydroxyandrostenedione and the IS were integrated for each injection by the chromatography data system. Any peak with improper integration was manually reintegrated. A linear regression equation weighted 1/x was calculated relating the response ratio (4-hydroxyandrostenedione/IS) (y) to the concentration of the vehicle/calibration standards (x). This regression equation and the response ratios

were used to calculate the concentration in each standard and formulation sample. The percent RE for each standard was calculated by subtracting the nominal value from the determined value, dividing by the nominal value, and then multiplying by 100. The percent RE for each formulation sample was calculated by subtracting the target value from the determined value, dividing by the target value, and then multiplying by 100. The average determined concentration, standard deviation, and percent RSD were calculated for the vehicle/ calibration standards and formulation samples when applicable.

6.6 Results

Specificity is shown by the representative overlaid chromatograms of the high and low standards, blank with IS and a blank presented in Figure 4.

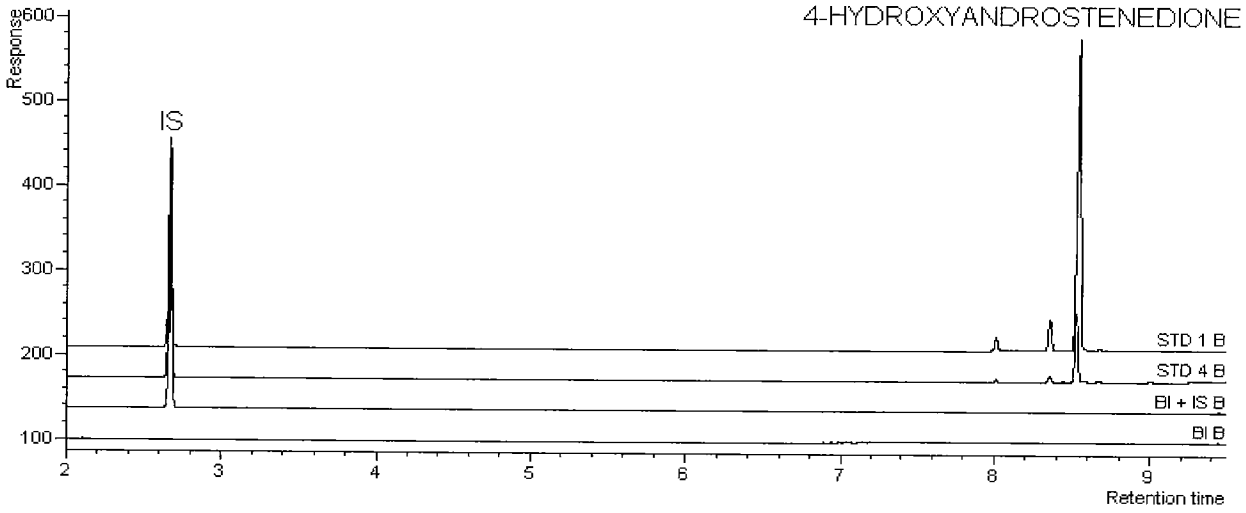

Figure 4 – Representative Overlaid Chromatograms of a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from Formulation Analysis

The regression analysis results of the vehicle/calibration standard curves indicated linearity and are shown in Table 6.

Table 6 – Regression Analysis Results

Slope	y-Intercept	Correlation Coefficient	Standard Error
0.0038	-0.0140	0.9999	0.0117
0.0035	-0.0037	1.000	0.0061
0.0036	-0.0251	0.9999	0.0100
0.0038	-0.0218	0.9999	0.0104

The results of the formulation analyses are shown in Table 7.

Table 7 – Formulation Analysis Results

Batch	Det'd Conc (mg/mL)			Avg Det'd Conc (mg/mL)	Avg % RE	% RSD
1-ASDN	3.005	3.022	3.005	3.011	-0.3	0.3
2-ASDN	3.056	3.089	3.049	3.065	1.4	0.7
3-ASDN	3.112	3.053	3.063	3.076	1.9	1.0
4-ASDN	2.943	2.945	2.950	2.946	-2.5	0.1

The formulations met acceptance criteria (RE within 10% of target and RSD of $\leq$ 10%).

6.7 Conclusions

The average concentration of the stock formulations and their percent RSD were within acceptance criteria. Therefore, the formulations were suitable for use.

7 ACKNOWLEDGMENTS

Analytical support for this work was provided by Sandy Runyon, Christina Zielinski, Tudor Fernando, Kevin Carrico, and Darren Brown. The report was written by Denise Contos. Review of the data and report for completeness and accuracy was performed by Maria Evascu.

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Chemical Repository Services for the EDSP
EPA Contract No. 68-W-01-023

1.0 TITLE PAGE

STUDY TITLE: ANALYSIS OF TEST SUBSTANCES FOR WORK ASSIGNMENTS 4-16 AND 4-17

Authors: Tim Fortman, Michael Cobb

Study Initiation Date: January 10, 2005

Study Completion Date: December 15, 2005

Performing Lab: EDSP Chemical Repository
Battelle Marine Research Operations
1529 West Sequim Bay Road
Sequim, WA 98382

Study Number: EDSP.416-01

Data Requirement: 40 CFR Part 160.105, 160.113

Submitted To: Dr. David P. Houchens
EDSP Program Manager
Battelle Columbus Operations
505 King Avenue
Columbus, OH, 43201-2693

Total Number of Pages: 111

2.0 STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentially is made for any information contained in this study on the basis of its falling within the scope of the United States Environmental Protection Agency Federal Insecticide, Fungicide, and Rodenticide Act Section 10(d) (1)(A), (B), or (C).

Company: Battelle

Company Agent: David P. Houchens, Ph.D.

Title: EDSP Program Manager

Signature: David P. Houchens Date: 12/16/05

3.0 STATEMENT OF COMPLIANCE

This study meets the requirements for 40 CFR Part 160, EPA FIFRA Good Laboratory Practices with the following exception:

The supplier purity claims for the test substances were verified by the Chemical Repository, but the purity claims of the reference substances (4-nonylphenol and 5-α-androstane) as characterized by the supplier were accepted and not verified. Characterization of both test and reference substances is a GLP requirement. This aspect of the study was not in compliance with GLPs.

Note: Protocol and method deviations occurred and are noted in Appendices B and D of this report.

Study Director: _____ 12-15-05
Michael Cobb — Date
Battelle – Marine Research Operations

Sponsor's Representative _____ 12/16/05
David Houchens, Ph.D. — Date
Battelle Columbus Operations

Submitter: _____ 12/16/05
Date

4.0 QUALITY ASSURANCE

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989. The dates of all audits and inspections and the dates of any findings were reported to the Study Director and Test Facility Management as follows:

ACTIVITY	DATE CONDUCTED	DATE REPORTED TO:	
		STUDY DIRECTOR	MANAGEMENT
In Process Inspection	Feb 8, 2005	Feb 8, 2005	Feb 8, 2005
Data	Mar 31, Apr 4,7, 2005	Apr 7, 2005	Apr 7, 2005
Draft Report	Aug 2 & 3, 2005	Aug 3, 2005	Aug 3, 2005
Final Report	Dec 15, 2005	Dec 15, 2005	Dec 15, 2005

Mary Ellen Lynn
Mary E. Lynn
Quality Assurance

12/15/05
Date

5.0 APPROVALS PAGE

Study Title: Analysis of Test Substances for Work Assignments 4-16 and 4-17

Submitted by: Battelle Marine Research Operations
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Approved by:

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EDSP Chemical Repository Study Director
Battelle – Marine Research Operations

Approved by:

Eric Crecelius — Date 12/15/05
Manager, EDSP Chemical Repository
Battelle – Marine Research Operations

Personnel participating in this study:

Analyst: Tim Fortman, Rebecca Wood

Chemical Repository Study Director: Michael Cobb

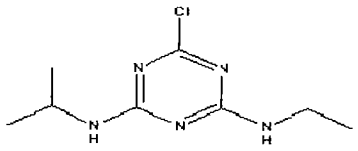
Experimental Dates:
Experimental Start 02/03/05
Experimental Termination 12/03/05

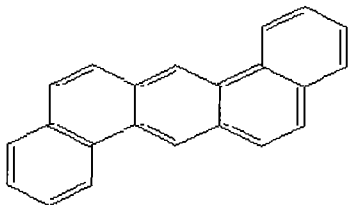
6.0 EXECUTIVE SUMMARY

Analysis Of Test Substances for Work Assignments 4-16 and 4-17

Atrazine, Dibenz(a,h)Anthracene, Dicofol, Fenarimol, 4-Nonylphenol and Prochloraz

Table 1. Study Test Substances

Parameter	Test Substance	Atrazine
Compound Name	Atrazine	
CAS #	1912-24-9	
Central File No.	2332-1	
Molecular Weight	215.72 g/mole	
Initial Receipt Date	12/14/04	
Expiration Date	10/07	
Supplier	Supelco/Chem Service	
Lot Number	328-137A	
Vendor Purity	98%	
Method	EDSP.G2-022	

Parameter	Test Substance	Dibenz(A,H)Anthracene
Compound Name	Dibenz(a,h)anthracene	
CAS #	53-70-3	
Central File No.	2319-1	
Molecular Weight	278.35 g/mole	
Initial Receipt Date	12/06/04	
Expiration Date	12/06/10	
Supplier	Sigma-Adrich	
Lot Number	11613BC	
Vendor Purity	97%	
Method	EDSP.G2-022	

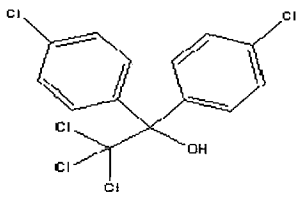
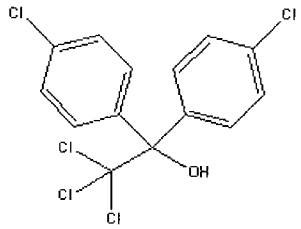
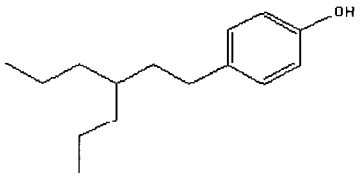
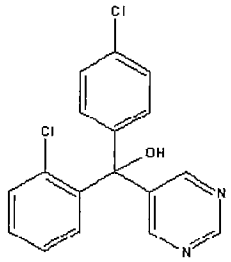
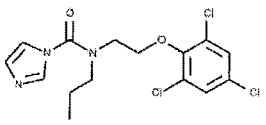
Parameter	Test Substance	Dicofol
Compound Name	Dicofol	
CAS #	115-32-2	
Central File No.	2334-1	
Molecular Weight	370.49 g/mole	
Initial Receipt Date	12/22/04	
Expiration Date	03/16/10	
Supplier	Sigma-Adrich	
Lot Number	4076X	
Vendor Purity	96.5%	
Method	EDSP.G2-025	

Table 1. (cont) Study Test Substances

Parameter	Test Substance	Dicofol
Compound Name	Dicofol	
CAS #	115-32-2	
Central File No.	2391-1	
Molecular Weight	370.49 g/mole	
Initial Receipt Date	04/11/05	
Expiration Date	02/08	
Supplier	Ultra Scientific	
Lot Number	RM00299	
Vendor Purity	96.5%	
Method	EDSP.G2-025	

Parameter	Test Substance	4-Nonylphenol
Compound Name	4-Nonylphenol	
CAS #	84852-15-3	
Central File No.	2331-1	
Molecular Weight	220.35 g/mole	
Initial Receipt Date	12/13/04	
Expiration Date	12/10	
Supplier	Acros Organics	
Lot Number	A0192712	
Vendor Purity	>98.5%	
Method	EDSP.G2-024	

Parameter	Test Substance	Fenarimol
Compound Name	Fenarimol	
CAS #	60168-88-9	
Central File No.	2335-1	
Molecular Weight	331.21 g/mole	
Initial Receipt Date	01/10/05	
Expiration Date	07/09	
Supplier	Supelco/Chem Service	
Lot Number	325-134C	
Vendor Purity	99%	
Method	EDSP.G2-022	

Parameter	Test Substance	Prochloraz
Compound Name	Prochloraz	
CAS #	67747-09-5	
Central File No.	2162-2	
Molecular Weight	376.67 g/mole	
Initial Receipt Date	05/20/04	
Expiration Date	02/14/08	
Supplier	Sigma-Aldrich/Riedel-de Haën	
Lot Number	2226x	
Vendor Purity	99.5%	
Method	EDSP. H4-023	

EXECUTIVE SUMMARY

The title compounds, atrazine, dibenz[a,h]anthracene, dicofol, fenarimol, 4-nonylphenol and prochloraz were analyzed in support of the Environmental Protection Agency (EPA) Placental and Recombinant Aromatase Assay Validation Work, Work Assignment 4-16/17.

The solubility of the test substances was determined to be acceptable at 0.1 M in dimethylsulfoxide (DMSO) (except for dibenz[a,h]anthracene which was tested at 0.01 M) for preparing formulations. See Table 2.

Table 2. Test Substance Solubility

Test Substance	Weight of Test Substance	Nominal conc. In mg/mL	Treatment	Time 0 Analysis Results, mg/mL	Recovery
Atrazine	0.2132 grams	21.32	Heated slightly on hot plate	20.31	95.2%
Dibenz[a,h]Anthracene	0.0278 grams	2.78	sonicated	2.79	100.5%
Dicofol	0.0368 grams	36.8	sonicated	33.72	91.6%
Fenarimol	0.3322 grams	33.22	sonicated	31.19	93.9%
4-Nonylphenol	0.2407 grams	24.07	sonicated	24.7	102.5%
Prochloraz	0.3797 grams	37.97	sonicated	37.5	98.7%

Test substance purity met study specifications as shown in Table 3. Note: EPA approval was obtained for dicofol purity.

Table 3. Test Substance Purity

TEST SUBSTANCE	SUPPLIER REPORTED PURITY	LOT NUMBER	CR DETERMINED PURITY
Atrazine	98%	328-137A	100.50%
Dibenz[a,h]Anthracene	97%	11613BC	97.84%
Dicofol	96.5%	4076X	95.30%
Dicofol	96.5%	RM00299	95.25%
Fenarimol	99%	LB325-134C	97.43%
4-Nonylphenol	98.5%	A0192712	97.25%
4-Nonylphenol*	98.5%	A0123226	98.81%
Prochloraz	99.5%	2226x	99.16%

*See footnote 1 page 23 for remark regarding this 4-nonylphenol

A formulation analysis method was developed and validated to analyze the test substances in DMSO at a concentration of 0.1M, except dibenz[a,h]anthracene which was 0.01 M. These methods were used to analyze samples for both formulation and formulation storage stability studies.

The storage stability study indicated that formulations of dicofol and 4-nonylphenol stored in sealed amber glass bottles and protected from light were stable for 14 days at approximately 5°C. The storage stability study indicated that formulations of atrazine, dibenz[a,h]anthracene, fenarimol and prochloraz stored in sealed amber glass bottles and protected from light were stable for a minimum of 14 days at room temperature – see Table 4.

Table 4 – Formulation Storage Stability Results

Test Substance	Average day 0 results (ug/mL)	Average day 14 results (ug/mL)	Recovery from nominal, day 0, %	Recovery from nominal, day 14, %	Nominal value (ug/mL)
Atrazine	20306	20382	95.24%	95.60%	21320
Dibenz[a,h]Anthracene	2794	2801	100.52%	100.74%	2780
Dicofol	33723	37969	91.64%	103.18%	36800
Fenarimol	31187	34657	93.88%	104.32%	33220
4-Nonylphenol	24663	23065	102.46%	95.82%	24070
Prochloraz	37487	39212	98.73%	103.27%	37970

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8.0 INTRODUCTION

The purpose of this work was to provide chemistry support activities for atrazine, dibenz[a,h]anthracene, dicofol, fenarimol, 4-nonylphenol and prochloraz on EPA Work Assignment 4-16/17, and consisted of:

- determining test substance solubility in DMSO
- developing and validating formulation analysis methods
- conducting storage stability studies over 14 days

This work was done at Battelle's Marine Research Operations by the Endocrine Disruptor Screening Program (EDSP) Chemical Repository (CR), 1529 W. Sequim Bay Road, Sequim, WA., 98382.

9.0 GENERAL METHODS

Methods of standard operation of the CR are currently addressed in Marine Science Laboratory (MSL) Standard Operating Proceedure (SOPs) numbered R-001 through R-017. These procedures address chemical procurement including procurement of controlled substances, when applicable, which have unique permitting, ordering, handling, inventory, and storage requirements; chemical receipt and chain of custody, chemical log-in and labeling, inventory, chemical storage; stock solution preparation, documentation and archiving; test solution preparation, documentation and shipping; chemical disposal, and CR maintenance over time. The quality assurance (QA) requirements for procurement of chemicals for use in the CR are addressed in the Quality Assurance Project Plan (QAPP) for the EDSP CR.

9.1 TEST SUBSTANCE PROCUREMENT

As requested by the EPA for WA 4-16/17, atrazine, (CAS No. 1912-24-9), dibenz[a,h]anthracene, (CAS No. 53-70-3), dicofol, (CAS No. 115-32-2) fenarimol, (CAS No. 60168-88-9), 4-nonylphenol, (CAS No. 84852-15-3), and prochloraz, (CAS No. 67747-09-5), were purchased by the CR. These materials were used for purity confirmation, formulation preparation, analysis development and validation, and stability analysis, as specified in 8.0 above (protocol EDSP.416-01). The chemicals were purchased from three suppliers (Table 5). The chemicals were logged into the Chemical Management System (CMS) and each was given a CMS barcode and unique log-in (central file) number as per the QAPP for the EDSP CR. The chemicals were stored in the CR in a clean, dry location away from direct sunlight at an appropriate temperature range as specified by the supplier.

Table 5. Study Test Substances

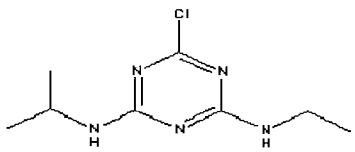
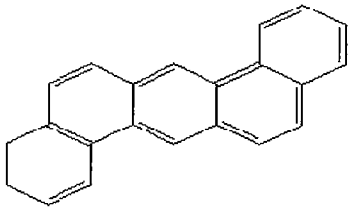
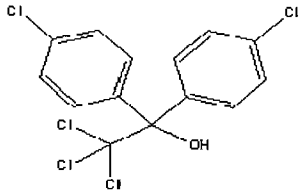
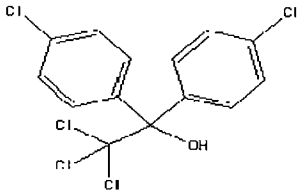
Parameter	Test Substance	Atrazine
Compound Name	Atrazine	
CAS #	1912-24-9	
Central File No.	2332-1	
Molecular Weight	215.72 g/mole	
Initial Receipt Date	12/14/04	
Expiration Date	10/07	
Supplier	Supelco/Chem Service	
Lot Number	328-137A	
Vendor Purity	98%	
Method	EDSP.G2-022	

Table 5. (cont.) Study Test Substances

Parameter	Test Substance	Dibenz(A,H)Anthracene
Compound Name	Dibenz(a,h)anthracene	
CAS #	53-70-3	
Central File No.	2319-1	
Molecular Weight	278.35 g/mole	
Initial Receipt Date	12/06/04	
Expiration Date	12/06/10	
Supplier	Sigma-Adrich	
Lot Number	11613BC	
Vendor Purity	97%	
Method	EDSP.G2-022	

Parameter	Test Substance	Dicofol
Compound Name	Dicofol	
CAS #	115-32-2	
Central File No.	2334-1	
Molecular Weight	370.49 g/mole	
Initial Receipt Date	12/22/04	
Expiration Date	03/16/10	
Supplier	Sigma-Adrich	
Lot Number	4076X	
Vendor Purity	96.5%	
Method	EDSP.G2-025	

Parameter	Test Substance	Dicofol
Compound Name	Dicofol	
CAS #	115-32-2	
Central File No.	2391-1	
Molecular Weight	370.49 g/mole	
Initial Receipt Date	04/11/05	
Expiration Date	02/08	
Supplier	Ultra Scientific	
Lot Number	RM00299	
Vendor Purity	96.5%	
Method	EDSP.G2-025	

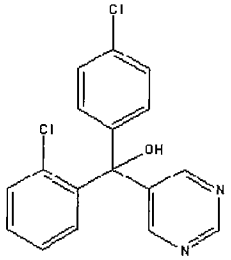
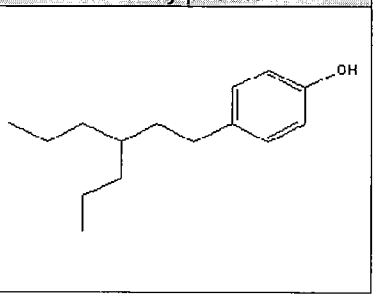
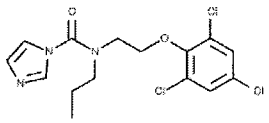
Parameter	Test Substance	Fenarimol
Compound Name	Fenarimol	
CAS #	60168-88-9	
Central File No.	2335-1	
Molecular Weight	331.21 g/mole	
Initial Receipt Date	01/10/05	
Expiration Date	07/09	
Supplier	Supelco/Chem Service	
Lot Number	325-134C	
Vendor Purity	99%	
Method	EDSP.G2-022	

Table 5. (cont.) Study Test Substances

Parameter	Test Substance	4-Nonylphenol
Compound Name	4-Nonylphenol	
CAS #	84852-15-3	
Central File No.	2331-1	
Molecular Weight	220.35 g/mole	
Initial Receipt Date	12/13/04	
Expiration Date	12/10	
Supplier	Acros Organics	
Lot Number	A0192712	
Vendor Purity	>98.5%	
Method	EDSP.G2-024	

Parameter	Test Substance	Prochloraz
Compound Name	Prochloraz	
CAS #	67747-09-5	
Central File No.	2162-2	
Molecular Weight	376.67 g/mole	
Initial Receipt Date	05/20/04	
Expiration Date	02/14/08	
Supplier	Sigma-Aldrich/Riedel-de Haën	
Lot Number	2226x	
Vendor Purity	99.5%	
Method	H4-023	

[redacted]

CERTIFICATE OF ANALYSIS

INVOICE #: CS255881
PO #: P337541

CATALOG #: PS-380

CAS #: 1912-24-9

DESCRIPTION: Atrazine

To re-order CHEM SERVICE products call
SUPELCO
800-247-6628 or 814-359-3441
or any local Supelco sales office

LOT #: 328-137A

PURITY: 98%

EXPIRATION DATE: 10/07

Chem Service, Inc. guarantees the purity of this chemical ± 0.5% deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Two or more of the following methods of analysis are used to determine purity: Melting point, refractive index, titration, FTIR, IR, TLC, GC/FID, GC/TCD, GC/ECD, GC/MS, HPLC or DSC.

Our standards are suitable for use with all EPA methods.

Certified By:

John Conrad

John Conrad
CSM/TC

Figure 1A. Atrazine Certificate of Analysis

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Dibenz[a,h]anthracene, 97%
Product Number	D3,140-0
Product Brand	ALDRICH
CAS Number	53-70-3
Molecular Formula	$C_{22}H_{14}$
Molecular Weight	278.35

TEST	SPECIFICATION	LOT 11613BC RESULTS
APPEARANCE	LIGHT YELLOW TO YELLOW CRYSTALLINE POWDER OR	YELLOW POWDER
INFRARED SPECTRUM	CONFORMS TO STRUCTURE AND STANDARD AS	CONFORMS TO STRUCTURE AND STANDARD
THIN-LAYER CHROMATOGRAPHY	CONSISTENT WITH 97% PURITY	CONSISTENT WITH 97% PURITY
SOLUBILITY	5% IN TOLUENE; CLEAR, COLORLESS TO LIGHT YELLOW, LIGHT GREEN OR LIGHT YELLOW-GREEN	5 MG/ML, TOLUENE; CLEAR FAINT YELLOW SOLUTION
QUALITY CONTROL ACCEPTANCE DATE		FEBRUARY, 2004

Ronnie J. Martin, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Figure 1B. Dibenz[a,h]Anthracene Certificate of Analysis

SIGMA-ALDRICH

Riedel-de Haën

CERTIFICATE OF ANALYSIS / INSPECTION CERTIFICATE 3.1.B acc. to EN10204

Sigma-Aldrich Laborchemikalien GmbH D-30918 Seelze
Telefon: 449 5137 8238-150

Seelze, 26.03.2004/616904

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production date: 16.Mar.2004
Min. shelf life: 16.Mar.2010

Article/Product: 36677 Batch : 4076X

Dicofol PESTANAL® (2,2,2-Trichloro- 1,1-bis(4-chlorophenyl)ethanol), 250 mg

Reference Material (RM)

1. General Information

Formula: C14H9Cl5O
CAS-No.: [115-32-2]
Usage : Acaricide

Molar mass: 370.49 g/Mole
Recomm. storage temp.: approx. 4 °C

The estimated relative error of a single measurement of the assay can be expected to be ± 1 % (confidence level 95 %, n=6).

2. Batch Analysis

Identity (NMR)	complying	
Assay (HPLC)	96.5	%
Melting range	71.8 - 72.8	°C
Date of Analysis	26.Mar.2004	

3. Advice and Remarks

- The minimum shelf life is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliquot portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
- We herewith confirm that the delivery is effected according to the technical delivery conditions agreed.
- The batch from which we delivered, showed the above-mentioned values.
- Particular properties of the products or the suitability for a particular area of application are not assured.
- We guarantee a proper quality within our General Conditions of Sales.

Sigma-Aldrich Laborchemikalien GmbH
Quality Assurance

(Dr. Gaede)
Works Inspector

Figure 1C. Dicofol Certificate of Analysis

ULTRA SCIENTIFIC
Analytical Solutions

Certificate of Analysis

Dicofol

Product Number: PST-391 **Expiration Date:** Feb-2008

Lot Number: RM00299 **Page:** 1 of 1

This reference material has been analyzed by high resolution gas chromatography or high performance liquid chromatography, and found to meet the specifications stated below. The uncertainty of the purity measurement is ± 0.5%.

Compound	CAS #	Purity
dicofol	000115-32-2	96.5%

Storage: May be stored at room temperature

Quality Endorsed Company ISO 9001 SAI Global Registered

ACCREDITED ISO 17025 Cert. No. 0851-01

250 Smith Street, North Kingstown, RI 02852 USA
401-294-9400 Fax: 401-295-2330
www.ultrasci.com

Dr. Edward Fitzgerald,
Senior Scientist

Figure 1D. Dicofol Certificate of Analysis

660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599
1-800-452-9994 • 1-610-692-3026 • Fax 1-610-692-8729
info@chemservice.com • www.chemservice.com

CERTIFICATE OF ANALYSIS

INVOICE #: CS257624
PO #: P339294

CATALOG #: PS-1073

CAS #: 60168-88-9

DESCRIPTION: Fenarimol

LOT #: 325-134C

PURITY: 99%

EXPIRATION DATE: 07/09

To re-order CHEM SERVICE products call
SUPELCO
800-247-6628 or 814-359-3441
or any local Supelco sales office
1800-0031

Chem Service, Inc. guarantees the purity of this chemical ± 0.5% deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Two or more of the following methods of analysis are used to determine purity: Melting point, refractive index, titration, FTIR, IR, TLC, GC/FID, GC/TCD, GC/ECD, GC/MS, HPLC or DSC.

Our standards are suitable for use with all EPA methods.

Certified By:

John Conrad

John Conrad
CSM/TC

Figure 1E. Fenarimol Certificate of Analysis

ACROS

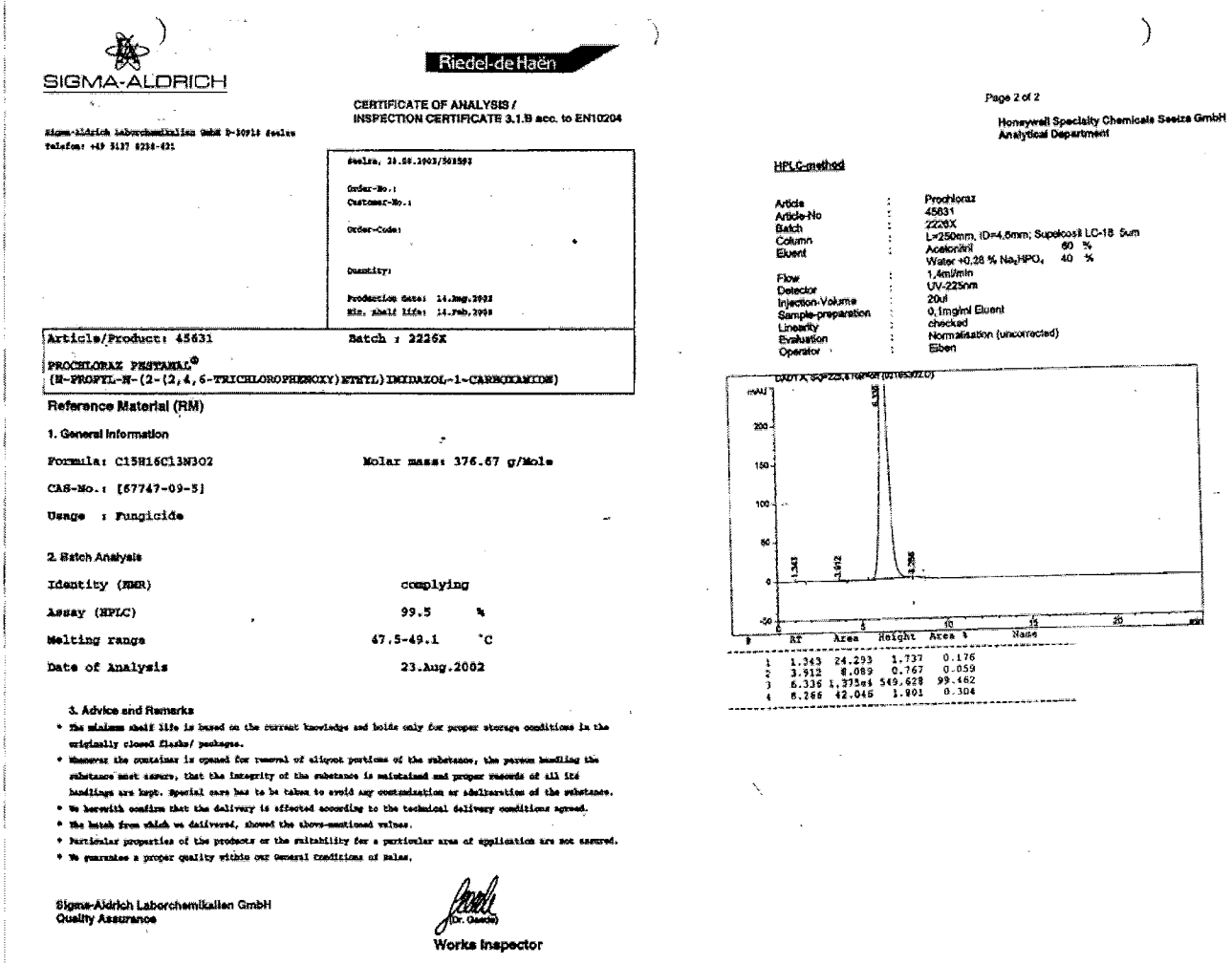
Certificate of Analysis

Product	41624-0000 4-NONYL-PHENOL, 99%, MIXTURE OF ISOMERS	General Product Data	
		Version	00
		CAS No	84852-15-3
		Molecular weight	220.35
		Molecular formula	C15 H24 O
		Linear formula	
		Flash point (°C)	141

Lot Specific Data **A0192712**

Appearance	Clear viscous liquid
Color scale	<50 APHA
Infrared spectrometry	authentic
Separat. techn. GC	>98.5 %
Water	<0.05 %
Refractive index	1.5119 (20°C, 589 nm)

Figure 1F. 4-Nonylphenol Certificate of Analysis



SIGMA-ALDRICH

Riedel-de Haën

CERTIFICATE OF ANALYSIS / INSPECTION CERTIFICATE 3.1.B acc. to EN10204

Page 2 of 2

Honeywell Specialty Chemicals Seelze GmbH
Analytical Department

Article/Product: 45631 Batch : 2226X

PROCHLORAZ PESTANAL®
(N-PROPYL-N-(2-(2,4,6-TRICHLOROPHENOXY)ETHYL)IMIDAZOL-1-CARBOXAMIDE)

Reference Material (RM)

1. General Information

Formula: C15H16Cl3N3O2 Molar mass: 376.67 g/Mole

CAS-No.: [67747-09-5]

Usage : Fungicide

2. Batch Analysis

Identity (NMR)	complying	
Assay (HPLC)	99.5	%
Melting range	47.5-49.1	°C
Date of Analysis	23.Aug.2002	

3. Advice and Remarks

- The minimum shelf life is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliquot portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
- We herewith confirm that the delivery is effected according to the technical delivery conditions agreed.
- The batch from which we delivered, showed the above-mentioned values.
- Particular properties of the products or the suitability for a particular area of application are not assured.
- We guarantee a proper quality within our General Conditions of Sales.

Sigma-Aldrich Laborchemikalien GmbH
Quality Assurance

Works Inspector

HPLC-method

Article	:	Prochloraz
Article-No	:	45631
Batch	:	2226X
Column	:	L=250mm, ID=4,6mm; Supelcosil LC-18 5um
Eluent	:	Acetonitril 60 %
		Water +0.28 % Na_2HPO_4 40 %
Flow	:	1,4ml/min
Detector	:	UV-225nm
Injection-Volume	:	20ul
Sample-preparation	:	0,1mg/ml Eluent
Linearity	:	checked
Evaluation	:	Normalisation (uncorrected)
Operator	:	Eiben

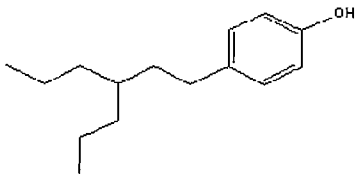
	Rt	Area	Height	Area %	Name
1	1.343	24.293	1.737	0.176	
2	3.912	8.089	0.767	0.059	
3	5.336	1,375e4	549.628	99.462	
4	8.286	42.045	1.991	0.304	

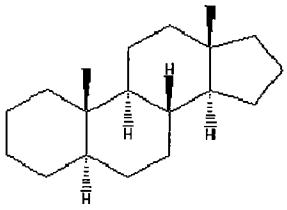
Figure 1G. Prochloraz Certificate of Analysis

9.1.1 Reference Substances Used

A second lot of 4-nonylphenol was used for verification of instrument calibration solutions. 5-α-androstane was used as an internal standard for gas chromatography analyses. See Table 6.

Table 6. Study Test Reference Substances

Parameter	Reference Substance	4-Nonylphenol
Compound Name	4-Nonylphenol	
CAS #	84852-15-3	
Central File No.	2305-1	
Molecular Weight	220.35 g/mole	
Initial Receipt Date	2/5/01	
Expiration Date	2/5/08	
Supplier	Acros Organics	
Lot Number	A0123226	
Vendor Purity	99.8%	
Method	EDSP.G2-022	

Parameter	Reference Substance	5-a-androstane
Compound Name	5-α-androstane	
CAS #	438-22-2	
Central File No.	2276-1	
Molecular Weight	260.46 g/mole	
Initial Receipt Date	10/12/04	
Expiration Date	10/12/09	
Supplier	Sigma	
Lot Number	054K4027	
Vendor Purity	99%	

SIGMA-ALDRICH

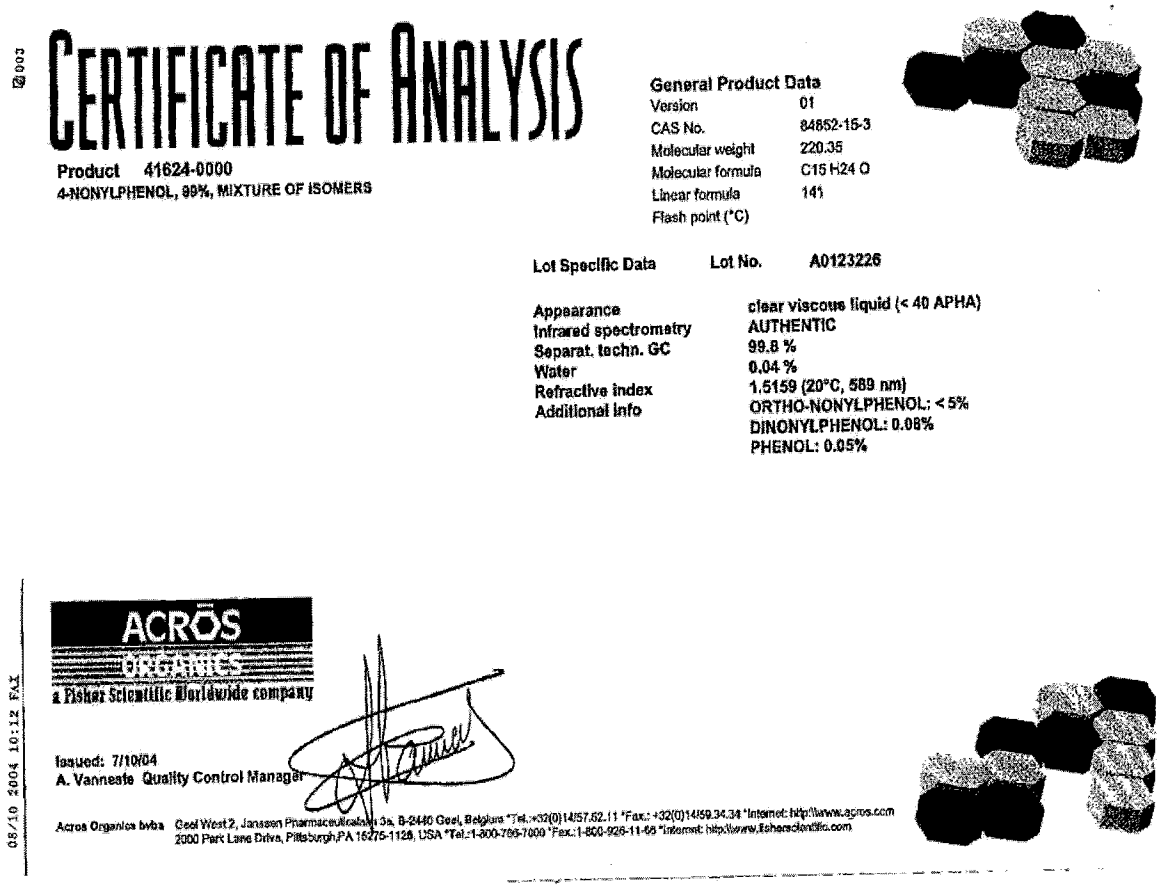
Certificate of Analysis

Product Name	5-α-Androstane
Product Number	A0887
Product Brand	SIGMA
CAS Number	438-22-2
Molecular Formula	$C_{19}H_{32}$
Molecular Weight	260.46

TEST	SPECIFICATION LOT 054K4027 RESULTS
APPEARANCE	WHITE POWDER
SOLUBILITY	CLEAR COLORLESS SOLUTION AT 50MG/ML IN CHLOROFORM
ELEMENTAL ANALYSIS	87.77% CARBON
PURITY BY GAS CHROMATOGRAPHY	99.6%
QC ACCEPTANCE DATE	JUNE 2004

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

Figure 1H. 5-α-Androstane Certificate of Analysis



CERTIFICATE OF ANALYSIS

Product 41624-0000
4-NONYLPHENOL, 99%, MIXTURE OF ISOMERS

General Product Data

Version	01
CAS No.	84852-15-3
Molecular weight	220.35
Molecular formula	C15 H24 O
Linear formula	141
Flash point (°C)	

Lot Specific Data Lot No. A0123226

Appearance	clear viscous liquid (< 40 APHA)
Infrared spectrometry	AUTHENTIC
Separat. techn. GC	99.8 %
Water	0.04 %
Refractive index	1.5159 (20°C, 589 nm)
Additional info	ORTHO-NONYLPHENOL: < 5% DINONYLPHENOL: 0.08% PHENOL: 0.05%

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Issued: 7/10/04
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08/10 2004 10:12 FAX

Figure 1l. 4-Nonylphenol Certificate of Analysis, Reference Material

9.2 SOLUBILITY STUDIES

Analysis of solubility study samplings and formulation preparations were done with a single combined calibration solution of atrazine, dibenz[a,h]anthracene, fenarimol, 4-nonylphenol and prochloraz to conserve expensive test materials and analysis time. The solutions were prepared in DMSO, at a concentration of 0.1 mole/liter (M), except dibenz[a,h]anthracene ,which was prepared at 0.01 M. Atrazine, dibenz[a,h]anthracene, fenarimol, 4-nonylphenol and prochloraz were weighed into a 10 mL volumetric flask and diluted to the mark with DMSO. Dicofol was weighed into a 1 mL vial and diluted with 1.0 mL DMSO. Solubility was indicated by both visual means and time 0 analysis results. Analysis result specifications were set at ±10% of nominal value to indicate solubility. Table 7 has the weights, conditions and results used to dissolve the test substances. These tests demonstrated that DMSO was an acceptable solvent for the 0.1M and the 0.01M formulations.

Table 7. Test Substance Solubility

Test Substance	Weight of Test Substance	Nominal conc. In mg/mL	Treatment	Time 0 Analysis Results (mg/mL)	Recovery
Atrazine	0.2132 grams	21.32	Heated slightly on hot plate	20.31	95.2%
Dibenz[a,h]Anthracene	0.0278 grams	2.78	sonicated	2.79	100.5%
Dicofol	0.0368 grams	36.8	sonicated	33.72	91.6%
Fenarimol	0.3322 grams	33.22	sonicated	31.19	93.9%
4-Nonylphenol	0.2407 grams	24.07	sonicated	24.7	102.5%
Prochloraz	0.3797 grams	37.97	sonicated	37.5	98.7%

9.3 TEST SUBSTANCE PURITY

Test substance purity was determined using gas chromatography flame Ionization detection (GC-FID) for atrazine, dibenz[a,h]anthracene, fenarimol, and dicofol. Prochloraz purity was determined using high performance liquid chromatography (HPLC) and 4-nonylphenol purity was determined using gas chromatography/mass spectrometry (GC/MS). Purity verification for the test substances was conducted by making a solution of each substance in the appropriate carrier. Method details are provided in section 9.5 and Tables 9 through 13. Each matrix was then run on the GC-FID, HPLC, or GC/MS. Carrier blanks were also analyzed for each test substance. The purity was determined by comparing the area of the peak associated with the substance of interest with the total area of all the peaks in the chromatogram. The areas associated with peaks common to the carrier blank were eliminated by subtraction. The percentage associated with the largest peak represented the purity of the test substance. This result was compared to the supplier's certificate of analysis/purity (Figures 1A – 1F). Since 4-nonylphenol is a mixture of isomers, purity determination required selection of specific fragments produced in the GC/MS analytical procedure that were common to all of the isomers. The peaks in the chromatogram that had retention times matching the common peaks were considered 4-nonylphenol. The area of the common peaks was divided by the area of all the peaks to determine purity.

9.4 TEST SUBSTANCE PURITY RESULTS

Purity analysis results demonstrated that all 6 test substances met the study protocol criteria of the CR determined purity values within ±3% of the supplier purity claims. The protocol requirements for supplier's purity claims were ≥97% (the EPA allowed a purity, determined by the manufacturer, of 96.5% for dicofol). See Table 8 for purity results.

Table 8. Test Substance Purity

TEST SUBSTANCE	SUPPLIER REPORTED PURITY	LOT NUMBER	CR DETERMINED PURITY
Atrazine	98%	328-137A	100.5%
Dibenz[a,h]Anthracene	97%	11613BC	97.84%
Dicofol	96.5%	4076X	95.30%
Dicofol	96.5%	RM00299	95.25%
Fenarimol	99%	325-134C	97.43%
4-Nonylphenol	98.5%	A0192712	97.25%
4-Nonylphenol[1]	98.5%	A0123226	98.81%
Prochloraz	99.5%	2226x	99.16%

[1]This compound was used as a test substance in WA 4-16, task 7 and WA 4-17, task 4 studies - see chemical repository study number EDSP.416-02.

9.5 METHOD DEVELOPMENT

This section describes the evaluation of methods developed to analyze atrazine, dibenz[a,h]anthracene, dicofol, fenarimol, 4-nonylphenol, and prochloraz in DMSO at target

concentrations of either 0.1M or 0.01M for the purity analyses and stability studies carried out for this work assignment. Method development for the test substances involved the evaluation of various chromatographic methods. The methods selected were ones which produced acceptable retention times for the major peak, apparent resolution of significant impurities and acceptable peak shape. The methods chosen are in listed in Table 9.

Table 9. Test Substance Methods

Test Substance	Method Employed	Compounds Analyzed Simultaneously
Atrazine	GC-FID	Dibenz[a,h]Anthracene, Fenarimol
Dibenz[a,h]Anthracene	GC-FID	Atrazine, Fenarimol
Dicofol	GC-FID	N/A
Fenarimol	GC-FID	Dibenz[a,h]Anthracene, Atrazine
4-Nonylphenol	GC-FID	N/A
Prochloraz	HPLC	N/A

9.5.1 Method Parameters

9.5.1.1 The GC parameters for atrazine, dibenz[a,h]anthracene and fenarimol, are presented in Table 10.

Table 10. GC-FID System Setup – Atrazine, Dibenz[a,h]Anthracene, Fenarimol

GC	Agilent 5890 (Palo Alto, CA)
Column	DB-5, 30 m × 0.25 mm (ID), 0.25 μm film thickness (J+W, Bellefonte, PA)
Carrier Gas Pressure	Helium at 18 PSI
Oven Temperature	100°C, hold for 1.5 minutes, increase at 15°C/minute to 320°C, hold 10 min.
Detector Type	Flame Ionization
Detector Gas Pressures	Hydrogen, 21 PSI. Air, 40 PSI
Detector Temperature	320°C
Injector Temperature	285°C
Injection Volume	1 μL
Run Time	25 minutes

9.5.1.2 The GC parameters for dicofol are presented in Table 11.

Table 11. GC-FID System Setup - Dicofol

GC	Agilent 5890 (Palo Alto, CA)
Column	DB-5, 30 m × 0.25 mm (ID), 0.25 μm film thickness (J+W, Bellefonte, PA)
Carrier Gas Pressure	Helium at 18 PSI
Oven Temperature	50°C, hold for 1.5 minutes, increase at 15°C/minute to 320°C, no hold time.
Detector Type	Flame Ionization
Detector Gas Pressures	Hydrogen, 21 PSI. Air, 40 PSI
Detector Temperature	320°C
Injector Temperature	210°C
Injection Volume	1 μL
Run Time	19.5 minutes

9.5.1.3 The GC parameters for 4-nonylphenol are presented in Table 12a and 12b.

Table 12a. GC-FID System Setup – 4-Nonylphenol

GC	Agilent 5890 (Palo Alto, CA)
Column	DB-5, 30 m × 0.25 mm (ID), 0.25 μm film thickness (J+W, Bellefonte, PA)
Carrier Gas Pressure	Helium at 18 PSI
Oven Temperature	50°C, hold for 1.5 minutes, increase at 15°C/minute to 320°C, hold 1 min.
Detector Type	Flame Ionization
Detector Gas Pressures	Hydrogen, 21 PSI. Air, 40 PSI
Detector Temperature	320°C
Injector Temperature	250°C
Injection Volume	1 μL
Run Time	20.5 minutes

Table 12b. GC-MS System Setup – 4-Nonylphenol (Purity Determination)

GC	Agilent 5890 (Palo Alto, CA)
Column	DB-5, 30 m × 0.25 mm (ID), 0.25 μm film thickness (J+W, Bellefonte, PA)
Carrier Gas Pressure	Helium at 9 PSI
Oven Temperature	50°C, hold for 1.5 minutes, increase at 15°C/minute to 320°C, hold 1 min.
Detector Type	Mass selective detector, Full Scan Mode
Detector Temperature	300°C
Injector Temperature	250°C
Injection Volume	1 μL
Run Time	21 minutes

9.5.1.4 The HPLC parameters for prochloraz are presented in Table 13.

Table 13. HPLC System Setup - Prochloraz

Instrument	Battelle MSL ID, HPLC1
Column	Reverse Phase, 250 X 4.6 mm Synergi RP80A (Phenomenex Torrance, CA)
Eluent	75% Acetonitrile, 25% Water
Eluent Flow Rate	1 mL/minute
Detector Type	UV/Vis., wavelength set at 204 mn
Column Oven Temperature	Ambient
Injector loop size	25 ul
Injection Volume	10 ul
Run Time	13 minutes

9.6 METHOD VALIDATION

Method validation for each test substance was accomplished by preparing calibration standards and calibration verification standards, testing system linearity and system recovery (using matrix spikes), and determining a method detection limit (MDL) for each analytical method employed.

9.6.1 Preparation of Standards, Atrazine, Dibenz[a,h]Anthracene and Fenarimol

9.6.1.1 Internal Standard

An internal standard was used with analyses done by GC-FID. The internal standard, 5-α-androstane, was prepared by weighing 0.0501 grams into a 50 ml volumetric flask, this was diluted to the mark with methylene chloride.

9.6.1.2 Stock Standards

Stock standards were prepared by accurately weighing about 50 mg of each test substance into a 50-mL volumetric flask and dissolving in and diluting to volume with the appropriate solvent for the analytical method. Table 14 shows details for this stock standard preparation.

Table 14. Stock Standard Preparations

Test Substance	Weight of Test Substance	Volume, mL	Concentration	Solvent
Atrazine	0.0494 grams	50	988 ug/mL	Methylene chloride
Dibenz[a,h]Anthracene	0.0505 grams	50	1010 ug/mL	Methylene chloride
Fenarimol	0.0501 grams	50	1002 ug/mL	Methylene chloride
5-α-Androstane	0.0501 grams	50	1002 ug/mL	Methylene chloride

9.6.1.3 Calibration Standards

Calibration standards were prepared so the calibration solutions were within the calibrated range of the instrument and the dilutions of the stability solutions were bracketed by calibration standards. Tables 14 and 15 show how standards were prepared. Briefly, the stock was pipetted into the volumetric flask and diluted to the mark with the appropriate solvent and mixed well.

9.6.1.4 Calibration Verification Standards

An independent standard was prepared to verify the calibration standards; Tables 16 and 17 list the specifics.

9.6.1.5 MDL and Spikes

Spike solutions were prepared by weighing an aliquot of the test material into a volumetric flask and diluting to the mark with DMSO (Table 18). An aliquot of each spike solution was then diluted to an appropriate level for an MDL solution (Table 19). Since the dibenz[a,h]anthracene stability solution was prepared at 0.01 M as compared to 0.1 M for the other test substances, this MDL was made separately. MDLs and spikes were prepared in a manner that mimics the sampling of the stability solutions. Atrazine and fenarimol spikes and MDLs were sampled by taking 0.005 mL of the spike or MDL solution, placing it in a 5 mL volumetric flask with 0.1 mL of the internal standard solution and adding methylene chloride to the 5 mL mark. Dibenz[a,h]anthracene spikes and MDLs were sampled by taking 0.025 mL of the spike or MDL, placing it into a 1.8 mL autosampler vial with 0.02 mL of the internal standard solution and diluting to 1 mL by adding 0.955 mL of methylene chloride. A blank was prepared for each dilution described above to test the procedure for contamination. These samples were run on the GC-FID as described in section 9.5.1 and Table 10 (GC conditions for atrazine, fenarimol and dibenz(a,h)anthracene).

Table 15.
Preparation of Calibration Standards for Atrazine, Dibenz[A,H]Anthracene and Fenarimol

Standard Name	Test Substance	Stock Standard Concentration	Stock Volume	Final Volume	Concentration	Solvent
WA 416/17-GC-16-A	Atrazine	988 ug/mL	5 mL	50 mL	98.8 ug/mL	Methylene Chloride
	Dibenz[a,h]Anthracene	1010 ug/mL	5 mL		101 ug/mL	
	Fenarimol	1002 ug/mL	5 mL		100 ug/mL	
	5-α-Androstane (IS)	1002 ug/mL	1 mL		20.0 ug/mL	
WA 416/17-GC-16-B	Atrazine	988 ug/mL	1 mL	50 mL	19.8 ug/mL	Methylene Chloride
	Dibenz[a,h]Anthracene	1010 ug/mL	1 mL		20.2 ug/mL	
	Fenarimol	1002 ug/mL	1 mL		20.0 ug/mL	
	5-α-Androstane (IS)	1002 ug/mL	1 mL		20.0 ug/mL	
WA 416/17-GC-16-C	Atrazine	988 ug/mL	0.25 mL	50 mL	4.94 ug/mL	Methylene Chloride
	Dibenz[a,h]Anthracene	1010 ug/mL	0.25 mL		5.05 ug/mL	
	Fenarimol	1002 ug/mL	0.25 mL		5.01 ug/mL	
	5-α-Androstane (IS)	1002 ug/mL	1 mL		20.0 ug/mL	
WA 416/17-GC-16-D	Atrazine	988 ug/mL	0.1 mL	100 mL	0.988 ug/mL	Methylene Chloride
	Dibenz[a,h]Anthracene	1010 ug/mL	0.1 mL		1.01 ug/mL	
	Fenarimol	1002 ug/mL	0.1 mL		1.00 ug/mL	
	5-α-Androstane (IS)	1002 ug/mL	2 mL		20.0 ug/mL	
WA 416/17-GC-16-E	Atrazine	988 ug/mL	0.05 mL	100 mL	0.494 ug/mL	Methylene Chloride
	Dibenz[a,h]Anthracene	1010 ug/mL	0.05 mL		0.505 ug/mL	
	Fenarimol	1002 ug/mL	0.05 mL		0.501 ug/mL	
	5-α-Androstane (IS)	1002 ug/mL	2 mL		20.0 ug/mL	

Table 16.
Preparation of Calibration Verification Stock Standards for Atrazine, Dibenz[a,h]Anthracene and Fenarimol

Test Substance	Weight of Test Substance	Volume, mL	Concentration	Solvent
Atrazine	0.0534 grams	50	1068 ug/mL	Hexane*
Dibenz[a,h]Anthracene	0.0518 grams	50	1036 ug/mL	Methylene Chloride
Fenarimol	0.0520 grams	50	1040 ug/mL	Hexane*
5-α-Androstane	0.0501 grams	50	1002 ug/mL	Methylene Chloride

* Use of Hexane was a Method Deviation that did not impact the analysis

Table 17.
Preparation of Calibration Verification Standards for Atrazine, Dibenz[a,h]Anthracene and Fenarimol

Standard Name	Test Substance	Stock Standard Concentration	Stock Volume	Final Volume	Concentration	Solvent
WA 416/17-GC-17	Atrazine	1068 ug/mL	0.25 mL	50 mL	5.34 ug/mL	Methylene Chloride
	Dibenz[a,h]Anthracene	1036 ug/mL	0.25 mL		5.18 ug/mL	
	Fenarimol	1040 ug/mL	0.25 mL		5.20 ug/mL	
	5-α-Androstane (IS)	1002 ug/mL	1 mL		20.04 ug/mL	

Table 18.
Preparation of Spike solutions for Atrazine, Dibenz[a,h]Anthracene and Fenarimol

Test Substance	Weight of Test Substance	Volume, mL	Concentration	Solvent
Atrazine	0.2270 grams	10	22.7 mg/mL	DMSO
Fenarimol	0.3340 grams	10	33.4 mg/mL	DMSO
Dibenz[a,h]Anthracene	0.0299 grams	10	2.99 mg/mL	DMSO

Table 19.
Preparation of MDL solutions for Atrazine, Dibenz[a,h]Anthracene and Fenarimol

Test Substance	Spike Concentration	Spike Volume	Final Volume	Concentration	Solvent
Atrazine	22.7 mg/mL	1 mL	10 mL	2.27 mg/mL	DMSO
Fenarimol	33.4 mg/mL	1 mL		3.34 mg/mL	
Dibenz[a,h]Anthracene	2.99 mg/mL	0.25 mL	10 mL	0.0748 mg/mL	DMSO

9.6.2 Preparation of Standards, Dicofol

9.6.2.1 Internal Standard

An internal standard was used with analyses done by GC-FID. The internal standard 5-α-androstane was prepared by weighing 0.0501 grams into a 50 ml volumetric flask; this was diluted to the mark with methylene chloride.

9.6.2.2 Stock Standards

Stock standard was prepared by accurately weighing about 50 mg of dicofol into a 50 mL volumetric flask and dissolving in and diluting to volume with the hexane. Table 20 shows details for this stock standard preparation.

Table 20. Preparation of Calibration Stock Standard for Dicofol

Test Substance	Weight of Test Substance	Volume, mL	Concentration	Solvent
Dicofol	0.0517 grams	50	1034 ug/mL	Hexane*

* Use of Hexane was a Method Deviation that did not impact the analysis

9.6.2.3 Calibration Standards

Calibration standards were prepared so the calibration solutions were within the linear range of the instrument and the dilutions of the stability solutions were bracketed by calibration standards. Tables 20 and 21 show how these standards were prepared. Briefly, the stock was pipetted into the volumetric flask, diluted to the mark with solvent, and mixed well.

9.6.2.4 Calibration Verification Standards

An independent standard was prepared to verify the calibration standards; Tables 22 and 23 have the preparation information.

9.6.2.5 MDL and Spikes

The spike solution was prepared by weighing an aliquot of the dicofol into a volumetric flask and diluting to the mark with DMSO. The MDL solution was prepared in a similar manner. See Table 24 for the details. MDLs and spikes were prepared in a manner that mimicked the sampling of the stability solution. The dicofol

spike and MDL solutions were sampled by taking 0.005 mL of the spike or MDL solution, placing it in a 5 mL volumetric flask with 0.1 mL of the internal standard solution and filling to the mark with methylene chloride. A blank was prepared for each dilution described above to test the procedure for contamination. These samples were run on the GC-FID as described in section 9.5.1 and Table 11 (GC conditions for dicofol).

Table 21. Preparation of Calibration Standards for Dicofol

Standard Name	Test Substance	Stock Standard Concentration	Stock Volume	Final Volume	Concentration	Solvent
WA 416-Dico-4-A	Dicofol	1034 ug/mL	5 mL	50 mL	103.4 ug/mL	Methylene Chloride
	5-α-Androstane (IS)	1002 ug/mL	1 mL		20.04 ug/mL	
WA 416-Dico-4-B	Dicofol	1034 ug/mL	1 mL	50 mL	20.68 ug/mL	Methylene Chloride
	5-α-Androstane (IS)	1000 ug/mL	1 mL		20.04 ug/mL	
WA 416-Dico-4-C	Dicofol	1034 ug/mL	0.25 mL	50 mL	5.35 ug/mL **	Methylene Chloride
	5-α-Androstane (IS)	1002 ug/mL	1 mL		20.04 ug/mL	
WA 416-Dico-4-D	Dicofol	1034 ug/mL	0.1 mL	100 mL	1.034 ug/mL	Methylene Chloride
	5-α-Androstane (IS)	1002 ug/mL	1 mL*		10.02 ug/mL*	
WA 416-Dico-4-E	Dicofol	1034 ug/mL	0.05 mL	100 mL	0.517 ug/mL	Methylene Chloride
	5-α-Androstane (IS)	1002 ug/mL	1 mL*		10.02 ug/mL*	

- * 1 mL of internal standard was used rather than 2 mL as specified in the Method
- ** The 5.35 value was inadvertently used in the curve, however, the actual calculated value is 5.17ug/ml, impact to data is minimal.

Table 22. Preparation of Calibration Verification Stock Standard for Dicofol

Test Substance	Weight of Test Substance	Volume	Concentration	Solvent
Dicofol	0.0507 grams	50 mL	1014 ug/mL	Methylene Chloride

Table 23. Preparation of Calibration Verification Standards for Dicofol

Standard Name	Test Substance	Stock Standard Concentration	Stock Volume	Final Volume	Concentration	Solvent
WA 416-Dico-8	Dicofol	1014 ug/mL	0.25 mL	50 mL	5.07 ug/mL	Methylene Chloride
-	5-α-Androstane (IS)	1002 ug/mL	1 mL		20.04 ug/mL	

Table 24. Preparation of MDL and Spike Solutions for Dicofol

Test Solution	Test Substance	Weight of Test Substance	Volume	Concentration	Solvent
Spike	Dicofol	0.3692 grams	10 mL	36.92 mg/mL	DMSO
MDL	Dicofol	0.0516 grams	25 mL	2.064 mg/mL	DMSO

9.6.3 Preparation of Standards, 4-Nonylphenol

9.6.3.1 Internal Standard

An internal standard was used with analyses done by GC-FID. The internal standard 5-α-androstane was prepared by weighing 0.0500 grams into a 50 mL volumetric flask; this was diluted to the mark with methylene chloride.

9.6.3.2 Stock Standards

Stock standards were prepared by accurately weighing about 50 mg of 4-nonylphenol into a 50 mL volumetric flask and dissolving in and diluting to volume

with the methylene chloride. Table 25 shows details for this stock standard preparation.

Table 25. Preparation of Calibration Stock Standard for 4-Nonylphenol

Test Substance	Weight of Test Substance	Volume	Concentration	Solvent
4-Nonylphenol	0.0535 grams	50 mL	1070 ug/mL	Methylene chloride

9.6.3.3 Calibration Standards

Calibration standards were prepared so the calibration solutions were within the calibrated range of the instrument and the dilutions of the stability solutions were bracketed by calibration standards; Tables 25 and 26 show how the standards were prepared. Briefly, the stock was pipetted into the volumetric flask and diluted to the mark with methylene chloride and mixed well.

9.6.3.4 Calibration Verification Standards

An independent standard was prepared to verify the calibration standards; Tables 27 and 28 detail this preparation.

9.6.3.5 MDL and Spikes

The spike solution was prepared by weighing an aliquot of the 4-nonylphenol into a volumetric flask and diluting to the mark with DMSO. The MDL solution was prepared in a similar manner. Table 29 shows the details. MDLs and spikes were prepared in a manner that mimics the sampling of the stability solution. The 4-nonylphenol spike and MDL were sampled by taking 0.005 mL of the spike or MDL solution, placing it in a 1.8 mL autosampler vial with 0.02 mL of the internal standard solution and adding 0.975 mL of methylene chloride. A blank was prepared for each dilution described above to test the procedure for contamination. These samples were run on the GC-FID as described in section 9.5.1 and Tables 12a and 12b (GC conditions for 4-nonylphenol).

Table 26. Preparation of Calibration Standards for 4-Nonylphenol

Standard Name	Test Substance	Stock Standard Concentration	Stock Volume	Final Volume	Concentration	Solvent
WA 416-nonyl-3-A	4-Nonylphenol	1070 ug/mL	10 mL	50 mL	214 ug/mL	Methylene Chloride
	5-α-Androstane (IS)	1000 ug/mL	1 mL		20.00 ug/mL	
WA 416-nonyl-3-B	4-Nonylphenol	1070 ug/mL	5 mL	50 mL	107 ug/mL	Methylene Chloride
	5-α-Androstane (IS)	1000 ug/mL	1 mL		20.00 ug/mL	
WA 416-nonyl-3-C	4-Nonylphenol	1070 ug/mL	2.5 mL	50 mL	53.5 ug/mL	Methylene Chloride
	5-α-Androstane (IS)	1000 ug/mL	1 mL		20.00 ug/mL	
WA 416-nonyl-3-D	4-Nonylphenol	1070 ug/mL	1 mL	50 mL	21.4 ug/mL	Methylene Chloride
	5-α-Androstane (IS)	1000 ug/mL	1 mL		20.00 ug/mL	
WA 416-nonyl-3-E	4-Nonylphenol	1070 ug/mL	0.5 mL	50 mL	10.7 ug/mL	Methylene Chloride
	5-α-Androstane (IS)	1000 ug/mL	1 mL		20.00 ug/mL	

Table 27. Preparation of Calibration Verification Stock Standard for 4-Nonylphenol

Test Substance	Weight of Test Substance	Volume	Concentration	Solvent
4-Nonylphenol (lot used, A0123226)	0.0556 grams	50 mL	1112 ug/mL	Hexane*

* Use of Hexane was a Method Deviation that did not impact the analysis

Table 28. Preparation of Calibration Verification Standards for 4-Nonylphenol

Standard Name	Test Substance	Stock Standard Concentration	Stock Volume	Final Volume	Concentration	Solvent
WA 416-nonyl-6	4-Nonylphenol	1112 ug/mL	2.5 mL	50 mL	55.6 ug/mL	Methylene Chloride
	5-α-Androstane (IS)	1000 ug/mL	1 mL		20.00 ug/mL	

Table 29. Preparation of MDL and Spike Solutions for 4-Nonylphenol

Test Solution	Test Substance	Weight of Test Substance	Volume	Concentration	Solvent
Spike	4-Nonylphenol	0.2497 grams	10 mL	24.97 mg/mL	DMSO
MDL	4-Nonylphenol	0.0329 grams	10 mL	3.29 mg/mL	DMSO

9.6.4 Preparation of Standards, Prochloraz

9.6.4.1Stock Standards

Stock standard was prepared by accurately weighing about 100 mg of prochloraz into a 50-mL volumetric flask and dissolving in and diluting to volume with the acetonitrile. Table 30 provides details for the stock standard preparation.

Table 30. Preparation of Calibration Stock Standard for Prochloraz

Test Substance	Weight of Test Substance	Volume	Concentration	Solvent
Prochloraz	0.1038 grams	50 mL	2076 ug/mL	Acetonitrile

9.6.4.2 Calibration Standards

Calibration standards were prepared so that the calibration solutions were within the calibrated range of the instrument and that the dilutions of the stability solutions were bracketed by calibration standards; Tables 30 and 31 describe how the standards were prepared. Briefly, the stock was pipetted into the volumetric flask and diluted to the mark with 75% acetonitrile:25% DI water and mixed well.

Table 31. Preparation of Calibration Standards for Prochloraz

Standard Name	Test Substance	Stock Standard Concentration	Stock Volume	Final Volume	Concentration	Solvent
Proclz-2B	Prochloraz	2076 ug/mL	11 mL	50 mL	456.72 ug/mL	75% Acetonitrile:25% DI H_2O
Proclz-2C	Prochloraz	2076 ug/mL	5 mL	50 mL	207.6 ug/mL	75% Acetonitrile:25% DI H_2O
Proclz-2D	Prochloraz	2076 ug/mL	2 mL	50 mL	83.04 ug/mL	75% Acetonitrile:25% DI H_2O
Proclz-2E	Prochloraz	2076 ug/mL	1 mL	50 mL	41.52 ug/mL	75% Acetonitrile:25% DI H_2O
Proclz-2F	Prochloraz	2076 ug/mL	0.5 mL	50 mL	20.76 ug/mL	75% Acetonitrile:25% DI H_2O

9.6.4.3 Calibration Verification Standards

An independent standard was prepared to verify the calibration standards; Table 32 and 33 provide the information on the preparation.

Table 32. Preparation of Calibration Verification Stock Standard for Prochloraz

Test Substance	Weight of Test Substance	Volume	Concentration	Solvent
Prochloraz	0.1047 grams	50 mL	2094 ug/mL	Acetonitrile

Table 33. Preparation of Calibration Verification Standards for Prochloraz

Standard Name	Test Substance	Stock Standard Concentration	Stock Volume	Final Volume	Concentration	Solvent
Proclz-7	Prochloraz	2094 ug/mL	2 mL	50 mL	83.76 ug/mL	75% Acetonitrile:25% DI H_2O

9.6.4.4 MDL and Spikes

The spike solution was prepared by weighing an aliquot of the prochloraz into a volumetric flask and diluting to the mark with DMSO. The MDL solution was prepared in a similar manner. Table 34 has the details. MDLs and spikes were prepared in a manner that mimics the sampling of the stability solution. The prochloraz spike and MDL solutions were sampled by taking 0.005 mL of the spike or MDL solution, placing it in a 1.8 mL vial with 0.995 mL of 75%acetonitrile:25% DI water, agitating it, and transferring it to an autosampler vial for the HPLC.

A blank was prepared for each dilution described above to test the procedure for contamination. These samples were run on the HPLC as described in section 9.5.1 and Table 13 (HPLC conditions for prochloraz).

Table 34. Preparation of MDL and Spike Solutions for Prochloraz

Test Solution	Test Substance	Weight of Test Substance	Volume	Concentration	Solvent
Spike	Prochloraz	0.3726 grams	10 mL	37.26 mg/mL	DMSO
MDL	Prochloraz	0.198 grams	50 mL	3.96 mg/mL	DMSO

9.6.5 Analysis

Each calibration standard, spike, MDL solution, and blank was transferred to individual autoinjector vials and the vials were sealed. Five spike solutions, 7 MDL solutions, and a blank were prepared. Single injections were made from each vial using the same chromatographic system and parameters determined during method development.

9.6.6 Calculations

The integration of the chromatograms by the chromatography data system (Varian Star Version 4.6 or Agilent G1701AA) was evaluated to assure it was correct in all chromatograms and manually reintegrated, if necessary. A linear or quadratic regression equation was calculated by the software and the internal standard was incorporated when used. The concentration of each spike, MDL, or blank was calculated by the software using the response of the analysis compared to the regression from the calibration. These values were adjusted for the dilution of the solution by the use of a dilution factor in Varian Star's sample table. These concentrations were used to calculate average concentrations, standard deviations, and percent relative standard deviation (RSD) as appropriate for the method verification test being performed.

9.6.7 Results

The methods used for each test substance were evaluated by an R^2 value obtained from the calibration curve, the MDL, determined by the students T times the standard deviation of seven replicate analyses at a low concentration (MDL solution), spike solution recoveries, and a blank. The R^2 value specification was set at $\geq$0.995. The blank result specification was set to be less than 3 times the MDL. Verification of the calibration standards was done by the use of an ICV, a solution made identically to the calibration standards but using a 2nd stock solution. The ICV value was required to be within 10% of the target value for HPLC analyses and 15% for GC analyses.

Table 35. Method Validation

Test Substance	R^2 value, T=0 calibration	MDL (ug/mL)	Blank Value (ug/mL)	Average Spike Recovery	ICV Recovery
Atrazine	1.000000	137.31	0.1270	99.08%	102.15%
Dibenz[a,h]Anthracene	0.999982	7.21	ND	100.67%	98.47%
Dicofol	0.999995	609.71	ND	100.51%	102.81%
Fenarimol	1.000000	271.18	ND	101.80%	104.18%
4-Nonylphenol	0.999977	252.76	ND	98.60%	102.75%
Prochloraz	0.999904	550.38	6.5578	101.80%	103.00%

ND Non-detect

9.6.8 Conclusions

All methods met acceptance criteria of acceptable spike recoveries and MDLs and acceptable blank values. The methods were suitable for the stability study and subsequent formulation analyses for which they were used.

10.0 FORMULATION STABILITY STUDIES

Formulation stability studies were conducted for atrazine, dibenz[a,h]anthracene, dicofol, fenarimol, 4-nonylphenol and prochloraz at the following concentrations: atrazine, 21.32 mg/mL; dibenz[a,h]anthracene, 2.78 mg/mL; dicofol, 36.8 mg/mL; fenarimol, 33.22 mg/mL; 4-nonylphenol, 24.07 mg/mL; and prochloraz, 37.97 mg/mL in DMSO. Stability was tested for 14 days in sealed, amber glass bottles. Atrazine, dibenz[a,h]anthracene, prochloraz and fenarimol were stored at room temperature, while dicofol and 4-nonly phenol were stored at ~5° Celsius.

10.1 STUDY DESIGN

A sample was analyzed on the day of preparation (Day 0) and Day 14. Day 0 sample analyses were used to verify solubility. Note; atrazine, fenarimol and dibenz[a,h] anthracene day 14 samples were analyzed the day after sampling due to equipment failure.

10.2 FORMULATION METHOD

Formulations were prepared on Day 0 of the storage stability study at the nominal concentrations, and shown in Table 36. The materials were weighed into 10 mL volumetric flasks, except dicofol, and diluted to volume with DMSO. Dicofol, due to the high cost and limited quantities, was weighed into a 1.8 mL vial and 1.0 mL of DMSO was pipetted into the vial using a volumetric pipette. The flasks and vial were sealed, then sonicated, and agitated to dissolve and mix the test substances. Due to limited solubility, atrazine in the DMSO was heated slightly on a hot plate to promote dissolution. The flask contents were then individually poured into 30 mL amber bottles, except dicofol, which was kept in the original container, and sealed. Samples for dicofol and 4-nonylphenol were stored at ~5° Celsius. Samples for atrazine, dibenz[a,h]anthracene, fenarimol, and prochloraz were stored at room temperature and not refrigerated; see deviation EDSP.416-01-D2.

Table 36. Formulation Preparations

Test Substance	Weight of Test Substance	Volumetric flask size, (Dicofol used a volumetric pipette for addition of DMSO)	Nominal concentration in mg/mL
Atrazine	0.2132 grams	10 mL	21.32
Dibenz[a,h]Anthracene	0.0278 grams	10 mL	2.78
Dicofol	0.0368 grams	1 mL	36.8
Fenarimol	0.3322 grams	10 mL	33.22
4-Nonylphenol	0.2407 grams	10 mL	24.07
Prochloraz	0.3797 grams	10 mL	37.97

Prior to storage on day 0, triplicate aliquots were removed from each container for analysis. For the day 14 analysis time, the bottles were removed from the refrigerator, if applicable, and allowed to come to room temperature prior to sampling in triplicate.

10.3 ANALYSIS METHOD

Atrazine, dibenz[a,h]anthracene, fenarimol, dicofol and 4-nonylphenol were analyzed by GC-FID. Briefly, an aliquot of each stability solution was removed from the stability container and placed into a appropriate sized container with an aliquot of the internal standard, and diluted with methylene chloride. For atrazine, fenarimol, and dicofol, 0.005 mL of the stability solution was employed along with 0.1 mL of the internal standard solution. These volumes were individually placed into 5 mL volumetric flasks and filled to the mark with methylene chloride. For dibenz[a,h]anthracene, 0.025 mL of the stability solution was added to a 1.8 mL vial along with 0.02 mL of the internal standard and 0.955 mL of methylene chloride. For 4-nonylphenol, 0.005 mL of the stability solution was added to a 1.8 mL vial along with 0.02 mL of the internal standard and 0.975 mL of methylene chloride. Prochloraz was analyzed by HPLC, UV/Vis employing 0.005 mL of the stability solution in a 1.8 mL vial combined with 0.995 mL of 75% acetonitrile:25% water solution. Solutions were analyzed as described in the methods section above.

10.4 RESULTS

The results from the storage stability study are shown in Table 37 and presented in chart format in Figure 2. All stability solutions remained within the target of less than 10% degradation over the term of the study. The time 0 sampling for dicofol, while acceptable, was lower than seen in the other chemicals; this chemical is very sensitive to the conditions of the GC. A new injection port liner was used to improve the recovery. Fenarimol also had a time 0 recovery slightly less than was seen in the other test substances; this may also have been due to the condition of the injection port.

Table 37. Formulation Storage Stability Results

Test Substance	Average day 0 results (ug/mL)	Average day 14 results (ug/mL)	Recovery from Nominal, day 0, %	Recovery from Nominal, day 14, %	Nominal value (ug/mL)
Atrazine	20306	20382	95.24%	95.60%	21320
Dibenz[a,h]Anthracene	2794	2801	100.52%	100.74%	2780
Dicofol	33723	37969	91.64%	103.18%	36800
Fenarimol	31187	34657	93.88%	104.32%	33220
4-Nonylphenol	24663	23065	102.46%	95.82%	24070
Prochloraz	37487	39212	98.73%	103.27%	37970

10.5 DISCUSSION AND CONCLUSIONS

The concentration of the samples stored for 14 days for all chemicals was within the target limit of 10% regardless of storage conditions. These data indicate the formulations were stable for 14 days (2 weeks).

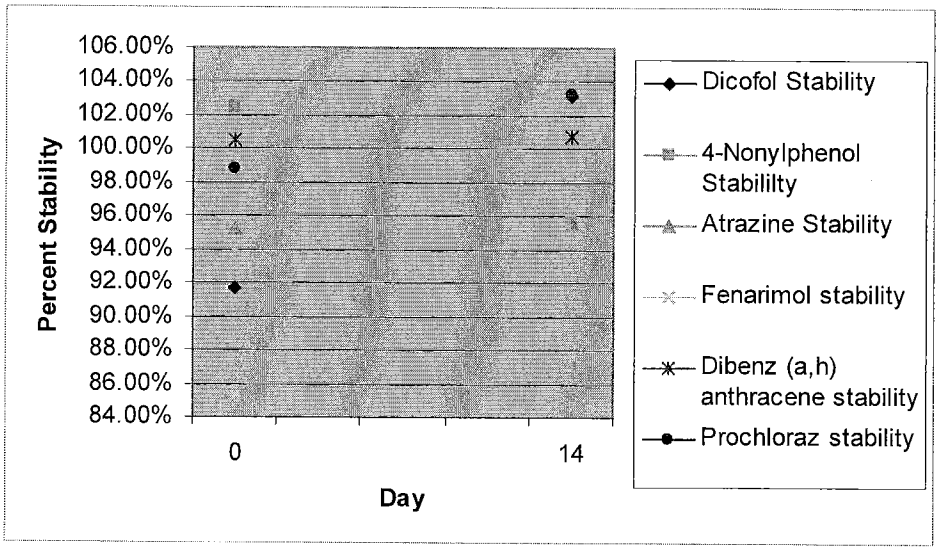

Figure 2. Two Week Stability Data

11.0 STABILITY SAMPLE PREPARATION

The stability sample for prochloraz was prepared and analyzed on 2-16-05 and 3-2-05 according to method No. EDSP.H4-023, *Analysis of prochloraz in dimethylsulfoxide using HPLC with UV/Vis detection.* Stability samples were prepared and analyzed on 2-8-05 and reinjected on 2-23-05 (15 days) for atrazine, dibenz[a,h]anthracene and fenarimol, according to method No. EDSP.G2-022, *Analysis of atrazine, dibenz[a,h]anthracene and fenarimol in dimethyl sulfoxide using GC with FID detection.* The stability sample for dicofol was prepared and analyzed on 3-4-05 and 3-18-05 according to method No. EDSP.G2-025, *Analysis of dicofol in dimethyl sulfoxide using GC with FID detection*. The stability samples for 4-nonylphenol was prepared and analyzed on 2-17-05 and 3-3-05 according to method No. EDSP.G2-024, *Analysis of 4-nonylphenol (mix of isomers) in dimethyl sulfoxide using GC with FID and MS detection.* This section describes the method, results, and conclusions.

11.1 ANALYSIS

Auto injector vials were filled with aliquots of each standard, blank and sample. A single injection was made from each vial using the GC or HPLC conditions from the validation. Representative chromatograms of the standards, blank with internal standard if applicable, and a blank are shown in Figures 3, 4, 5, and 6.

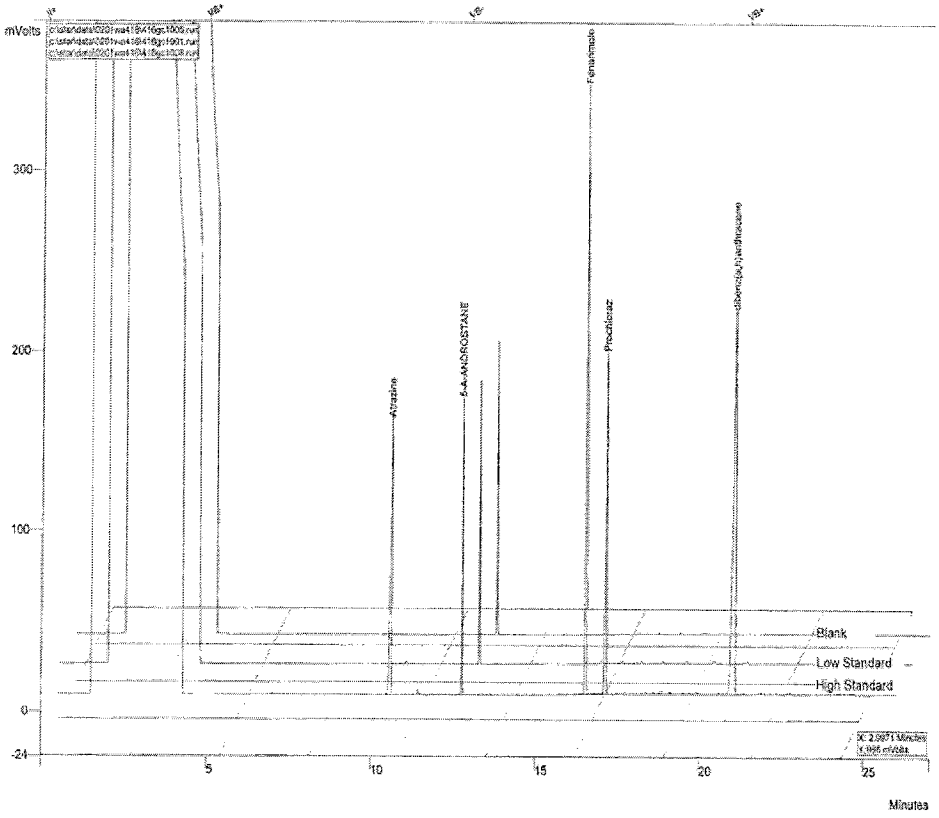

Figure 3. Calibration Standards and Blank for Atrazine, Fenarimol and Dibenz[a,h]Anthracene, (internal standard 5-α-Androstane also shown)

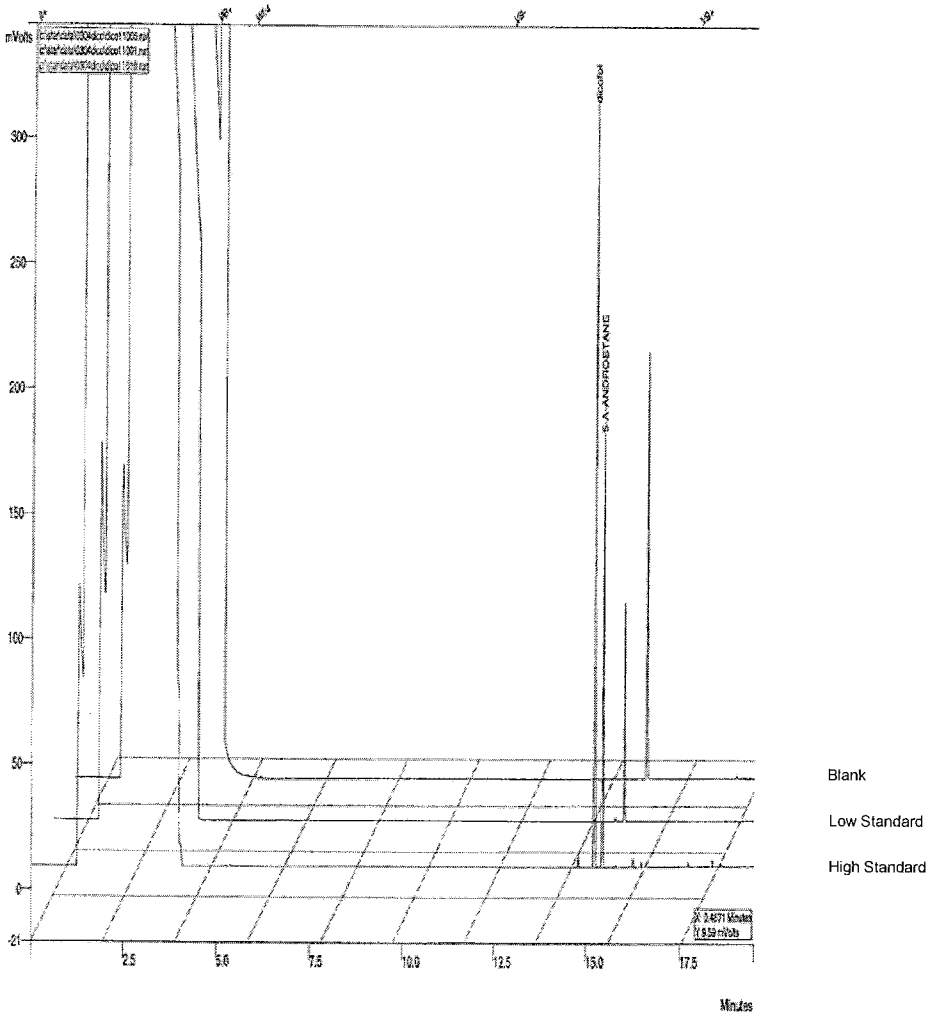

Figure 4. Chromatograms for Dicofol with internal standard 5-α-Androstane

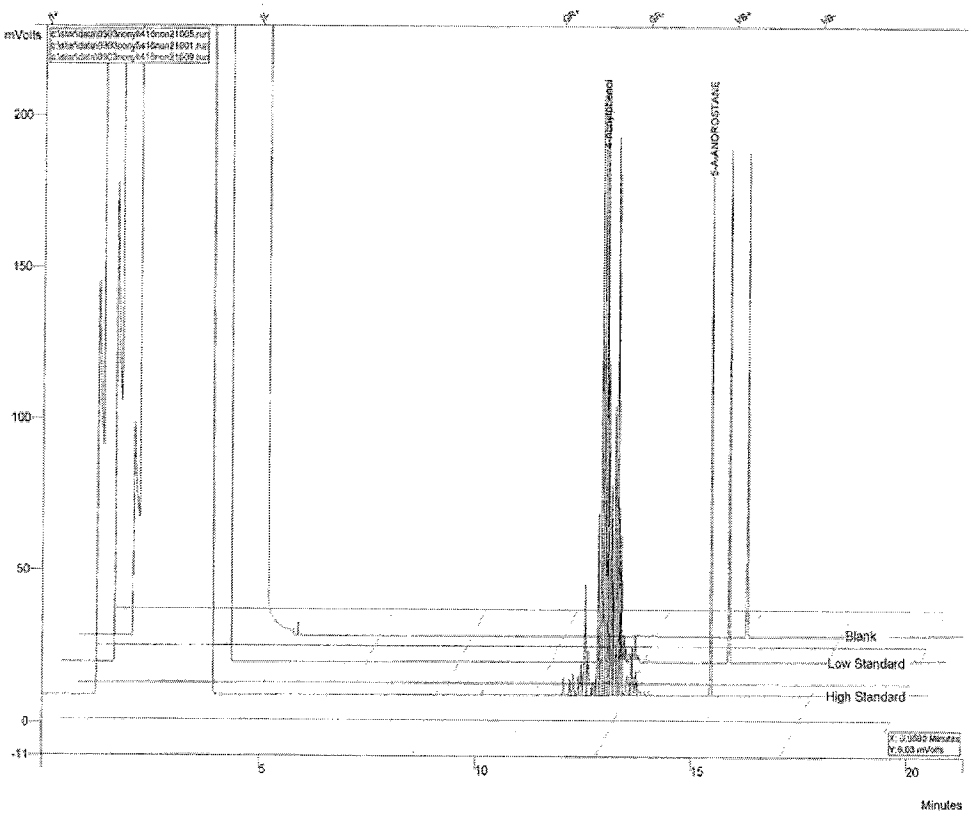

Figure 5. Chromatograms for 4-Nonylphenol, includes internal standard 5-α-Androstane

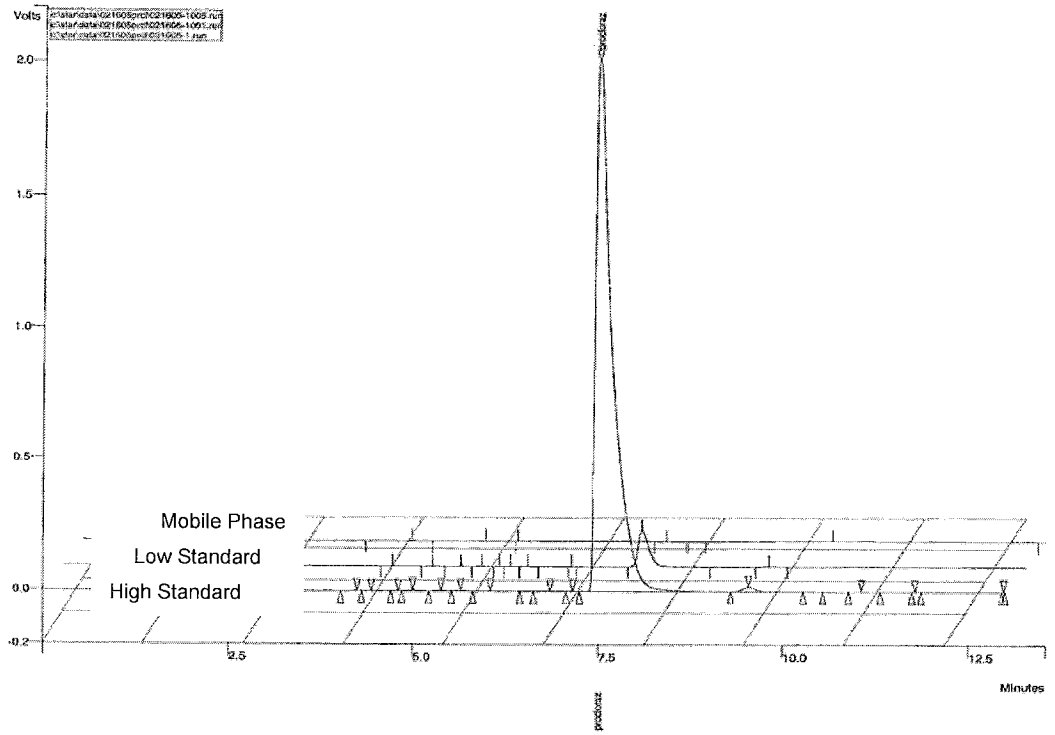

Figure 6. Chromatograms for Prochloraz

11.2 CALCULATIONS

The peaks for each test substance and when used, the internal standard (IS), were integrated for each injection by the chromatography data system. Any peak with improper integration was manually reintegrated. A regression equation was calculated by the software relating the response of the test substance to the concentration of the calibration standards. If applicable, the internal standard was incorporated into response factor calculations. This regression equation and the response ratios were used to calculate the concentration in each quality control and formulation sample. The percent recovery for each quality control sample was calculated by dividing the determined value by the nominal value, and then multiplying by 100. The percent recovery for each formulation sample was calculated by dividing the determined value by the target value and then multiplying by 100. The average determined concentration and relative standard deviation were calculated for the quality control and formulation samples when applicable.

11.3 RESULTS

The results of the stability sample analyses are shown in Table 37. The standard curves and the results of the regression analyses are shown in Figures 7 through 12. The stability sample formulations met acceptance criteria (within 10% of target).

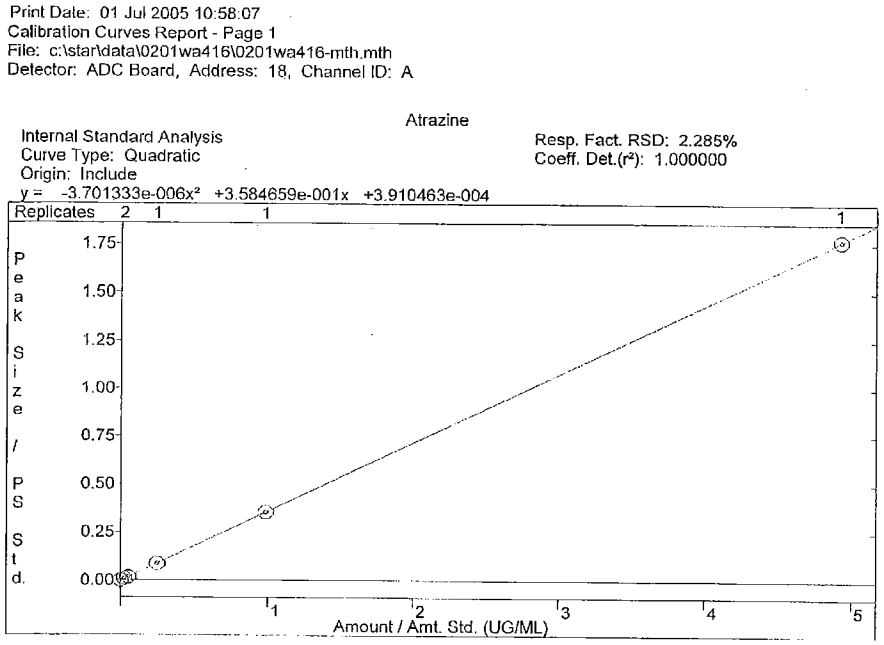

Figure 7. Atrazine Calibration Curve

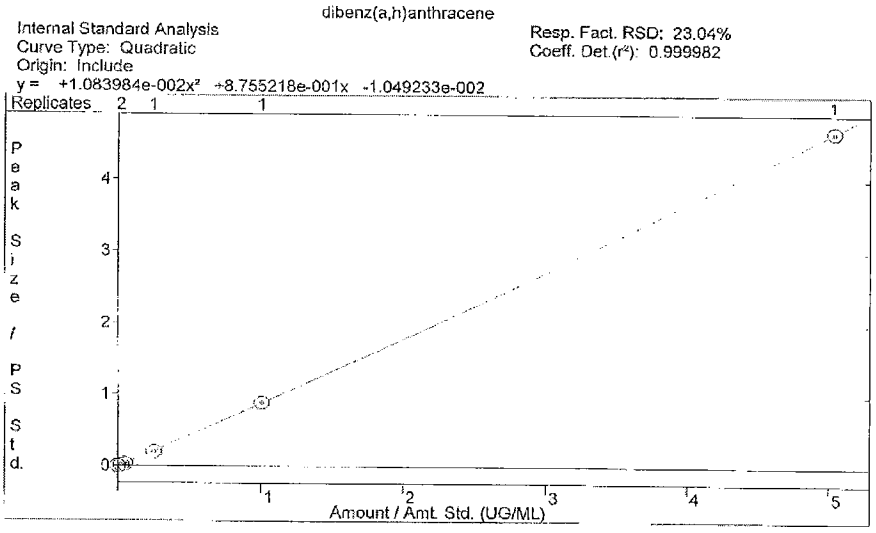

Figure 8. Dibenz[a,h]Anthracene Calibration Curve

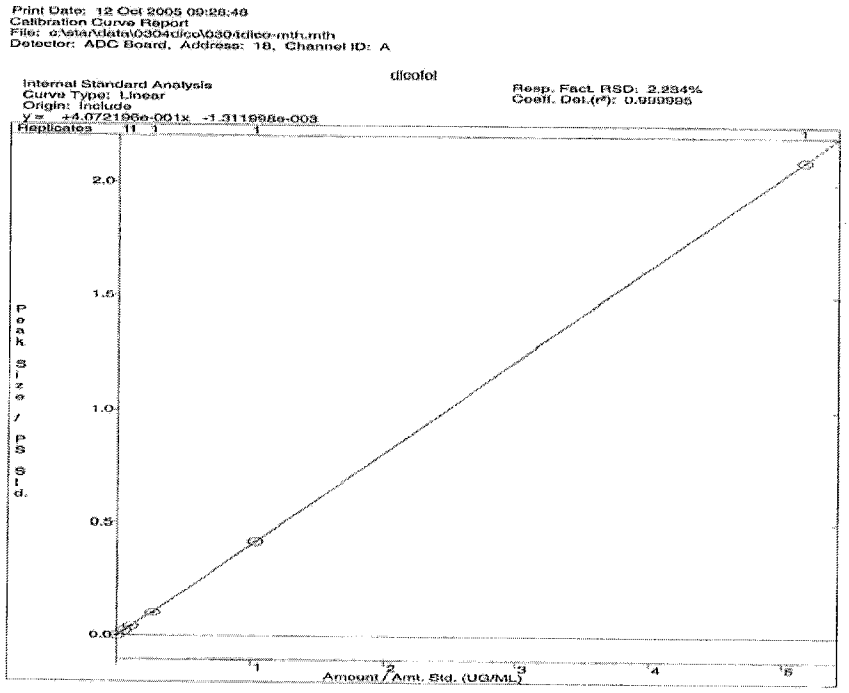

Figure 9. Dicofol Calibration Curve

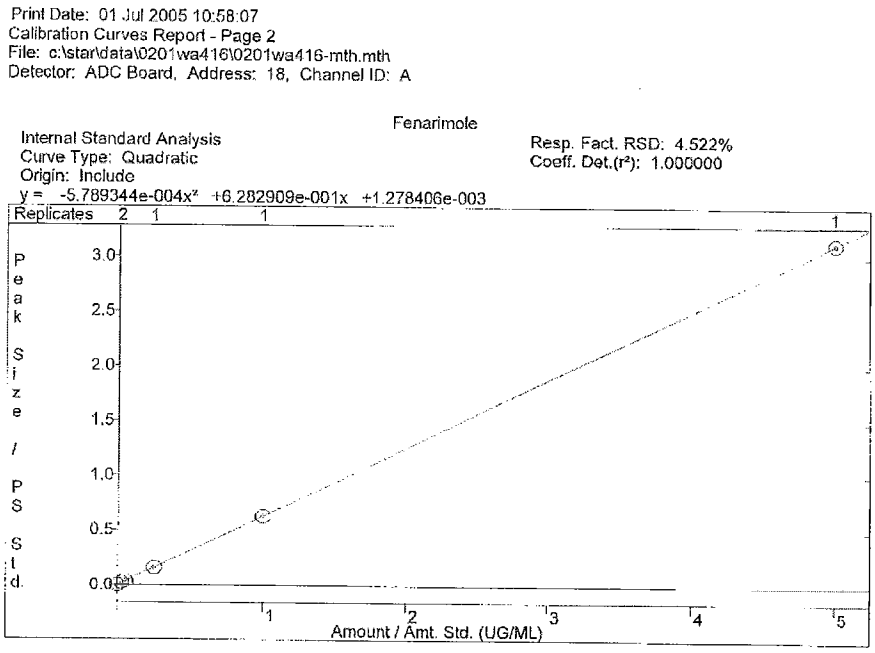

Figure 10. Fenarimol Calibration Curve

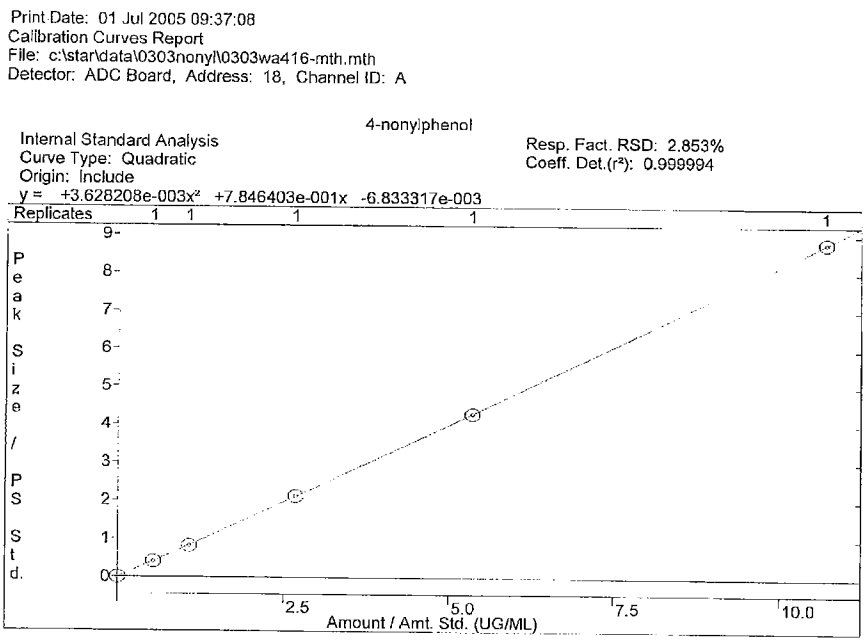

Figure 11. 4-Nonylphenol Calibration Curve

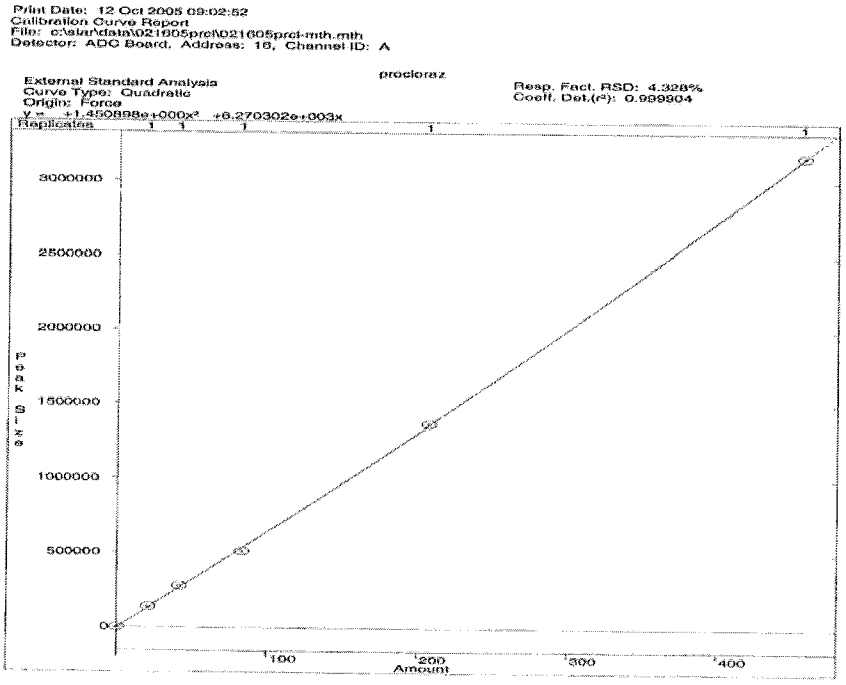

Figure 12. Prochloraz Calibration Curve

11.4 CONCLUSIONS

The average concentration of the stock formulations and their percent relative standard deviation were within acceptance criteria. Therefore these materials are suitable for use with the Environmental Protection Agency (EPA) Placental and Recombinant Aromatase Assay Validation Work, Work Assignment 4-16/17.

12.0 ARCHIVING

Archive samples of the test substances employed in this study will be maintained in the EDSP Chemical Repository for the shelf life indicated on the chemical label.

The protocol, any amendments, all records and the final report generated as a result of this study will be transported to and maintained for archival purposes at the following address:

PNNL Records Management
540 Fifth Street
Richland, WA 99352
PH: 509.375.2340

APPENDIX A

Study Protocol: Purity and Stability of Test Substances for Work Assignment 4-16 and 4-17, EDSP Study Number: EDSP.416-01 (includes Amendments 1-3)

Study Protocol:
Analysis of Test Substances for Work Assignments 4-16 and 4-17
EDSP Study Number: EDSP.416-01

Study Objective:

The following tasks will be carried out for the (6) six test *Chemicals* identified in Table 1:

1. Demonstrate a viable formulation for each of the *Chemicals*, at the *Stock Solution Concentrations* listed in Table 2, in the specified carrier (100% DMSO).
2. Develop and validate an analytical method for each of the Chemicals over the concentration range needed to measure the target stock concentration.
3. Determine the stability of the *Chemicals* dissolved in 100% DMSO (at the concentrations specified in Table 2), over a 14 day period.
4. Provide a report documenting the results on the above studies.

This study is in support of EPA contract number 68-W-01-023, MSL Work Assignment Number 4-16, *Placental Aromatase Validation Study* and MSL Work Assignment Number 4-17, *Recombinant Aromatase Validation Study*

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Address of Sponsor's Representative

Battelle
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Ph: (614) 424-3564
FAX (614) 458-3564
Email: houchensd@battelle.org

TABLE 1
Test Substance Abbreviations:

Chemical	Abbreviation
Atrazine	Atrz
Dibenz [A,H] Anthracene	DiBzAnt
Dicofol	Dicofol
4-Nonylphenol	4-NonF
Fenarimol	Fenrl
Prochloraz	Procl z

TABLE 2
Test Substance Specifications:

Chemical Name	Atrz	DiBzAnt	Dicofol	Fenrl	4-NonF	Proclz
Manufacturer	Supelco/Chem Service	Sigma	Sigma/Riedel	Supelco/Chem Service	Acros Organics	Sigma/Riedel
CAS #	1912-24-9	53-70-3	115-32-2	60168-88-9	84852-15-3	67747-09-5
Lot #	328-137A	11613BC	4076X	LB301-123B	A0192712	2226X
Purity req.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 96.5% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.
Mfg. Purity Claim	98%	97%	96.5%	99%	98.5%	99.5%
Target Concentration Stock Solution	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Duration Stability Study	14 days	14 days	14 days	14 days	14 days	14 days
Concentrations for Stability Study	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Carrier (Vehicle)	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO
Analytical Method	TBA	TBA	TBA	TBA	TBA	TBA

TBA = To Be Amended

Proposed experimental start and termination dates:

Start Date – January 15, 2005
Termination Date – March 1, 2005

Definitions:

Test Substance: The Test Substances are the 6 chemicals listed in Table 2 above. The Test Substances are the subject chemicals of the studies described in this protocol.

Reference Substance: The Reference Substances are identical chemicals to the Test Substances and may be from the same manufacturer and lot, or purchased as different lots and/or possibly from separate manufacturers than the Test Substances. The source, purity, and lot number of reference substances will be documented in the data and reported. Regardless of the source, the Reference Substance solutions will be made up separately from the Test Substance solutions. The Reference Substances are used for the calibration standards in the analytical methods referenced in Table 2 above. A reference substance can also be a material used to facilitate the analysis of the test substance, such as, an internal standard.

Experimental Design:

- Substance purity will be verified using HPLC, GC/MS or GC/FID as appropriate, and should be within ±3% of the value provided on the Certificate of Analysis by the

manufacturer. To use substances with values that fall outside this ±3% range or are less than 95% pure, written pre-approval must be secured from the designated EPA work assignment manager.

- Solubility of the substances will be assessed visually in the carrier (100% DMSO) at the stock formulation concentration (see Table 2). The accuracy of attaining the target concentration for the formulations will be verified in triplicate using the analytical methods referenced in Table 2. Acceptable accuracy will be ±10 percent of the target concentration.
- Stability test solutions – Stability testing will be carried out at the stock concentration level (as specified in Table 2) in sealed, amber glass bottles, stored refrigerated at approximately 5°C. A single sample will be analyzed on the day of preparation (Day 0) and after 14 days of storage. Nominal concentrations to be tested in DMSO are delineated in Table 2 but the actual concentrations used for testing will be within ±10 percent of the target concentration.
- Storage and Labeling Requirements of Formulations – Stock formulations will be stored refrigerated (~5°C). Minimally, containers will be labeled with the name of the test substance, the date of preparation, the formulation concentration, and the study number.
- Testing Schedule – Samples will be analyzed the day of collection from the test formulation
- Replicates – 3 aliquots per sample tested at each analysis time point.
- Sampling schedule. – Samples will be collected for analysis at initiation of the stability study (on day of formulation preparation), then on Day 14.
- For details of the analytical methods see the substance specific method cited in Table 2.

Data Analysis:

The stability data for the 14 day data point (average of triplicate determinations) for each of the six chemicals will be compared to the average of the time zero point for each respective chemical (initiation of stability study). Percent variation from the zero point (Day 0) will be used to determine instability. Acceptable stability will be defined as the concentration not degrading more than 10 percent from the Day 0 concentration determination, which will allow the amount of time that can pass to be determined after preparation of a formulation before a formulation prepared at 100% of the target degrades to within 90% of the target.

Acceptance Criteria:

No average 14 day stability value will deviate beyond ±10% of the zero value. The Work Assignment Study Director will be consulted for a recommended course of action for any data found outside the ±10% acceptance range.

Regulatory requirements:

This study will be conducted in compliance with EPA FIFRA Good Laboratory Practices (40CFR, Part 160). An EDSP QA representative will inspect the study at least once while in-progress and will audit the data and final report.

Report:

A final report covering the following information for all 6 chemicals will be issued to the Sponsor Representative (Dr. David Houchens, EDSP Program Manager), who will then forward the report to the testing laboratories:

Title Page

Executive Summary

Table of Contents

Introduction

General Methods
- Chemical Procurement
- Formulation Preparation (Methods)
- Stability Testing Plan Design and Detail
- Analytical Method

Results
- Formulation Analysis
- Analytical Method Validation
- Formulation Stability

Conclusions

Appendix
- Manufacturer's Certificates of Analysis
- Stability Testing Protocol
- List of Protocol Amendments

Analytical Results of Stability Testing

Records to be maintained:

The protocol, any amendments, all records and the final report generated as a result of this study will be transported to and maintained in the EDSP Data Coordination Center for archival purposes at the following address:

EDSP Data Coordination Center
Battelle Columbus Operations (PNL)
505 King Ave
Columbus, OH 43201

Chemical Repository Study Director _____ Michael Cobb — Date 1/10/05

Chemical Repository Manager _____ Eric Crecelius, Ph.D. — Date 1/13/05

Sponsor Representative _____ David Houchens, Ph. D. — Date 1/26/05

PROTOCOL AMENDMENT
STUDY NUMBER: EDSP.416-01
AMENDMENT NUMBER: A-1

ENDOCRINE DISRUPTOR SCREENING PROGRAM AMENDMENT REPORT

STUDY NUMBER: EDSP.416-01				DATE: March 16, 2005	
AMENDMENT NUMBER: A-1				WAL/STUDY DIRECTOR:	
NOTEBOOK NUMBER: N/A				~~Michael Blanton~~/Michael Cobb	
TITLE OF STUDY: Analysis of Test Substances for Work Assignments 4-16 and 4-17				Jerry Johnson Printed name TJ 3-18-05	
QAPP/PROTOCOL ID: Work Assignment 4-16 and 4-17					
AMENDMENT RELATING TO:					
[ ]	QAPP	[ ]	QMP	[x]	Protocol
[ ]	SOP	[ ]	Method		

ORIGINAL DOCUMENT SPECIFICATIONS:

Entries that are earmarked for change in this amendment are presented as bold, underlined, and italicized in ***Georgia Font***

1. Address

Address of Sponsor's Representative:

Battelle ***550*** King Avenue
Columbus, Ohio 43201-2693
Ph: (614) 424-3564
FAX (614) 458-3564
Email: houchensd@battelle.org

2. Amending Table 2 with methods.

TABLE 2
Test Substance Specifications:

Chemical Name	Atrz	DiBzAnt	Dicofol	Fenrl	4-NonΦ	Prociz
Manufacturer	Supelco/Chem Service	Sigma	Sigma/Riedel	Supelco/Chem Service	Acros Organics	Sigma/Riedel
CAS #	1912-24-9	53-70-3	115-32-2	60168-88-9	84852-15-3	67747-09-5
Lot #	328-137A	11613BC	4076X	LB301-123B	A0192712	2226X
Purity req.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 96.5% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.
Mfg. Purity Claim	98%	97%	96.5%	99%	98.5%	99.5%
Target Concentration Stock Solution	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Duration Stability Study	14 days	14 days	14 days	14 days	14 days	14 days
Concentrations for Stability Study	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Carrier (Vehicle)	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO
Analytical Method	***TBA***	***TBA***	***TBA***	***TBA***	***TBA***	***TBA***

TBA = To Be Amended

DATE: March 16, 2005

3. Revision of records archiving location.

The protocol, any amendments, all records and the final report generated as a result of this study will be ***transported to and maintained in the EDSP Data Coordination Center for archival purposes at the following address:***

EDSP Data Coordination Center
Battelle Columbus Operations (PNL)
505 King Ave
Columbus, OH 43201

AMENDMENTS:

1. Address

Address of Sponsor's Representative:

Battelle 505 King Avenue
Columbus, Ohio 43201-2693
Ph: (614) 424-3564
FAX (614) 458-3564
Email: houchensd@battelle.org

2. Table 2

TABLE 2
Test Substance Specifications:

Chemical Name	Atrz	DiBzAnt	Dicofol	Fenrl	4-NonΦ	Proclz
Manufacturer	Supelco/Chem Service	Sigma	Sigma/Riedel	Supelco/Chem Service	Acros Organics	Sigma/Riedel
CAS #	1912-24-9	53-70-3	115-32-2	60168-88-9	84852-15-3	67747-09-5
Lot #	328-137A	11613BC	4076X	LB301-123B	A0192712	2226X
Purity req.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 96.5% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.
Mfg. Purity Claim	98%	97%	96.5%	99%	98.5%	99.5%
Target Concentration Stock Solution	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Duration Stability Study	14 days	14 days	14 days	14 days	14 days	14 days
Concentrations for Stability Study	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Carrier (Vehicle)	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO
Analytical Method	EDSP.G2-020	EDSP.G2-020	EDSP.G2-025	EDSP.G2-020	EDSP.G2-022	EDSP.H4-021

3. Archiving location

The protocol, any amendments, all records and the final report generated as a result of this study will be maintained on a temporary basis by the Chemical Repository Study Director at the Marine

DATE: March 16, 2005

Research Operations facility in Sequim, WA until the study is completed and the records are transferred for archival purposes to:

PNNL Records Management
540 Fifth Street
Richland, WA 99352
PH: 509.375.2340

REASON FOR CHANGE:

1. The street address of the Battelle facility in Columbus was entered incorrectly in the original document.

2. The methods were not completed when the protocol was written – this amendment incorporates the recently documented methods into the protocol.

3. The location of storage for the protocol, amendments, records, and final report was incorrectly identified in the original protocol document.

Approval:

Work Assignment Leader	Jerry D. Johnson	Date 3-25-05
Study Director		Date 4-1-05
EDSP QA Representative	Mary E. Lynn	Date 4/4/05
MSL Laboratory Director	by S. R. Atlas	Date 4/20/05
EDSP Program Management	Dick P. Hamilton	Date 3/28/05
EDSP Battelle QAM	Kimi Pollock	Date 3-18-05

DATE: March 16, 2005

PROTOCOL AMENDMENT
STUDY NUMBER: EDSP.416-01
AMENDMENT NUMBER: A-2

ENDOCRINE DISRUPTOR SCREENING PROGRAM AMENDMENT REPORT

STUDY NUMBER: EDSP.416-01					DATE: May 3, 2005
AMENDMENT NUMBER: A-2					WAL/STUDY DIRECTOR:
NOTEBOOK NUMBER: N/A					Jerry Johnson/Michael Cobb
TITLE OF STUDY: Analysis of Test Substances for Work Assignments 4-16 and 4-17					
QAPP/PROTOCOL ID: Work Assignment 4-16 and 4-17					
AMENDMENT RELATING TO:					
[ ]	QAPP	[ ]	QMP	[x]	Protocol
[ ]	SOP	[ ]	Method		

ORIGINAL DOCUMENT SPECIFICATIONS:

- Substance purity will be verified using HPLC, GC/MS or GC/FID as appropriate, and should be within ±3% of the value provided on the Certificate of Analysis by the manufacturer. To use substances with values that fall outside this ±3% range or are less than 95% pure, written pre-approval must be secured from the designated EPA work assignment manager.

AMENDMENTS:

Delete the experimental section regarding substance purity.

REASON FOR CHANGE:

The protocol was originally prepared as a standard GLP study where purity verification is a routine test parameter. According to our study file, on September 21, 2004, EPA Work Assignment Manager, Gary E. Timm, directed the Battelle Work Assignment Leader (Dr. Jerry Johnson) to carry this study out without the standard purity verification, relying on the stated purity provided by the manufacturer. This parameter was removed from the study objective section and the report section but inadvertently left in the experimental section of the protocol.

Approval:

Work Assignment Leader	Jerry D Johnson	Date 5-6-05
Study Director		Date 8-3-05
EDSP QA Representative	Mary Ellyn	Date 8/3/05
MSL Laboratory Director		Date 8/3/05
EDSP Program Management		Date 5/9/05
EDSP Battelle QAM		Date 5-4-05

cc: Send final approved copies to:
MSL QA Manager
EDSP Battelle QAM

DATE: March 16, 2005

PROTOCOL AMENDMENT
STUDY NUMBER: EDSP.416-01
AMENDMENT NUMBER: A-3

ENDOCRINE DISRUPTOR SCREENING PROGRAM AMENDMENT REPORT

STUDY NUMBER: EDSP.416-01				DATE: August 15, 2005	
AMENDMENT NUMBER: A-3				WAL/STUDY DIRECTOR:	
NOTEBOOK NUMBER: N/A				Jerry Johnson/Michael Cobb	
TITLE OF STUDY: Analysis of Test Substances for Work Assignments 4-16 and 4-17					
QAPP/PROTOCOL ID: Work Assignment 4-16 and 4-17					
AMENDMENT RELATING TO:					
[ ]	QAPP	[ ]	QMP	[x]	Protocol
[ ]	SOP	[ ]	Method		

ORIGINAL DOCUMENT SPECIFICATIONS:

Entries that are earmarked for change in this amendment are presented as bold, underlined, and italicized in ***Georgia Font***

Amending Table 2 with correct method numbers.

TABLE 2
Test Substance Specifications:

Chemical Name	Atrz	DiBzAnt	Dicofol	Fenrl	4-NonΦ	Proclz
Manufacturer	Supelco/Chem Service	Sigma	Sigma/Riedel	Supelco/Chem Service	Acros Organics	Sigma/Riedel
CAS #	1912-24-9	53-70-3	115-32-2	60168-88-9	84852-15-3	67747-09-5
Lot #	328-137A	11613BC	4076X	LB301-123B	A0192712	2226X
Purity req.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 96.5% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.
Mfg. Purity Claim	98%	97%	96.5%	99%	98.5%	99.5%
Target Concentration Stock Solution	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Duration Stability Study	14 days	14 days	14 days	14 days	14 days	14 days
Concentrations for Stability Study	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Carrier (Vehicle)	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO
Analytical Method	***EDSP.G2-020***	***EDSP.G2-020***	EDSP.G2-025	***EDSP.G2-020***	***EDSP.G2-022***	***EDSP.H4-021***

DATE: March 16, 2005

PROTOCOL AMENDMENT
STUDY NUMBER: EDSP.416-01
AMENDMENT NUMBER: A-3

AMENDMENT:

Table 2

TABLE 2
Test Substance Specifications:

Chemical Name	Atrz	DiBzAnt	Dicofol	Fenrl	4-NonΦ	Proclz
Manufacturer	Supelco/Chem Service	Sigma	Sigma/Riedel	Supelco/Chem Service	Acros Organics	Sigma/Riedel
CAS #	1912-24-9	53-70-3	115-32-2	60168-88-9	84852-15-3	67747-09-5
Lot #	328-137A	11613BC	4076X	LB301-123B	A0192712	2226X
Purity req.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 96.5% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.
Mfg. Purity Claim	98%	97%	96.5%	99%	98.5%	99.5%
Target Concentration Stock Solution	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Duration Stability Study	14 days	14 days	14 days	14 days	14 days	14 days
Concentrations for Stability Study	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Carrier (Vehicle)	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO
Analytical Method	EDSP.G2-022	EDSP.G2-022	EDSP.G2-025	EDSP.G2-022	EDSP.G2-024	EDSP.H4-023

REASON FOR CHANGE:

1. The methods were incorrectly identified by method number when entered into Table 2 when generating amendment 1 to the protocol – this current amendment incorporates the correct methods numbers into the protocol.

Approval:

Work Assignment Leader _____ Date 8-16-05

Study Director _____ Date 8-16-05

EDSP QA Representative _____ Date 8/19/05

MSL Laboratory Director _____ Date 8/19/05

EDSP Program Management _____ Date 8-16-05

EDSP Battelle QAM _____ Date 8-16-05

cc: Send final approved copies to:
MSL QA Manager
EDSP Battelle QAM

DATE: March 16, 2005

APPENDIX B

EDSP.416-01 Protocol Deviations

ENDOCRINE DISRUPTOR SCREENING PROGRAM DEVIATION FORM

STUDY NUMBER: EDSP.416-01	WAL/ CHEMICAL REPOSITORY STUDY DIRECTOR: Jerry Johnson/Michael Cobb for WA 4-16
DEVIATION NUMBER: EDSP.416-01-D1	Date: 07/22/05
TITLE OF STUDY: Analysis of Test Substances for Work Assignments 4-16 and 4-17 EDSP Study Number: EDSP.416-01	Document deviated from: Protocol EDSP.416-01

DEVIATION RELATING TO:

[ ]	QAPP	[ ]	QMP	[X]	Protocol
[ ]	SOP	[ ]	Method	[ ]	Miscellaneous Documentation

ORIGINAL DOCUMENT SPECIFICATIONS:

1. Data Analysis – Protocol EDSP.416-01

Data Analysis:

The stability data for the 14 day data point (average of triplicate determinations) for each of the six chemicals will be compared to the average of the time zero point for each respective chemical (initiation of stability study). Percent variation from the zero point (Day 0) will be used to determine instability. Acceptable stability will be defined as the concentration not degrading more than 10 percent from the Day 0 concentration determination, which will allow the amount of time that can pass to be determined after preparation of a formulation before a formulation prepared at 100% of the target degrades to within 90% of the target.

2. Lot number specified for Fenarimol – Protocol EDSP.416-01

Table 2 indicates the lot of fenarimol employed for the study would be number LB301-123B with a supplier purity claim of 98.5%

TABLE 2
Test Substance Specifications:

Chemical Name	Atrz	DiBzAnt	Dicofol	Fenrl	4-NonF	Proclz
Manufacturer	Supelco/Chem Service	Sigma	Sigma/Riedel	Supelco/Chem Service	Acros Organics	Sigma/Riedel
CAS #	1912-24-9	53-70-3	115-32-2	60168-88-9	84852-15-3	67747-09-5
Lot #	328-137A	11613BC	4076X	LB301-123B	A0192712	2226X
Purity req.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 96.5% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.	≥ 97% per mfg.
Mfg. Purity Claim	98%	97%	96.5%	99%	98.5%	99.5%
Target Concentration Stock Solution	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Duration Stability Study	14 days	14 days	14 days	14 days	14 days	14 days
Concentrations for Stability Study	0.1 M	0.01 M	0.1 M	0.1 M	0.1 M	0.1 M
Carrier (Vehicle)	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO	100% DMSO
Analytical Method	EDSP.G2-020	EDSP.G2-020	EDSP.G2-025	EDSP.G2-020	EDSP.G2-022	EDSP.H4-021

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DEVIATION:

1. The stability data for each data point (average of triplicate determinations) was compared to the nominal concentration of the stability solutions prepared at the beginning of the study. Percent variation from nominal was used to determine stability. Acceptable stability was defined as the concentration not varying more than 10% from the nominal value.
2. The actual lot number for the fenarimol employed in the study was LB325-134C with a supplier purity claim of 99%

REASON/IMPACT:

1. Analyst followed typical method used for monitoring stability in previous studies (stability variation relative to nominal) rather than the original approach described in the protocol.
2. The protocol was written in December and January (2004-2005) when the received the first order of fenarimol. The fenarimol received was lot number LB301-123B, but it was a partial shipment with the remainder of the shipment on backorder. When the backordered material arrived in January, it was from a second lot, number LB325-134C. The material from lot LB301-123B was returned for replacement with a single lot number, but the protocol was not amended with the change.

PROPOSED CORRECTIVE ACTION AND SCHEDULE FOR COMPLETION:

None, beyond this documentation.

ACTIONS TO PREVENT RECURRENCE:

1. Insure clarity with analysts on precise contents of protocol prior to start of study and track that the protocol is followed throughout the study.
2. Implement appropriate protocol amendments on a timely basis.

Approval:

Work Assignment Leader Jerry Johnson, Ph.D., DABT		Date 7-28-05
Study Director Michael Cobb		Date 8-4-05
EDSP QA Representative Mary Lynn	Mary E Lynn	Date 8/15/05
MSL Laboratory Director Dick Ecker	RM Ecker	Date 8/16/05
EDSP Program Management David Houchens, Ph.D.	David P. Houchens	Date 8/2/05
EDSP Battelle QAM Terri Pollock	Terri L Pollock	Date 7-28-05

cc: Send final approved copies to: MSL QA Manager, EDSP Battelle QAM

2

ENDOCRINE DISRUPTOR SCREENING PROGRAM DEVIATION FORM

STUDY NUMBER: EDSP.416-01				EDSP Chemical Repository Work Assignment Leader/Study Director : Jerry Johnson/Michael Cobb	
DEVIATION NUMBER: EDSP.4-416-01-D2				Date: 10/12/05	
TITLE OF Protocol: Analysis of Test Substances for Work Assignment 4-16 and 4-17				Document deviated from: Protocol EDSP.416-01	
DEVIATION RELATING TO:					
[ ]	QAPP	[ ]	QMP	[X]	Protocol
[ ]	SOP	[ ]	Method	[ ]	Miscellaneous Documentation

ORIGINAL DOCUMENT SPECIFICATIONS

Experimental Design

Third bullet point under the Experimental Design section of the protocol: Stability test solutions – Stability testing will be carried out at the stock concentration level (as specified in Table 2) in sealed, amber glass bottles, stored refrigerated at approximately 5°C. A single sample will be analyzed on the day of preparation (Day 0) and after 14 days of storage.

DEVIATION

1. Stability samples for atrazine, dibenz [a,h] anthracene, fenarimol and prochloraz were stored at room temperature and not refrigerated at approximately 5°C.
2. One set of samples was analyzed the day following sample collection.

REASON/IMPACT

1. Technician error in setting up the study and incorrect storage conditions for the stability samples for the aforementioned chemicals. Room temperature storage is generally considered a more rigorous test of stability than refrigerated storage. The fact that the samples were stable at room temperature suggests that samples stored refrigerated would minimally be as stable. No negative impact.

2. Samples generally are set up on an autosampler and allowed to run unattended. In this instance the run was set up to run overnight, upon inspection the following morning, it was noted that the run had failed due to a CCV outside acceptable limits. Using the same set of samples, maintenance was performed and the GC was restarted and the run was allowed to complete. All QC requirements were met in the 2nd run and the stability samples were within the allowable window. No impact to the data.

PROPOSED CORRECTIVE ACTION AND SCHEDULE FOR COMPLETION:

None beyond this documentation.

ACTIONS TO PREVENT RECURRENCE:

1. A semi-annual currency training session for all Chemical Repository (CR) personnel will be scheduled by the CR Study Director. During this training, all SOPs relevant to the trainee will be reviewed.

2. The GC system periodically malfunctions or is in need of unscheduled maintenance. Although the incident of these occurrences is rare, it is the reason that QA/QC samples are run with the analysis. If instrument malfunction reoccurs, a deviation will be written.

Approval:

Work Assignment Leader
Jerry Johnson, Ph.D., DABT — Date 10-14-05

Study Director
Michael Cobb — Date 10-21-05

EDSP QA Representative
Mary Lynn — Date 10/21/05

MSL Laboratory Director
Dick Ecker — Date 21 OCT 05

EDSP Program Management
David Houchens, Ph.D. — Date 10/18/05

EDSP Battelle QAM
Terri Pollock — Date 10-14-05

cc: Send final approved copies to: MSL QA Manager, EDSP Battelle QAM

ENDOCRINE DISRUPTOR SCREENING PROGRAM DEVIATION FORM

STUDY NUMBER: EDSP.416-01				EDSP Chemical Repository Work Assignment Leader/Study Director : Jerry Johnson/Michael Cobb	
DEVIATION NUMBER: EDSP.416-01-D3				Date: 12/02/05	
TITLE OF Protocol: Analysis of Test Substances for Work Assignment 4-16 and 4-17				Document deviated from: Protocol EDSP.416-01	
DEVIATION RELATING TO:					
[ ]	QAPP	[ ]	QMP	[X]	Protocol
[ ]	SOP	[ ]	Method	[ ]	Miscellaneous Documentation

ORIGINAL DOCUMENT SPECIFICATIONS

1. Protocol Deviation EDSP.416-01-D1 stated that: "The actual lot number for the fenarimol employed in the study was LB325-134C with a supplier purity claim of 99%."
2. Under the Experimental Design section, the protocol stated:
   - Substance purity will be verified using HPLC, GC/MS or GC/FID as appropriate, and should be within ±3% of the value provided on the Certificate of Analysis by the manufacturer. To use substances with values that fall outside this ±3% range or are less than 95% pure, written pre-approval must be secured from the designated EPA work assignment manager.

   Protocol Amendment A-2 states: "Delete the experimental section regarding substance purity."

DEVIATION

1. Protocol Deviation EDSP.416-01-D1 incorrectly identified the fenarimol lot number. The correct lot number is 325-134C.
2. The Amendment indicating that the purity determinations should be deleted from the protocol instructions was written after the purity determinations were already carried out. The stability/purity report will include purity results.

REASON/IMPACT

1. The LB letters used in front of the lot number in the deviation were based on their use as part of the lot number in correspondence from the supplier, but are not included on the test substance bottle or on the certificate of analysis. No negative impact.

2. The Work Assignment Leader indicated in a communication prior to start of the study that purity determination would not be required. The Work Assignment Leader was not a required signature on the protocol, and the purity determination instructions were inadvertently included in the protocol per standard GLP requirements. The purity determinations were carried out as specified in the protocol at the time of the analysis. No impact on validity of study.

PROPOSED CORRECTIVE ACTION AND SCHEDULE FOR COMPLETION:

None

EDSP Protocol Deviation Number EDSP.416-01-D3 Page 2 of 2

ACTIONS TO PREVENT RECURRENCE:

The work assignment leader will receive a copy of all protocols for review prior to approvals.

Approval:

Work Assignment Leader Jerry Johnson, Ph.D., DABT	Jerry D. Johnson	Date 12-2-05
Study Director Michael Cobb		Date 12-12-05
EDSP QA Representative Mary Lynn	Mary Lynn	Date 12/8/05
MSL Laboratory Director Dick Ecker	R M Ecker	Date 12/8/05
EDSP Program Management David Houchens, Ph.D.	Dil F Houchens	Date 12/3/05
EDSP Battelle QAM Terri Pollock	Terri Y Pollock	Date 12-2-05

cc: Send final approved copies to: MSL QA Manager, EDSP Battelle QAM

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APPENDIX C
EDSP .416-01 Analytical Methods

Four method documents are included for analysis of the test substances shown in Appendix E:

Test Substance	Method
Atrazine	EDSP.G2-022-00
Dibenz[a,h]Anthracene	EDSP.G2-022-00
Fenarimol	EDSP.G2-022-00
Prochloraz	EDSP.H4-023-00
4-Nonylphenol	EDSP.G2-024-00
Dicofol	EDSP.G2-025-00

These documents were the methods originally developed, validated, and utilized within the Chemical Repository for the generation of the study results documented in this report.

NOTE: Since all 4 methods utilize the same 6 data collection forms, for this document, the forms are only provided at the end of the first method to minimize duplication.

Battelle
The Business of Innovation

Marine Sciences Laboratory

EFFECTIVE DATE: 2-7-05

Method # EDSP.G2-022-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

ANALYSIS OF ATRAZENE, DIBENZ (A,H) ANTHRACENE AND FENARIMOLE IN DIMETHYL SULFOXIDE USING GC WITH FID DETECTION

Approvals:		
AUTHOR: Tim Fortman	Tim Fortman	2-7-05
	Signature	Date
TECHNICAL REVIEWER: Rebecca Wood	Rebecca Wood	2/7/05
	Signature	Date
CHEMICAL REPOSITORY DIRECTOR: Michael Cobb		2/7/05
	Signature	Date

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ANALYSIS OF ATRAZENE, DIBENZ (A,H) ANTHRACENE AND FENARIMOLE IN DIMETHYL SULFOXIDE USING GC WITH FID DETECTION

1.0 SCOPE AND APPLICATION

This method describes the determination of Atrazine (CAS# 1912-24-9), Dibenz (a,h) Anthracine (CAS# 53-70-3) and Fenarimole (CAS# 60168-88-9) in Dimethyl sulfoxide using GC with FID detection. The method was developed for use in the analysis of Atrazine, Dibenz (a,h) Anthracine and Fenarimole for the EDSP program.

2.0 DEFINITIONS

Initial Calibration Verification (ICV)	A standard made from a neat material different from the material used to make the calibration standards. Used to verify the calibration solutions. If none are available, a 2nd standard from the same neat material is acceptable.
Continuing Calibration Verification (CCV)	A mid level calibration standard run after every 10 samples to ensure the instrument is in calibration.
Internal Standard (IS)	A compound in solution added to every sample to adjust for variation (the internal standard is also in the calibration solutions).

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation.
Analyst - analysis, calculations
QA Manager or Representative - data verification

4.0 ANALYSIS

4.1 Hardware and Reagents

- Balance capable of weighing to 0.0001 g
- Gas chromatograph (Agilent 5890 or equivalent) with an FID (Flame ionization detector) and an autosampler.
- J+W DB-5 30m X 0.25 mm X 0.25 um film thickness GC column or equivalent.
- Dichloromethane, GC-MS grade or better.
- Atrazine (CAS# 1912-24-9), Dibenz (a,h) Anthracine (CAS# 53-70-3), Fenarimol (CAS# 60168-88-9) and 5a Androstane, (CAS# 438-22-2) 98% purity or better.
- 1.8 ml autosampler vials
- 25, 50 and 100 ml amber glass bottles.
- Helium for carrier gas, Hydrogen, Breathing Air and Nitrogen for FID flame.
- Software for collection of data from FID detector, Varian Star Software, vers. 6.2.
- Variable positive displacement Pipettors, to pipette 0.1 ml and 0.010 ml.
- Varity of volumetric flasks.
- ~~Volumetric flasks~~
- Dimethyl Sulfoxide

4.2 GC conditions

4.2.1 The GC should be set up as per manufacturer specifications, for the system presently in use, the carrier gas pressure is set to 18 PSI, split vent at 89 ml/min, septum purge set at 6.9 ml/min. The FID (flame ionization detector) gasses are set at; air, 300 ml/min, hydrogen, 46 ml/min and nitrogen at 43 ml/min.

4.3 Calibration Solution

4.3.1 A 5 point curve is used to calibrate the GC over a range that will bracket the concentration in the stability tests. To start a stock of all chemicals to be included in the calibration are made at a concentration of about 1000 ug/ml. ~0.05 grams is weighed into a 50 ml volumetric flask and diluted to the mark with dichloromethane. Record exact information on the organic standard preparation form (attachment 1) and give the solution a unique identifying label. Pour the solution into an appropriate size amber vial with a Teflon lined lid. Stability of the calibration solutions should be verified at the end of the test, by the analysis of a new (freshly made) solution made from the neat material and compared to the calibration solutions.

4.3.2 An internal standard is incorporated with this analysis. The chemical 5 a Androstane is used as the internal standard and a ~1000 ug/ml solution is made as described in 4.3.1.

4.3.2 Serially dilute the solution made in 4.3.1 to make standards ranging from 0.5 ug/ml to 100 ug/ml. The internal standard should be the same concentration in all the standards, for this series of solutions, 50 and 100 ml. volumes are used for each, so 1 ml of the 1000 ug/ml internal standard solution is added to the 50 ml vol. flask and 2 ml to the 100 ml vol. flask for a target concentration of 20 ug/ml. This

calibration targets these concentrations; 0.5 ug/ml, 1 ug/ml, 5 ug/ml, 20 ug/ml and 100 ug/ml. Include all information in the standards preparation form (see attachment 1).

4.4 GC Setup

4.4.1 The GC equipment has the following components, an autosampler, GC oven, detector (FID) and a data collection system. The autosampler is set to inject 1 ul and the injection port is set to 285 deg. C. The oven conditions are; initial temp. 100 deg. C., hold for 1.5 min., ramp at 15 deg. per minute to 320 deg. and hold for 10 min. The FID gasses are described in section 4.2.1, the detector temp. is set at 320 deg. C.

4.4.2 The GC is set in the splitless mode and the split vent valve is opened at 2.5 minutes. Split vent and septum purge settings are listed in section 4.2.1.

4.4.3 The column used is a J+W DB-5 30M X 0.25 mm X 0.25 um film thickness. Flow thru the column is controlled by the head pressure which is set at 18 PSI (see section 4.2.1).

4.4.4 The FID detector conditions are described in section 4.2.1. The conditions on the present system are controlled by valves set up for the detector, the supply gasses are set up as follows, Hydrogen pressure, 21 PSI, Air pressure, 58 PSI and nitrogen pressure 34 PSI.

4.4.5 The data system used is Varian's Star software, version 6.2. The system is set up to start automatically with the injection of the sample. The run time is set to 25 minutes. Bunch rate is set to 8 and signal to noise set at 100. Different data systems will employ different nomenclature for the same settings, see software manuals for information on how to optimize the software.

4.4.6 The system used is composed of several different components from different manufacturers. The method printouts will only contain information pertaining to the calibration and quantitation of the unknowns, in order to ensure that the conditions used are recorded, an EDSP GC Analysis Form should be filled out (attachment 2). Also, much of this information should be included in the "notes" tab on Varian Star's method.

4.5 Analysis

4.5.2 Prior to the analysis of any samples linearity must be demonstrated. A 5 point curve is run (minimum of a 4 point curve is needed), the 0 point not considered a calibration point. An r^2 value of greater than 0.995 is necessary before analysis can begin.

4.5.3 Once the calibration is done, if possible it must be verified with an initial calibration verification sample (ICV). An independent solution is made using a different lot of the WA 4-16/17 chemicals when available, if not, use of the existing lot is OK. These solutions are diluted to the proper concentration so that it is within the calibration range. This solution is run and the value obtained should be within 15% of the expected value.

4.5.4 After the calibration is verified, a continuing calibration verification (CCV) sample is run. This sample is usually one of the mid level calibration solutions. The value obtained should be within 15% of the expected value. A CCV should be run after every 10 samples.

~~4.5.5 A blank should be prepared with each sampling. A blank is prepared for each dilution. For example if 0.005 ml of sample is taken and diluted with the~~

typo - repeated on next page MM 11/2/05

4.5.5 A blank should be prepared with each sampling. A blank is prepared for each dilution. For example if 0.005 ml of sample is taken and diluted with the dichloromethane, a blank using 0.005 ml of dimethyl sulfoxide is treated as the sample. The blank should be < 3X MDL.

4.5.6 Method Detection Limit (MDL) is determined by preparing a sample at a low concentration, using similar techniques as used to analyze the stability solution. This is done 7 times and the MDL is the students T (3.143 for 7 replicates) times the standard deviation of the seven replicate runs. An MDL should be performed prior to the analysis of any sample. Samples with no peak or less than the MDL will be reported as the MDL and flagged with a "U.

4.6 Purity

4.6.1 The confirmation of purity is a required component of EDSP studies per the Chemical Repository (CR) QAPP (Ver 2, 01/07/05). Purity analysis will be performed unless the CR is instructed not to do so by the sponsor (EPA) due to technical or budgetary reasons. The Chemical Characterization SOP# MSL-R-009-00 has additional information. Purity is determined by running a solution of the material that is at or near the top of the demonstrated linearity of the system. The solution contains only the chemical of interest, no internal standard or other chemicals, dissolved in dichloromethane. This solution is injected onto the GC and all the peaks in the chromatogram are summarized. The peak corresponding to the chemical of interest is then compared to all the other peaks and the purity is the area of the chemical peak divided by the sum of the total area in the chromatogram (presented as a percentage). A blank dichloromethane is run prior to the purity run and the peaks in the purity run that correlate to peaks in the blank run are eliminated from the calculation (this includes the solvent peak). This purity should be 98% or greater and should compare favorably to the purity from the vendor. See example purity form: attachment 3.

5.0 STABILITY AND FORMULATION VERIFICATION

5.1 Stability for WA 4-16 and WA4-17 chemicals is to run for 14 days. Stability is prepared by weighing an aliquot of each chemical into 10 milliliters of dimethyl sulfoxide and storing the solution in a 30 milliliter amber bottle (attachment 4). The details for the stability are contained in the Study Protocol. Target concentration is 0.1 M, for Atrazine, 21.6 mg/ml, Fenarimole, 33.1 mg/ml and 0.01 M for dibenzo (a,h) anthracine or 2.78 mg/ml. Sampling and analysis will be done 2 times over the course of the stability study.

5.2 Sampling is done by removing an aliquot of the sample and diluting it into dichloromethane with an aliquot of the internal standard. For solutions at 0.1 M, 0.005 ml is added to a 5 ml volumetric flask along with 0.1 ml of the internal standard (1000 ug/ml 5 a androstane) and diluted to mark with dichloromethane. An aliquot of this is placed into a a 1.8 ml autosampler vial for analysis. For the 0.01 M solution, 0.025 ml is placed into a 1.8 mL autosampler vial with 0.02 ml of the internal standard (1000 ug/ml 5a Androstane) and 0.955 mL of dichloromethane. The vials are capped and are ready for GC analysis.

5.3 For an example of the form used for the stability preparation and sampling see attachments ~~4 and~~ 5.

5.4 Samples should be analyzed on the day of sampling, but if this is not possible, samples should be stored at 4 deg. C. until analysis. If samples are not analyzed on the day of sampling, the actual analysis date and storage conditions shall be documented.

5.5 Stability solutions are stored in the same conditions as the neat material. For this study, all are stored at room temperature.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 Prior to analysis of any samples, the instrument is calibrated with a minimum of a 4 point curve. Internal standard calculations will be performed. All calculations will be done using Varian's Star chromatography software, version 6.2. This software allows the input of a multiplier, so that any dilutions will be included with the software calculations. For example, for Atrazine stability, 0.005 ml of the 10 ml sample solution is diluted to 5 ml, a multiplier of 1000 is used so that the output from the software will give values that reflect the concentration in the diluted solution. Calibration curve fits can be set to either linear or non-linear (quadratic fit), past experience indicates that even though the calibration meets linearity criteria, the quantification is improved with a non-linear fit.

6.2 Prior to the tabulation of the data, each chromatogram and report printed by the software will be initialed and dated for GLP compliance.

7.0 QUALITY CONTROL

7.1 A blank is prepared with each sampling, this blank is dimethyl sulfoxide processed identically to the stability solution. If background levels are sufficiently high (i.e., greater than 3 x MDL), this value may be subtracted from the values obtained for samples analyzed with that batch. Processing of these samples is very straight forward, therefore spikes are optional.

7.2 An initial calibration verification (ICV) standard will be analyzed following the calibration curve. This ICV is made from a second source when available, but if a second source is not available, the same source is used. Continuing calibration verification standards (CCVs) will be analyzed after every 10 samples. A CCV is a mid point calibration solution. If CCV variation exceeds a 15% difference from expected, samples will be re-run with acceptable calibration criteria.

7.7 An internal standard is added to each sample, recovery of this internal standard should be ≥80%.

8.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of chemicals, including the following:

- Gloves, protective clothing and safety glasses should be worn when handling samples and chemicals.

9.0 TRAINING REQUIREMENTS

9.1 All staff performing this analysis should first read this procedure and conduct their first analysis under the supervision of a staff member who has had previous experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work. Documentation of training will be performed in accordance with MSL-A-006, Marine Sciences Laboratory Training.

10.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

MSL-Q-007-04 Procedure for Determining Method Detection Limits

Federal Register (CFR Part 136 Appendix B).

Table 1. Summary of Data Quality Objectives and Corrective Actions

Quality Control Sample Type	Data Quality Objective (DQO)	Corrective Action
Procedural Blank one/batch	Less than 3 x MDL	Re-extract and analyze sample batch. If batch can not be re-extracted and analyzed, "B" flag all samples that are in the batch. Investigate sources of blank contamination.
Calibration curve acceptability	r^2 values greater than or equal to 0.995	If r^2 value is outside of criterion, re-analyze calibration standards, if r^2 is still out, perform instrument maintenance and/or remake calibration standards and rerun calibration samples.
Initial calibration verification (ICV) standard; one/batch	+/- 15 % of true value	Re-calibrate. Must meet DQO in order to continue processing samples.
Internal Standard Recovery	+/- 20 % of true value	Re-sample and rerun
Continuing calibration verification standards; one every 10th sample analyzed	+/- 15 % of true value	Re-run CCV, if still not acceptable, re-calibrate and reanalyze affected samples.
Replicate sample precision; triplicates will be analyzed for stability, duplicate for in-life	Precision: 30% as relative standard deviation (RSD) or relative percent deviation (RPD)	If RSD or RPD is not acceptable, resample and reanalyze. If reanalysis data are still not acceptable, then "*" flag the values.
Blank or Matrix Spike and spike duplicate, one set per batch	+/- 20% of true value	If recoveries are unacceptable, check the spike solution to ensure it has not degraded, also check pipettes to ensure they are delivering accurate volumes.

[a] DQO is based on limited sample analysis as part of method development experience, and may require adjustment when more experience with the method is available.

Table 2. Data Qualifiers[a]

U	The analyte was detected below the MDL. Note: Samples with no peaks are reported as zero.
B	Samples associated with procedural blank contamination.
*	QC sample data that does not meet the DQO acceptability criterion.
Q	The data are questionable.
D	Sample diluted for analysis. (note: this procedure outlines the dilution of the samples, data will not be D flagged unless diluted other than indicated in this SOP)

[a] Additional data qualifiers may be added as necessary.

attachment 1

Organic Standard Preparation

Page_____ of _____ STD ID: _____

Prep. Date: _____

Exp. Date: _____

Stock Used and Lot #	Stock Exp. Date/Date Rec.	Stock/Chemical Name	Stock Conc.	Stock Vol./Wt.	Diluted To:	Solvent Mfg, Exp. date & Lot #	Standard Concentration

Solution Prepared By: _____ If Balance used, Balance # _____

Storage Location _____

Purpose of Standard _____

Storage Temp. _____

Liquids handled by (circle ones used) Graduated cylinder Syringe Disposable pipette Vol. Flask

Pipettes used: _____

attachment 2

Mobile Phase Preparation

Solution Name: _____ Prep. Date: _____

Study # _____ Exp. Date: _____

Stock Used and Lot #	Amount (g or ml)	Stock Used and Lot #	Amount (g or ml)	Stock Used and Lot #	Amount (g or ml)	Prepared By: (Intials and Date)

If Balance used, Balance # _____ Storage Location _____

Purpose of Solution _____ Storage Temp. _____

Liquids handled by (circle ones used) Graduated cylinder Syringe Disposable pipette Vol. Flask

Pipettes used: _____

attachment 3

EDSP HPLC Analysis Form

Date _____ Work Study # and Analysis _____

Instrument Used _____
Detector Used _____
(UV/Vis) Wavelength _____
(Fluorescense) Wavelengths Excitation _____ Emission _____
Mobile Phase A _____
Mobile Phase B _____
Loop Size _____
Sample size _____

Desired Flow rate _____
Measured Flow Rate Flow rate _____

Column Used _____
Serial # _____
Pressure _____

Gradient Program, Step #	% Mobile Phase A	% Mobile Phase B	Time

Number of Samples Run _____ Initials and Date _____

attachment 4

EDSP Purity

Chemical _____ CF# _____

Lot # _____

Instrument Used _____

Standard Prep Name	Date Prepared	Analysis Date	Data File Name	Blank File Name

Area, compound peak	Area Impurity + Compound Peaks	Area Peaks In Blank	Purity *

*** Purity Calculation:** Area Compound Peak / ([Area Impurity Peaks + Compound Peaks] - Area Peaks In Blank)

attachment 5

Stability Solution Preparation

Study Protocol _____ Date: _____

Stock/Chemical Name _____

Manufacturer and Lot # _____

Stock Exp. Date/Date Rec. _____

Diluent + Lot _____

Dose

Dose Name	Stock/Chemical Name	Stock Wt.	Diluted To:	Concentration	Prep. Date:

Solution Prepared By: _____ Balance used, # _____ Storage Location _____

Treatment Conditions Sonicate to dissolve or agitate until dissolved Storage Temp. _____

Liquids handled by (circle ones used) Graduated cylinder Syringe Disposable pipette Vol. Flask

attachment c

EDSP Sampling

Chemical Lot # _____ CF# _____

Sampling Interval _____ days

Sampling Date _____ Analysis Date _____

Sampler _____

Method used for analysis

Sample Name	Volume Taken	Volume Diluent	Diluent used	Sample Type	Multiplier used	Comments

Liquids handled by (circle ones used) Graduated cylinder Syringe Disposable pipette Vol. Flask

Pipettes used: _____

Diluents used + Exp. Date _____

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Marine Sciences Laboratory

EFFECTIVE DATE: 02-16-05

Method # EDSP.H4-023-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

ANALYSIS OF PROCLORAZ IN DIMETHYLSULFOXIDE USING HPLC WITH UV/VIS DETECTION

Approvals:		
AUTHOR: Rebecca Wood	Rebecca Wood	2-16-05
	Signature	Date
TECHNICAL REVIEWER: Tim Fortman	Tim Fortman	2-16-05
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	E Crecelius	2-16-05
	Signature	Date

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ANALYSIS OF PROCHLORAZ IN DIMETHYL SULFOXIDE USING HPLC WITH UV/VIS DETECTION

1.0 SCOPE AND APPLICATION

This method describes the determination of Prochloraz in dimethyl sulfoxide (DMSO) using HPLC/UV/Vis detection. The method was developed for use in the analysis of Prochloraz for the EDSP program. The eluent used is an Acetonitrile/Water solution.

2.0 DEFINITIONS

Initial Calibration Verification (ICV)	A standard made from a neat material different from the material used to make the calibration standards. Used to verify the calibration solutions.
Continuing Calibration Verification (CCV)	A mid level calibration standard run after every 10 samples to ensure the instrument is in calibration.

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation.
Analyst - analysis, calculations
QA Manager or Representative - data verification

4.0 ANALYSIS

4.1 Hardware and Reagents

- Balance capable of weighing to 0.0001 g
- High performance liquid chromatograph (Perkin Elmer 250 pump, with a Gilson 294 liquid autosampler) with UV/Vis detector (Waters 486 detector) set at wavelength 204nm.
- Phenomenex Synergi 4µ hydro-RP 80A 250 X 4.6 mm HPLC column Serial # 258206-4.
- Acetonitrile, HPLC grade or better.
- Dimethyl sulfoxide (DMSO), reagent grade.
- Prochloraz, 97% purity or better.
- 1.8 mL vials
- Autosampler vial for Gilson Autosampler.
- Helium for sparging eluents.
- Software for collection of data from UV/Vis detector, Varian Star Software, vers. 6.2.
- 1 liter amber bottle with Teflon lined lid.
- Variable positive displacement Pipettors, to pipette 0.005 mL.
- 47 mm glass filter apparatus for filtration of HPLC eluent, i.e. Micro Filtration System with 300 mL reservoir.

4.2 HPLC Mobile Phase (Eluent)

4.2.1 The pump used is a binary gradient pump, one reservoir is filled with Acetonitrile while the other is filled with DI water, the 75:25 eluent is then made by programming the pump to take 75% acetontrile and 25% water.

4.3 Calibration Solution

4.3.1 A 5 point curve is used to calibrate the HPLC over a range that will bracket the concentration in the stability tests. To start, a stock is made at a concentration of about 2000 ug/mL. Approximately 0.0500 grams is weighed into a 25 mL volumetric flask and diluted to the mark with Acetontrile. Record exact information on the standards preparation form (attachment 1) and give the solution a unique identifying label (include contents, date prepared, prepared by, work assignment number). Pour the solution into an appropriate size amber vial with a Teflon lined lid. Stability of the calibration solutions should be verified at the end of the test by the analysis of a new (freshly made) solution prepared from the neat material and compared to the calibration solutions.

4.3.2 Serially dilute the solution made in 4.3.1 to make standards ranging from 20 ug/mL to 450 ug/mL using a solution that will mimic the eluent, 75% Acetonitrile, 25% water (use Mobile Phase Preparation form to document the preparation of the eluent [diluent] see attachment 2 for example form). Record all information in the standards preparation form (see attachment 1).

4.4 HPLC Setup

4.4.1 The HPLC equipment has 5 main components, a pump, autosampler, HPLC column, detector and data system. The pump is set up to pump at 1.0 mL/min. The mobile phase (eluent) is purged using helium (H_2) for about 15 minutes prior to running the system. The pump is primed as per instrument instructions and the flow directed thru the HPLC system. The pump run time should be set to 13 minutes.

4.4.2 The autosampler is set up to inject 10 ul. A 25 ul loop is installed. See instrument manual for setup details. The autosampler is then set to flush the contaminated surfaces with Acetonitrile.

4.4.3 The column used is a Phenomenex Synergi 4µ hydro RP 80A 250 X 4.6 mm HPLC column Serial # 258206-4. Pressure limit on the column is 3000 PSI, adjust pump so pressure limit will shut the pump off prior to damaging the column.

4.4.4 The detector is a UV/Vis detector set to a wavelength of 204 nm. The detector is attached to the data collection system by way of the analog output from the 1 volt full scale (integrator) terminal.
The data system used is Varian's Star software, version 6.2. The system is set up to start automatically with the injection of the sample. The run time is set to 13 minutes. Data is collected at 10 Hz. Calibration samples are run prior to analysis and the software is used to calculate the unknowns. See software manuals for setup.

4.4.5 This system is composed of several different components from different manufacturers. The method printouts will only contain information pertaining to the calibration and quantitation of the unknowns, in order to ensure that the conditions

used are recorded, an EDSP HPLC Analysis Form should be filled out (attachment 3). Also, much of this information should be included in the "notes" tab on the Varian Star method.

4.5 Analysis

4.5.1 Solutions run on the HPLC should have similar composition to the eluent. For example, the solution for Prochloraz has 0.005 mL of the solution placed into a 1.8 mL vial with 0.995 mL of the eluent. Using a transfer pipette, the solution is mixed then transferred to a 1 mL autosampler vial designed for the Gilson autosampler.

4.5.2 Prior to the analysis of any samples, linearity must be demonstrated. A 5 point curve is run (minimum of a 4 point curve is needed). An r^2 value of greater than 0.995 is necessary before analysis can begin.

4.5.3 Once the calibration is done, if possible, it should be verified with an initial calibration verification sample (ICV). An independent solution is made using a different lot of the Prochloraz and diluted to the proper concentration so that it is within the calibration range. This solution is run and the value obtained should be within +/−10% of the expected value.

4.5.4 After the calibration is verified, a continuing calibration verification (CCV) sample is run. This sample is usually one of the mid level calibration solutions. The value obtained should be within +/−10% of the expected value. A CCV should be run after every 10 samples.

4.5.5 A blank should be prepared with each sampling. For example, if a 0.005 mL of sample is taken and diluted with the eluent, a blank DMSOI using 0.005 mL is treated as the sample. The blank should be < 3X MDL.

4.5.6 Method Detection Limit (MDL) is determined by preparing a sample at a low concentration, using similar techniques as used to analyze the stability solution. This is done 7 times and the MDL is the students T (3.143 for 7 replicates) times the standard deviation of the seven replicate runs. An MDL should be performed prior to the analysis of any sample for ~~Dexamethasone~~ prochloraz. Samples with no peak or less than the MDL will be reported as the MDL and flagged with a "U.

Must remove inadvertently left in from previous method

4.6 Purity

4.6.1 All EDSP studies require confirmation of purity unless a written waiver is received from the EPA. The Chemical Characterization SOP# MSL-R-009-00 has additional information. Purity is determined by running a solution of the material that is at or near the top of the demonstrated linearity of the system. All the peaks in the chromatogram are summarized. The peak corresponding to the Prochloraz is then compared to all the other peaks and the purity is the area of the Prochloraz peak divided by the sum of the total area in the chromatogram (presented as a percentage). A blank is run prior to the purity run and the peaks in the purity run that correlate to peaks in the blank run are eliminated from the calculation. This purity should be 97% or greater and should compare favorably to the purity from the vendor. Note: the limitation of using a UV/Vis detector for purity is that one cannot be certain that the impurities will absorb at the same wavelength. This purity is an estimation. See example purity form: attachment 4.

5.0 STABILITY

5.1 Stability for Prochloraz is to run for 14 days. Stability is prepared by weighing an aliquot of Prochloraz into a 30 milliliter amber bottle with 10 milliliters of DMSO (attachment 5). The details for the stability are contained in the Study Protocol. Target concentration is 0.1 M, or about 37.7 mg/mL. Sampling and analysis will be done 8 times over the course of the stability study. 2 typo rmb 12/7/05

5.2 Sampling is done by removing an aliquot of the sample and diluting it into a solution similar to the mobile phase. 0.005 mL of the solution is placed into a 1.8 mL autosampler vial with 0.995 mL of the mobile phase (see 4.2.1). Using a transfer pipette, mix the solution in the vial then transfer it to a 1 mL autosampler vial that works with the autosampler.

5.3 For an example of the form used for the stability preparation and sampling see attachments 5 and 6.

5.4 Samples should be analyzed on the day of sampling, but if this is not possible, samples should be stored at 4 deg. C. until analysis. If samples are not analyzed in the day of sampling, the actual analysis date and storage conditions shall be documented.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 Prior to analysis of any samples, the instrument is calibrated with a minimum of a 4 point curve. External standard calculations will be performed. All calculations will be done using Varian's Star chromatography software, version 6.2. This software allows the input of a multiplier, so that any dilutions will be included with the software calculations. For example, for Prochloraz stability, 0.005 mL of the stock is diluted with 0.995 mL of eluent and a multiplier of 200 is used so that the output from the software will give values that reflect the concentration in the stability solution. Calibration curve fits can be set to non-linear (quadratic fit) or a linear fit.

6.2 Prior to the tabulation of the data, each chromatogram and report printed by the software will be initialed and dated for GLP compliance.

7.0 QUALITY CONTROL

A blank is prepared with each sampling, this blank is DMSO processed identically to the stability solution. If background levels are sufficiently high (i.e., greater than 3 x MDL), this value may be subtracted from the values obtained for samples analyzed with that batch. Processing of these samples is very straight forward, therefore spikes are optional. Whenever available, an initial calibration verification (ICV) standard (made from a second source, not the same source as the calibration standards) will be analyzed following the calibration curve. Continuing calibration verification standards (CCVs) will be analyzed after every 10 samples. If CCV variation exceeds a +/-10% difference from expected, samples will be re-run with acceptable calibration criteria.

Table1. Summary of Data Quality Objectives and Corrective Actions

Quality Control Sample Type	Data Quality Objective[a] (DQO)	Corrective Action
Procedural Blank one/batch	Less than 3 x MDL	Re-extract and analyze sample batch. If batch can not be re-extracted and analyzed, "B" flag all samples that are in the batch. Investigate sources of blank contamination.
Calibration curve acceptability	r^2 values greater than or equal to 0.995	If r^2 value is outside of criterion, re-analyze calibration standards, if r^2 is still out, perform instrument maintenance and/or remake calibration standards and rerun calibration samples.
Initial calibration verification (ICV) standard; one/batch	+/−10 % of true value	Re-calibrate. Must meet DQO in order to continue processing samples.
Continuing calibration verification standards; one every 10^{th} sample analyzed	+/−10 % of true value	Re-run CCV, if still not acceptable, re-calibrate and reanalyze affected samples.
Replicate sample precision; triplicates will be analyzed	Precision: 30% as relative standard deviation (RSD)	If RSD is not acceptable, resample and reanalyze. If reanalysis data are still not acceptable, then "*" flag the values.
Blank or Matrix Spike and spike duplicate, one set per batch	+/−15% of true value	If recoveries are unacceptable, check the spike solution to ensure it has not degraded, also check pipettes to ensure they are delivering accurate volumes.

[a] DQO is based on limited sample analysis as part of method development experience, and may require adjustment when more experience with the method is available.

Table 2. Data Qualifiers[a]

U	The analyte was detected below the MDL. Note: Samples with no peaks are reported as zero.
B	Samples associated with procedural blank contamination.
*	QC sample data that does not meet the DQO acceptability criterion.
Q	The data are questionable.
D	Sample diluted for analysis. (note: this procedure outlines the dilution of the samples, data will not be D flagged unless diluted other than indicated in this SOP)

[a] Additional data qualifiers may be added as necessary.

8.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of chemicals, including the following:

- Gloves, protective clothing and safety glasses should be worn when handling samples and chemicals.

9.0 TRAINING REQUIREMENTS

9.1 All staff performing this analysis should first read this procedure and conduct their first analysis under the supervision of a staff member who has had previous experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work. Documentation of training will be performed in accordance with MSL-A-006, Marine Sciences Laboratory Training.

9.2 All staff should have received training in the handling of chemicals and the use of fume hoods.

10.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

MSL-Q-007-04 Procedure for Determining Method Detection Limits

Federal Register (CFR Part 136 Appendix B).

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Marine Sciences Laboratory

EFFECTIVE DATE: 2-17-05

Method # EDSP.G2-024-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

ANALYSIS OF 4-NONYLPHENOL (MIX OF ISOMERS) IN DIMETHYL SULFOXIDE USING GC WITH FID AND MS DETECTION

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ANALYSIS OF 4-NONYLPHENOL (MIX OF ISOMERS) IN DIMETHYL SULFOXIDE USING GC WITH FID AND MS DETECTION

Supposed to be CAS# 84852-15-5
Deviation written #EDSP.G2.024-05-02
TMB 12/8/05

1.0 SCOPE AND APPLICATION

This method describes the determination of 4-nonylphenol (CAS# 104-40-5) in Dimethyl sulfoxide using GC with FID and MS detection. Stability and verification of formulation will be done using the GC-FID, purity will be done on the GC-MS.

2.0 DEFINITIONS

Initial Calibration Verification (ICV)	A standard made from a neat material different from the material used to make the calibration standards. Used to verify the calibration solutions. If an alternative source of material is not available, a 2nd standard prepared from the primary neat material is acceptable.
Continuing Calibration Verification (CCV)	A mid level calibration standard run after every 10 samples to ensure the instrument is in calibration.
Internal Standard (IS)	A compound in solution added to every sample (except purity sample) to adjust for variation (the internal standard is also in the calibration solutions).

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation.
Analyst - analysis, calculations
QA Manager or Representative - data verification

4.0 ANALYSIS

4.1 Hardware and Reagents

- Balance capable of weighing to 0.0001 g
- Gas chromatograph (Agilent 5890 or equivalent) with an FID (Flame ionization detector) and a mass selective detector (agilent 5970 or equivalent) and an autosampler.
- J+W DB-5 30m X 0.25 mm X 0.25 µm film thickness GC column or equivalent.
- Hexane, GC-MS grade ~~or better~~.
- 4-nonylphenol (CAS# 104-40-5) and 5α Androstane, (CAS# 438-22-2) 98% purity or better.
- 1.8 mL autosampler vials
- 25, 50 and 100 mL amber glass bottles.
- Helium for carrier gas, Hydrogen, Breathing Air and Nitrogen for FID flame.
- Software for collection of data from FID detector, Varian Star Software, version 6.2 and MS software, Agilent G1701AA.
- Variable positive displacement Pipettors, to pipette 0.1 mL and 0.010 mL.
- Varity of volumetric flasks.
- Volumetric flasks
- Dimethyl Sulfoxide

4.2 GC Setup (FID)

4.2.1 The GC equipment has the following components, an autosampler, GC oven, detector (FID) and a data collection system. The autosampler is set to inject 1 µL and the injection port is set to 250° C. The oven conditions are; initial temp. 50° C., hold for 1.5 min., ramp at 15° C. per minute to 320° C and hold for 1 min. The detector temperature is set at 320° C.

4.2.2 The GC is set in the splitless mode and the split vent valve is opened at 2.5 minutes. Split vent is set at 89 mL/min and septum purge is set at 6.9 mL/min.

4.2.3 The column used is a J+W DB-5 30M X 0.25 mm X 0.25 µm film thickness. Flow thru the column is controlled by the head pressure which is set at 18 PSI.

4.2.4 The FID detector conditions are controlled by valves set up for the detector, the supply gasses are set up as follows, Hydrogen pressure, 21 PSI, Air pressure, 58 PSI and nitrogen pressure 34 PSI.

4.2.5 The data system used is Varian's Star software, version 6.2. The system is set up to start automatically with the injection of the sample. The run time is set to 21 minutes. Bunch rate is set to 8 and signal to noise set at 100. Different data systems will employ different nomenclature for the same settings, see software manuals for information on how to optimize the software.

4.2.6 The system used is composed of several different components from different manufacturers. The method printouts will only contain information pertaining to the calibration and quantitation of the unknowns, in order to ensure that the conditions used are recorded, an EDSP GC Analysis Form should be filled out (attachment 2). Also, much of this information should be included in the "notes" tab on Varian's Star method.

4.3 GC Setup (MS)

4.3.1 The GC is set with the following parameters. The autosampler is set to inject 1 µL and the injection port is set to 250° C. The oven conditions are; initial temp. 50° C., hold for 1.5 min., ramp at 15° C per minute to 320° C and hold for 1 min. The transfer line temperature is set at 300° C.

4.3.2 The GC is set in the splitless mode and the split vent valve is opened at 1.5 minutes. Split vent is set at 99 mL/min and septum purge is set at 1.8 mL/min.

4.3.3 The column used is a J+W DB-5 30M X 0.25 mm X 0.25 µm film thickness. Flow thru the column is controlled by the head pressure which is set at 9 PSI.

4.3.4 The GC and MS detector conditions are controlled by the software, it is operated in full scan, scanning from 30 amu to 300 amu. Solvent delay is set at 5 minutes. Scans per second are set at 2.91, threshold is set at 500 and EM voltage set at 94 volts above autotune value.

4.3.5 The GC-MS is used for purity determination and not quantitative analysis, no calibration curve is needed for this.

4.3.6 The GC-MS is tuned using PFTBA and the internal tuning program. The values obtained from a scan of the PFTBA should meet the criteria in table 1.

4.3.7 An air/water check is done to ensure no leaks in the system which can degrade the source. Nitrogen (ion 28) and water (ion 18) abundance should be less than 10% of the PFTBA ion 69 abundance.

TABLE 1. PFTBA TUNING CRITERIA

M/Z	ION ABUNDANCE[a]
69 (base peak)	100%
219	30-60%
502	≈1%

[a] Relative to base peak m/z 69

4.4 Calibration Solution (FID)

4.4.1 A 5 point curve is used to calibrate the GC over a range that will bracket the concentration in the stability tests. To start a stock of the 4-nonylphenol is made at a concentration of about 1000 µg/mL. ~0.05 grams is weighed into a 50 mL volumetric flask and diluted to the mark with methylene chloride. Record exact information on the organic standard preparation form (attachment 1) and give the solution a unique identifying label. Pour the solution into an appropriate size amber vial with a Teflon lined lid. Stability of the calibration solutions should be verified at the end of the test, by the analysis of a new (freshly made) solution made from the neat material and compared to the calibration solutions.

4.4.2 An internal standard is incorporated with this analysis. The chemical 5 a Androstane is used as the internal standard and a ~1000 µg/mL solution is made as described in 4.4.1.

4.4.3 Serially dilute the solution made in 4.4.1 to make standards ranging from 10 µg/mL to 200 µg/mL. The internal standard should be the same concentration in all the standards, for this series of solutions, 50 ml volume is used for each, so 1 mL of the 1000 µg/mL internal standard solution is added to the 50 mL vol. flask for a target concentration of 20 µg/mL. This calibration targets these concentrations; 10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL and 200 µg/mL. Include all information in the standards preparation form (see attachment 1).

4.5 Analysis

4.5.2 Prior to the analysis of any samples linearity must be demonstrated. A 5 point curve is run (minimum of a 4 point curve is needed), the 0 point not considered a calibration point. An r^2 value of greater than 0.995 is necessary before analysis can begin.

4.5.3 This material is a mix of isomers and quantification is done summarizing the peaks. Varian star software has a function called, grouping which summarizes all the peaks within a specified window. The 4-nonylphenol isomers all elute within 2 minutes. A window using this grouping function of 2 minutes is set with the midpoint of the window at the center of the group of peaks. The software then reports one number for this group and the midpoint is entered into the software at the peaks retention time.

4.5.4 Once the calibration is done, if possible it must be verified with an initial calibration verification sample (ICV). An independent solution is made using a different lot of the 4-nonyl-phenol when available, if not, use of the existing lot is OK. This solution is diluted to the proper concentration so that it is within the calibration range. This solution is run and the value obtained should be within 15% of the expected value.

4.5.5 After the calibration is verified, a continuing calibration verification (CCV) sample is run. This sample is usually one of the mid level calibration solutions. The value obtained should be within 15% of the expected value. A CCV should be run after every 10 samples.

4.5.6 A blank should be prepared with each sampling. A blank is prepared for each dilution. For example if 0.005 mL of sample is taken and diluted with the dichloromethane, a blank using 0.005 mL of dimethyl sulfoxide is treated as the sample. The blank should be < 3X MDL.

4.5.7 Method Detection Limit (MDL) is determined by preparing a sample at a low concentration, using similar techniques as used to analyze the stability solution. This is done 7 times and the MDL is the students T (3.143 for 7 replicates) times the standard deviation of the seven replicate runs. An MDL should be performed prior to the analysis of any sample. Samples with no peak or less than the MDL will be reported as the MDL and flagged with a "U.

4.6 Purity

4.6.1 The confirmation of purity is a required component of EDSP studies per the Chemical Repository (CR) QAPP (Ver 2, 01/07/05). Purity analysis will be performed unless the CR is instructed not to do so by the sponsor (EPA) due to technical or budgetary reasons. The Chemical Characterization SOP# MSL-R-009-00 has additional information. Purity is determined by running a solution of the material that is concentrated enough that a sufficient signal is seen on the GC-MS in full scan mode. The solution contains only the chemical of interest, no internal standard or other chemicals, dissolved in methylene chloride. This solution is

injected onto the GC and run with the parameters described in section 4.3. The 4-nonylphenol is a mix of isomers. In order to determine which peaks are associated with 4-nonlyphenol and which are impurities, the mass spectra obtained is assessed.

4.6.2 The TIC (total Ion Chromatogram) is integrated and a list obtained of all peaks. This TIC includes all fragmentation ions. The isomers of 4-nonylphenol have variable fragmentation patterns but several fragments will be common to all the isomers. Ion 107 and ion 220 are chosen to represent the 4-nonylphenol isomers. Fragment 107 is from the cleavage of the carbon carbon bond between the 1st and 2nd carbon atom on the nonyl chain. This forms the common fragment of 4-methylphenol, ion 107. The other ion chosen is the molecular ion 220.

4.6.3 The software is used to extract ion 107 from the TIC. The 107 ion chromatogram is then integrated and a list obtained of all peaks. This is printed out. The software is again used to extract the 220 ion from the TIC and this is integrated and the list of peaks printed out. The peaks are tabulated so that peaks of the same retention time are listed together for the TIC, ion 107 and ion 220 (a retention time window of 0.02 minutes is used to determine if the retention times are the same). The peaks in the TIC that have retention times matching both the ion 107 and ion 220 chromatograms are considered 4-nonylphenol peaks, these areas are summed and considered the 4-nonylphenol. Peaks in the TIC that have one or both ions missing are considered impurities, these areas are summed and are considered the impurities.

4.6.4 The area of the peaks in the TIC that are associated with the the 4-nonylphenol peaks is divided by The area of all the peaks in the TIC (summation of both the impurities and the 4-nonylphenol, presented as a percentage). A blank dichloromethane is run prior to the purity run and the peaks in this TIC of this blank are summed and subtracted from the 4-nonylphenol TIC area total. This purity should be 97% or greater and should compare favorably to the purity from the vendor.

5.0 STABILITY AND FORMULATION VERIFICATION

5.1 Stability for WA 4-16 chemicals is to run for 14 days. Stability is prepared by weighing an aliquot of the 4-nonylphenol into 10 milliliters of dimethyl sulfoxide and storing the solution in a 30 milliliter amber bottle. The details for the stability are contained in the Study Protocol. Target concentration is 0.1 M or 22 mg/mL. Sampling and analysis will be done 2 times over the course of the stability study, T=0 and day 14.

5.2 Sampling is done by removing an aliquot of the sample and diluting it into dichloromethane with an aliquot of the internal standard. For 4-nonylphenol, 0.005 mL is placed into a 1.8 mL autosampler vial with 0.02 mL of the internal standard (1000 ug/mL 5α Androstane) and 0.975 mL of dichloromethane. The vials are capped and are ready for GC analysis.

5.3 Samples should be analyzed on the day of sampling, but if this is not possible, samples should be stored at 4° C. until analysis. If samples are not analyzed on the day of sampling, the actual analysis date and storage conditions shall be documented.

5.4 Stability solutions are stored at 4° C.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 Prior to analysis of any samples, the instrument is calibrated with a minimum of a 4 point curve. Internal standard calculations will be performed. All nonpurity calculations will be done using Varian's Star chromatography software, version 6.2. This software allows the input of a multiplier, so that any dilutions will be included with the software calculations. For example, for this stability, 0.005 mL of the 10 mL sample solution is diluted to 1 mL, a multiplier of 200 is used so that the output from the software will give values that reflect the concentration in the diluted solution. Calibration curve fits can be set to either linear or non-linear (quadratic fit), past experience indicates that even though the calibration meets linearity criteria, the quantification is improved with a non-linear fit.

6.2 Prior to the tabulation of the data, each chromatogram and report printed by the software will be initialed and dated for GLP compliance.

7.0 QUALITY CONTROL

7.1 A blank is prepared with each sampling, this blank is dimethyl sulfoxide processed identically to the stability solution. If background levels are sufficiently high (i.e., greater than 3 x MDL), this value may be subtracted from the values obtained for samples analyzed with that batch. Processing of these samples is very straight forward, therefore spikes are optional.

7.2 An initial calibration verification (ICV) standard will be analyzed following the calibration curve. This ICV is made from a second source when available, but if a second source is not available, the same source is used. Continuing calibration verification standards (CCVs) will be analyzed after every 10 samples. A CCV is a mid point calibration solution. If CCV variation exceeds a 15% difference from expected, samples will be re-run with acceptable calibration criteria.

7.7 An internal standard is added to each sample, recovery of this internal standard should be ≤ ±80%.

8.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of chemicals, including the following:

- Gloves, protective clothing and safety glasses should be worn when handling samples and chemicals.

9.0 TRAINING REQUIREMENTS

9.1 All staff performing this analysis should first read this procedure and conduct their first analysis under the supervision of a staff member who has had previous experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work. Documentation of training will be performed in accordance with MSL-A-006, Marine Sciences Laboratory Training.

9.2 All staff should have received training in the handling of chemicals and the use of fume hoods.

10.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

MSL-Q-007-04 Procedure for Determining Method Detection Limits

Federal Register (CFR Part 136 Appendix B).

Table 1. Summary of Data Quality Objectives and Corrective Actions

Quality Control Sample Type	Data Quality Objective (DQO)	Corrective Action
Procedural Blank one/batch	Less than 3 x MDL	Re-extract and analyze sample batch. If batch can not be re-extracted and analyzed, "B" flag all samples that are in the batch. Investigate sources of blank contamination.
Calibration curve acceptability	r^2 values greater than or equal to 0.995	If r^2 value is outside of criterion, re-analyze calibration standards, if r^2 is still out, perform instrument maintenance and/or remake calibration standards and rerun calibration samples.
Initial calibration verification (ICV) standard; one/batch	+ / - 15 % of true value	Re-calibrate. Must meet DQO in order to continue processing samples.
Internal Standard Recovery	+ / - 20 % of true value	Re-sample and rerun
Continuing calibration verification standards; one every 10th sample analyzed	+/ - 15 % of true value	Re-run CCV, if still not acceptable, re-calibrate and reanalyze affected samples.
Replicate sample precision; triplicates will be analyzed for stability, duplicate for in-life	Precision: 30% as relative standard deviation (RSD) or relative percent deviation (RPD)	If RSD or RPD is not acceptable, resample and reanalyze. If reanalysis data are still not acceptable, then "*" flag the values.
Blank or Matrix Spike and spike duplicate, one set per batch	+/- 20% of true value	If recoveries are unacceptable, check the spike solution to ensure it has not degraded, also check pipettes to ensure they are delivering accurate volumes.

[a] DQO is based on limited sample analysis as part of method development experience, and may require adjustment when more experience with the method is available.

Table 2. Data Qualifiers[a]

U	The analyte was detected below the MDL. Note: Samples with no peaks are reported as zero.
B	Samples associated with procedural blank contamination.
*	QC sample data that does not meet the DQO acceptability criterion.
Q	The data are questionable.
D	Sample diluted for analysis. (note: this procedure outlines the dilution of the samples, data will not be D flagged unless diluted other than indicated in this SOP)

[a] Additional data qualifiers may be added as necessary.

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Method # EDSP.G2-025-00

Battelle Pacific Northwest National Laboratories
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ANALYSIS OF DICOFOL IN DIMETHYL SULFOXIDE USING GC WITH FID DETECTION

Approvals:		
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	Signature	Date
TECHNICAL REVIEWER: Rebecca Wood	Rebecca Wood	3-4-05
	Signature	Date
CHEMICAL REPOSITORY DIRECTOR: Michael Cobb		03-04-05
	Signature	Date

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ANALYSIS OF DICOFOL IN DIMETHYL SULFOXIDE USING GC WITH FID DETECTION

1.0 SCOPE AND APPLICATION

This method describes the determination of Dicofol (CAS# 115-32-2) in Dimethyl sulfoxide using GC with FID detection. The method was developed for use in the analysis of Dicofol for the EDSP program.

2.0 DEFINITIONS

Initial Calibration Verification (ICV)	A standard made from a neat material different from the material used to make the calibration standards. Used to verify the calibration solutions. If none are available, a 2nd standard from the same neat material is acceptable.
Continuing Calibration Verification (CCV)	A mid level calibration standard run after every 10 samples to ensure the instrument is in calibration.
Internal Standard (IS)	A compound in solution added to every sample to adjust for variation (the internal standard is also in the calibration solutions).

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation.
Analyst - analysis, calculations
QA Manager or Representative - data verification

4.0 ANALYSIS

4.1 Hardware and Reagents

- Balance capable of weighing to 0.0001 g
- Gas chromatograph (Agilent 5890 or equivalent) with an FID (Flame ionization detector) and an autosampler.
- J+W DB-5 30m X 0.25 mm X 0.25 um film thickness GC column or equivalent.
- Dichloromethane, GC-MS grade or better.
- Dicofol (CAS# 115-32-2) and 5aAndrostane, (CAS# 438-22-2) 98% purity or better.
- 1.8 ml autosampler vials
- 25, 50 and 100 ml amber glass bottles.
- Helium for carrier gas, Hydrogen, Breathing Air and Nitrogen for FID flame.
- Software for collection of data from FID detector, Varian Star Software, vers. 6.2.
- Variable positive displacement Pipettors, to pipette 0.1 ml and 0.010 ml.
- Varity of volumetric flasks.
- ~~Volumetric flasks~~ duplicated from above
- Dimethyl Sulfoxide typo mb 1/8/05

4.2 GC conditions

4.2.1 The GC should be set up as per manufacturer specifications, for the system presently in use, the carrier gas pressure is set to 18 PSI, split vent at 89 ml/min, septum purge set at 6.9 ml/min. The FID (flame ionization detector) gasses are set at; air, 300 ml/min, hydrogen, 46 ml/min and nitrogen at 43 ml/min.

4.3 Calibration Solution

4.3.1 A 5 point curve is used to calibrate the GC over a range that will bracket the concentration in the stability tests. To start a stock of dicofol is made at a concentration of about 1000 ug/ml. ~0.05 grams is weighed into a 50 ml volumetric flask and diluted to the mark with dichloromethane. Record exact information on the organic standard preparation form (attachment 1) and give the solution a unique identifying label. Pour the solution into an appropriate size amber vial with a Teflon lined lid. Stability of the calibration solutions should be verified at the end of the test, by the analysis of a new (freshly made) solution made from the neat material and compared to the calibration solutions.

4.3.2 An internal standard is incorporated with this analysis. The chemical 5 a Androstane is used as the internal standard and a ~1000 ug/ml solution is made as described in 4.3.1.

4.3.2 Serially dilute the dicofol solution made in 4.3.1 to make standards ranging from 0.5 ug/ml to 100 ug/ml. The internal standard should be the same concentration in all the standards, for this series of solutions, 50 and 100 ml. volumes are used for each, so 1 ml of the 1000 ug/ml internal standard solution is added to the 50 ml vol. flask and 2 ml to the 100 ml vol. flask for a target concentration of 20 ug/ml. This calibration targets these concentrations; 0.5 ug/ml, 1 ug/ml, 5 ug/ml, 20 ug/ml

and 100 ug/ml. Include all information in the standards preparation form (see attachment 1).

4.4 GC Setup

4.4.1 The GC equipment has the following components, an autosampler, GC oven, detector (FID) and a data collection system. The autosampler is set to inject 1 ul and the injection port is set to 210 deg. C. The oven conditions are; initial temp. 50 deg. C., hold for 1.5 min., ramp at 15 deg. per minute to 320 deg. with no hold time. The FID gasses are described in section 4.2.1, the detector temp. is set at 320 deg. C.

4.4.2 The GC is set in the splitless mode and the split vent valve is opened at 2.5 minutes. Split vent and septum purge settings are listed in section 4.2.1.

4.4.3 The column used is a J+W DB-5 30M X 0.25 mm X 0.25 um film thickness. Flow thru the column is controlled by the head pressure which is set at 18 PSI (see section 4.2.1).

4.4.4 The FID detector conditions are described in section 4.2.1. The conditions on the present system are controlled by valves set up for the detector, the supply gasses are set up as follows, Hydrogen pressure, 21 PSI, Air pressure, 58 PSI and nitrogen pressure 34 PSI.

4.4.5 The data system used is Varian's Star software, version 6.2. The system is set up to start automatically with the injection of the sample. The run time is set to 19.5 minutes. Bunch rate is set to 8 and signal to noise set at 100. Different data systems will employ different nomenclature for the same settings, see software manuals for information on how to optimize the software.

4.4.6 The system used is composed of several different components from different manufacturers. The method printouts will only contain information pertaining to the calibration and quantitation of the unknowns, in order to ensure that the conditions used are recorded, an EDSP GC Analysis Form should be filled out (attachment 2). Also, much of this information should be included in the "notes" tab on Varian Star's method.

4.5 Analysis

4.5.2 Prior to the analysis of any samples linearity must be demonstrated. A 5 point curve is run (minimum of a 4 point curve is needed), the 0 point not considered a calibration point. An r^2 value of greater than 0.995 is necessary before analysis can begin.

4.5.3 Once the calibration is done, if possible it must be verified with an initial calibration verification sample (ICV). An independent solution is made using a different lot of the dicofol when available, if not, use of the existing lot is OK. This solution is diluted to the proper concentration so that it is within the calibration range. This solution is run and the value obtained should be within 15% of the expected value.

4.5.4 After the calibration is verified, a continuing calibration verification (CCV) sample is run. This sample is usually one of the mid level calibration solutions. The value obtained should be within 15% of the expected value. A CCV should be run after every 10 samples.

4.5.5 A blank should be prepared with each sampling. A blank is prepared for each dilution. For example if 0.005 ml of sample is taken and diluted with the dichloromethane, a blank using 0.005 ml of dimethyl sulfoxide is treated as the sample. The blank should be < 3X MDL.

4.5.6 Method Detection Limit (MDL) is determined by preparing a sample at a low concentration, using similar techniques as used to analyze the stability solution. This is done 7 times and the MDL is the students T (3.143 for 7 replicates) times the standard deviation of the seven replicate runs. An MDL should be performed prior to the analysis of any sample. Samples with no peak or less than the MDL will be reported as the MDL and flagged with a "U.

4.6 Purity

4.6.1 The confirmation of purity is a required component of EDSP studies per the Chemical Repository (CR) QAPP (Ver 2, 01/07/05). Purity analysis will be performed unless the CR is instructed not to do so by the sponsor (EPA) due to technical or budgetary reasons. The Chemical Characterization SOP# MSL-R-009-00 has additional information. Purity is determined by running a solution of the material that is at or near the top of the demonstrated linearity of the system. The solution contains only the test material, no internal standard or other chemicals, dissolved in dichloromethane. This solution is injected onto the GC and all the peaks in the chromatogram are summarized. The peak corresponding to the chemical of interest is then compared to all the other peaks and the purity is the area of the chemical peak divided by the sum of the total area in the chromatogram (presented as a percentage). A blank dichloromethane is run prior to the purity run and the peaks in the purity run that correlate to peaks in the blank run are eliminated from the calculation (this includes the solvent peak). This purity should be 96% or greater and should compare favorably to the purity from the vendor. See example purity form: attachment 3.

5.0 STABILITY AND FORMULATION VERIFICATION

5.1 Stability for WA 4-16 and WA4-17 chemicals is to run for 14 days. Stability is prepared by weighing an aliquot of the test substance into 1 milliliter of dimethyl sulfoxide and storing the solution in a 1.8 milliliter vial at 4 degress centigrade (attachment 4). The details for the stability are contained in the Study Protocol. Target concentration is 0.1 M, or for dicofol, 37 mg/ml. Sampling and analysis will be done 2 times over the course of the stability study.

5.2 Sampling is done by removing an aliquot of the sample and diluting it into dichloromethane with an aliquot of the internal standard. For dicofol, 0.005 ml is added to a 5 ml volumetric flask along with 0.1 ml of the internal standard (1000 ug/ml 5 a androstane) and diluted to mark with dichloromethane. An aliquot of this is placed into a a 1.8 ml autosampler vial for analysis. The vials are capped and are ready for GC analysis.

5.3 For an example of the form used for the stability preparation and sampling see attachments ~~4 and~~ 5.

5.4 Samples should be analyzed on the day of sampling, but if this is not possible, samples should be stored at 4 deg. C. until analysis. If samples are not analyzed on the day of sampling, the actual analysis date and storage conditions shall be documented.

5.5 Stability solutions are stored at 4 degrees centrigrade.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 Prior to analysis of any samples, the instrument is calibrated with a minimum of a 4 point curve. Internal standard calculations will be performed. All calculations will be done using

Varian's Star chromatography software, version 6.2. This software allows the input of a multiplier, so that any dilutions will be included with the software calculations. For example, for dicofol stability, 0.005 ml of the 10 ml sample solution is diluted to 5 ml, a multiplier of 1000 is used so that the output from the software will give values that reflect the concentration in the diluted solution. Calibration curve fits can be set to either linear or non-linear (quadratic fit), past experience indicates that even though the calibration meets linearity criteria, the quantification is improved with a non-linear fit.

6.2 Prior to the tabulation of the data, each chromatogram and report printed by the software will be initialed and dated for GLP compliance.

7.0 QUALITY CONTROL

7.1 A blank is prepared with each sampling, this blank is dimethyl sulfoxide processed identically to the stability solution. If background levels are sufficiently high (i.e., greater than 3 x MDL), this value may be subtracted from the values obtained for samples analyzed with that batch. Processing of these samples is very straight forward, therefore spikes are optional.

7.2 An initial calibration verification (ICV) standard will be analyzed following the calibration curve. This ICV is made from a second source when available, but if a second source is not available, the same source is used. Continuing calibration verification standards (CCVs) will be analyzed after every 10 samples. A CCV is a mid point calibration solution. If CCV variation exceeds a 15% difference from expected, samples will be re-run with acceptable calibration criteria.

7.7 An internal standard is added to each sample, recovery of this internal standard should be ≥80%.

8.0 SAFETY

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9.0 TRAINING REQUIREMENTS

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9.2 All staff should have received training in the handling of chemicals and the use of fume hoods.

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Federal Register (CFR Part 136 Appendix B).

Table 1. Summary of Data Quality Objectives and Corrective Actions

Quality Control Sample Type	Data Quality Objective (DQO)	Corrective Action
Procedural Blank one/batch	Less than 3 x MDL	Re-extract and analyze sample batch. If batch can not be re-extracted and analyzed, "B" flag all samples that are in the batch. Investigate sources of blank contamination.
Calibration curve acceptability	r^2 values greater than or equal to 0.995	If r^2 value is outside of criterion, re-analyze calibration standards, if r^2 is still out, perform instrument maintenance and/or remake calibration standards and rerun calibration samples.
Initial calibration verification (ICV) standard; one/batch	+ / - 15 % of true value	Re-calibrate. Must meet DQO in order to continue processing samples.
Internal Standard Recovery	+ / - 20 % of true value	Re-sample and rerun
Continuing calibration verification standards; one every 10th sample analyzed	+/ - 15 % of true value	Re-run CCV, if still not acceptable, re-calibrate and reanalyze affected samples.
Replicate sample precision; triplicates will be analyzed for stability, duplicate for in-life	Precision: 30% as relative standard deviation (RSD) or relative percent deviation (RPD)	If RSD or RPD is not acceptable, resample and reanalyze. If reanalysis data are still not acceptable, then "*" flag the values.
Blank spike or Matrix Spike and spike duplicate, one set per batch	+/- 20% of true value	If recoveries are unacceptable, check the spike solution to ensure it has not degraded, also check pipettes to ensure they are delivering accurate volumes.

[a] DQO is based on limited sample analysis as part of method development experience, and may require adjustment when more experience with the method is available.

Table 2. Data Qualifiers[a]

U	The analyte was detected below the MDL. Note: Samples with no peaks are reported as zero.
B	Samples associated with procedural blank contamination.
*	QC sample data that does not meet the DQO acceptability criterion.
Q	The data are questionable.
D	Sample diluted for analysis. (note: this procedure outlines the dilution of the samples, data will not be D flagged unless diluted other than indicated in this SOP)

[a] Additional data qualifiers may be added as necessary.

APPENDIX D
EDSP .416-01 Analytical Method Deviations

The following method deviations were filed:

1. EDSP.G2-022-00-D1 - The protocol specified refrigerated storage of the stability formulations, the method specified room temperature storage. For the stability studies in associated with the protocol, the samples were stored refrigerated. No impact to project or data.
2. EDSP.G2-022-00-D2 - The original spec described a 25 µL loop install in the system, the loop size used was 100 µL. There was no impact on the analysis.
3. EDSP.G2-022-00-D5 – Several standards were made with hexane, instead of the specified solvent, methylene chloride. The hexane was used for some of the standards during method development causing no problems so they were used in the studies as well. No impact.
4. EDSP.G2-022-00-D6 – Pressure for air on the FID detector was set at 40 psi instead of the specified 58 psi. The setting was in error but the performance specifications were met so there was no impact on the data.
5. EDSP.G2-024-00-D1 - Pressure for air on the FID detector was set at 40 psi instead of the specified 58 psi. The setting was in error but the performance specifications were met so there was no impact on the data.
6. EDSP.G2-024-00-D2 – Under scope and application, the test substance 4-nonylphenol was identified by an incorrect CAS number, the correct CAS number is 84852-15-3. No impact on data as the correct material was used in the study.
7. EDSP.G2-025-00-D1 – In prepping the dicofol standards, an internal standard volume of 1 mL was incorrectly used instead of 2 mL. The actual volume (1 mL) was used in generation of the calibration curve and had no adverse impact on the data.
8. EDSP.G2-025-00-D2 - Pressure for air on the FID detector was set at 40 psi instead of the specified 58 psi. The setting was in error but the performance specifications were met so there was no impact on the data.

APPENDIX E
ANALYTICAL RESULTS OF STABILITY TESTING

(Note: Calculations were conducted at full precision in a spreadsheet.)

Table E1. Atrazine Stability Results in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	Atrazine (ug/mL)	Average (ug/mL)	Recovery	RSD
21320	Atzr-1 R-1	2/8/2005	19977	20306	95.2%	2.9%
21320	Atzr-1 R-2	2/8/2005	19956			
21320	Atzr-1 R-3	2/8/2005	20986			
21320	Atzr-2 R-1	2/23/2005	20043	20382	95.6%	1.7%
21320	Atzr-2 R-2	2/23/2005	20717			
21320	Atzr-2 R-3	2/23/2005	20385			

Table E2. Fenarimol Stability Results in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	Fenarimol (ug/mL)	Average (ug/mL)	Recovery	RSD
33220	Fenrl-1 R-1	2/8/2005	31337	31187	93.9%	2.0%
33220	Fenrl-1 R-2	2/8/2005	31715			
33220	Fenrl-1 R-3	2/8/2005	30509			
33220	Fenrl-2 R-1	2/23/2005	33004	34657	104.3%	4.7%
33220	Fenrl-2 R-2	2/23/2005	34671			
33220	Fenrl-2 R-3	2/23/2005	36295			

Table E3. Dibenz(a,h)anthracene Stability Results in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	Dibenz(a,h) anthracene (ug/mL)	Average (ug/mL)	Recovery	RSD
2780	DiBzAnt-1 R1	2/8/2005	2786	2794	100.5%	0.4%
2780	DiBzAnt-1 R2	2/8/2005	2805			
2780	DiBzAnt-1 R3	2/8/2005	2792			
2780	DiBzAnt-2 R1	2/23/2005	2798	2801	100.7%	0.1%
2780	DiBzAnt-2 R2	2/23/2005	2803			
2780	DiBzAnt-2 R3	2/23/2005	2801			

Table E4. Prochloraz Stability Results in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	Prochloraz (ug/mL)	Average (ug/mL)	Recovery	RSD
37970	proclz-6B R-1	2/16/2005	36864	37487	98.7%	2.9%
37970	proclz-6B R-2	2/16/2005	36871			
37970	proclz-6B R-3	2/16/2005	38726			
37970	proclz-6(2) R-1	3/2/2005	38448	39212	103.3%	2.2%
37970	proclz-6(2) R-2	3/2/2005	40167			
37970	proclz-6(2) R-3	3/2/2005	39020			

Table E5. 4-Nonylphenol Stability Results in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	4-Nonylphenol, ug/mL	Average (ug/mL)	Recovery	RSD
24070	4Nonyl stab 1 R-1	2/17/2005	23686	24663	102.5%	7.6%
24070	4Nonyl stab 1 R-2	2/17/2005	26822			
24070	4Nonyl stab 1 R-3	2/17/2005	23481			
24070	4Nonyl stab 2 R-1	3/3/2005	22182	23065	95.8%	3.7%
24070	4Nonyl stab 2 R-2	3/3/2005	23111			
24070	4Nonyl stab 2 R-3	3/3/2005	23902			

Table E6. Dicofol Stability Results in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	Dicofol (ug/mL)	Average (ug/mL)	Recovery	RSD
36800	Dico Stab 1 R-1	3/4/2005	33722	33723	91.6%	1.7%
36800	Dico Stab 1 R-2	3/4/2005	33158			
36800	Dico Stab 1 R-3	3/4/2005	34289			
36800	Dico Stab 2 R-1	3/18/2005	36879	37969	103.2%	2.8%
36800	Dico Stab 2 R-2	3/18/2005	39035			
36800	Dico Stab 2 R-3	3/18/2005	37994			

Table E7. Calibration Verification Data for Atrazine

Standard Name	Date	Expected Atrazine (ug/mL)	Measured Atrazine (ug/mL)	Recovery
WA416-17-GC-17 ICV	2/8/2005	5.34	5.41	101.3%
WA416-17-GC-16C CCV	2/8/2005	4.94	4.93	99.7%
WA416-17-GC-16C CCV	2/8/2005	4.94	5.00	101.2%
WA416-17-GC-16C CCV	2/8/2005	4.94	4.99	101.1%
WA416-17-GC-17 ICV	2/23/2005	5.34	5.55	103.9%
WA416-17-GC-21 CV	2/23/2005	5.08	5.07	99.9%
WA416-17-GC-16C CCV	2/23/2005	4.94	5.04	102.1%
WA416-17-GC-16C CCV	2/23/2005	4.94	5.08	102.8%
WA416-17-GC-16C CCV	2/23/2005	4.94	5.13	103.8%

Table E8. Calibration Verification Data for Fenarimol

Sandard Name	Date	Expected Fenarimol (ug/mL)	Measured Fenarimol (ug/mL)	Recovery
WA416-17-GC-17 ICV	2/8/2005	5.20	5.49	105.6%
WA416-17-GC-16C CCV	2/8/2005	5.01	5.13	102.4%
WA416-17-GC-16C CCV	2/8/2005	5.01	4.97	99.3%
WA416-17-GC-16C CCV	2/8/2005	5.01	4.92	98.1%
WA416-17-GC-17 ICV	2/23/2005	5.20	5.51	106.0%
WA416-17-GC-21 CV	2/23/2005	5.02	5.31	105.7%
WA416-17-GC-16C CCV	2/23/2005	5.01	5.22	104.2%
WA416-17-GC-16C CCV	2/23/2005	5.01	5.40	107.8%
WA416-17-GC-16C CCV	2/23/2005	5.01	5.36	106.9%

Table E9. Calibration Verification Data for Dibenz(a,h)anthracene

Standard Name	Date	Expected Dibenz(a, h) anthracene (ug/mL)	Measured Dibenz(a, h) anthracene (ug/mL)	Recovery
WA416-17-GC-17 ICV	2/8/2005	5.18	5.47	105.6%
WA416-17-GC-16C CCV	2/8/2005	5.05	5.13	101.7%
WA416-17-GC-16C CCV	2/8/2005	5.05	5.01	99.2%
WA416-17-GC-16C CCV	2/8/2005	5.05	4.74	93.8%
WA416-17-GC-17 ICV	2/23/2005	5.18	5.46	105.3%
WA416-17-GC-21 CV	2/23/2005	5.14	5.24	101.9%
WA416-17-GC-16C CCV	2/23/2005	5.05	5.08	100.5%
WA416-17-GC-16C CCV	2/23/2005	5.05	5.13	101.6%
WA416-17-GC-16C CCV	2/23/2005	5.05	5.12	101.3%

Table E10. Calibration Verification Data for Prochloraz

Standard Name	Date	Expected Prochloraz (ug/mL)	Measured Prochloraz (ug/mL)	Recovery
WA416-proclz-7 ICV	2/16/2005	83.8	86.3	103.0%
WA416-proclz-2D CCV	2/16/2005	83.0	81.2	97.8%
WA416-proclz-2D CCV	2/16/2005	83.0	78.1	94.1%
WA416-proclz-2D CCV	2/16/2005	83.0	79.2	95.4%
WA416-proclz-2D CCV	2/16/2005	83.0	89.0	107.2%
WA416-proclz-7 ICV	3/2/2005	83.8	86.6	103.4%
WA416-proclz-9 ICV	3/2/2005	83.2	86.4	103.8%
WA416-proclz-2D CCV	3/2/2005	83.0	84.6	101.9%
WA416-proclz-2D CCV	3/2/2005	83.0	79.4	95.6%

Table E11. Calibration Verification Data for 4-nonylphenol

Standard Name	Date	Expected 4-nonylphenol (ug/mL)	Measured 4-nonylphenol (ug/mL)	Recovery
WA416-nonyl-6 ICV	2/17/2005	55.6	57.1	102.7%
WA416-nonyl-3 C CCV	2/17/2005	53.5	54.0	100.9%
WA416-nonyl-3C CCV	2/17/2005	53.5	53.5	100.1%
WA416-nonyl-3C CCV	2/17/2005	53.5	54.3	101.5%
WA416-nonyl-6 ICV	3/3/2005	55.6	58.6	105.3%
WA416-nonyl-8 CV	3/3/2005	50.9	53.5	105.0%
WA416-nonyl-3 C CCV	3/3/2005	53.5	55.3	103.4%
WA416-nonyl-3C CCV	3/3/2005	53.5	57.1	106.8%

Table E12. Calibration Verification Data for Dicofol

Standard Name	Date	Expected Dicofol (ug/mL)	Measured Dicofol (ug/mL)	Recovery
WA416-Dico-8 ICV	3/4/2005	5.07	5.21	102.8%
WA416-Dico-4 C CCV5	3/4/2005	5.17	5.15	99.5%
WA416-Dico-4 C CCV5	3/4/2005	5.17	5.05	97.8%
WA416-Dico-4 C CCV5	3/4/2005	5.17	4.98	96.3%
WA416-Dico-8 ICV	3/18/2005	5.07	5.51	108.7%
WA416-Dico-10 CV	3/18/2005	5.09	5.49	107.9%
WA416-Dico-4 C CCV5	3/18/2005	5.17	5.30	102.6%
WA416-Dico-4 C CCV5	3/18/2005	5.17	5.17	100.0%

Table E13. Spike Recovery Data for Stability Analyses

Compound	Target Conc. (mg/L)	Sample ID	Date	Measured (mg/L)	Recovery
Atrazine	22700	Spike Mix MV R1	2/3/2005	22095	97.3%
Atrazine	22700	Spike Mix MV R2	2/3/2005	23018	101.4%
Atrazine	22700	Spike Mix MV R3	2/3/2005	22792	100.4%
Atrazine	22700	Spike Mix MV R4	2/3/2005	22115	97.4%
Atrazine	22700	Spike Mix MV R5	2/3/2005	22433	98.8%
Atrazine	22700	Spike Mix R1	2/8/2005	20840	91.8%
Atrazine	22700	Spike Mix R2	2/8/2005	22009	97.0%
Atrazine	22700	Spike Mix R3	2/23/2005	21812	96.1%
Atrazine	22700	Spike Mix R4	2/23/2005	22580	99.5%
Fenarimol	33400	Spike Mix MV R1	2/3/2005	32779	98.1%
Fenarimol	33400	Spike Mix MV R2	2/3/2005	34290	102.7%
Fenarimol	33400	Spike Mix MV R3	2/3/2005	34507	103.3%
Fenarimol	33400	Spike Mix MV R4	2/3/2005	34711	103.9%
Fenarimol	33400	Spike Mix MV R5	2/3/2005	33725	101.0%
Fenarimol	33400	Spike Mix R1	2/8/2005	31562	94.5%
Fenarimol	33400	Spike Mix R2	2/8/2005	32137	96.2%
Fenarimol	33400	Spike Mix R3	2/23/2005	33344	99.8%
Fenarimol	33400	Spike Mix R4	2/23/2005	34354	102.9%
Dibenz(a,h)anthracene	2990	Spike Benz R1	2/3/2005	2950	98.7%
Dibenz(a,h)anthracene	2990	Spike Benz R2	2/3/2005	3007	100.6%
Dibenz(a,h)anthracene	2990	Spike Benz R3	2/3/2005	3057	102.2%
Dibenz(a,h)anthracene	2990	Spike Benz R4	2/3/2005	3011	100.7%
Dibenz(a,h)anthracene	2990	Spike Benz R5	2/3/2005	3024	101.2%
Dibenz(a,h)anthracene	2990	Spike DiBzAnt R1	2/8/2005	2872	96.1%
Dibenz(a,h)anthracene	2990	Spike DiBzAnt R2	2/8/2005	2948	98.6%
Dibenz(a,h)anthracene	2990	Spike DiBzAnt R3	2/23/2005	2900	97.0%
Dibenz(a,h)anthracene	2990	Spike DiBzAnt R4	2/23/2005	2969	99.3%
Prochloraz	37260	Spike R-1	2/16/2005	37739	101.3%
Prochloraz	37260	Spike R-2	2/16/2005	38288	102.8%
Prochloraz	37260	Spike R-3	2/16/2005	37671	101.1%
Prochloraz	37260	Spike R-4	2/16/2005	38319	102.8%
Prochloraz	37260	Spike R-5	2/16/2005	37633	101.0%
Prochloraz	37260	Spike R6	3/2/2005	41756	112.1%
4-Nonylphenol	24970	Spike nonyl R-1	2/17/2005	24050	96.3%
4-Nonylphenol	24970	Spike nonyl R-2	2/17/2005	26173	104.8%
4-Nonylphenol	24970	Spike nonyl R-3	2/17/2005	24814	99.4%
4-Nonylphenol	24970	Spike nonyl R-4	2/17/2005	24243	97.1%
4-Nonylphenol	24970	Spike nonyl R-5	2/17/2005	23818	95.4%
4-Nonylphenol	24970	Spike nonyl R-6	3/3/2005	22790	91.3%
4-Nonylphenol	24970	Spike nonyl R-7	3/3/2005	23089	92.5%
Dicofol	36920	Spike R-1	3/4/2005	35786	96.9%
Dicofol	36920	Spike R-2	3/4/2005	37780	102.3%
Dicofol	36920	Spike R-3	3/4/2005	38141	103.3%
Dicofol	36920	Spike R-4	3/4/2005	37612	101.9%
Dicofol	36920	Spike R-5	3/4/2005	36219	98.1%
Dicofol	36920	Spike R-6	3/18/2005	37190	100.7%
Dicofol	36920	Spike R-7	3/18/2005	38712	104.9%

Table E14. Method Blank Results

Compound	Blank 1/T=O (ug/mL)	Blank 2/T=14 (ug/mL)	Method Detection Limit (ug/mL)
Atrazine	ND	ND	137
Fenarimol	ND	ND	271
Dibenz(a,h)anthracene	ND	19.3	7.21
Prochloraz	ND	ND	550
4-Nonylphenol	ND	ND	253
Dicofol	ND	ND	610

ND Non-detect; compound was not detected at or above detection limit shown

Table E15. Internal Standard Recovery 2-8-05 Analyses

Sample	Recovery
Mix Blank 1	94.6%
Spike Mix R1	96.1%
Spike Mix R2	95.4%
Atzr-1 R-1	95.3%
Atzr-1 R-2	95.1%
Atzr-1 R-3	95.3%
Fenrl-1 R-1	94.8%
Fenrl-1 R-2	95.4%
Fenrl-1 R-3	94.5%
DiBzAnt Blank 1	96.6%
Spike DiBzAnt R1	96.8%
Spike DiBzAnt R2	97.7%
DiBzAnt-1 R1	99.5%
DiBzAnt-1 R2	99.6%
DiBzAnt-1 R3	97.6%
WA416-17-GC-17 ICV	98.1%
WA416-17-GC-16C CCV	101.6%
WA416-17-GC-16C CCV	105.2%
WA416-17-GC-16C CCV	105.9%

Table E16. Internal Standard Recovery 2-17-05 Analyses

Sample	Recovery
MDL nonyl R-1	95.7%
MDL nonyl R-2	98.0%
MDL nonyl R-3	102.0%
MDL nonyl R-4	99.9%
MDL nonyl R-5	98.3%
MDL nonyl R-6	99.2%
MDL nonyl R-7	99.4%
4Nonyl stab 1 R-1	100.3%
4Nonyl stab 1 R-2	98.4%
4Nonyl stab 1 R-3	100.6%
MDL Blank 1	101.2%
4Nonyl stab Blk 1	100.2%
Spike nonyl R-1	101.9%
Spike nonyl R-2	99.4%
Spike nonyl R-3	103.0%
Spike nonyl R-4	103.7%
Spike nonyl R-5	100.4%
WA416-nonyl-6 ICV	100.3%
WA416-nonyl-3 C CCV	100.4%
WA416-nonyl-3C CCV	103.7%
WA416-nonyl-3C CCV	104.5%

Table E17. Internal Standard Recovery 2-23-05 Analyses

Sample	Recovery
Atzr-2 R-1	100.5%
Atzr-2 R-2	97.5%
Atzr-2 R-3	98.8%
Fenrl-2 R-1	101.8%
Fenrl-2 R-2	95.4%
Fenrl-2 R-3	98.6%
DiBzAnt-2 R1	105.3%
DiBzAnt-2 R2	106.5%
DiBzAnt-2 R3	104.2%
Mix Blank 2	94.9%
DiBzAnt Blank 2	97.1%
Spike Mix R3	98.3%
Spike Mix R4	98.0%
Spike DiBzAnt R3	102.6%
Spike DiBzAnt R4	100.7%
WA416-17-GC-17 ICV	96.2%
WA416-17-GC-21 CV	98.5%
WA416-17-GC-16C CCV	101.1%
WA416-17-GC-16C CCV	100.8%
WA416-17-GC-16C CCV	107.0%

Table E18. Internal Standard Recovery 3-3-05 Analyses

Sample	Recovery
4Nonyl stab 2 R-1	98.1%
4Nonyl stab 2 R-2	98.8%
4Nonyl stab 2 R-3	100.4%
4Nonyl stab Blk 2	95.5%
Spike nonyl R-6	96.4%
Spike nonyl R-7	98.6%
WA416-nonyl-6 ICV	99.9%
WA416-nonyl-8 CV	97.5%
WA416-nonyl-3 C CCV	100.3%
WA416-nonyl-3C CCV	103.8%

Table E19. Internal Standard Recovery 3-4-05 Analyses

Sample	Recovery
MDL R-1	97.3%
MDL R-2	97.3%
MDL R-3	103.8%
MDL R-4	98.9%
MDL R-5	103.2%
MDL R-6	102.4%
MDL R-7	114.8%
Dico Stab 1 R-1	97.9%
Dico Stab 1 R-2	97.8%
Dico Stab 1 R-3	98.4%
MDL Blank	98.2%
Dico stability blk1	97.1%
Spike R-1	94.2%
Spike R-2	95.1%
Spike R-3	95.1%
Spike R-4	95.0%
Spike R-5	95.4%
WA416-Dico-5 ICV	98.1%
WA416-Dico-4 C CCV5	97.6%
WA416-Dico-4 C CCV5	100.2%
WA416-Dico-4 C CCV5	100.5%

Table E20. Internal Standard Recovery 3-18-05 Analyses

Sample	Recovery
Dico Stab 2 R-1	101.4%
Dico Stab 2 R-2	100.4%
Dico Stab 2 R-3	100.3%
Dico stability blk2	98.5%
Spike R-6	100.3%
Spike R-7	98.5%
WA416-Dico-8 ICV	92.4%
WA416-Dico-10 CV	101.5%
WA416-Dico-4 C CCV5	103.6%
WA416-Dico-4 C CCV5	107.6%

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Chemical Repository Services for the EDSP
EPA Contract No. 68-W-01-023

1.0 TITLE PAGE

Report Title: Formulation of Test Substances in DMSO, Concentration Verification, Stability, and Shipping for Work Assignments 4-16 and 4-17

Author: Michael Cobb

Report Completion Date: December 15, 2005

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Work Assignment: 4-16 and 4-17

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Total Number of Pages: 105

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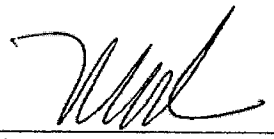
2.0 STATEMENT OF COMPLIANCE

This portion of the study meets the requirements for 40 CFR Part 160, EPA FIFRA Good Laboratory Practices with the following exceptions:

The supplier purity claims for the test substances were verified by the Chemical Repository, but the purity claims of the reference substances (4-nonylphenol and 5-α-androstane) as characterized by the supplier were accepted and not verified. Characterization of both test and reference substances is a GLP requirement. This aspect of the study was not in compliance with GLPs.

Documentation for preparing prochloraz samples on July 25, 2005 was not recorded on the day of preparation. Based on close correlation of results on July 25 and the subsequent analyses, this lack of timely documentation does not impact the results or interpretation of the data.

Note: Method deviations occurred and are noted in Appendix G of this report.

Chemical Repository Principal Investigator: 

Michael Cobb
Battelle – Marine Science Laboratory

12-15-05
Date

3.0 QUALITY ASSURANCE

This portion of the study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989. The dates of all audits and inspections and the dates of any findings were reported to the MSL Chemical Repository (CR) Principal Investigator (PI) and Management and Study Director and Test Facility Management were as follows:

ACTIVITY	DATE CONDUCTED	DATE REPORTED TO:	
		MSL CR PI & MANAGEMENT	STUDY DIRECTOR & MANAGEMENT
In Process Inspection Preparation of atrazine and Dicofol solutions for analysis	Sept 9, 2005	Sept 13, 2005	Oct 3, 2005
Data & Draft Report	Oct 14, 2005	Oct 17, 2005	Nov 3, 2005
Final Report	Dec 15, 2005	Dec 15, 2005	Dec 15, 2005

Mary E Lynn

Mary E. Lynn
Quality Assurance

12/15/05
Date

4.0 APPROVALS PAGE

Study Title: Formulation of Test Substances in DMSO, Concentration Verification, Stability, and Shipping for Work Assignments 4-16 and 4-17

Submitted by: Battelle – Marine Research Operations
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Michael E. Cobb — 12/15/05 Date
EDSP Chemical Repository Principal Investigator
Battelle – Marine Research Operations

Approved by:

Eric Crecelius — 12/15/05 Date
Eric Crecelius
Manager, EDSP Chemical Repository
Battelle – Marine Research Operations

Personnel participating in this study:

Formulation Preparation: Rhonda Karls
Analyst: Tim Fortman, Rebecca Wood
Chemical Repository PI: Michael Cobb

Experimental Dates:

Date of first formulation preparation: July 25, 2005

Date of last analysis: October 3, 2005

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6.0 INTRODUCTION

Dr. Jerry Johnson, Work Assignment Leader (WAL) for Work Assignments 4-16 and 4-17 issued a directive for the Endocrine Disruptor Screening Program (EDSP) Chemical Repository (CR) to prepare and ship a group of test substances each formulated in the carrier dimethylsulfoxide (DMSO).

The directive follows: *Below is a tentative preparation and assay conduct schedule.*

1) *Please review the dates planned for the preparations and let me know if this will be acceptable. A volume of 50 mL will be prepared for each formulation. Of this 50 mL, 10 mL will be sent to each of four labs (40 mL) and the remainder (~10 mL) will be retained at the CR for possible future analysis in case the stability needs to be extended.*

2) *Please confirm that the concentration values (mg/mL and M) are correct according to the formulation preparation and stability results.*

3) *Please confirm that the stability information is current.*

4) *Please confirm that if 50 mL of each reference chemical is prepared at the concentration listed (including the quantity of chemical needed for analysis, standards, etc.) that there is enough chemical available in the inventory to do so.*

5) *Please note that the formulation labels will have to contain the code below in order for the labs to run the chemicals blind. The actual information may be sent to the labs, but at the labs, someone other than the technicians performing the assay will need to unpack the shipment.*

Prep/Analyze/Ship[†]	Labs Conduct Assay	Chemical Code No.	Chemical	Concentration mg/mL	Vehicle	Stability
Week of July 25	Weeks of August 1 and August 8	3	Prochloraz	38.0 (0.1 M)	100% DMSO	14 days
		6	Fenarimol	33.2 (0.1 M)	100% DMSO	14 days
Week of August 29	Weeks of Sept 5 and Sept 12	9	Dicofol	36.8 (0.1 M)	100% DMSO	14 days
		10	Atrazine	21.3 (0.1 M)	100% DMSO	14 days
Week of Sept 12	Weeks of Sept 19 and Sept 26	4	4-Nonylphenol	24.1 (0.1 M)	100% DMSO	14 days
		5	Dibenz[a,h]Anthracene	2.78 (0.01 M)	100% DMSO	14 days

[†]Column one indicates schedule for initial Chemical Repository Tasks.

In addition to the initial formulation preparation and concentration verification (analysis) work, the CR was also charged with checking stability of the test solutions four weeks after preparation. During the assignment, Dr. Johnson requested additional stability checks as recorded in the data.

7.0 GENERAL METHODS

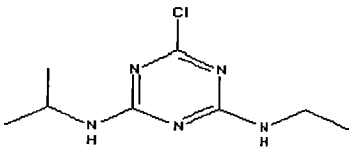
Methods of standard operation of the CR are currently addressed in MSL SOPs numbered R-001 through R-017. These procedures address chemical procurement, ordering, handling, inventory, and storage requirements; chemical receipt and chain of custody, chemical log-in and labeling, inventory, chemical storage; stock solution preparation, documentation and archiving; test solution preparation, formulation analysis, documentation and shipping; chemical disposal, and

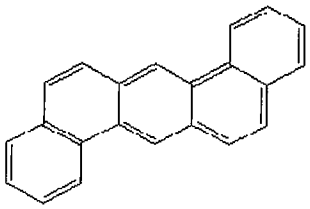
CR maintenance over time. The requirements for procurement of chemicals for use in the CR are addressed in the Quality Assurance Project Plan (QAPP) for EDSP Chemical Repository.

7.1 TEST SUBSTANCE PROCUREMENT

The test substances and vehicle for this study were in inventory at the time of the above directive so no additional procurement was required. Each of the chemicals is maintained in the CR inventory in a clean, dry location, and at a temperature as specified in the individual test substance material safety data sheets (MSDS). Table 1 provides the chemical name, structure, and facts pertinent to the study regarding each of the test substances. Table 2 provides parallel information regarding the study vehicle.

Table 1. Study Test Substances

Parameter	Test Substance	Atrazine
Compound Name	Atrazine	
CAS #	1912-24-9	
Central File No.	2332-1	
Molecular Weight	215.72 g/mole	
Initial Receipt Date	12/14/04	
Expiration Date	10/07	
Supplier	Supelco/Chem Service	
Lot Number	328-137A	
Vendor Purity	98%	
Method	EDSP.G2-022	

Parameter	Test Substance	Dibenz(A,H)Anthracene
Compound Name	Dibenz[a,h]Anthracene	
CAS #	53-70-3	
Central File No.	2319-1	
Molecular Weight	278.35 g/mole	
Initial Receipt Date	12/06/04	
Expiration Date	12/06/10	
Supplier	Sigma	
Lot Number	11613BC	
Vendor Purity	97%	
Method	EDSP.G2-022	

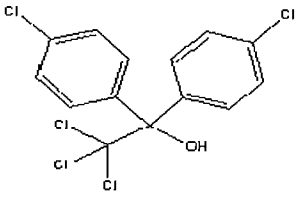
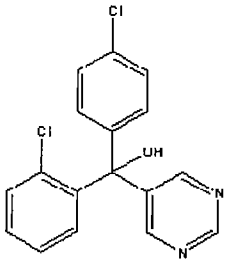
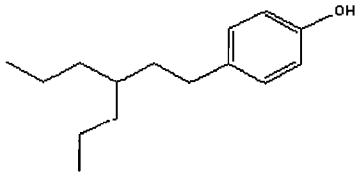
Parameter	Test Substance	Dicofol
Compound Name	Dicofol	
CAS #	115-32-2	
Central File No.	2391-1	
Molecular Weight	370.49 g/mole	
Initial Receipt Date	4/11/05	
Expiration Date	Feb. 08	
Supplier	Ultra Scientific	
Lot Number	RM00299	
Vendor Purity	96.5%	
Method	EDSP.G2-025	

Table 1. Study Test Substances (continued)

Parameter	Test Substance	Fenarimol
Compound Name	Fenarimol	
CAS #	60168-88-9	
Central File No.	2335-1	
Molecular Weight	331.21 g/mole	
Initial Receipt Date	01/10/05	
Expiration Date	07/09	
Supplier	Supelco/Chem Service	
Lot Number	325-134C	
Vendor Purity	99%	
Method	EDSP.G2-022	

Parameter	Test Substance	4-Nonylphenol
Compound Name	4-Nonylphenol	
CAS #	84852-15-3	
Central File No.	2305-1	
Molecular Weight	220.35 g/mole	
Initial Receipt Date	02/05/01	
Expiration Date	02/08	
Supplier	Acros Organics	
Lot Number	A0123226	
Vendor Purity	99.8%	
Method	EDSP.G2-024	

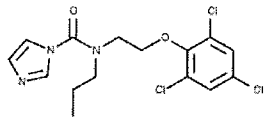
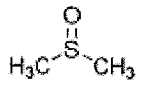
Parameter	Test Substance	Prochloraz
Compound Name	Prochloraz	
CAS #	67747-09-5	
Central File No.	2162-2	
Molecular Weight	376.67 g/mole	
Initial Receipt Date	05/20/04	
Expiration Date	02/14/08	
Supplier	Sigma/Riedel	
Lot Number	2226X	
Vendor Purity	99.5%	
Method	EDSP. H4-023	

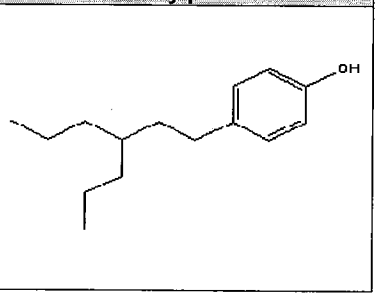
Table 2. Study Vehicle

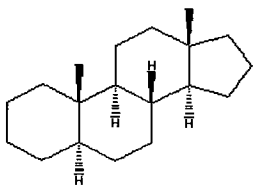
Parameter	Carrier	Dimethylsulfoxide
Compound Name	Dimethylsulfoxide	
CAS #	67-68-5	
Central File No.	Not Applicable	
Initial Receipt Date	01/20/05	
Expiration Date	January 2010	
Supplier	Sigma-Aldrich	
Lot Number	00743BD	

Reference Substances

5-α-androstane was used as an internal standard for gas chromatography analyses. 4-Nonylphenol is the only analyte where a lot other than the test substance was used as a reference substance (Table 3).

Table 3. Reference Substances

Parameter	Reference Substance	4-Nonylphenol
Compound Name	4-Nonylphenol	
CAS #	84852-15-3	
Central File No.	2331	
Molecular Weight	220.35 g/mole	
Initial Receipt Date	12/13/04	
Expiration Date	12/2010	
Supplier	Acros Organics	
Lot Number	A0192712	
Vendor Purity	>98.5%	
Method	EDSP.G2-024	

Parameter	Reference Substance	5-α-androstane
Compound Name	5-α-androstane	
CAS #	438-22-2	
Central File No.	2276-1	
Molecular Weight	260.46 g/mole	
Initial Receipt Date	10/12/04	
Expiration Date	10/12/09	
Supplier	Sigma-Aldrich	
Lot Number	054K4027	
Vendor Purity	99%	

7.2 TEST SUBSTANCE FORMULATION

Per the directive provided by Dr. Johnson, formulations of each of the test substances were prepared as specified. For each formulation prepared, a *Chemical Repository Stock and Sample Preparation Worksheet* was generated. Worksheets for the six formulations are provided in Appendix B.

Appropriate quantities of each chemical (see Table 4) were weighed to the nearest 0.1 mg into individually tared, clean[1] dry amber glass bottles. 50 mL of DMSO was added to each bottle and the bottles were capped and vortexed and checked visually for test substance dissolution. Five of the six test substances went into solution during this step of the process. The sixth test substance, atrazine, did not appear to be dissolved at this stage. The capped bottle was then placed in a sonicator for ~15 minutes but still appeared as a cloudy suspension after this step. The atrazine suspension was allowed to sit overnight in the dark at room temperature and then the following morning heated to ~40°C on a hot plate for 10 minutes. The formulation was then visually inspected and the atrazine appeared to be in solution

Table 4. Formulations

Test Substance	Mass Added grams	Vehicle Mass in mg (mL)	Conc. mg/mL*	Conc. molarity
Atrazine	1.0786	54.800 (50 mL)	21.572	0.100
Dibenz[a,h]Anthracene	0.1392	54.810 (50 mL)	2.783	0.010
Dicofol	1.8525	54.800 (50 mL)	37.050	0.100
Fenarimol	1.6564	54.800 (50 mL)	33.128	0.100
4-Nonylphenol	1.1018	54.800 (50 mL)	22.036	0.100
Prochloraz	1.8836	54.800 (50 mL)	37.672	0.100

*Concentrations are mg test substance per mL DMSO (vehicle)

[1] Prior to use, bottles are ashed for a minimum of 8 hours at 400°C per the CR QAPP.

7.3 FORMULATION CONCENTRATION VERIFICATION

The six formulations were prepared on the schedule presented in the directive in the introduction, Section 6.0, except for dicofol and atrazine, which were prepared a week earlier than scheduled. To assure the early preparation did not impact the contract labs, an additional stability measurement was included in the schedule. Verification of the prep concentrations and follow-on stability analyses of the formulations were carried out using the methods (Table 5) developed for the initial chemistry purity/stability work on WA 4-16/17 carried out by the CR. Results for the formulation concentration verification and follow-on stability assessments are provided in Table 6.

Table 5. Analytical Methods

Test Substance	Atrazine	Dibenz[a,h] anthracene	Dicofol	Fenarimol	4-Nonylphenol	Prochloraz
Analysis Method	EDSP.G2-022	EDSP.G2-022	EDSP.G2-025	EDSP.G2-022	EDSP.G2-024	EDSP.H4-023

Table 6. Formulation Concentration Verification and Stability

Test Substance	Atrazine	Dibenz[A,H] Anthracene	Dicofol	Fenarimol	4-Nonylphenol	Prochloraz
[Expected] mg/mL	21.572	2.783	37.050	33.128	22.036	37.672
Day 0 Accuracy*(date)	92.5%(8-23)	97.7%(9-14)	91.0%(8-23)	91. 8%(7-26)	99.0%(9-13)	101.9%(7-25)
Extended Stability*(date)	92.8%(9-9)	97.0%(10-3)	94.8%(9-9)	90.5%(8-24)	97.0%(10-3)	96.7%(8-25)
Additional Stability*(date)	91.7%(9-19)	-	96.0%(9-20)	-	-	97.1%(9-26)

*(actual/expected) X 100

7.4 FORMULATION SHIPPING

Formulations were aliquoted for shipping into appropriately cleaned/ashed amber bottles as documented in Table 7.

Table 7. Formulation Aliquots Shipped to Labs

Test Substance	Vol. (mL) Shipped to Research Triangle Institute (RTI)	Vol. (mL) Shipped to WIL Research Labs	Vol. (mL) Shipped to In Vitro Technologies	Vol. (mL) Shipped to Battelle Columbus	Vol. (mL) Retained By CR	Storage Condition of CR Retained Aliquot
Atrazine	10	10	10	10	10	~5°C
Dibenz[a,h]Anthracene	10	10	10	10	10	Room Temperature
Dicofol	10	10	10	10	10	~5°C
Fenarimol	10	10	10	10	10	Room Temperature
4-Nonylphenol	10	10	10	10	10	2-8°C
Prochloraz	10	10	10	10	10	Room Temperature

Each bottle was labeled with the: test substance code name and concentration in DMSO, volume provided, expiration date of formulation prepared by, appropriate storage temperature, and preparation date. Each shipment included the formulations as listed in Table 7, a chain of custody (CoC), a letter describing the shipment and directions for signing and returning the CoC,

and a self-addressed stamped envelope for the return of the CoC. Each shipment also contained a sealed envelope containing an MSDS for each test substance provided, a certificate of analysis (CoA) for each test substance provided, and a letter to the laboratory safety officer with the test substance name associated with each formulation code number to allow the formulation code to be broken in case of a laboratory accident with the blinded solutions.

All formulations were shipped via Fed Ex overnight as described on each CoC document. Recipient labs were instructed to store the formulations per the CoC specifications between usages.

7.5 ARCHIVING

Samples of the six test solutions retained by the CR were stored as noted in Table 7 for follow-on stability evaluations or for provision to study labs as required.

Archive samples of the test substances employed in this study will be maintained in the EDSP Chemical Repository for the shelf life indicated on the chemical label.

The protocol, any amendments provided, all records, and the final report generated as a result of this study will be transported to and maintained for archival purposes at the following address:

PNNL Records Management
540 Fifth Street
Richland, WA 99352
PH: 509.375.2340

APPENDIX A

SUPPLIER'S CERTIFICATES OF TEST AND REFERENCE SUBSTANCE ANALYSIS/PURITY

[redacted]

660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599
1-800-452-9994 • 1-610-692-3026 • Fax 1-610-692-8729
info@chemservice.com • www.chemservice.com

CERTIFICATE OF ANALYSIS

INVOICE #: CS255881
PO #: P337541

CATALOG #: PS-380

CAS #: 1912-24-9

DESCRIPTION: Atrazine

To re-order CHEM SERVICE products call
SUPELCO
800-247-6628 or 814-359-3441
or any local Supelco sales office
T800-0031

LOT #: 328-137A

PURITY: 98%

EXPIRATION DATE: 10/07

Chem Service, Inc. guarantees the purity of this chemical ± 0.5% deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Two or more of the following methods of analysis are used to determine purity: Melting point, refractive index, titration, FTIR, IR, TLC, GC/FID, GC/TCD, GC/ECD, GC/MS, HPLC or DSC.

Our standards are suitable for use with all EPA methods.

Certified By:

John Conrad

John Conrad
CSM/TC

ISO 9001
Certificate Number: 31610

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Dibenz[a,h]anthracene, 97%
Product Number	D3,140-0
Product Brand	ALDRICH
CAS Number	53-70-3
Molecular Formula	$C_{22}H_{14}$
Molecular Weight	278.35

TEST	SPECIFICATION	LOT 11613BC RESULTS
APPEARANCE	LIGHT YELLOW TO YELLOW CRYSTALLINE POWDER OR	YELLOW POWDER
INFRARED SPECTRUM	CONFORMS TO STRUCTURE AND STANDARD AS	CONFORMS TO STRUCTURE AND STANDARD
THIN-LAYER CHROMATOGRAPHY	CONSISTENT WITH 97% PURITY	CONSISTENT WITH 97% PURITY
SOLUBILITY	5% IN TOLUENE; CLEAR, COLORLESS TO LIGHT YELLOW, LIGHT GREEN OR LIGHT YELLOW-GREEN	5 MG/ML, TOLUENE; CLEAR FAINT YELLOW SOLUTION
QUALITY CONTROL ACCEPTANCE DATE		FEBRUARY, 2004

Ronnie J. Martin, Supervisor
Quality Control
Milwaukee, Wisconsin USA

ULTRA SCIENTIFIC
Analytical Solutions

Certificate of Analysis

Dicofol

Product Number: PST-391 **Expiration Date:** Feb-2008

Lot Number: RM00299 **Page:** 1 of 1

This reference material has been analyzed by high resolution gas chromatography or high performance liquid chromatography, and found to meet the specifications stated below. The uncertainty of the purity measurement is ± 0.5%.

Compound	CAS #	Purity
dicofol	000115-32-2	96.5%

Storage: May be stored at room temperature

Quality System
Quality Endorsed Company ISO 9001 SAI Global Registered

ACCREDITED
ISO 17025 Cert. No. 0851-01

250 Smith Street, North Kingstown, RI 02852 USA
401-294-9400 Fax: 401-295-2330
www.ultrasci.com

Dr. Edward Fitzgerald,
Senior Scientist

660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599
1-800-452-9994 • 1-610-692-3026 • Fax 1-610-692-8729
info@chemservice.com • www.chemservice.com

CERTIFICATE OF ANALYSIS

INVOICE #: CS257624
PO #: P339294

CATALOG #: PS-1073

CAS #: 60168-88-9

DESCRIPTION: Fenarimol

LOT #: 325-134C

PURITY: 99%

EXPIRATION DATE: 07/09

To re-order CHEM SERVICE products call
SUPELCO
800-247-6628 or 814-359-3441
or any local Supelco sales office
T800-0031

Chem Service, Inc. guarantees the purity of this chemical ± 0.5% deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Two or more of the following methods of analysis are used to determine purity: Melting point, refractive index, titration, FTIR, IR, TLC, GC/FID, GC/TCD, GC/ECD, GC/MS, HPLC or DSC.

Our standards are suitable for use with all EPA methods.

Certified By:

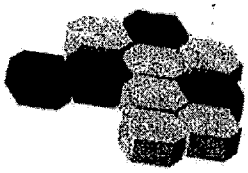
John Conrad

John Conrad
CSM/TC

ISO 9001
Certificate Number: 31610

CERTIFICATE OF ANALYSIS

Product 41624-0000
4-NONYLPHENOL, 99%, MIXTURE OF ISOMERS



General Product Data

Version	01
CAS No.	84852-15-3
Molecular weight	220.35
Molecular formula	C15 H24 O
Linear formula	141
Flash point (°C)	

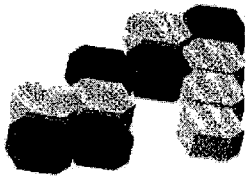
Lot Specific Data **Lot No. A0123226**

Appearance	clear viscous liquid (< 40 APHA)
Infrared spectrometry	AUTHENTIC
Separat. techn. GC	99.8 %
Water	0.04 %
Refractive index	1.5159 (20°C, 589 nm)
Additional info	ORTHO-NONYLPHENOL: < 5% DINONYLPHENOL: 0.08% PHENOL: 0.05%

ACROS ORGANICS
a Fisher Scientific Worldwide company

Issued: 7/10/04
A. Vanneste Quality Control Manager

Acros Organics bvba Geel West 2, Janssen Pharmaceuticalaan 3a, B-2440 Geel, Belgium *Tel.:+32(0)14/57.52.11 *Fax.: +32(0)14/59.34.34 *Internet: http://www.acros.com
2000 Park Lane Drive, Pittsburgh,PA 15275-1126, USA *Tel.:1-800-766-7000 *Fax.:1-800-926-11-66 *Internet: http://www.fishersci3llc.com



08/10 2004 10:12 FAX 003

SIGMA-ALDRICH

Riedel-de Haën

Sigma-Aldrich Laborchemikalien GmbH D-30918 Seelze
Telefon: +49 5137 8258-831

CERTIFICATE OF ANALYSIS /
INSPECTION CERTIFICATE 3.1.B acc. to EN10204

Seelze, 23.08.2002/363592

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production date: 14.Aug.2002
Min. shelf life: 14.Feb.2004

Article/Product: 45631 Batch : 2226X

PROCHLORAZ PESTANAL®
(N-PROPYL-N-(2-(2,4,6-TRICHLOROPHENOXY)ETHYL)IMIDAZOL-1-CARBOXAMIDE)

Reference Material (RM)

1. General Information

Formula: C15H16Cl3N3O2 Molar mass: 376.67 g/Mole

CAS-No.: [67747-09-5]

Usage : Fungicide

2. Batch Analysis

Identity (NMR)	complying	
Assay (HPLC)	99.5	%
Melting range	47.5-49.1	°C
Date of Analysis	23.Aug.2002	

3. Advice and Remarks

- The minimum shelf life is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliquot portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
- We herewith confirm that the delivery is effected according to the technical delivery conditions agreed.
- The batch from which we delivered, showed the above-mentioned values.
- Particular properties of the product or the suitability for a particular area of application are not assured.
- We guarantee a proper quality within our General conditions of Sales.

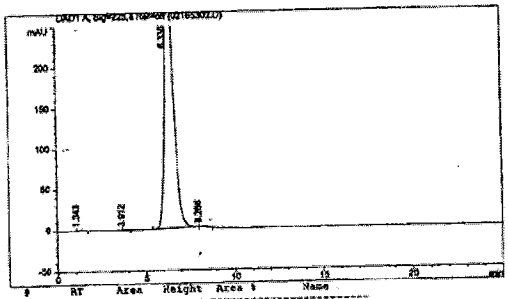
Sigma-Aldrich Laborchemikalien GmbH
Quality Assurance

(Dr. Gaede)
Works Inspector

Honeywell Specialty Chemicals Seelze GmbH
Analytical Department

HPLC-method

Article	:	Prochloraz
Article-No	:	45631
Batch	:	2226X
Column	:	L=250mm, ID=4,6mm; Supelcosil LC-18 5um
Eluent	:	Acetonitril 60 %
		Water +0.28 % Na_2HPO_4 40 %
Flow	:	1,4ml/min
Detector	:	UV-225nm
Injection-Volume	:	20ul
Sample-preparation	:	0,1mg/ml Eluent
Linearity	:	checked
Evaluation	:	Normalisation (uncorrected)
Operator	:	Eiben



#	RT	Area	Height	Area %	Name
1	1.343	24.293	1.737	0.176	
2	3.912	8.089	0.767	0.059	
3	6.336	1.375e4	549.628	99.462	
4	8.266	42.046	1.801	0.304	

Certificate of Analysis

Product41624-0000
4-NONYLPHENOL, 99%, MIXTURE OF ISOMERS

General Product Data	
Version	00
CAS No	84852-15-3
Molecular weight	220.35
Molecular formula	C15 H24 O
Linear formula	
Flash point (°C)	141

Lot Specific Data **A0192712**

Appearance	clear viscous liquid
Color scale	<50 APHA
Infrared spectrometry	authentic
Separat. techn. GC	>98.5 %
Water	<0.05 %
Refractive index	1.5119 (20°C, 589 nm)

APPENDIX B

CHEMICAL REPOSITORY STOCK AND SAMPLE PREPARATIONS WORKSHEETS

Chemical Repository Stock and Sample Preparation Worksheet

Fill In Data (Initial or circle "shaded" items to signify completion)

Neat Chemical				
Date:	7/22/2005	Cert. of Analysis:	(Y) or N	
Analyst:	RK	MSDS:	(Y) or N	
Chemical Name:	Prochloraz	Purity:	Min, 99.5% per mfg.	
Bottle CF Number:	2162-2	Building:	MSL-5	
CMS Barcode:	Several - See below	Room:	219	
Supplier Name:	Sigma-Aldrich	Location:	Cabinet #1, Shelf #3	
Lot Number:	2226X	CAS:	67747-09-5	
Date Received:	5/20/2004			
Expiration Date:	Feb-08			
Formula Weight:	376.67			

SIGMA-ALDRICH / RIEDEL-de Haën
RK 12-1-05

253067 253072 253070 253069 253071 253066 253065 253083 253068 252970 253074

Solvent			
Date:	7/22/2005	Building:	MSL-5
Analyst:	RK	Room:	219
Solvent Name:	Dimethyl sulfoxide	Location:	Flammable Cabinet
Supplier Name:	Sigma-Aldrich	CAS:	67-68-5
Lot Number:	00743BD	Catalog Number:	472301-2L
Date Received:	6/3/2005	CMS Barcode:	253353
Specific Gravity:	1.0960	Expiration Date:	Jun-10

Stock Preparation			
Date:	7/25/2005	Balance Information:	
Analyst:	RK	Manufacturer:	Sartorius
Stock Barcode:	Code 3	Model:	B3100P
Expiration Date:	8/7/2005	Serial Number:	1120122767
Target Molarity (mM):	100.0	Calibration Interval:	12 mo.
Volume Needed (mL):	50.00	Last Calibration:	10/18/2004
Target Chemical Mass (g):	1.8834	Calibration Check:	(Y) or N
Target Solvent Mass (g):	54.8073	(documented in Calibration	
Actual Molarity Achieved (mM):	100.0133	Notebook; room MSL-5, 219)	
Step 1: Tare Stock Bottle:	✓		
Add Chemical:	✓		
Actual Chemical Mass (g):	1.88360		
Step 2: Tare Stock Bottle again:	✓		
Add Solvent:	✓	Chemical Disolved in Solvent:	(Y) or N
Actual Solvent Mass (g):	54.8000		

Formulas Used

$$\text{Target Chemical Mass (g)} = \text{Target Molarity (Moles/L)} \times \text{Volume Needed (L)} \times \text{FW (g/Mole)}$$

$$\text{Target Solvent Mass (g)} = \frac{(\text{Actual Chemical Mass (g)} \times \text{Solvent Density (g/L)})}{(\text{Target Molarity (Moles/L)} \times \text{Chemical FW (g/Mole)})}$$

$$\text{Actual Molarity (Moles/L)} = \frac{\left(\frac{\text{Actual Chemical Mass (g)}}{\text{Chemical FW (g/Mole)}}\right)}{\left(\frac{\text{Actual Solvent Mass (g)}}{\text{Solvent Density g/L)}}\right)}$$

Note: Approximately 10 mL of chemical stock was retained and stored @ Room Temperature in Room 219, MSL 5 in drawer labeled EDSP RK 10-19-05

WA 4-16, TASK 8

Chemical Repository Stock and Sample Preparation Worksheet

Fill in Data (Initial or circle "shaded" items to signify completion)

Neat Chemical

Date:	8/22/2005	Cert. of Analysis:	(Y) or N
Analyst:	RKarle	MSDS:	(Y) or N
Chemical Name:	Atrazine	Purity:	Min. 98% per mfg.
Bottle CF Number:	2332-1	Building:	MSL-5
CMS Barcode:	253,212	Room:	219
Supplier Name:	Chem Service	Location:	Cabinet #1, Shelf #1
Lot Number:	328-137A	CAS:	1912-24-9
Date Received:	12/14/2004		
Expiration Date:	~~Oct-05~~ 10/07	RK 12-1-05 COMPUTER ENTRY ERROR	
Formula Weight:	215.72		

Solvent

Date:	8/22/2005	Building:	MSL-5
Analyst:	RKarle	Room:	219
Solvent Name:	Dimethyl sulfoxide	Location:	Flammable Cabinet
Supplier Name:	Sigma-Aldrich	CAS:	67-68-5
Lot Number:	00743BD	Catalog Number:	472301-2L
Date Received:	6/3/2005	CMS Barcode:	253353
Specific Gravity:	1.0960	Expiration Date:	Jun-10

Stock Preparation

Date:	8/22/2005	Balance Information:	
Analyst:	RKarle	Manufacturer:	Sartorius
Stock Barcode:	Code 10	Model:	B3100P
Expiration Date:	9/5/2005	Serial Number:	1120122767
Target Molarity (mM):	100.0	Calibration Interval:	12 mo.
Volume Needed (mL):	50.00	Last Calibration:	10/18/2004
Target Chemical Mass (g):	1.0786	Calibration Check:	(Y) or N
Target Solvent Mass (g):	54.8000	(documented in Calibration Notebook; room MSL-5, 219)	
Actual Molarity Achieved (mM):	100.0000		

Step 1:	Tare Stock Bottle:	✓	Sonicated 10-15 minutes and chemical is still visable. Swirling, cloud
	Add Chemical:	✓	
	Actual Chemical Mass (g):	1.07860	
Step 2:	Tare Stock Bottle again:	✓	Chemical Disolved in Solvent: * (Y) or N ✓
	Add Solvent:	✓	
	Actual Solvent Mass (g):	54.8000	

Formulas Used

$$\text{Target Chemical Mass (g)} = \text{Target Molarity (Moles/L)} \times \text{Volume Needed (L)} \times \text{FW (g/Mole)}$$

$$\text{Target Solvent Mass (g)} = \frac{(\text{Actual Chemical Mass (g)} \times \text{Solvent Density (g/L)})}{(\text{Target Molarity (Moles/L)} \times \text{Chemical FW (g/Mole)})}$$

$$\text{Actual Molarity (Moles/L)} = \frac{\left(\frac{\text{Actual Chemical Mass (g)}}{\text{Chemical FW (g/Mole)}}\right)}{\left(\frac{\text{Actual Solvent Mass (g)}}{\text{Solvent Density g/L)}}\right)}$$

Note: Approximately 10 mL of stock was retained and stored @ ~5°C in Fridge #1, Room 219, MSL 5, RKarle 10-19-05

* 8-23-05 - Due to initial insolubility of the Atrazine, the formulation was allowed to sit in the dark @ room temperature overnight then heated to ~40°C for 10 minutes on a hot plate. The material was then visually inspected and the atrazine appeared to be in solution. RK

Chemical Repository Stock and Sample Preparation Worksheet

Fill in Data (initial or circle "shaded" items to signify completion)

Neat Chemical				
Date:	9/13/2005	Cert. of Analysis:	(Y) or N	
Analyst:	RK	MSDS:	(Y) or N	
Chemical Name:	Dibenz[a,h]anthracene	Purity:	97% per mfg.	
Bottle CF Number:	2319-1	Building:	MSL-5	
CMS Barcode:	254404-254433	Room:	219	
Supplier Name:	Sigma	Location:	Cabinet #1, Shelf #2	
Lot Number:	11613BC	CAS:	53-70-3	
Date Received:	12/6/2004			
Expiration Date:	Dec-10			
Formula Weight:	278.35			

Solvent			
Date:	9/13/2005	Building:	MSL-5
Analyst:	RK	Room:	219
Solvent Name:	Dimethyl sulfoxide	Location:	Flammable Cabinet
Supplier Name:	Sigma-Aldrich	CAS:	67-68-5
Lot Number:	00743BD	Catalog Number:	472301-2L
Date Received:	6/3/2005	CMS Barcode:	253353
Specific Gravity:	1.0960	Expiration Date:	Jun-10

Stock Preparation			
Date:	9/13/2005	Balance Information:	
Analyst:	RK	Manufacturer:	Sartorius
Stock Barcode:	Code 5	Model:	B3100P
Expiration Date:	9/28/2005	Serial Number:	1120122767
Target Molarity (mM):	10.0	Calibration Interval:	12 mo.
Volume Needed (mL):	50.00	Last Calibration:	10/18/2004
Target Chemical Mass (g):	0.1392	Calibration Check:	(Y) or N
Target Solvent Mass (g):	54.8098	(documented in Calibration	
Actual Molarity Achieved (mM):	10.0000	Notebook; room MSL-5, 219)	

Step 1:	Tare Stock Bottle:	✓
	Add Chemical:	✓
	Actual Chemical Mass (g):	0.13920
Step 2:	Tare Stock Bottle again:	✓
	Add Solvent:	✓
	Actual Solvent Mass (g):	54.8100

Chemical Disolved in Solvent: (Y) or N

Formulas Used

$$\text{Target Chemical Mass (g)} = \text{Target Molarity (Moles/L)} \times \text{Volume Needed (L)} \times \text{FW (g/Mole)}$$

$$\text{Target Solvent Mass (g)} = \frac{(\text{Actual Chemical Mass (g)} \times \text{Solvent Density (g/L)})}{(\text{Target Molarity (Moles/L)} \times \text{Chemical FW (g/Mole)})}$$

$$\text{Actual Molarity (Moles/L)} = \frac{\left(\frac{\text{Actual Chemical Mass (g)}}{\text{Chemical FW (g/Mole)}}\right)}{\left(\frac{\text{Actual Solvent Mass (g)}}{\text{Solvent Density g/L)}}\right)}$$

Note: Approximately 10 ml of chemical stock was retained and stored @ Room Temperature in Rm 219, MSL5 in drawer labeled EDSP. RK 10-19-05

WA 4-16, TASK 8

Chemical Repository Stock and Sample Preparation Worksheet

Fill in Data (Initial or circle "shaded" items to signify completion)

Neat Chemical

Date:	8/22/2005	Cert. of Analysis:	(Y) or N
Analyst:	RKarls	MSDS:	(Y) or N
Chemical Name:	Dicofol	Purity:	Min, 96.5% per mfg.
Bottle CF Number:	2391-1	Building:	MSL-5
CMS Barcode:	253313	Room:	219
Supplier Name:	Ultra	Location:	Cabinet #1, Shelf #1
Lot Number:	RM00299	CAS:	115-32-2
Date Received:	4/11/2005		
Expiration Date:	Feb-08		
Formula Weight:	370.49		

Solvent

Date:	8/22/2005	Building:	MSL-5
Analyst:	RKarls	Room:	219
Solvent Name:	Dimethyl sulfoxide	Location:	Flammable Cabinet
Supplier Name:	Sigma-Aldrich	CAS:	67-68-5
Lot Number:	00743BD	Catalog Number:	472301-2L
Date Received:	6/3/2005	CMS Barcode:	253353
Specific Gravity:	1.0960	Expiration Date:	Jun-10

Stock Preparation

Date:	8/22/2005	Balance Information:	
Analyst:	RKarls	Manufacturer:	Sartorius
Stock Barcode:	Code 9	Model:	B3100P
Expiration Date:	9/5/2005	Serial Number:	1120122767
Target Molarity (mM):	100.0	Calibration Interval:	12 mo.
Volume Needed (mL):	50.00	Last Calibration:	10/18/2004
Target Chemical Mass (g):	1.8525	Calibration Check:	(Y) or N
Target Solvent Mass (g):	54.8015	(documented in Calibration Notebook; room MSL-5, 219)	
Actual Molarity Achieved (mM):	100.0027		

Step 1:	Tare Stock Bottle:	✓
	Add Chemical:	✓
	Actual Chemical Mass (g):	1.85250
Step 2:	Tare Stock Bottle again:	✓
	Add Solvent:	✓
	Actual Solvent Mass (g):	54.8000

Chemical Disolved in Solvent: (Y) or N

Formulas Used

$$\text{Target Chemical Mass (g)} = \text{Target Molarity (Moles/L)} \times \text{Volume Needed (L)} \times \text{FW (g/Mole)}$$

$$\text{Target Solvent Mass (g)} = \frac{(\text{Actual Chemical Mass (g)} \times \text{Solvent Density (g/L)})}{(\text{Target Molarity (Moles/L)} \times \text{Chemical FW (g/Mole)})}$$

$$\text{Actual Molarity (Moles/L)} = \frac{\left(\frac{\text{Actual Chemical Mass (g)}}{\text{Chemical FW (g/Mole)}}\right)}{\left(\frac{\text{Actual Solvent Mass (g)}}{\text{Solvent Density g/L)}}\right)}$$

Note: Approximately 10 mL (Cstock) of chemical was retained and stored @ ~5°C in Fridge #1, Room 219, MSL 5. RKarls 10-19-05

Chemical Repository Stock and Sample Preparation Worksheet

Fill in Data (Initial or circle "shaded" items to signify completion)

Neat Chemical				
Date:	7/22/2005	Cert. of Analysis:	(Y) or N	
Analyst:	RK	MSDS:	(Y) or N	
Chemical Name:	Fenarimol	Purity:	Min. 99% per mfg.	
Bottle CF Number:	2335-1	Building:	MSL-5	
CMS Barcode:	Several - See below	Room:	219	
Supplier Name:	Supelco	Location:	Cabinet #1, Shelf #3	
Lot Number:	LB325-134C	CAS:	60168-88-9	
Date Received:	1/10/2005			
Expiration Date:	Jul-09			
Formula Weight:	331.21			

254459 254471 254472 254465 254482 254467 254481 254461 254454 254475 254464

Solvent			
Date:	7/22/2005	Building:	MSL-5
Analyst:	RK	Room:	219
Solvent Name:	Dimethyl sulfoxide	Location:	Flammable Cabinet
Supplier Name:	Sigma-Aldrich	CAS:	67-68-5
Lot Number:	00743BD	Catalog Number:	472301-2L
Date Received:	6/3/2005	CMS Barcode:	253353
Specific Gravity:	1.0960	Expiration Date:	~~Jun-06~~ 06/10

RL 12-1-05 COMPUTER ENTRY ERROR

Stock Preparation			Balance Information:	
Date:	7/25/2005		Manufacturer:	Sartorius
Analyst:	RK		Model:	B3100P
Stock Barcode:	Code 6		Serial Number:	1120122767
Expiration Date:	8/7/2005		Calibration Interval:	12 mo.
Target Molarity (mM):	100.0		Last Calibration:	10/18/2004
Volume Needed (mL):	50.00		Calibration Check:	(Y) or N
Target Chemical Mass (g):	1.6561		(documented in Calibration	
Target Solvent Mass (g):	54.8116		Notebook; room MSL-5, 219)	
Actual Molarity Achieved (mM):	100.0211			
Step 1: Tare Stock Bottle:	✓			
Add Chemical:	✓			
Actual Chemical Mass (g):	1.65640			
Step 2: Tare Stock Bottle again:	✓		Chemical	
Add Solvent:	✓		Dissolved	(Y) or N
Actual Solvent Mass (g):	54.8000		in Solvent:	

Formulas Used

$$\text{Target Chemical Mass (g)} = \text{Target Molarity (Moles/L)} \times \text{Volume Needed (L)} \times \text{FW (g/Mole)}$$

$$\text{Target Solvent Mass (g)} = \frac{(\text{Actual Chemical Mass (g)} \times \text{Solvent Density (g/L)})}{(\text{Target Molarity (Moles/L)} \times \text{Chemical FW (g/Mole)})}$$

$$\text{Actual Molarity (Moles/L)} = \frac{\left(\frac{\text{Actual Chemical Mass (g)}}{\text{Chemical FW (g/Mole)}}\right)}{\left(\frac{\text{Actual Solvent Mass (g)}}{\text{Solvent Density g/L)}}\right)}$$

Note: Approximately 10 mL of chemical stock was retained and stored @ Room Temperature in Room 219, MSL 5 in drawer Labeled EDSP. RK 10-19-05

Chemical Repository Stock and Sample Preparation Worksheet

Fill In Data (Initial or circle "shaded" items to signify completion)

Neat Chemical				
Date:	9/13/2005	Cert. of Analysis:	(Y) or N	
Analyst:	RK	MSDS:	(Y) or N	
Chemical Name:	4-Nonylphenol	Purity:	99.8% per mfg.	
Bottle CF Number:	2305-1	Building:	MSL-5	
CMS Barcode:	173899	Room:	219	
Supplier Name:	Acros	Location:	Cabinet #1, Shelf #1	
Lot Number:	A01232261 ~~A01232261~~ ~~A01927101~~ RK 12-1-05 RF	CAS:	84852-15-3	
Date Received:	2/5/2001			
Expiration Date:	Feb-08			
Formula Weight:	220.35			

RK 12-1-05 CC

Solvent			
Date:	9/13/2005	Building:	MSL-5
Analyst:	RK	Room:	219
Solvent Name:	Dimethyl sulfoxide	Location:	Flammable Cabinet
Supplier Name:	Sigma-Aldrich	CAS:	67-68-5
Lot Number:	00743BD	Catalog Number:	472301-2L
Date Received:	6/3/2005	CMS Barcode:	253353
Specific Gravity:	1.0960	Expiration Date:	Jun-10

Stock Preparation			Balance Information:	
Date:	9/13/2005		Manufacturer:	Sartorius
Analyst:	RK		Model:	B3100P
Stock Barcode:	Code 4		Serial Number:	1120122767
Expiration Date:	9/29/2005		Calibration Interval:	12 mo.
Target Molarity (mM):	100.0		Last Calibration:	10/18/2004
Volume Needed (mL):	50.00		Calibration Check:	(Y) or N
Target Chemical Mass (g):	1.1018		(documented in Calibration	
Target Solvent Mass (g):	54.8025		Notebook; room MSL-5, 219)	
Actual Molarity Achieved (mM):	100.0045			
Step 1: Tare Stock Bottle:	✓			
Add Chemical:	✓			
Actual Chemical Mass (g):	1.10180			
Step 2: Tare Stock Bottle again:	✓		Chemical	
Add Solvent:	✓		Dissolved	(Y) or N
Actual Solvent Mass (g):	54.8000		In Solvent:	

Formulas Used

$$\text{Target Chemical Mass (g)} = \text{Target Molarity (Moles/L)} \times \text{Volume Needed (L)} \times \text{FW (g/Mole)}$$

$$\text{Target Solvent Mass (g)} = \frac{(\text{Actual Chemical Mass (g)} \times \text{Solvent Density (g/L)})}{(\text{Target Molarity (Moles/L)} \times \text{Chemical FW (g/Mole)})}$$

$$\text{Actual Molarity (Moles/L)} = \frac{\left(\frac{\text{Actual Chemical Mass (g)}}{\text{Chemical FW (g/Mole)}}\right)}{\left(\frac{\text{Actual Solvent Mass (g)}}{\text{Solvent Density g/L}}\right)}$$

Note: approximately 10 mL of stock was retained and stored @ ~2-8°C in Fridge #1, Room 219, MSL5. RK 10-19-05

APPENDIX C

CHAIN OF CUSTODY DOCUMENTS

EDSP CHAIN OF CUSTODY RECORD

Dr. Jim Mathews / Ms. Sherry Black

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

(FED Ex) UPS Other

CONDITIONS: (circle one)

Ice Dry Ice Cold Packs (Ambient)

RECOMMENDED STORAGE CONDITIONS: (circle one)

(RT) 4°C -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observations, Instructions
1	STOCK CODE: 3 COMMENTS: 10 mL of 100 mM solution		1	Store at RT
2	STOCK CODE: 6 COMMENTS: 10 mL of 100 mM solution		1	Store at RT

Relinquished By:

M. McCarthy 7/27/05 1500
Signature Date Time

M McCARTHY BATTELLE
Printed Name Company

Received By:

7-28-05
Signature Date Time

J.E. LARSON RTI
Printed Name Company

VERIFIED COPY

INT. JL DATE 8-1-05

EDSP CHAIN OF CUSTODY RECORD

Dr. Jennifer Wohlever / Mr. Justin Godsey

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

(FED Ex) UPS Other

CONDITIONS: (circle one)

Ice Dry Ice Cold Packs (Ambient)

RECOMMENDED STORAGE CONDITIONS: (circle one)

(RT) 4°C -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observations, Instructions
1	STOCK CODE: 3 COMMENTS: 10 mL of 100 mM solution		1	Store at RT
2	STOCK CODE: 6 COMMENTS: 10 mL of 100 mM solution		1	Store at RT

Relinquished By:

Signature Date Time

M-McCARTHY Battelle

Printed Name Company

Received By:

Pete Resnis 7-28-05 1130 hrs

Signature Date Time

PETE RESNIS WIL RESEARCH

Printed Name Company

Certified Copy

Init: Date: 7.28.05

EDSP CHAIN OF CUSTODY RECORD

Dr. Neil Jensen / Mr. Tim Moeller

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

(FED Ex) UPS Other

CONDITIONS: (circle one)

Ice Dry Ice Cold Packs (Ambient)

RECOMMENDED STORAGE CONDITIONS: (circle one)

(RT) 4°C -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observations, Instructions
1	STOCK CODE: 3 COMMENTS: 10 mL of 100 mM solution		1	Store at RT
2	STOCK CODE: 6 COMMENTS: 10 mL of 100 mM solution		1	Store at RT

Relinquished By:

Signature Date Time

7/27/05 1500

M. McCARTHY BATTELLE

Printed Name Company

Received By:

Signature Date Time

29 Jul 05 1530

Tony Tasem Invitro

Printed Name Company

EDSP CHAIN OF CUSTODY RECORD

Dr. Bozena Lusiak / Mr. Tom Deck

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

(FED Ex) UPS Other

CONDITIONS: (circle one)

Ice Dry Ice Cold Packs (Ambient)

RECOMMENDED STORAGE CONDITIONS: (circle one)

(RT) 4°C -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observations, Instructions
1	STOCK CODE: 3 COMMENTS: 10 mL of 100 mM solution	7-28-05	1	Store at RT
2	STOCK CODE: 6 COMMENTS: 10 mL of 100 mM solution	7-28-05	1	Store at RT

Relinquished By:

M. McCarthy 7/27/05 1500
Signature Date Time

M. McCARTHY BATTELLE
Printed Name Company

Received By:

Shawn Norton 7-28-05 1:00pm
Signature Date Time

Shawn Norton Battelle Memorial
Printed Name Company

EDSP CHAIN OF CUSTODY RECORD

Dr. Jim Mathews / Ms. Sherry Black

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16, Task 8

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

(FED Ex) UPS Other

CONDITIONS: (circle one)

Ice Dry Ice (Cold Packs) Ambient

RECOMMENDED STORAGE CONDITIONS: (circle one)

RT (2-8°C) -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observations, Instructions
1	STOCK CODE: 9 COMMENTS: 10 mL of 100 mM solution		1	Store at ~5C
2	STOCK CODE: 10 COMMENTS: 10 mL of 100 mM solution		1	Store at ~5C

Relinquished By:

M. Mathews 8/24/05 1500
Signature Date Time

M. McArthur BATTELLE
Printed Name Company

Received By:

Randy A Price 8-25-05
Signature Date Time

Randy Price RTI
Printed Name Company

VERIFIED COPY

INT. RP DATE 8-31-05

EDSP CHAIN OF CUSTODY RECORD

Dr. Jennifer Wohlever / Mr. Justin Godsey

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16, Task 8

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

FED Ex UPS Other

CONDITIONS: (circle one)

Ice Dry Ice Cold Packs Ambient

RECOMMENDED STORAGE CONDITIONS: (circle one)

RT 2-8°C -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observations, Instructions
1	STOCK CODE: 9 COMMENTS: 10 mL of 100 mM solution		1	Store at ~5C
2	STOCK CODE: 10 COMMENTS: 10 mL of 100 mM solution		1	Store at ~5C

Relinquished By:

M. McMartin 8/24/05 1500
Signature Date Time

M. McMartin Battelle
Printed Name Company

Received By:

Pete Resnis 8-25-05 1140
Signature Date Time

Pete Resnis WIL Research
Printed Name Company

CERTIFIED COPY

DATE 8-25-05 INITIALS BC

RECEIVED
AUG 25 2005

EDSP CHAIN OF CUSTODY RECORD

Dr. Neil Jensen / Mr. Tim Moeller

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16, Task 8

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

(FED Ex) UPS Other

CONDITIONS: (circle one)

Ice Dry Ice (Cold Packs) Ambient

RECOMMENDED STORAGE CONDITIONS: (circle one)

RT (2-8°C) -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observations, Instructions
1	STOCK CODE: 9 COMMENTS: 10 mL of 100 mM solution		1	Store at ~5C
2	STOCK CODE: 10 COMMENTS: 10 mL of 100 mM solution		1	Store at ~5C

Relinquished By:

Signature Date 8/24/05 Time 1500

M. McCarthy BATTELLE

Printed Name Company

Received By:

Not Signed & returned M. Cobb

Signature Date Time 16/08/05

Printed Name Company

EDSP CHAIN OF CUSTODY RECORD

Dr. Bozena Lusiak / Mr. Tom Deck

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16, Task 8

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

(FED Ex) UPS Other

CONDITIONS: (circle one)

Ice Dry Ice (Cold Packs) Ambient

RECOMMENDED STORAGE CONDITIONS: (circle one)

RT (2-8°C) -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observations, Instructions
1	STOCK CODE: 9 COMMENTS: 10 mL of 100 mM solution		1	Store at ~5C
2	STOCK CODE: 10 COMMENTS: 10 mL of 100 mM solution		1	Store at ~5C

Relinquished By:

Signature Date Time

Printed Name Company

Received By:

Shawn Norton 8-26-05 12:45pm

Signature Date Time

Shawn Norton Battelle Memorial

Printed Name Company

EDSP CHAIN OF CUSTODY RECORD

Dr. Jim Mathews/Ms. Sherry Black

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

FED Ex UPS Other

CONDITIONS: (circle one)

Ice Dry Ice Cold Packs Ambient

RECOMMENDED STORAGE CONDITIONS: (circle one)

RT 4°C -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observation & Instructions
1	STOCK CODE: 4 COMMENTS: 10 mL of 0.1M solution		1	Store at 2-8°C
2	STOCK CODE: 5 COMMENTS: 10 mL of 0.01M solution		1	Store at 2-8°C

Relinquished By:

Signature 9/14/05 Date 1510 Time

MCCARTHY Printed Name BATTELLE Company

Received By:

Don Hubbard 9-15-05 Date 2:30 p.m. Time

DON HUBBARD Printed Name RTI INTERNATIONAL Company

VERIFIED COPY
INT. DH DATE 9-16-05

EDSP CHAIN OF CUSTODY RECORD

Dr. Jennifer Wohlever / Mr. Justin Godsey

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

(FED Ex) UPS Other

CONDITIONS: (circle one)

Ice Dry Ice (Cold Packs) Ambient

RECOMMENDED STORAGE CONDITIONS: (circle one)

RT (4°C) -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observations, Instructions
1	STOCK CODE: 4 COMMENTS: 10 mL of 0.1M solution		1	Store at 2-8°C
2	STOCK CODE: 5 COMMENTS: 10 mL of 0.01M solution		1	Store at 2-8°C

Relinquished By:

[signature] 9/14/05 1510
Signature Date Time

MC MARTIN BATTELLE
Printed Name Company

Received By:

[signature] 9-15-05 1255
Signature Date Time

PETE RESNIS WIL RESEARCH LABS
Printed Name Company

Certified Copy
Init: AR **Date:** 9-15-05

EDSP CHAIN OF CUSTODY RECORD

Dr. Neil Jensen/Mr. Tim Moeller

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

FED Ex UPS Other

CONDITIONS: (circle one)

Ice Dry Ice Cold Packs Ambient

RECOMMENDED STORAGE CONDITIONS: (circle one)

RT 4°C -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observation & Instructions
1	STOCK CODE: 4 COMMENTS: 10 mL of 0.1M solution		1	Store at 2-8°C
2	STOCK CODE: 5 COMMENTS: 10 mL of 0.01M solution		1	Store at 2-8°C

Relinquished By:

Signature 9/14/05 1510
Date Time

McCARTHY BATTELLE
Printed Name Company

Received By:

Signature 9/15/05 9a
Date Time

Tony Twen IUT
Printed Name Company

EDSP CHAIN OF CUSTODY RECORD

Dr. Bozena Lusiak/Mr. Tom Deck

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 4-16

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

FED Ex UPS Other

CONDITIONS: (circle one)

Ice Dry Ice Cold Packs Ambient

RECOMMENDED STORAGE CONDITIONS: (circle one)

RT 4°C -10°C -20°C -80°C

	CHEMICALS: 1X10 mL each 100% DMSO	Receipt Date	# of containers	Observations, Instructions
1	STOCK CODE: 4 COMMENTS: 10 mL of 0.1M solution	9/15/05	1	Store at 2-8°C
2	STOCK CODE: 5 COMMENTS: 10 mL of 0.01M solution	9/15/05	1	Store at 2-8°C

Relinquished By:

Signature: [signature] Date: 9/14/05 Time: 1510

Printed Name: MCCARTHY Company: BATTELLE

Received By:

Signature: [signature] Date: 9/15/05 Time: 12:45PM

Printed Name: LesleeSingh Company: Battelle

APPENDIX D

SHIPPING LETTERS

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr Jim Mathews / Ms. Sherry Black
Research Triangle Institute
3040 Cornwallis Road
Research Triangle Park, NC 27709
Tele: (919) 541-6270

July 27, 2005

Dear Dr. Mathews and Ms. Black:

Re: Provision of WA 4-16 test chemicals in 100% DMSO.

Enclosed:

Item #	Containers	Contents	Vol.	Code
1	Brown Glass	Test compound in DMSO	1 X 10 mL	3
2	Brown Glass	Test compound in DMSO	1 X 10 mL	6

Copies of the MSDS' and Certificates of Analysis are provided in a sealed envelope for the safety officer as well as the information associating a chemical name and concentration with each code. Also find a Chain of Custody form and a self addressed stamped envelope (SASE). Please sign, date, time, and print your name on the Chain of Custody Form and send a copy back to me in the SASE. Since this is a blind study, the MSDS' and Certificates of Analysis should be restricted to and maintained by the Safety Officer. Code identities of the test compounds are provided on each of the MSDS documents for decoding in case of contamination.

If you have any questions, please feel free to contact me via, email, phone, or fax.

Sincerely,

Rhonda K. Karls for Michael E. Cobb

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr. Jennifer Wohlever / Mr. Justin Godsey
WIL Laboratories
1407 George Road
Ashland, OH 44805-9281
Tele: (419) 289-8700

July 27, 2005

Dear Dr. Wohlever and Mr. Godsey:

Re: Provision of WA 4-16 test chemicals in 100% DMSO.

Enclosed:

Item #	Containers	Contents	Vol.	Code
1	Brown Glass	Test compound in DMSO	1 X 10 mL	3
2	Brown Glass	Test compound in DMSO	1 X 10 mL	6

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Sincerely,

Rhonda K. Karls for Michael E. Cobb

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr. Neil Jensen / Mr. Tim Moeller
In Vitro Technologies, Inc.
1450 South Rolling Road
Baltimore, MD 21227
Tele: (410) 455-1242

July 27, 2005

Dear Dr. Jensen and Mr. Moeller:

Re: Provision of WA 4-16 test chemicals in 100% DMSO.

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Sincerely,

Rhonda K. Karls for Michael E. Cobb

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr. Bozena Lusiak / Mr. Tom Deck
Battelle Memorial Institute
651 West Fifth Avenue
Columbus, OH 43201-2693
Tele : (614) 424-4366

July 27, 2005

Dear Dr. Lusiak and Mr. Deck:

Re: Provision of WA 4-16 test chemicals in 100% DMSO.

Enclosed:

Item #	Containers	Contents	Vol.	Code
1	Brown Glass	Test compound in DMSO	1 X 10 mL	3
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Sincerely,

Rhonda K. Karls for Michael E. Cobb

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr Jim Mathews / Ms. Sherry Black
Research Triangle Institute
3040 Cornwallis Road
Research Triangle Park, NC 27709
Tele: (919) 541-6270

August 24, 2005

Dear Dr. Mathews and Ms. Black:

Re: Provision of WA 4-16, Task 8 test chemicals in 100% DMSO.

Enclosed:

Item #	Containers	Contents	Vol.	Code
1	Brown Glass	Test compound in DMSO	1 X 10 mL	9
2	Brown Glass	Test compound in DMSO	1 X 10 mL	10

Copies of the MSDS' and Certificates of Analysis are provided in a sealed envelope for the safety officer as well as the information associating a chemical name and concentration with each code. Also find a Chain of Custody form and a self addressed stamped envelope (SASE). Please sign, date, time, and print your name on the Chain of Custody Form and send a copy back to me in the SASE. Since this is a blind study, the MSDS' and Certificates of Analysis should be restricted to and maintained by the Safety Officer. Code identities of the test compounds are provided on each of the MSDS documents for decoding in case of contamination.

If you have any questions, please feel free to contact me via, email, phone, or fax.

Sincerely,

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr. Jennifer Wohlever / Mr. Justin Godsey
WIL Laboratories
1407 George Road
Ashland, OH 44805-9281
Tele: (419) 289-8700

August 24, 2005

Dear Dr. Wohlever and Mr. Godsey:

Re: Provision of WA 4-16, Task 8 test chemicals in 100% DMSO.

Enclosed:

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Sincerely,

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr. Neil Jensen / Mr. Tim Moeller
In Vitro Technologies, Inc.
1450 South Rolling Road
Baltimore, MD 21227
Tele: (410) 455-1242

August 24, 2005

Dear Dr. Jensen and Mr. Moeller:

Re: Provision of WA 4-16, Task 8 test chemicals in 100% DMSO.

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If you have any questions, please feel free to contact me via, email, phone, or fax.

Sincerely,

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr. Bozena Lusiak / Mr. Tom Deck
Battelle Memorial Institute
651 West Fifth Avenue
Columbus, OH 43201-2693
Tele : (614) 424-4366

August 24, 2005

Dear Dr. Lusiak and Mr. Deck:

Re: Provision of WA 4-16, Task 8 test chemicals in 100% DMSO.

Enclosed:

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Sincerely,

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr Jim Mathews/Ms. Sherry Black
Research Triangle Institute
3040 Cornwallis Road
Research Triangle Park, NC 27709
Tele: (919) 541-6270

September 14, 2005

Dear Dr. Mathews and Ms. Black:

Re: Provision of WA 4-16 test chemicals in 100% DMSO.

Enclosed:

Item #	Containers	Contents	Vol.	Code
1	Brown Glass	Test compound in DMSO	1 X 10 mL	4
2	Brown Glass	Test compound in DMSO	1 X 10 mL	5

Copies of the MSDS' and Certificates of Analysis are provided in a sealed envelope for the safety officer as well as the information associating a chemical name and concentration with each code. Also find a Chain of Custody form and a self addressed stamped envelope (SASE). Please sign, date, time, and print your name on the Chain of Custody Form and send a copy back to me in the SASE. Since this is a blind study, the MSDS' and Certificates of Analysis should be restricted to and maintained by the Safety Officer. Code identities of the test compounds are provided on each of the MSDS documents for decoding in case of contamination.

If you have any questions, please feel free to contact me via, email, phone, or fax.

Sincerely,

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr. Jennifer Wohlever /Mr. Justin Godsey
WIL Laboratories
1407 George Road
Ashland, OH 44805-9281
Tele: (419) 289-8700

September 14, 2005

Dear Dr. Wohlever and Mr. Godsey:

Re: Provision of WA 4-16 test chemicals in 100% DMSO.

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1	Brown Glass	Test compound in DMSO	1 X 10 mL	4
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If you have any questions, please feel free to contact me via, email, phone, or fax.

Sincerely,

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr. Neil Jensen/Mr. Tim Moeller
In Vitro Technologies, Inc.
1450 South Rolling Road
Baltimore, MD 21227
Tele: (410) 455-1242

September 14, 2005

Dear Dr. Jensen and Mr. Moeller:

Re: Provision of WA 4-16 test chemicals in 100% DMSO.

Enclosed:

Item #	Containers	Contents	Vol.	Code
1	Brown Glass	Test compound in DMSO	1 X 10 mL	4
2	Brown Glass	Test compound in DMSO	1 X 10 mL	5

Copies of the MSDS' and Certificates of Analysis are provided in a sealed envelope for the safety officer as well as the information associating a chemical name and concentration with each code. Also find a Chain of Custody form and a self addressed stamped envelope (SASE). Please sign, date, time, and print your name on the Chain of Custody Form and send a copy back to me in the SASE. Since this is a blind study, the MSDS' and Certificates of Analysis should be restricted to and maintained by the Safety Officer. Code identities of the test compounds are provided on each of the MSDS documents for decoding in case of contamination.

If you have any questions, please feel free to contact me via, email, phone, or fax.

Sincerely,

Michael E. Cobb
Battelle

cc: Blanton, Michael

Battelle
. . . Putting Technology To Work

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road,
Sequim WA 98382
Attn: Michael E. Cobb
Ph: (360) 681-4580
Fax (360) 681-3699
Email: michael.cobb@pnl.gov

Dr. Bozena Lusiak/Mr. Tom Deck
Battelle Memorial Institute
651 West Fifth Avenue
Columbus, OH 43201-2693
Tele : (614) 424-4366

September 14, 2005

Dear Dr. Lusiak and Mr. Deck:

Re: Provision of WA 4-16 test chemicals in 100% DMSO.

Enclosed:

Item #	Containers	Contents	Vol.	Code
1	Brown Glass	Test compound in DMSO	1 X 10 mL	4
2	Brown Glass	Test compound in DMSO	1 X 10 mL	5

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If you have any questions, please feel free to contact me via, email, phone, or fax.

Sincerely,

Michael E. Cobb
Battelle

cc: Blanton, Michael

APPENDIX E

SAFETY OFFICERS LETTERS

SAFETY OFFICER

Enclosed please find MSDS' and Certificates of Analysis for the 2 chemicals provided as solutions to your laboratory. Please communicate any handling hazards indicated in the MSDS' to lab personnel while maintaining the blinded study. Each MSDS has the bar code information hand printed at the top of the first page to allow decoding for your use in case a problem arises requiring immediate dissemination of safety information.

#	Chemical	CAS #	Purity Mfg.	MW	Conc. In 100% EtOH	MFG.	MSL Code
1	Prochloraz	67747-09-5	99.5%	376.67	100 mM	Sigma	Code 3
2	Fenarimol	60168-88-9	99%	331.21	100 mM	Supelco	Code 6

SAFETY OFFICER

Enclosed please find MSDS' and Certificates of Analysis for the 2 chemicals provided as solutions to your laboratory. Please communicate any handling hazards indicated in the MSDS' to lab personnel while maintaining the blinded study. Each MSDS has the bar code information hand printed at the top of the first page to allow decoding for your use in case a problem arises requiring immediate dissemination of safety information.

#	Chemical	CAS #	Purity Mfg.	MW	Conc. In 100% EtOH	MFG.	MSL Code
1	Difocol	115-32-2	96.5%	370.49	100 mM	Ultra	Code 9
2	Atrazine	1912-24-9	98%	215.72	100 mM	Supelco	Code 10

SAFETY OFFICER

Enclosed please find MSDS' and Certificates of Analysis for the 2 chemicals provided as solutions to your laboratory. Please communicate any handling hazards indicated in the MSDS' to lab personnel while maintaining the blinded study. Each MSDS has the bar code information hand printed at the top of the first page to allow decoding for your use in case a problem arises requiring immediate dissemination of safety information.

#	Chemical	CAS #	Purity Mfg.	MW	Conc. in 100% EtOH	MFG.	MSL Code
1	4-Nonylphenol	84852-15-3	99.8%	220.35	0.1M	Acros	Code 4
2	Dibenz[a,h]anthracene	53-70-3	97%	278.35	0.01M	Sigma	Code 5

APPENDIX F

Analytical Methods

Note: The 6 attachments at the end of each of the methods are identical, so these 6 sheets were only included in the first method to eliminate repetition in the report.

Battelle
The Business of Innovation

Marine Sciences Laboratory

EFFECTIVE DATE: 2-7-05

Method # EDSP.G2-022-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

ANALYSIS OF ATRAZENE, DIBENZ (A,H) ANTHRACENE AND FENARIMOL IN DIMETHYL SULFOXIDE USING GC WITH FID DETECTION

Approvals:		
AUTHOR: Tim Fortman	Tim Fortman	2-7-05
	Signature	Date
TECHNICAL REVIEWER: Rebecca Wood	Rebecca Wood	2/7/05
	Signature	Date
CHEMICAL REPOSITORY DIRECTOR: Michael Cobb		2/7/05
	Signature	Date

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ANALYSIS OF ATRAZENE, DIBENZ (A,H) ANTHRACENE AND FENARIMOLE IN DIMETHYL SULFOXIDE USING GC WITH FID DETECTION

1.0 SCOPE AND APPLICATION

This method describes the determination of Atrazine (CAS# 1912-24-9), Dibenz (a,h) Anthracine (CAS# 53-70-3) and Fenarimole (CAS# 60168-88-9) in Dimethyl sulfoxide using GC with FID detection. The method was developed for use in the analysis of Atrazine, Dibenz (a,h) Anthracine and Fenarimole for the EDSP program.

2.0 DEFINITIONS

Initial Calibration Verification (ICV)	A standard made from a neat material different from the material used to make the calibration standards. Used to verify the calibration solutions. If none are available, a 2nd standard from the same neat material is acceptable.
Continuing Calibration Verification (CCV)	A mid level calibration standard run after every 10 samples to ensure the instrument is in calibration.
Internal Standard (IS)	A compound in solution added to every sample to adjust for variation (the internal standard is also in the calibration solutions).

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation.
Analyst - analysis, calculations
QA Manager or Representative - data verification

4.0 ANALYSIS

4.1 Hardware and Reagents

- Balance capable of weighing to 0.0001 g
- Gas chromatograph (Agilent 5890 or equivalent) with an FID (Flame ionization detector) and an autosampler.
- J+W DB-5 30m X 0.25 mm X 0.25 um film thickness GC column or equivalent.
- Dichloromethane, GC-MS grade or better.
- Atrazine (CAS# 1912-24-9), Dibenz (a,h) Anthracine (CAS# 53-70-3), Fenarimol (CAS# 60168-88-9) and 5a Androstane, (CAS# 438-22-2) 98% purity or better.
- 1.8 ml autosampler vials
- 25, 50 and 100 ml amber glass bottles.
- Helium for carrier gas, Hydrogen, Breathing Air and Nitrogen for FID flame.
- Software for collection of data from FID detector, Varian Star Software, vers. 6.2.
- Variable positive displacement Pipettors, to pipette 0.1 ml and 0.010 ml.
- Varity of volumetric flasks.
- ~~Volumetric flasks~~
- Dimethyl Sulfoxide

4.2 GC conditions

4.2.1 The GC should be set up as per manufacturer specifications, for the system presently in use, the carrier gas pressure is set to 18 PSI, split vent at 89 ml/min, septum purge set at 6.9 ml/min. The FID (flame ionization detector) gasses are set at; air, 300 ml/min, hydrogen, 46 ml/min and nitrogen at 43 ml/min.

4.3 Calibration Solution

4.3.1 A 5 point curve is used to calibrate the GC over a range that will bracket the concentration in the stability tests. To start a stock of all chemicals to be included in the calibration are made at a concentration of about 1000 ug/ml. ~0.05 grams is weighed into a 50 ml volumetric flask and diluted to the mark with dichloromethane. Record exact information on the organic standard preparation form (attachment 1) and give the solution a unique identifying label. Pour the solution into an appropriate size amber vial with a Teflon lined lid. Stability of the calibration solutions should be verified at the end of the test, by the analysis of a new (freshly made) solution made from the neat material and compared to the calibration solutions.

4.3.2 An internal standard is incorporated with this analysis. The chemical 5 a Androstane is used as the internal standard and a ~1000 ug/ml solution is made as described in 4.3.1.

4.3.2 Serially dilute the solution made in 4.3.1 to make standards ranging from 0.5 ug/ml to 100 ug/ml. The internal standard should be the same concentration in all the standards, for this series of solutions, 50 and 100 ml. volumes are used for each, so 1 ml of the 1000 ug/ml internal standard solution is added to the 50 ml vol. flask and 2 ml to the 100 ml vol. flask for a target concentration of 20 ug/ml. This

calibration targets these concentrations; 0.5 ug/ml, 1 ug/ml, 5 ug/ml, 20 ug/ml and 100 ug/ml. Include all information in the standards preparation form (see attachment 1).

4.4 GC Setup

4.4.1 The GC equipment has the following components, an autosampler, GC oven, detector (FID) and a data collection system. The autosampler is set to inject 1 ul and the injection port is set to 285 deg. C. The oven conditions are; initial temp. 100 deg. C., hold for 1.5 min., ramp at 15 deg. per minute to 320 deg. and hold for 10 min. The FID gasses are described in section 4.2.1, the detector temp. is set at 320 deg. C.

4.4.2 The GC is set in the splitless mode and the split vent valve is opened at 2.5 minutes. Split vent and septum purge settings are listed in section 4.2.1.

4.4.3 The column used is a J+W DB-5 30M X 0.25 mm X 0.25 um film thickness. Flow thru the column is controlled by the head pressure which is set at 18 PSI (see section 4.2.1).

4.4.4 The FID detector conditions are described in section 4.2.1. The conditions on the present system are controlled by valves set up for the detector, the supply gasses are set up as follows, Hydrogen pressure, 21 PSI, Air pressure, 58 PSI and nitrogen pressure 34 PSI.

4.4.5 The data system used is Varian's Star software, version 6.2. The system is set up to start automatically with the injection of the sample. The run time is set to 25 minutes. Bunch rate is set to 8 and signal to noise set at 100. Different data systems will employ different nomenclature for the same settings, see software manuals for information on how to optimize the software.

4.4.6 The system used is composed of several different components from different manufacturers. The method printouts will only contain information pertaining to the calibration and quantitation of the unknowns, in order to ensure that the conditions used are recorded, an EDSP GC Analysis Form should be filled out (attachment 2). Also, much of this information should be included in the "notes" tab on Varian Star's method.

4.5 Analysis

4.5.2 Prior to the analysis of any samples linearity must be demonstrated. A 5 point curve is run (minimum of a 4 point curve is needed), the 0 point not considered a calibration point. An r^2 value of greater than 0.995 is necessary before analysis can begin.

4.5.3 Once the calibration is done, if possible it must be verified with an initial calibration verification sample (ICV). An independent solution is made using a different lot of the WA 4-16/17 chemicals when available, if not, use of the existing lot is OK. These solutions are diluted to the proper concentration so that it is within the calibration range. This solution is run and the value obtained should be within 15% of the expected value.

4.5.4 After the calibration is verified, a continuing calibration verification (CCV) sample is run. This sample is usually one of the mid level calibration solutions. The value obtained should be within 15% of the expected value. A CCV should be run after every 10 samples.

~~4.5.5 A blank should be prepared with each sampling. A blank is prepared for each dilution. For example if 0.005 ml of sample is taken and diluted with the~~

typo - repeated on next page MW 11/2/05

4.5.5 A blank should be prepared with each sampling. A blank is prepared for each dilution. For example if 0.005 ml of sample is taken and diluted with the dichloromethane, a blank using 0.005 ml of dimethyl sulfoxide is treated as the sample. The blank should be < 3X MDL.

4.5.6 Method Detection Limit (MDL) is determined by preparing a sample at a low concentration, using similar techniques as used to analyze the stability solution. This is done 7 times and the MDL is the students T (3.143 for 7 replicates) times the standard deviation of the seven replicate runs. An MDL should be performed prior to the analysis of any sample. Samples with no peak or less than the MDL will be reported as the MDL and flagged with a "U.

4.6 Purity

Typo mb 12/2/05

4.6.1 The confirmation of purity is a required component of EDSP studies per the Chemical Repository (CR) QAPP (Ver 2.1 01/07/05). Purity analysis will be performed unless the CR is instructed not to do so by the sponsor (EPA) due to technical or budgetary reasons. The Chemical Characterization SOP# MSL-R-009-00 has additional information. Purity is determined by running a solution of the material that is at or near the top of the demonstrated linearity of the system. The solution contains only the chemical of interest, no internal standard or other chemicals, dissolved in dichloromethane. This solution is injected onto the GC and all the peaks in the chromatogram are summarized. The peak corresponding to the chemical of interest is then compared to all the other peaks and the purity is the area of the chemical peak divided by the sum of the total area in the chromatogram (presented as a percentage). A blank dichloromethane is run prior to the purity run and the peaks in the purity run that correlate to peaks in the blank run are eliminated from the calculation (this includes the solvent peak). This purity should be 98% or greater and should compare favorably to the purity from the vendor. See example purity form: attachment 3.

5.0 STABILITY AND FORMULATION VERIFICATION

5.1 Stability for WA 4-16 and WA4-17 chemicals is to run for 14 days. Stability is prepared by weighing an aliquot of each chemical into 10 milliliters of dimethyl sulfoxide and storing the solution in a 30 milliliter amber bottle (attachment #). The details for the stability are contained in the Study Protocol. Target concentration is 0.1 M, for Atrazine, 21.6 mg/ml, Fenarimole, 33.1 mg/ml and 0.01 M for dibenzo (a,h) anthracine or 2.78 mg/ml. Sampling and analysis will be done 2 times over the course of the stability study.

#5 Typo 12/2/05 mb

5.2 Sampling is done by removing an aliquot of the sample and diluting it into dichloromethane with an aliquot of the internal standard. For solutions at 0.1 M, 0.005 ml is added to a 5 ml volumetric flask along with 0.1 ml of the internal standard (1000 ug/ml 5 a androstane) and diluted to mark with dichloromethane. An aliquot of this is placed into a a 1.8 ml autosampler vial for analysis. For the 0.01 M solution, 0.025 ml is placed into a 1.8 mL autosampler vial with 0.02 ml of the internal standard (1000 ug/ml 5a Androstane) and 0.955 mL of dichloromethane. The vials are capped and are ready for GC analysis.

5.3 For an example of the form used for the stability preparation and sampling see attachments ~~4 and~~ 5. and 6 typo MB 12/2/05

5.4 Samples should be analyzed on the day of sampling, but if this is not possible, samples should be stored at 4 deg. C. until analysis. If samples are not analyzed on the day of sampling, the actual analysis date and storage conditions shall be documented.

5.5 Stability solutions are stored in the same conditions as the neat material. For this study, all are stored at room temperature.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 Prior to analysis of any samples, the instrument is calibrated with a minimum of a 4 point curve. Internal standard calculations will be performed. All calculations will be done using Varian's Star chromatography software, version 6.2. This software allows the input of a multiplier, so that any dilutions will be included with the software calculations. For example, for Atrazine stability, 0.005 ml of the 10 ml sample solution is diluted to 5 ml, a multiplier of 1000 is used so that the output from the software will give values that reflect the concentration in the diluted solution. Calibration curve fits can be set to either linear or non-linear (quadratic fit), past experience indicates that even though the calibration meets linearity criteria, the quantification is improved with a non-linear fit.

6.2 Prior to the tabulation of the data, each chromatogram and report printed by the software will be initialed and dated for GLP compliance.

7.0 QUALITY CONTROL

7.1 A blank is prepared with each sampling, this blank is dimethyl sulfoxide processed identically to the stability solution. If background levels are sufficiently high (i.e., greater than 3 x MDL), this value may be subtracted from the values obtained for samples analyzed with that batch. Processing of these samples is very straight forward, therefore spikes are optional.

7.2 An initial calibration verification (ICV) standard will be analyzed following the calibration curve. This ICV is made from a second source when available, but if a second source is not available, the same source is used. Continuing calibration verification standards (CCVs) will be analyzed after every 10 samples. A CCV is a mid point calibration solution. If CCV variation exceeds a 15% difference from expected, samples will be re-run with acceptable calibration criteria.

7.7 An internal standard is added to each sample, recovery of this internal standard should be ≥80%.

8.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of chemicals, including the following:

- Gloves, protective clothing and safety glasses should be worn when handling samples and chemicals.

9.0 TRAINING REQUIREMENTS

9.1 All staff performing this analysis should first read this procedure and conduct their first analysis under the supervision of a staff member who has had previous experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work. Documentation of training will be performed in accordance with MSL-A-006, Marine Sciences Laboratory Training.

10.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

MSL-Q-007-04 Procedure for Determining Method Detection Limits

Federal Register (CFR Part 136 Appendix B).

Table 1. Summary of Data Quality Objectives and Corrective Actions

Quality Control Sample Type	Data Quality Objective (DQO)	Corrective Action
Procedural Blank one/batch	Less than 3 x MDL	Re-extract and analyze sample batch. If batch can not be re-extracted and analyzed, "B" flag all samples that are in the batch. Investigate sources of blank contamination.
Calibration curve acceptability	r^2 values greater than or equal to 0.995	If r^2 value is outside of criterion, re-analyze calibration standards, if r^2 is still out, perform instrument maintenance and/or remake calibration standards and rerun calibration samples.
Initial calibration verification (ICV) standard; one/batch	+ / - 15 % of true value	Re-calibrate. Must meet DQO in order to continue processing samples.
Internal Standard Recovery	+ / - 20 % of true value	Re-sample and rerun
Continuing calibration verification standards; one every 10th sample analyzed	+/ - 15 % of true value	Re-run CCV, if still not acceptable, re-calibrate and reanalyze affected samples.
Replicate sample precision; triplicates will be analyzed for stability, duplicate for in-life	Precision: 30% as relative standard deviation (RSD) or relative percent deviation (RPD)	If RSD or RPD is not acceptable, resample and reanalyze. If reanalysis data are still not acceptable, then "*" flag the values.
Blank or Matrix Spike and spike duplicate, one set per batch	+/- 20% of true value	If recoveries are unacceptable, check the spike solution to ensure it has not degraded, also check pipettes to ensure they are delivering accurate volumes.

[a] DQO is based on limited sample analysis as part of method development experience, and may require adjustment when more experience with the method is available.

Table 2. Data Qualifiers[a]

U	The analyte was detected below the MDL. Note: Samples with no peaks are reported as zero.
B	Samples associated with procedural blank contamination.
*	QC sample data that does not meet the DQO acceptability criterion.
Q	The data are questionable.
D	Sample diluted for analysis. (note: this procedure outlines the dilution of the samples, data will not be D flagged unless diluted other than indicated in this SOP)

[a] Additional data qualifiers may be added as necessary.

attachment 1

Page_____ of _____ STD ID: _____

Organic Standard Preparation

Prep. Date: _____

Exp. Date: _____

Stock Used and Lot #	Stock Exp. Date/Date Rec.	Stock/Chemical Name	Stock Conc.	Stock Vol./Wt.	Diluted To:	Solvent Mfg, Exp. date & Lot #	Standard Concentration

Solution Prepared By: _____ If Balance used, Balance # _____

Storage Location _____

Purpose of Standard _____

Storage Temp. _____

Liquids handled by (circle ones used) Graduated cylinder Syringe Disposable pipette Vol. Flask

Pipettes used: _____

attachment 2

Mobile Phase Preparation

Solution Name: _____ Prep. Date: _____

Study # _____ Exp. Date: _____

Stock Used and Lot #	Amount (g or ml)	Stock Used and Lot #	Amount (g or ml)	Stock Used and Lot #	Amount (g or ml)	Prepared By: (Intials and Date)

If Balance used, Balance # _____ Storage Location _____

Purpose of Solution _____ Storage Temp. _____

Liquids handled by (circle ones used) Graduated cylinder Syringe Disposable pipette Vol. Flask

Pipettes used: _____

attachment 3

EDSP HPLC Analysis Form

Date _____ Work Study # and Analysis _____

Instrument Used _____
Detector Used _____
(UV/Vis) Wavelength _____
(Fluorescense) Wavelengths Excitation _____ Emission _____
Mobile Phase A _____
Mobile Phase B _____
Loop Size _____
Sample size _____

Desired Flow rate _____
Measured Flow Rate Flow rate _____

Column Used _____
Serial # _____
Pressure _____

Gradient Program, Step #	% Mobile Phase A	% Mobile Phase B	Time

Number of Samples Run _____ Initials and Date _____

Attachment 4

EDSP Purity

Chemical _____ CF# _____

Lot # _____

Instrument Used _____

Standard Prep Name	Date Prepared	Analysis Date	Data File Name	Blank File Name

Area, compound peak	Area Impurity + Compound Peaks	Area Peaks in Blank	Purity *

* **Purity Calculation:** Area Compound Peak / ([Area Impurity Peaks + Compound Peaks] - Area Peaks in Blank)

attachment 5

Stability Solution Preparation

Study Protocol _____ Date: _____

Stock/Chemical Name _____

Manufacturer and Lot # _____

Stock Exp. Date/Date Rec. _____

Diluent + Lot _____

Dose

Dose Name	Stock/Chemical Name	Stock Wt.	Diluted To:	Concentration	Prep. Date:

Solution Prepared By: _____ Balance used, # _____ Storage Location _____

Treatment Conditions Sonicate to dissolve or agitate until dissolved Storage Temp. _____

Liquids handled by (circle ones used) Graduated cylinder Syringe Disposable pipette Vol. Flask

attachment 6

EDSP Sampling

Chemical _____ CF# _____

Lot # _____

Sampling Interval _____ days

Sampling Date _____ Analysis Date _____

Sampler _____

Method used for analysis

Sample Name	Volume Taken	Volume Diluent	Diluent used	Sample Type	Multiplier used	Comments

Liquids handled by (circle ones used) Graduated cylinder Syringe Disposable pipette Vol. Flask

Pipettes used: _____

Diluents used + Exp. Date _____

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Marine Sciences Laboratory

EFFECTIVE DATE: 02-16-05

Method # EDSP.H4-023-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

ANALYSIS OF PROCLORAZ IN DIMETHYLSULFOXIDE USING HPLC WITH UV/VIS DETECTION

Approvals:		
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ANALYSIS OF PROCHLORAZ IN DIMETHYL SULFOXIDE USING HPLC WITH UV/VIS DETECTION

1.0 SCOPE AND APPLICATION

This method describes the determination of Prochloraz in dimethyl sulfoxide (DMSO) using HPLC/UV/Vis detection. The method was developed for use in the analysis of Prochloraz for the EDSP program. The eluent used is an Acetonitrile/Water solution.

2.0 DEFINITIONS

Initial Calibration Verification (ICV)	A standard made from a neat material different from the material used to make the calibration standards. Used to verify the calibration solutions.
Continuing Calibration Verification (CCV)	A mid level calibration standard run after every 10 samples to ensure the instrument is in calibration.

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation.
Analyst - analysis, calculations
QA Manager or Representative - data verification

4.0 ANALYSIS

4.1 Hardware and Reagents

- Balance capable of weighing to 0.0001 g
- High performance liquid chromatograph (Perkin Elmer 250 pump, with a Gilson 294 liquid autosampler) with UV/Vis detector (Waters 486 detector) set at wavelength 204nm.
- Phenomenex Synergi 4µ hydro-RP 80A 250 X 4.6 mm HPLC column Serial # 258206-4.
- Acetonitrile, HPLC grade or better.
- Dimethyl sulfoxide (DMSO), reagent grade.
- Prochloraz, 97% purity or better.
- 1.8 mL vials
- Autosampler vial for Gilson Autosampler.
- Helium for sparging eluents.
- Software for collection of data from UV/Vis detector, Varian Star Software, vers. 6.2.
- 1 liter amber bottle with Teflon lined lid.
- Variable positive displacement Pipettors, to pipette 0.005 mL.
- 47 mm glass filter apparatus for filtration of HPLC eluent, i.e. Micro Filtration System with 300 mL reservoir.

4.2 HPLC Mobile Phase (Eluent)

4.2.1 The pump used is a binary gradient pump, one reservoir is filled with Acetonitrile while the other is filled with DI water, the 75:25 eluent is then made by programming the pump to take 75% acetontrile and 25% water.

4.3 Calibration Solution

4.3.1 A 5 point curve is used to calibrate the HPLC over a range that will bracket the concentration in the stability tests. To start, a stock is made at a concentration of about 2000 ug/mL. Approximately 0.0500 grams is weighed into a 25 mL volumetric flask and diluted to the mark with Acetontrile. Record exact information on the standards preparation form (attachment 1) and give the solution a unique identifying label (include contents, date prepared, prepared by, work assignment number). Pour the solution into an appropriate size amber vial with a Teflon lined lid. Stability of the calibration solutions should be verified at the end of the test by the analysis of a new (freshly made) solution prepared from the neat material and compared to the calibration solutions.

4.3.2 Serially dilute the solution made in 4.3.1 to make standards ranging from 20 ug/mL to 450 ug/mL using a solution that will mimic the eluent, 75% Acetonitrile, 25% water (use Mobile Phase Preparation form to document the preparation of the eluent [diluent] see attachment 2 for example form). Record all information in the standards preparation form (see attachment 1).

4.4 HPLC Setup

4.4.1 The HPLC equipment has 5 main components, a pump, autosampler, HPLC column, detector and data system. The pump is set up to pump at 1.0 mL/min. The mobile phase (eluent) is purged using helium (H_2) for about 15 minutes prior to running the system. The pump is primed as per instrument instructions and the flow directed thru the HPLC system. The pump run time should be set to 13 minutes.

4.4.2 The autosampler is set up to inject 10 ul. A 25 ul loop is installed. See instrument manual for setup details. The autosampler is then set to flush the contaminated surfaces with Acetonitrile.

4.4.3 The column used is a Phenomenex Synergi 4µ hydro RP 80A 250 X 4.6 mm HPLC column Serial # 258206-4. Pressure limit on the column is 3000 PSI, adjust pump so pressure limit will shut the pump off prior to damaging the column.

4.4.4 The detector is a UV/Vis detector set to a wavelength of 204 nm. The detector is attached to the data collection system by way of the analog output from the 1 volt full scale (integrator) terminal.
The data system used is Varian's Star software, version 6.2. The system is set up to start automatically with the injection of the sample. The run time is set to 13 minutes. Data is collected at 10 Hz. Calibration samples are run prior to analysis and the software is used to calculate the unknowns. See software manuals for setup.

4.4.5 This system is composed of several different components from different manufacturers. The method printouts will only contain information pertaining to the calibration and quantitation of the unknowns, in order to ensure that the conditions

used are recorded, an EDSP HPLC Analysis Form should be filled out (attachment 3). Also, much of this information should be included in the "notes" tab on the Varian Star method.

4.5 Analysis

4.5.1 Solutions run on the HPLC should have similar composition to the eluent. For example, the solution for Prochloraz has 0.005 mL of the solution placed into a 1.8 mL vial with 0.995 mL of the eluent. Using a transfer pipette, the solution is mixed then transferred to a 1 mL autosampler vial designed for the Gilson autosampler.

4.5.2 Prior to the analysis of any samples, linearity must be demonstrated. A 5 point curve is run (minimum of a 4 point curve is needed). An r^2 value of greater than 0.995 is necessary before analysis can begin.

4.5.3 Once the calibration is done, if possible, it should be verified with an initial calibration verification sample (ICV). An independent solution is made using a different lot of the Prochloraz and diluted to the proper concentration so that it is within the calibration range. This solution is run and the value obtained should be within +/−10% of the expected value.

4.5.4 After the calibration is verified, a continuing calibration verification (CCV) sample is run. This sample is usually one of the mid level calibration solutions. The value obtained should be within +/−10% of the expected value. A CCV should be run after every 10 samples.

4.5.5 A blank should be prepared with each sampling. For example, if a 0.005 mL of sample is taken and diluted with the eluent, a blank DMSOI using 0.005 mL is treated as the sample. The blank should be < 3X MDL.

4.5.6 Method Detection Limit (MDL) is determined by preparing a sample at a low concentration, using similar techniques as used to analyze the stability solution. This is done 7 times and the MDL is the students T (3.143 for 7 replicates) times the standard deviation of the seven replicate runs. An MDL should be performed prior to the analysis of any sample for ~~Dexamethasone~~ Prochloraz. Samples with no peak or less than the MDL will be reported as the MDL and flagged with a "U.

Multiplier inadvertently left in from previous method

4.6 Purity

4.6.1 All EDSP studies require confirmation of purity unless a written waiver is received from the EPA. The Chemical Characterization SOP# MSL-R-009-00 has additional information. Purity is determined by running a solution of the material that is at or near the top of the demonstrated linearity of the system. All the peaks in the chromatogram are summarized. The peak corresponding to the Prochloraz is then compared to all the other peaks and the purity is the area of the Prochloraz peak divided by the sum of the total area in the chromatogram (presented as a percentage). A blank is run prior to the purity run and the peaks in the purity run that correlate to peaks in the blank run are eliminated from the calculation. This purity should be 97% or greater and should compare favorably to the purity from the vendor. Note: the limitation of using a UV/Vis detector for purity is that one cannot be certain that the impurities will absorb at the same wavelength. This purity is an estimation. See example purity form: attachment 4.

5.0 STABILITY

5.1 Stability for Prochloraz is to run for 14 days. Stability is prepared by weighing an aliquot of Prochloraz into a 30 milliliter amber bottle with 10 milliliters of DMSO (attachment 5). The details for the stability are contained in the Study Protocol. Target concentration is 0.1 M, or about 37.7 mg/mL. Sampling and analysis will be done 8 times over the course of the stability study. 2 typo TMB 12/2/05

5.2 Sampling is done by removing an aliquot of the sample and diluting it into a solution similar to the mobile phase. 0.005 mL of the solution is placed into a 1.8 mL autosampler vial with 0.995 mL of the mobile phase (see 4.2.1). Using a transfer pipette, mix the solution in the vial then transfer it to a 1 mL autosampler vial that works with the autosampler.

5.3 For an example of the form used for the stability preparation and sampling see attachments 5 and 6.

5.4 Samples should be analyzed on the day of sampling, but if this is not possible, samples should be stored at 4 deg. C. until analysis. If samples are not analyzed in the day of sampling, the actual analysis date and storage conditions shall be documented.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 Prior to analysis of any samples, the instrument is calibrated with a minimum of a 4 point curve. External standard calculations will be performed. All calculations will be done using Varian's Star chromatography software, version 6.2. This software allows the input of a multiplier, so that any dilutions will be included with the software calculations. For example, for Prochloraz stability, 0.005 mL of the stock is diluted with 0.995 mL of eluent and a multiplier of 200 is used so that the output from the software will give values that reflect the concentration in the stability solution. Calibration curve fits can be set to non-linear (quadratic fit) or a linear fit.

6.2 Prior to the tabulation of the data, each chromatogram and report printed by the software will be initialed and dated for GLP compliance.

7.0 QUALITY CONTROL

A blank is prepared with each sampling, this blank is DMSO processed identically to the stability solution. If background levels are sufficiently high (i.e., greater than 3 x MDL), this value may be subtracted from the values obtained for samples analyzed with that batch. Processing of these samples is very straight forward, therefore spikes are optional. Whenever available, an initial calibration verification (ICV) standard (made from a second source, not the same source as the calibration standards) will be analyzed following the calibration curve. Continuing calibration verification standards (CCVs) will be analyzed after every 10 samples. If CCV variation exceeds a +/-10% difference from expected, samples will be re-run with acceptable calibration criteria.

Table1. Summary of Data Quality Objectives and Corrective Actions

Quality Control Sample Type	Data Quality Objective[a] (DQO)	Corrective Action
Procedural Blank one/batch	Less than 3 x MDL	Re-extract and analyze sample batch. If batch can not be re-extracted and analyzed, "B" flag all samples that are in the batch. Investigate sources of blank contamination.
Calibration curve acceptability	r^2 values greater than or equal to 0.995	If r^2 value is outside of criterion, re-analyze calibration standards, if r^2 is still out, perform instrument maintenance and/or remake calibration standards and rerun calibration samples.
Initial calibration verification (ICV) standard; one/batch	+/−10 % of true value	Re-calibrate. Must meet DQO in order to continue processing samples.
Continuing calibration verification standards; one every 10th sample analyzed	+/−10 % of true value	Re-run CCV, if still not acceptable, re-calibrate and reanalyze affected samples.
Replicate sample precision; triplicates will be analyzed	Precision: 30% as relative standard deviation (RSD)	If RSD is not acceptable, resample and reanalyze. If reanalysis data are still not acceptable, then "*" flag the values.
Blank or Matrix Spike and spike duplicate, one set per batch	+/−15% of true value	If recoveries are unacceptable, check the spike solution to ensure it has not degraded, also check pipettes to ensure they are delivering accurate volumes.

[a] DQO is based on limited sample analysis as part of method development experience, and may require adjustment when more experience with the method is available.

Table 2. Data Qualifiers[a]

U	The analyte was detected below the MDL. Note: Samples with no peaks are reported as zero.
B	Samples associated with procedural blank contamination.
*	QC sample data that does not meet the DQO acceptability criterion.
Q	The data are questionable.
D	Sample diluted for analysis. (note: this procedure outlines the dilution of the samples, data will not be D flagged unless diluted other than indicated in this SOP)

[a] Additional data qualifiers may be added as necessary.

8.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of chemicals, including the following:

- Gloves, protective clothing and safety glasses should be worn when handling samples and chemicals.

9.0 TRAINING REQUIREMENTS

9.1 All staff performing this analysis should first read this procedure and conduct their first analysis under the supervision of a staff member who has had previous experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work. Documentation of training will be performed in accordance with MSL-A-006, Marine Sciences Laboratory Training.

9.2 All staff should have received training in the handling of chemicals and the use of fume hoods.

10.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

MSL-Q-007-04 Procedure for Determining Method Detection Limits

Federal Register (CFR Part 136 Appendix B).

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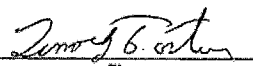
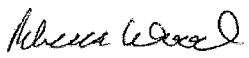
Marine Sciences Laboratory

EFFECTIVE DATE: 2-17-05

Method # EDSP.G2-024-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

ANALYSIS OF 4-NONYLPHENOL (MIX OF ISOMERS) IN DIMETHYL SULFOXIDE USING GC WITH FID AND MS DETECTION

Approvals:		
AUTHOR: Tim Fortman		2-17-05
	Signature	Date
TECHNICAL REVIEWER: Rebecca Wood		2-17-05
	Signature	Date
CHEMICAL REPOSITORY DIRECTOR: Michael Cobb		2-17-05
	Signature	Date

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ANALYSIS OF 4-NONYLPHENOL (MIX OF ISOMERS) IN DIMETHYL SULFOXIDE USING GC WITH FID AND MS DETECTION

Supposed to be CAS# 84852-15-5
Deviation written #EDSP.G2.024.05-D2
DMB 12/8/05

1.0 SCOPE AND APPLICATION

This method describes the determination of 4-nonylphenol (CAS# 104-40-5) in Dimethyl sulfoxide using GC with FID and MS detection. Stability and verification of formulation will be done using the GC-FID, purity will be done on the GC-MS.

2.0 DEFINITIONS

Initial Calibration Verification (ICV)	A standard made from a neat material different from the material used to make the calibration standards. Used to verify the calibration solutions. If an alternative source of material is not available, a 2nd standard prepared from the primary neat material is acceptable.
Continuing Calibration Verification (CCV)	A mid level calibration standard run after every 10 samples to ensure the instrument is in calibration.
Internal Standard (IS)	A compound in solution added to every sample (except purity sample) to adjust for variation (the internal standard is also in the calibration solutions).

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation.
Analyst - analysis, calculations
QA Manager or Representative - data verification

4.0 ANALYSIS

4.1 Hardware and Reagents

- Balance capable of weighing to 0.0001 g
- Gas chromatograph (Agilent 5890 or equivalent) with an FID (Flame ionization detector) and a mass selective detector (agilent 5970 or equivalent) and an autosampler.
- J+W DB-5 30m X 0.25 mm X 0.25 µm film thickness GC column or equivalent.
- Hexane, GC-MS grade ~~or better~~ suggested to be CAS# 84852-15-3 Deviation written EDSP.416.024.00-02 mm 12/18/05
- 4-nonylphenol (CAS# 104-40-5) and 5α Androstane, (CAS# 438-22-2) 98% purity or better.
- 1.8 mL autosampler vials
- 25, 50 and 100 mL amber glass bottles.
- Helium for carrier gas, Hydrogen, Breathing Air and Nitrogen for FID flame.
- Software for collection of data from FID detector, Varian Star Software, version 6.2 and MS software, Agilent G1701AA.
- Variable positive displacement Pipettors, to pipette 0.1 mL and 0.010 mL.
- Varity of volumetric flasks.
- Volumetric flasks
- Dimethyl Sulfoxide

4.2 GC Setup (FID)

4.2.1 The GC equipment has the following components, an autosampler, GC oven, detector (FID) and a data collection system. The autosampler is set to inject 1 µL and the injection port is set to 250° C. The oven conditions are; initial temp. 50° C., hold for 1.5 min., ramp at 15° C. per minute to 320° C and hold for 1 min. The detector temperature is set at 320° C.

4.2.2 The GC is set in the splitless mode and the split vent valve is opened at 2.5 minutes. Split vent is set at 89 mL/min and septum purge is set at 6.9 mL/min.

4.2.3 The column used is a J+W DB-5 30M X 0.25 mm X 0.25 µm film thickness. Flow thru the column is controlled by the head pressure which is set at 18 PSI.

4.2.4 The FID detector conditions are controlled by valves set up for the detector, the supply gasses are set up as follows, Hydrogen pressure, 21 PSI, Air pressure, 58 PSI and nitrogen pressure 34 PSI.

4.2.5 The data system used is Varian's Star software, version 6.2. The system is set up to start automatically with the injection of the sample. The run time is set to 21 minutes. Bunch rate is set to 8 and signal to noise set at 100. Different data systems will employ different nomenclature for the same settings, see software manuals for information on how to optimize the software.

4.2.6 The system used is composed of several different components from different manufacturers. The method printouts will only contain information pertaining to the calibration and quantitation of the unknowns, in order to ensure that the conditions used are recorded, an EDSP GC Analysis Form should be filled out (attachment 2). Also, much of this information should be included in the "notes" tab on Varian's Star method.

4.3 GC Setup (MS)

4.3.1 The GC is set with the following parameters. The autosampler is set to inject 1 µL and the injection port is set to 250° C. The oven conditions are; initial temp. 50° C., hold for 1.5 min., ramp at 15° C per minute to 320° C and hold for 1 min. The transfer line temperature is set at 300° C.

4.3.2 The GC is set in the splitless mode and the split vent valve is opened at 1.5 minutes. Split vent is set at 99 mL/min and septum purge is set at 1.8 mL/min.

4.3.3 The column used is a J+W DB-5 30M X 0.25 mm X 0.25 µm film thickness. Flow thru the column is controlled by the head pressure which is set at 9 PSI.

4.3.4 The GC and MS detector conditions are controlled by the software, it is operated in full scan, scanning from 30 amu to 300 amu. Solvent delay is set at 5 minutes. Scans per second are set at 2.91, threshold is set at 500 and EM voltage set at 94 volts above autotune value.

4.3.5 The GC-MS is used for purity determination and not quantitative analysis, no calibration curve is needed for this.

4.3.6 The GC-MS is tuned using PFTBA and the internal tuning program. The values obtained from a scan of the PFTBA should meet the criteria in table 1.

4.3.7 An air/water check is done to ensure no leaks in the system which can degrade the source. Nitrogen (ion 28) and water (ion 18) abundance should be less than 10% of the PFTBA ion 69 abundance.

TABLE 1. PFTBA TUNING CRITERIA

M/Z	ION ABUNDANCE[a]
69 (base peak)	100%
219	30-60%
502	≈1%

[a] Relative to base peak m/z 69

4.4 Calibration Solution (FID)

4.4.1 A 5 point curve is used to calibrate the GC over a range that will bracket the concentration in the stability tests. To start a stock of the 4-nonylphenol is made at a concentration of about 1000 µg/mL. ~0.05 grams is weighed into a 50 mL volumetric flask and diluted to the mark with methylene chloride. Record exact information on the organic standard preparation form (attachment 1) and give the solution a unique identifying label. Pour the solution into an appropriate size amber vial with a Teflon lined lid. Stability of the calibration solutions should be verified at the end of the test, by the analysis of a new (freshly made) solution made from the neat material and compared to the calibration solutions.

4.4.2 An internal standard is incorporated with this analysis. The chemical 5 a Androstane is used as the internal standard and a ~1000 µg/mL solution is made as described in 4.4.1.

4.4.3 Serially dilute the solution made in 4.4.1 to make standards ranging from 10 µg/mL to 200 µg/mL. The internal standard should be the same concentration in all the standards, for this series of solutions, 50 ml volume is used for each, so 1 mL of the 1000 µg/mL internal standard solution is added to the 50 mL vol. flask for a target concentration of 20 µg/mL. This calibration targets these concentrations; 10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL and 200 µg/mL. Include all information in the standards preparation form (see attachment 1).

4.5 Analysis

4.5.2 Prior to the analysis of any samples linearity must be demonstrated. A 5 point curve is run (minimum of a 4 point curve is needed), the 0 point not considered a calibration point. An r^2 value of greater than 0.995 is necessary before analysis can begin.

4.5.3 This material is a mix of isomers and quantification is done summarizing the peaks. Varian star software has a function called, grouping which summarizes all the peaks within a specified window. The 4-nonylphenol isomers all elute within 2 minutes. A window using this grouping function of 2 minutes is set with the midpoint of the window at the center of the group of peaks. The software then reports one number for this group and the midpoint is entered into the software at the peaks retention time.

4.5.4 Once the calibration is done, if possible it must be verified with an initial calibration verification sample (ICV). An independent solution is made using a different lot of the 4-nonyl-phenol when available, if not, use of the existing lot is OK. This solution is diluted to the proper concentration so that it is within the calibration range. This solution is run and the value obtained should be within 15% of the expected value.

4.5.5 After the calibration is verified, a continuing calibration verification (CCV) sample is run. This sample is usually one of the mid level calibration solutions. The value obtained should be within 15% of the expected value. A CCV should be run after every 10 samples.

4.5.6 A blank should be prepared with each sampling. A blank is prepared for each dilution. For example if 0.005 mL of sample is taken and diluted with the dichloromethane, a blank using 0.005 mL of dimethyl sulfoxide is treated as the sample. The blank should be < 3X MDL.

4.5.7 Method Detection Limit (MDL) is determined by preparing a sample at a low concentration, using similar techniques as used to analyze the stability solution. This is done 7 times and the MDL is the students T (3.143 for 7 replicates) times the standard deviation of the seven replicate runs. An MDL should be performed prior to the analysis of any sample. Samples with no peak or less than the MDL will be reported as the MDL and flagged with a "U.

4.6 Purity

4.6.1 The confirmation of purity is a required component of EDSP studies per the Chemical Repository (CR) QAPP (Ver 2, 01/07/05). Purity analysis will be performed unless the CR is instructed not to do so by the sponsor (EPA) due to technical or budgetary reasons. The Chemical Characterization SOP# MSL-R-009-00 has additional information. Purity is determined by running a solution of the material that is concentrated enough that a sufficient signal is seen on the GC-MS in full scan mode. The solution contains only the chemical of interest, no internal standard or other chemicals, dissolved in methylene chloride. This solution is

injected onto the GC and run with the parameters described in section 4.3. The 4-nonylphenol is a mix of isomers. In order to determine which peaks are associated with 4-nonlyphenol and which are impurities, the mass spectra obtained is assessed.

4.6.2 The TIC (total Ion Chromatogram) is integrated and a list obtained of all peaks. This TIC includes all fragmentation ions. The isomers of 4-nonylphenol have variable fragmentation patterns but several fragments will be common to all the isomers. Ion 107 and ion 220 are chosen to represent the 4-nonylphenol isomers. Fragment 107 is from the cleavage of the carbon carbon bond between the 1st and 2nd carbon atom on the nonyl chain. This forms the common fragment of 4-methylphenol, ion 107. The other ion chosen is the molecular ion 220.

4.6.3 The software is used to extract ion 107 from the TIC. The 107 ion chromatogram is then integrated and a list obtained of all peaks. This is printed out. The software is again used to extract the 220 ion from the TIC and this is integrated and the list of peaks printed out. The peaks are tabulated so that peaks of the same retention time are listed together for the TIC, ion 107 and ion 220 (a retention time window of 0.02 minutes is used to determine if the retention times are the same). The peaks in the TIC that have retention times matching both the ion 107 and ion 220 chromatograms are considered 4-nonylphenol peaks, these areas are summed and considered the 4-nonylphenol. Peaks in the TIC that have one or both ions missing are considered impurities, these areas are summed and are considered the impurities.

4.6.4 The area of the peaks in the TIC that are associated with the the 4-nonylphenol peaks is divided by The area of all the peaks in the TIC (summation of both the impurities and the 4-nonylphenol, presented as a percentage). A blank dichloromethane is run prior to the purity run and the peaks in this TIC of this blank are summed and subtracted from the 4-nonylphenol TIC area total. This purity should be 97% or greater and should compare favorably to the purity from the vendor.

5.0 STABILITY AND FORMULATION VERIFICATION

5.1 Stability for WA 4-16 chemicals is to run for 14 days. Stability is prepared by weighing an aliquot of the 4-nonylphenol into 10 milliliters of dimethyl sulfoxide and storing the solution in a 30 milliliter amber bottle. The details for the stability are contained in the Study Protocol. Target concentration is 0.1 M or 22 mg/mL. Sampling and analysis will be done 2 times over the course of the stability study, T=0 and day 14.

5.2 Sampling is done by removing an aliquot of the sample and diluting it into dichloromethane with an aliquot of the internal standard. For 4-nonylphenol, 0.005 mL is placed into a 1.8 mL autosampler vial with 0.02 mL of the internal standard (1000 ug/mL 5α Androstane) and 0.975 mL of dichloromethane. The vials are capped and are ready for GC analysis.

5.3 Samples should be analyzed on the day of sampling, but if this is not possible, samples should be stored at 4° C. until analysis. If samples are not analyzed on the day of sampling, the actual analysis date and storage conditions shall be documented.

5.4 Stability solutions are stored at 4° C.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 Prior to analysis of any samples, the instrument is calibrated with a minimum of a 4 point curve. Internal standard calculations will be performed. All nonpurity calculations will be done using Varian's Star chromatography software, version 6.2. This software allows the input of a multiplier, so that any dilutions will be included with the software calculations. For example, for this stability, 0.005 mL of the 10 mL sample solution is diluted to 1 mL, a multiplier of 200 is used so that the output from the software will give values that reflect the concentration in the diluted solution. Calibration curve fits can be set to either linear or non-linear (quadratic fit), past experience indicates that even though the calibration meets linearity criteria, the quantification is improved with a non-linear fit.

6.2 Prior to the tabulation of the data, each chromatogram and report printed by the software will be initialed and dated for GLP compliance.

7.0 QUALITY CONTROL

7.1 A blank is prepared with each sampling, this blank is dimethyl sulfoxide processed identically to the stability solution. If background levels are sufficiently high (i.e., greater than 3 x MDL), this value may be subtracted from the values obtained for samples analyzed with that batch. Processing of these samples is very straight forward, therefore spikes are optional.

7.2 An initial calibration verification (ICV) standard will be analyzed following the calibration curve. This ICV is made from a second source when available, but if a second source is not available, the same source is used. Continuing calibration verification standards (CCVs) will be analyzed after every 10 samples. A CCV is a mid point calibration solution. If CCV variation exceeds a 15% difference from expected, samples will be re-run with acceptable calibration criteria.

7.7 An internal standard is added to each sample, recovery of this internal standard should be ≤ ±80%.

8.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of chemicals, including the following:

- Gloves, protective clothing and safety glasses should be worn when handling samples and chemicals.

9.0 TRAINING REQUIREMENTS

9.1 All staff performing this analysis should first read this procedure and conduct their first analysis under the supervision of a staff member who has had previous experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work. Documentation of training will be performed in accordance with MSL-A-006, Marine Sciences Laboratory Training.

9.2 All staff should have received training in the handling of chemicals and the use of fume hoods.

10.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

MSL-Q-007-04 Procedure for Determining Method Detection Limits

Federal Register (CFR Part 136 Appendix B).

Table 1. Summary of Data Quality Objectives and Corrective Actions

Quality Control Sample Type	Data Quality Objective (DQO)	Corrective Action
Procedural Blank one/batch	Less than 3 x MDL	Re-extract and analyze sample batch. If batch can not be re-extracted and analyzed, "B" flag all samples that are in the batch. Investigate sources of blank contamination.
Calibration curve acceptability	r^2 values greater than or equal to 0.995	If r^2 value is outside of criterion, re-analyze calibration standards, if r^2 is still out, perform instrument maintenance and/or remake calibration standards and rerun calibration samples.
Initial calibration verification (ICV) standard; one/batch	+ / - 15 % of true value	Re-calibrate. Must meet DQO in order to continue processing samples.
Internal Standard Recovery	+ / - 20 % of true value	Re-sample and rerun
Continuing calibration verification standards; one every 10th sample analyzed	+/ - 15 % of true value	Re-run CCV, if still not acceptable, re-calibrate and reanalyze affected samples.
Replicate sample precision; triplicates will be analyzed for stability, duplicate for in-life	Precision: 30% as relative standard deviation (RSD) or relative percent deviation (RPD)	If RSD or RPD is not acceptable, resample and reanalyze. If reanalysis data are still not acceptable, then "*" flag the values.
Blank or Matrix Spike and spike duplicate, one set per batch	+/- 20% of true value	If recoveries are unacceptable, check the spike solution to ensure it has not degraded, also check pipettes to ensure they are delivering accurate volumes.

[a] DQO is based on limited sample analysis as part of method development experience, and may require adjustment when more experience with the method is available.

Table 2. Data Qualifiers[a]

U	The analyte was detected below the MDL. Note: Samples with no peaks are reported as zero.
B	Samples associated with procedural blank contamination.
*	QC sample data that does not meet the DQO acceptability criterion.
Q	The data are questionable.
D	Sample diluted for analysis. (note: this procedure outlines the dilution of the samples, data will not be D flagged unless diluted other than indicated in this SOP)

[a] Additional data qualifiers may be added as necessary.

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EFFECTIVE DATE: 3-4-05

Method # EDSP.G2-025-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

ANALYSIS OF DICOFOL IN DIMETHYL SULFOXIDE USING GC WITH FID DETECTION

Approvals:		
AUTHOR: Tim Fortman	Tim Fortman	3-4-05
	Signature	Date
TECHNICAL REVIEWER: Rebecca Wood	Rebecca Wood	3-4-05
	Signature	Date
CHEMICAL REPOSITORY DIRECTOR: Michael Cobb	MC	03-04-05
	Signature	Date

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ANALYSIS OF DICOFOL IN DIMETHYL SULFOXIDE USING GC WITH FID DETECTION

1.0 SCOPE AND APPLICATION

This method describes the determination of Dicofol (CAS# 115-32-2) in Dimethyl sulfoxide using GC with FID detection. The method was developed for use in the analysis of Dicofol for the EDSP program.

2.0 DEFINITIONS

Initial Calibration Verification (ICV)	A standard made from a neat material different from the material used to make the calibration standards. Used to verify the calibration solutions. If none are available, a 2nd standard from the same neat material is acceptable.
Continuing Calibration Verification (CCV)	A mid level calibration standard run after every 10 samples to ensure the instrument is in calibration.
Internal Standard (IS)	A compound in solution added to every sample to adjust for variation (the internal standard is also in the calibration solutions).

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation.
Analyst - analysis, calculations
QA Manager or Representative - data verification

4.0 ANALYSIS

4.1 Hardware and Reagents

- Balance capable of weighing to 0.0001 g
- Gas chromatograph (Agilent 5890 or equivalent) with an FID (Flame ionization detector) and an autosampler.
- J+W DB-5 30m X 0.25 mm X 0.25 um film thickness GC column or equivalent.
- Dichloromethane, GC-MS grade or better.
- Dicofol (CAS# 115-32-2) and 5aAndrostane, (CAS# 438-22-2) 98% purity or better.
- 1.8 ml autosampler vials
- 25, 50 and 100 ml amber glass bottles.
- Helium for carrier gas, Hydrogen, Breathing Air and Nitrogen for FID flame.
- Software for collection of data from FID detector, Varian Star Software, vers. 6.2.
- Variable positive displacement Pipettors, to pipette 0.1 ml and 0.010 ml.
- Varity of volumetric flasks.
- ~~Volumetric flasks~~ duplicated from above typo mb 1/18/05
- Dimethyl Sulfoxide

4.2 GC conditions

4.2.1 The GC should be set up as per manufacturer specifications, for the system presently in use, the carrier gas pressure is set to 18 PSI, split vent at 89 ml/min, septum purge set at 6.9 ml/min. The FID (flame ionization detector) gasses are set at; air, 300 ml/min, hydrogen, 46 ml/min and nitrogen at 43 ml/min.

4.3 Calibration Solution

4.3.1 A 5 point curve is used to calibrate the GC over a range that will bracket the concentration in the stability tests. To start a stock of dicofol is made at a concentration of about 1000 ug/ml. ~0.05 grams is weighed into a 50 ml volumetric flask and diluted to the mark with dichloromethane. Record exact information on the organic standard preparation form (attachment 1) and give the solution a unique identifying label. Pour the solution into an appropriate size amber vial with a Teflon lined lid. Stability of the calibration solutions should be verified at the end of the test, by the analysis of a new (freshly made) solution made from the neat material and compared to the calibration solutions.

4.3.2 An internal standard is incorporated with this analysis. The chemical 5 a Androstane is used as the internal standard and a ~1000 ug/ml solution is made as described in 4.3.1.

4.3.2 Serially dilute the dicofol solution made in 4.3.1 to make standards ranging from 0.5 ug/ml to 100 ug/ml. The internal standard should be the same concentration in all the standards, for this series of solutions, 50 and 100 ml. volumes are used for each, so 1 ml of the 1000 ug/ml internal standard solution is added to the 50 ml vol. flask and 2 ml to the 100 ml vol. flask for a target concentration of 20 ug/ml. This calibration targets these concentrations; 0.5 ug/ml, 1 ug/ml, 5 ug/ml, 20 ug/ml

and 100 ug/ml. Include all information in the standards preparation form (see attachment 1).

4.4 GC Setup

4.4.1 The GC equipment has the following components, an autosampler, GC oven, detector (FID) and a data collection system. The autosampler is set to inject 1 ul and the injection port is set to 210 deg. C. The oven conditions are; initial temp. 50 deg. C., hold for 1.5 min., ramp at 15 deg. per minute to 320 deg. with no hold time. The FID gasses are described in section 4.2.1, the detector temp. is set at 320 deg. C.

4.4.2 The GC is set in the splitless mode and the split vent valve is opened at 2.5 minutes. Split vent and septum purge settings are listed in section 4.2.1.

4.4.3 The column used is a J+W DB-5 30M X 0.25 mm X 0.25 um film thickness. Flow thru the column is controlled by the head pressure which is set at 18 PSI (see section 4.2.1).

4.4.4 The FID detector conditions are described in section 4.2.1. The conditions on the present system are controlled by valves set up for the detector, the supply gasses are set up as follows, Hydrogen pressure, 21 PSI, Air pressure, 58 PSI and nitrogen pressure 34 PSI.

4.4.5 The data system used is Varian's Star software, version 6.2. The system is set up to start automatically with the injection of the sample. The run time is set to 19.5 minutes. Bunch rate is set to 8 and signal to noise set at 100. Different data systems will employ different nomenclature for the same settings, see software manuals for information on how to optimize the software.

4.4.6 The system used is composed of several different components from different manufacturers. The method printouts will only contain information pertaining to the calibration and quantitation of the unknowns, in order to ensure that the conditions used are recorded, an EDSP GC Analysis Form should be filled out (attachment 2). Also, much of this information should be included in the "notes" tab on Varian Star's method.

4.5 Analysis

4.5.2 Prior to the analysis of any samples linearity must be demonstrated. A 5 point curve is run (minimum of a 4 point curve is needed), the 0 point not considered a calibration point. An r^2 value of greater than 0.995 is necessary before analysis can begin.

4.5.3 Once the calibration is done, if possible it must be verified with an initial calibration verification sample (ICV). An independent solution is made using a different lot of the dicofol when available, if not, use of the existing lot is OK. This solution is diluted to the proper concentration so that it is within the calibration range. This solution is run and the value obtained should be within 15% of the expected value.

4.5.4 After the calibration is verified, a continuing calibration verification (CCV) sample is run. This sample is usually one of the mid level calibration solutions. The value obtained should be within 15% of the expected value. A CCV should be run after every 10 samples.

4.5.5 A blank should be prepared with each sampling. A blank is prepared for each dilution. For example if 0.005 ml of sample is taken and diluted with the dichloromethane, a blank using 0.005 ml of dimethyl sulfoxide is treated as the sample. The blank should be < 3X MDL.

4.5.6 Method Detection Limit (MDL) is determined by preparing a sample at a low concentration, using similar techniques as used to analyze the stability solution. This is done 7 times and the MDL is the students T (3.143 for 7 replicates) times the standard deviation of the seven replicate runs. An MDL should be performed prior to the analysis of any sample. Samples with no peak or less than the MDL will be reported as the MDL and flagged with a "U.

4.6 Purity

4.6.1 The confirmation of purity is a required component of EDSP studies per the Chemical Repository (CR) QAPP (Ver 2, 01/07/05). Purity analysis will be performed unless the CR is instructed not to do so by the sponsor (EPA) due to technical or budgetary reasons. The Chemical Characterization SOP# MSL-R-009-00 has additional information. Purity is determined by running a solution of the material that is at or near the top of the demonstrated linearity of the system. The solution contains only the test material, no internal standard or other chemicals, dissolved in dichloromethane. This solution is injected onto the GC and all the peaks in the chromatogram are summarized. The peak corresponding to the chemical of interest is then compared to all the other peaks and the purity is the area of the chemical peak divided by the sum of the total area in the chromatogram (presented as a percentage). A blank dichloromethane is run prior to the purity run and the peaks in the purity run that correlate to peaks in the blank run are eliminated from the calculation (this includes the solvent peak). This purity should be 96% or greater and should compare favorably to the purity from the vendor. See example purity form: attachment 3.

5.0 STABILITY AND FORMULATION VERIFICATION

5.1 Stability for WA 4-16 and WA4-17 chemicals is to run for 14 days. Stability is prepared by weighing an aliquot of the test substance into 1 milliliter of dimethyl sulfoxide and storing the solution in a 1.8 milliliter vial at 4 degress centigrade (attachment 4). The details for the stability are contained in the Study Protocol. Target concentration is 0.1 M, or for dicofol, 37 mg/ml. Sampling and analysis will be done 2 times over the course of the stability study.

5.2 Sampling is done by removing an aliquot of the sample and diluting it into dichloromethane with an aliquot of the internal standard. For dicofol, 0.005 ml is added to a 5 ml volumetric flask along with 0.1 ml of the internal standard (1000 ug/ml 5 a androstane) and diluted to mark with dichloromethane. An aliquot of this is placed into a a 1.8 ml autosampler vial for analysis. The vials are capped and are ready for GC analysis.

5.3 For an example of the form used for the stability preparation and sampling see attachments ~~4 and~~ 5.4 & 6

5.4 Samples should be analyzed on the day of sampling, but if this is not possible, samples should be stored at 4 deg. C. until analysis. If samples are not analyzed on the day of sampling, the actual analysis date and storage conditions shall be documented.

5.5 Stability solutions are stored at 4 degrees centrigrade.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 Prior to analysis of any samples, the instrument is calibrated with a minimum of a 4 point curve. Internal standard calculations will be performed. All calculations will be done using

Varian's Star chromatography software, version 6.2. This software allows the input of a multiplier, so that any dilutions will be included with the software calculations. For example, for dicofol stability, 0.005 ml of the 10 ml sample solution is diluted to 5 ml, a multiplier of 1000 is used so that the output from the software will give values that reflect the concentration in the diluted solution. Calibration curve fits can be set to either linear or non-linear (quadratic fit), past experience indicates that even though the calibration meets linearity criteria, the quantification is improved with a non-linear fit.

6.2 Prior to the tabulation of the data, each chromatogram and report printed by the software will be initialed and dated for GLP compliance.

7.0 QUALITY CONTROL

7.1 A blank is prepared with each sampling, this blank is dimethyl sulfoxide processed identically to the stability solution. If background levels are sufficiently high (i.e., greater than 3 x MDL), this value may be subtracted from the values obtained for samples analyzed with that batch. Processing of these samples is very straight forward, therefore spikes are optional.

7.2 An initial calibration verification (ICV) standard will be analyzed following the calibration curve. This ICV is made from a second source when available, but if a second source is not available, the same source is used. Continuing calibration verification standards (CCVs) will be analyzed after every 10 samples. A CCV is a mid point calibration solution. If CCV variation exceeds a 15% difference from expected, samples will be re-run with acceptable calibration criteria.

7.7 An internal standard is added to each sample, recovery of this internal standard should be ≥80%.

8.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of chemicals, including the following:

- Gloves, protective clothing and safety glasses should be worn when handling samples and chemicals.

9.0 TRAINING REQUIREMENTS

9.1 All staff performing this analysis should first read this procedure and conduct their first analysis under the supervision of a staff member who has had previous experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work. Documentation of training will be performed in accordance with MSL-A-006, Marine Sciences Laboratory Training.

9.2 All staff should have received training in the handling of chemicals and the use of fume hoods.

10.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

MSL-Q-007-04 Procedure for Determining Method Detection Limits

Federal Register (CFR Part 136 Appendix B).

Table 1. Summary of Data Quality Objectives and Corrective Actions

Quality Control Sample Type	Data Quality Objective (DQO)	Corrective Action
Procedural Blank one/batch	Less than 3 x MDL	Re-extract and analyze sample batch. If batch can not be re-extracted and analyzed, "B" flag all samples that are in the batch. Investigate sources of blank contamination.
Calibration curve acceptability	r^2 values greater than or equal to 0.995	If r^2 value is outside of criterion, re-analyze calibration standards, if r^2 is still out, perform instrument maintenance and/or remake calibration standards and rerun calibration samples.
Initial calibration verification (ICV) standard; one/batch	+ / - 15 % of true value	Re-calibrate. Must meet DQO in order to continue processing samples.
Internal Standard Recovery	+ / - 20 % of true value	Re-sample and rerun
Continuing calibration verification standards; one every 10th sample analyzed	+/ - 15 % of true value	Re-run CCV, if still not acceptable, re-calibrate and reanalyze affected samples.
Replicate sample precision; triplicates will be analyzed for stability, duplicate for in-life	Precision: 30% as relative standard deviation (RSD) or relative percent deviation (RPD)	If RSD or RPD is not acceptable, resample and reanalyze. If reanalysis data are still not acceptable, then "*" flag the values.
Blank spike or Matrix Spike and spike duplicate, one set per batch	+/- 20% of true value	If recoveries are unacceptable, check the spike solution to ensure it has not degraded, also check pipettes to ensure they are delivering accurate volumes.

[a] DQO is based on limited sample analysis as part of method development experience, and may require adjustment when more experience with the method is available.

Table 2. Data Qualifiers[a]

U	The analyte was detected below the MDL. Note: Samples with no peaks are reported as zero.
B	Samples associated with procedural blank contamination.
*	QC sample data that does not meet the DQO acceptability criterion.
Q	The data are questionable.
D	Sample diluted for analysis. (note: this procedure outlines the dilution of the samples, data will not be D flagged unless diluted other than indicated in this SOP)

[a] Additional data qualifiers may be added as necessary.

APPENDIX G

Analytical Method Deviations

The following method deviations were filed:

1. EDSP.G2-022-00-D2 - The original spec described a 25 µL loop install in the system, the loop size used was 100 µL. There was no impact on the analysis.
2. EDSP.G2-022-00-D4 – The final CCV sample failed during the analysis and a rerun also failed, a third rerun was at the limit. Appeared to be related to performance of the autosampler. The analysis was repeated.
3. EDSP.G2-022-00-D5 – Several standards were made with hexane, instead of the specified solvent, methylene chloride. The hexane was used for some of the standards during method development causing no problems so they were used in the studies as well. No impact.
4. EDSP.G2-022-00-D6 – Pressure for air on the FID detector was set at 40 psi instead of the specified 58 psi. The setting was in error but the performance specifications were met so there was no impact on the data.
5. EDSP.G2-022-00-D7 – The internal standard recovery specification for dibenz[a,h]anthracene was set at ±20% and final CCV internal standard recovery was 125%. Results obtained for the analyte of interest were 101.4% so there was no apparent impact on the data.
6. EDSP.H4-023-00-D1 – The original method specified a binary gradient pump using acetonitrile and DI water at a 75:25 ratio. The new instrument employed allowed more flexibility and a pump set with 75% acetonitrile, 23% water, and 2% 10mM phosphate buffer was used. Calibration and analysis QA.QC samples passed, indicating that there was no impact on the data.
7. EDSP.G2-024-00-D1 - Pressure for air on the FID detector was set at 40 psi instead of the specified 58 psi. The setting was in error but the performance specifications were met so there was no impact on the data.
8. EDSP.G2-024-00-D2 – Under scope and application, the test substance 4-nonylphenol was identified by an incorrect CAS number, the correct CAS number is 84852-15-3. No impact on data as the correct material was used in the study.
9. EDSP.G2-025-00-D1 – In prepping the dicofol standards, an internal standard volume of 1 mL was incorrectly used instead of 2 mL. The actual volume (1 mL) was used in generation of the calibration curve and had no adverse impact on the data.
10. EDSP.G2-025-00-D2 - Pressure for air on the FID detector was set at 40 psi instead of the specified 58 psi. The setting was in error but the performance specifications were met so there was no impact on the data.

APPENDIX H

ANALYTICAL DATA TABLES

ANALYTICAL RESULTS OF CONCENTRATION VERIFICATION TESTING

(Note: Calculations were conducted at full precision in a spreadsheet.)

Table H1. Results for Atrazine in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	Atrazine (ug/mL)	Average (ug/mL)	Recovery	RSD
21572	0823 Atzr R-1	8/23/2005	19582	19951	92.5%	1.7%
21572	0823 Atzr R-2	8/23/2005	20030			
21572	0823 Atzr R-3	8/23/2005	20240			
21572	0909 Atzr R-1	9/9/2005	20389	20029	92.8%	1.7%
21572	0909 Atzr R-2	9/9/2005	19728			
21572	0909 Atzr R-3	9/9/2005	19969			
21572	0919 Atzr R-1	9/19/2005	19267	19785	91.7%	2.3%
21572	0919 Atzr R-2	9/19/2005	20062			
21572	0919 Atzr R-3	9/19/2005	20026			

Table H2. Results for Fenarimol in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	Fenarimol (ug/mL)	Average (ug/mL)	Recovery	RSD
33128	Fenrl-in-life R-1	7/26/2005*	29943	30397	91.8%	2.4%
33128	Fenrl-in-life R-2	7/26/2005*	31247			
33128	Fenrl-in-life R-3	7/26/2005*	30001			
33128	0824 Fenrl inlf R1	8/24/2005	29409	29966	90.5%	3.1%
33128	0824 Fenrl inlf R2	8/24/2005	29437			
33128	0824 Fenrl inlf R3	8/24/2005	31053			

*Samples were prepared on 7/25/05 but due to instrument usage, samples were stored overnight refrigerated and analyzed on 7/26/05

Table H3. Results for Dibenz(a,h)anthracene in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	Dibenz(a,h) anthracene (ug/mL)	Average (ug/mL)	Recovery	RSD
2783	0913 DiBzAnt R1	* 9/14/2005	2727	2719	97.7%	1.5%
2783	0913 DiBzAnt R2	9/14/2005	2757			
2783	0913 DiBzAnt R3	9/14/2005	2674			
2783	1003 DiBzAnt R1	10/3/2005	2608	2701	97.0%	3.0%
2783	1003 DiBzAnt R2	10/3/2005	2749			
2783	1003 DiBzAnt R3	10/3/2005	2745			

*NOTE: Samples initially run on 9/13/05 and rerun on 9/14/05 because of a failed CCV on 9/13/05. GC liner was changed between analyses

Table H4. Results for Prochloraz in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	Prochloraz (ug/mL)	Average (ug/mL)	Recovery	RSD
37672	072405 R1	7/25/2005	31449	38602	102.5%	16.1%
37672	072405 R2	7/25/2005	41967			
37672	072405 R3	7/25/2005	42392			
37672	072405 R4	7/25/2005	36250	38132	101.2%	7.3%
37672	072405 R5	7/25/2005	41333			
37672	072405 R6	7/25/2005	36812			
37672	082505 proclz R1	8/25/2005	35565	36432	96.7%	2.2%
37672	082505 proclz R2	8/25/2005	37130			
37672	082505 proclz R3	8/25/2005	36602			
37672	0926 proclz R1	9/26/2005	35559	36562	97.1%	2.4%
37672	0926 proclz R2	9/26/2005	36893			
37672	0926 proclz R3	9/26/2005	37235			

Table H5. Results for 4-Nonylphenol in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	4-Nonylphenol, ug/mL	Average (ug/mL)	Recovery	RSD
22036	091Nonyl R-1	9/13/2005	21505	21821	99.0%	3.9%
22036	091Nonyl R-2	9/13/2005	22776			
22036	091Nonyl R-3	9/13/2005	21181			
22036	1003Nonyl R-1	10/3/2005	21974	21380	97.0%	2.5%
22036	1003Nonyl R-2	10/3/2005	21219			
22036	1003Nonyl R-3	10/3/2005	20948			

Table H6. Results for Dicofol in DMSO (ug/mL)

Target Conc. (ug/mL)	Sample ID	Date	Dicofol (ug/mL)	Average (ug/mL)	Recovery	RSD
37050	0823 Dico R-1	8/23/2005[1]	34114	33707	91.0%	2.9%
37050	0823 Dico R-2	8/23/2005[1]	34407			
37050	0823 Dico R-3	8/23/2005[1]	32599			
37050	0909 Dico R-1	9/9/2005	34644	35126	94.8%	2.9%
37050	0909 Dico R-2	9/9/2005	36297			
37050	0909 Dico R-3	9/9/2005	34436			
37050	0919 Dico R-1	9/20/2005[2]	35326	35557	96.0%	2.4%
37050	0919 Dico R-2	9/20/2005[2]	36513			
37050	0919 Dico R-3	9/20/2005[2]	34832			

[1]Samples prepared on 8/22/05 but due to instrument usage, samples were stored overnight refrigerated and analyzed on 8/23/05
[2]Samples prepared on 9/19/05, but due to instrument usage, samples were stored overnight refrigerated and analyzed on 9/20/05

Table H7. Calibration Verification Data for Atrazine

Sample Name	Date	Expected Atrazine (ug/mL)	Measured Atrazine (ug/mL)	Recovery
WA416-17-GC-17 ICV	8/23/2005	5.34	5.49	102.8%
WA416-17-GC-27 CV	8/23/2005	5.01	4.91	98.0%
WA416-17-GC-16C CCV	8/23/2005	4.94	5.09	103.0%
WA416-17-GC-16C CCV	8/23/2005	4.94	5.09	103.1%
WA416-17-GC-17 ICV	9/9/2005	5.34	5.46	102.3%
WA416-17-GC-27 CV	9/9/2005	5.01	4.85	96.7%
WA416-17-GC-16C CCV	9/9/2005	4.94	4.99	100.9%
WA416-17-GC-16C CCV	9/9/2005	4.94	4.97	100.7%
WA416-17-GC-17 ICV	9/19/2005	5.34	5.41	101.3%
WA416-17-GC-27 CV	9/19/2005	5.01	4.82	96.3%
WA416-17-GC-16C CCV	9/19/2005	4.94	5.01	101.4%
WA416-17-GC-16C CCV	9/19/2005	4.94	5.04	102.0%

Table H8. Calibration Verification Data for Fenarimol

Sample Name	Date	Expected Fenarimol (ug/mL)	Measured Fenarimol (ug/mL)	Recovery
WA416-17-GC-17 ICV	7/26/2005	5.20	5.11	98.2%
WA416-17-GC-23 CV	7/26/2005	5.04	5.72	113.5%
WA416-17-GC-16C CCV	7/26/2005	5.01	4.78	95.4%
WA416-17-GC-25 CV	7/26/2005	5.04	4.44	88.1%
WA416-17-GC-16C CCV	7/26/2005	5.01	4.77	95.3%
WA416-17-GC-17 ICV	8/24/2005	5.20	5.13	98.7%
WA416-17-GC-23 CV	8/24/2005	5.04	5.73	113.7%
WA416-17-GC-16C CCV	8/24/2005	5.01	4.72	94.2%
WA416-17-GC-16C CCV	8/24/2005	5.01	4.81	96.0%

Table H9. Calibration Verification Data for Dibenz(a,h)anthracene

Sample Name	Date	Expected Dibenz(a,h) anthracene (ug/mL)	Measured Dibenz(a,h) anthracene (ug/mL)	Recovery
WA416-17-GC-17 ICV	9/14/2005	5.18	5.48	105.9%
WA416-17-GC-29 CV	9/14/2005	5.04	5.13	101.9%
WA416-17-GC-16C CCV	9/14/2005	5.05	5.12	101.4%
WA416-17-GC-16C CCV	9/14/2005	5.05	5.12	101.4%
WA416-17-GC-17 ICV	10/3/2005	5.18	5.42	104.7%
WA416-17-GC-29 CV	10/3/2005	5.04	5.09	101.0%
WA416-17-GC-16C CCV	10/3/2005	5.05	5.08	100.6%
WA416-17-GC-16C CCV	10/3/2005	5.05	5.10	101.0%

Table H10. Calibration Verification Data for Prochloraz

Sample Name	Date	Expected Prochloraz (ug/mL)	Measured Prochloraz (ug/mL)	Recovery
WA416-proclz-9 ICV	7/25/2005	83.2	95.1	114.3%
WA416-proclz-9 ICV	7/25/2005	83.2	91.2	109.6%
WA416-proclz-2D CCV	7/25/2005	83.0	86.0	103.6%
WA416-proclz-9 ICV	7/25/2005	83.2	88.5	106.4%
WA416-proclz-2D CCV	7/25/2005	83.0	84.3	101.5%
WA416-proclz-2D CCV	7/25/2005	83.0	96.7	116.3%
WA416-proclz-2D CCV	7/25/2005	83.0	91.8	110.5%
WA416-proclz-9 ICV	8/25/2005	83.2	83.3	100.1%
WA416-proclz-13 CV	8/25/2005	80.8	80.6	99.8%
WA416-proclz-2D CCV	8/25/2005	83.0	77.0	92.8%
WA416-proclz-2D CCV	8/25/2005	83.0	77.0	92.7%
WA416-proclz-9 ICV	9/26/2005	83.2	83.5	100.4%
WA416-proclz-13 CV	9/26/2005	80.8	80.8	100.0%
WA416-proclz-2D CCV	9/26/2005	83.0	77.4	93.3%
WA416-proclz-2D CCV	9/26/2005	83.0	77.4	93.2%

Table H11. Calibration Verification Data for 4-Nonylphenol

Sample Name	Date	Expected 4-nonylphenol (ug/mL)	Measured 4-nonylphenol (ug/mL)	Recovery
WA416-nonyl-6 ICV	9/13/2005	55.6	56.8	102.1%
WA416-nonyl-10 CV	9/13/2005	52.2	52.0	99.6%
WA416-nonyl-3 C CCV	9/13/2005	53.5	53.8	100.6%
WA416-nonyl-3C CCV	9/13/2005	53.5	53.9	100.7%
WA416-nonyl-6 ICV	10/3/2005	55.6	57.2	102.8%
WA416-nonyl-10 CV	10/3/2005	52.2	52.1	99.9%
WA416-nonyl-3 C CCV	10/3/2005	53.5	54.3	101.4%
WA416-nonyl-3C CCV	10/3/2005	53.5	54.5	102.0%

Table H12. Calibration Verification Data for Dicofol

Sample Name	Date	Expected Dicofol (ug/mL)	Measured Dicofol (ug/mL)	Recovery
WA416-Dico-8 ICV	8/23/2005	5.07	5.23	103.2%
WA416-Dico-12 CV	8/23/2005	5.03	5.06	100.6%
WA416-Dico-4 C CCV5	8/23/2005	5.17	5.21	100.7%
WA416-Dico-4 C CCV5	8/23/2005	5.17	4.75	91.9%
WA416-Dico-8 ICV	9/9/2005	5.07	5.34	105.3%
WA416-Dico-12 CV	9/9/2005	5.03	5.09	101.2%
WA416-Dico-4 C CCV5	9/9/2005	5.17	5.45	105.4%
WA416-Dico-4 C CCV5	9/9/2005	5.17	5.26	101.7%
WA416-Dico-8 ICV	9/20/2005	5.07	5.33	105.2%
WA416-Dico-12 CV	9/20/2005	5.03	5.06	100.7%
WA416-Dico-4 C CCV5	9/20/2005	5.17	5.28	102.1%
WA416-Dico-4 C CCV5	9/20/2005	5.17	4.97	96.1%

Table H13. Spike Recovery Data for Stability Analyses

Compound	Target Conc. (mg/L)	Sample ID	Date	Measured (mg/L)	Recovery
Fenarimol	30000	Spike fenrl R-5	7/26/2005	26611	88.7%
Fenarimol	30000	0824 Fen Spike	8/24/2005	27000	90.0%
Prochloraz	37260	Spike R7	7/25/2005	40206	107.9%
Prochloraz	37260	0825 spike 1	8/25/2005	37147	99.7%
Prochloraz	37260	0926 spike 1	9/26/2005	38259	102.7%
4-Nonylphenol	24970	0913 Spike 1	9/13/2005	20083	80.4%
4-Nonylphenol	24970	0913 Spike 2	9/13/2005	24772	99.2%
4-Nonylphenol	24970	1003 Spike 1	10/3/2005	23098	92.5%
4-Nonylphenol	24970	1003 Spike 2	10/3/2005	24801	99.3%
Dicofol	36920	0823 dico Spike1	8/23/2005	38399	104.0%
Dicofol	36920	0823 dico Spike2	8/23/2005	41197	111.6%
Dicofol	36920	0909 dico Spike1	9/9/2005	41363	112.0%
Dicofol	36920	0909 dico Spike2	9/9/2005	39111	105.9%
Dicofol	36920	0919 dico Spike1	9/20/2005	36942	100.1%
Dicofol	36920	0919 dico Spike2	9/20/2005	42807	116.0%

Table H14. Method Blank Results

Compound	Blank 1 (ug/mL)	Blank 2 (ug/mL)	Blank 3 (ug/mL)	Method Detection Limit (ug/mL)
Atrazine	ND	ND	ND	137
Fenarimol	ND	216	--	271
Dibenz(a,h)anthracene	ND	ND	--	7.21
Prochloraz	ND	937	ND	550
4-Nonylphenol	ND	ND	--	253
Dicofol	ND	ND	ND	610

ND Non-detect; compound was not detected at or above detection limit shown

Table H15. Internal Standard Recovery 7-26-05 Analyses

Sample	Recovery
WA416-17-GC-17 ICV	98.7%
WA416-17-GC-23 CV	96.9%
WA416-17-GC-16C CCV	106.7%
Fen Blank	99.0%
Spike fenrl R-5	98.9%
Fenrl-in-life R-1	99.0%
Fenrl-in-life R-2	97.4%
Fenrl-in-life R-3	96.8%
WA416-17-GC-23 CV	100.1%
WA416-17-GC-16C CCV	106.9%

Table H16. Internal Standard Recovery 8-23-05 Analyses

Sample	Recovery
WA416-17-GC-17 ICV	100.0%
WA416-17-GC-27 CV	102.3%
WA416-17-GC-16C CCV	104.2%
0823 Blank	98.7%
0823 Atzr R-1	100.4%
0823 Atzr R-2	101.5%
0823 Atzr R-3	103.8%
WA416-17-GC-16C CCV	107.4%
WA416-Dico-8 ICV	108.4%
WA416-Dico-12 CV	100.6%
WA416-Dico-4 C CCV5	100.0%
0823 blank dico 1	95.9%
0823 dico Spike1	99.9%
0823 dico Spike2	99.4%
0823 Dico R-1	99.0%
0823 Dico R-2	97.1%
0823 Dico R-3	100.6%
WA416-Dico-4 C CCV5	101.9%

Table H17. Internal Standard Recovery 8-24-05 Analyses

Sample	Recovery
WA416-17-GC-17 ICV	98.5%
WA416-17-GC-23 CV	96.2%
WA416-17-GC-16C CCV	105.8%
0824 Fen Blank	97.5%
0824 Fen Spike	101.3%
0824 Fenrl inlf R1	100.6%
0824 Fenrl inlf R2	100.0%
0824 Fenrl inlf R3	100.4%
WA416-17-GC-16C CCV	105.7%

Table H18. Internal Standard Recovery 9-9-05 Analyses

Sample	Recovery
WA416-17-GC-17 ICV	99.7%
WA416-17-GC-27 CV	101.1%
WA416-17-GC-16C CCV	103.5%
0909 Blank	93.8%
0909 Atzr R-1	95.6%
0909 Atzr R-2	97.7%
0909 Atzr R-3	97.0%
WA416-17-GC-16C CCV	104.4%
WA416-Dico-8 ICV	97.6%
WA416-Dico-12 CV	100.6%
WA416-Dico-4 C CCV5	101.5%
0909 blank dico 1	95.9%
0909 dico Spike1	97.5%
0909 dico Spike2	98.0%
0909 Dico R-1	97.5%
0909 Dico R-2	97.3%
0909 Dico R-3	98.0%
WA416-Dico-4 C CCV5	103.7%

Table H19. Internal Standard Recovery 9-13-05 and 9-14-05 Analyses

Sample	Recovery
WA416-17-GC-17 ICV	107.4%
WA416-17-GC-29 CV	90.4%
WA416-17-GC-16C CCV	115.2%
0913DiBzAnt Blank	89.7%
0913 DiBzAnt R1	91.3%
0913 DiBzAnt R2	88.6%
0913 DiBzAnt R3	91.7%
WA416-17-GC-16C CCV	125.6% *
WA416-nonyl-6 ICV	98.8%
WA416-nonyl-10 CV	100.9%
WA416-nonyl-3 C CCV	101.4%
0913 nonyl blank	96.4%
0913 Spike 1	101.2%
0913 Spike 2	102.9%
091Nonyl R-1	101.5%
091Nonyl R-2	101.5%
091Nonyl R-3	102.5%
WA416-nonyl-3C CCV	107.3%

* This recovery outside acceptable limits, since this is a calibration verification sample and the value for the compound was acceptable, this recovery does not impact the data.

Table H20. Internal Standard Recovery 9-20-05 Analyses*

Sample	Recovery
WA416-17-GC-17 ICV	98.3%
WA416-17-GC-27 CV	102.0%
WA416-17-GC-16C CCV	103.9%
0919 Blank	98.6%
0919 Atzr R-1	99.1%
0919 Atzr R-2	99.6%
0919 Atzr R-3	99.2%
WA416-17-GC-16C CCV	103.4%
WA416-Dico-8 ICV	99.0%
WA416-Dico-12 CV	100.7%
WA416-Dico-4 C CCV5	101.7%
0919 blank dico 1	100.6%
0919 dico Spike1	100.1%
0919 dico Spike2	99.5%
0919 Dico R-1	101.2%
0919 Dico R-2	98.5%
0919 Dico R-3	100.7%
WA416-Dico-4 C CCV5	103.5%

*Samples prepared on 09/19/05, but due to instrument usage, 5samples were stored overnight refrigerated and analyzed on 09/20/05

Table H21. Internal Standard Recovery 10-3-05 Analyses

Sample	Recovery
WA416-17-GC-17 ICV	99.8%
WA416-17-GC-29 CV	100.1%
WA416-17-GC-16C CCV	105.1%
1003DiBzAnt Blank	101.1%
1003 DiBzAnt R1	101.3%
1003 DiBzAnt R2	101.3%
1003 DiBzAnt R3	101.8%
WA416-17-GC-16C CCV	105.2%
WA416-nonyl-6 ICV	100.0%
WA416-nonyl-10 CV	101.1%
WA416-nonyl-3 C CCV	101.8%
1003 nonyl blank	98.3%
1003 Spike 1	99.6%
1003 Spike 2	97.8%
1003Nonyl R-1	98.4%
1003Nonyl R-2	99.8%
1003Nonyl R-3	101.7%
WA416-nonyl-3C CCV	103.7%

Table H22. Calibration Linearity Verification

Date	Compound	R^2 Value
08/23/2005	Atrazine	1.0000
09/09/2005	Atrazine	1.0000
09/19/2005	Atrazine	1.0000
09/14/2005	Dibenz[a,h]Anthracene	1.0000
10/03/2005	Dibenz[a,h]Anthracene	1.0000
08/23/2005	Dicofol	1.0000
09/09/2005	Dicofol	0.9996
09/20/2005	Dicofol	0.9975
07/26/2005	Fenarimol	1.0000
08/24/2005	Fenarimol	1.0000
09/13/2005	4-nonylphenol	1.0000
10/03/2005	4-nonylphenol	1.0000
07/25/2005	Prochloraz	0.9998
08/25/2005	Prochloraz	0.9996
09/26/2005	Prochloraz	0.9997

APPENDIX G

QUALITY ASSURANCE PROJECT PLAN

1.0 TITLE AND APPROVAL

Quality Assurance Project Plan (QAPP)
For Work Assignment 4-16
Placental Aromatase Validation Study

Task 7. Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories

for

EPA CONTRACT NUMBER 68-W-01-023

June 7, 2005

SIGNATURE PAGE

Quality Assurance Project Plan for WA 4-16, Task 7
Placental Aromatase Validation Study
EPA CONTRACT NUMBER 68-W-01-023

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4.0 PROJECT ORGANIZATION

The U.S. Environmental Protection Agency (EPA) is implementing the Endocrine Disruptor Screening Program (EDSP). To support this program, the EPA has contracted with Battelle to provide comprehensive toxicological and ecotoxicological testing services, including chemical, analytical, statistical, and quality assurance (QA)/quality control (QC) support, to assist EPA in developing, standardizing, and validating a suite of *in vitro*, mammalian, and ecotoxicological screens and tests for identifying and characterizing endocrine effects through exposure to pesticides, industrial chemicals, and environmental contaminants. The studies conducted will be used to develop, standardize and validate methods, prepare appropriate guidance documents for peer review of the methods, and develop technical guidance and test guidelines in support of the Office of Prevention, Pesticides and Toxic Substances regulatory programs. The validation studies will be conducted under the EDSP Quality Management Plan (QMP), study protocols, applicable Quality Assurance Project Plans (QAPPs), relevant program and facility Standard Operating Procedures (SOPs), guidance documents, and Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practice Standards (GLPs).

One of the assays recommended for validation and consideration for inclusion in the screening program is the aromatase assay. A Detailed Review Paper (DRP) was prepared for the U.S. EPA in 2002 to review the scientific basis of the aromatase assay and examine assays reported in the literature used to measure the effect of chemical substances on aromatase.

Prevalidation studies on the aromatase assay (Work Assignment [WA] 2-24) were conducted to optimize the microsomal aromatase assay protocol for human placental 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 runs and partial inhibition curves were addressed in a supplemental prevalidation study (WA 4-10).

The objectives of this work assignment are to use the now optimized assay: (1) to obtain intra- and interlaboratory assay variability estimates by conducting experiments at multiple laboratories, (2) to conduct microsome preparation and analysis experiments at multiple laboratories, and (3) to test up to 10 reference chemicals with different modes of action in order to evaluate assay relevance.

This work assignment is composed of multiple studies that will be conducted by the lead laboratory (Research Triangle Institute International [RTI], Research Triangle Park, NC) and three participating laboratories (Battelle, Columbus, OH; In Vitro Technologies, Baltimore, MD; WIL Research Laboratories, LLC, Ashland, OH). This QAPP will address the work to be conducted in Task 7 of the work assignment.

A summary of the work assignment organization is shown in Figure 4-1.

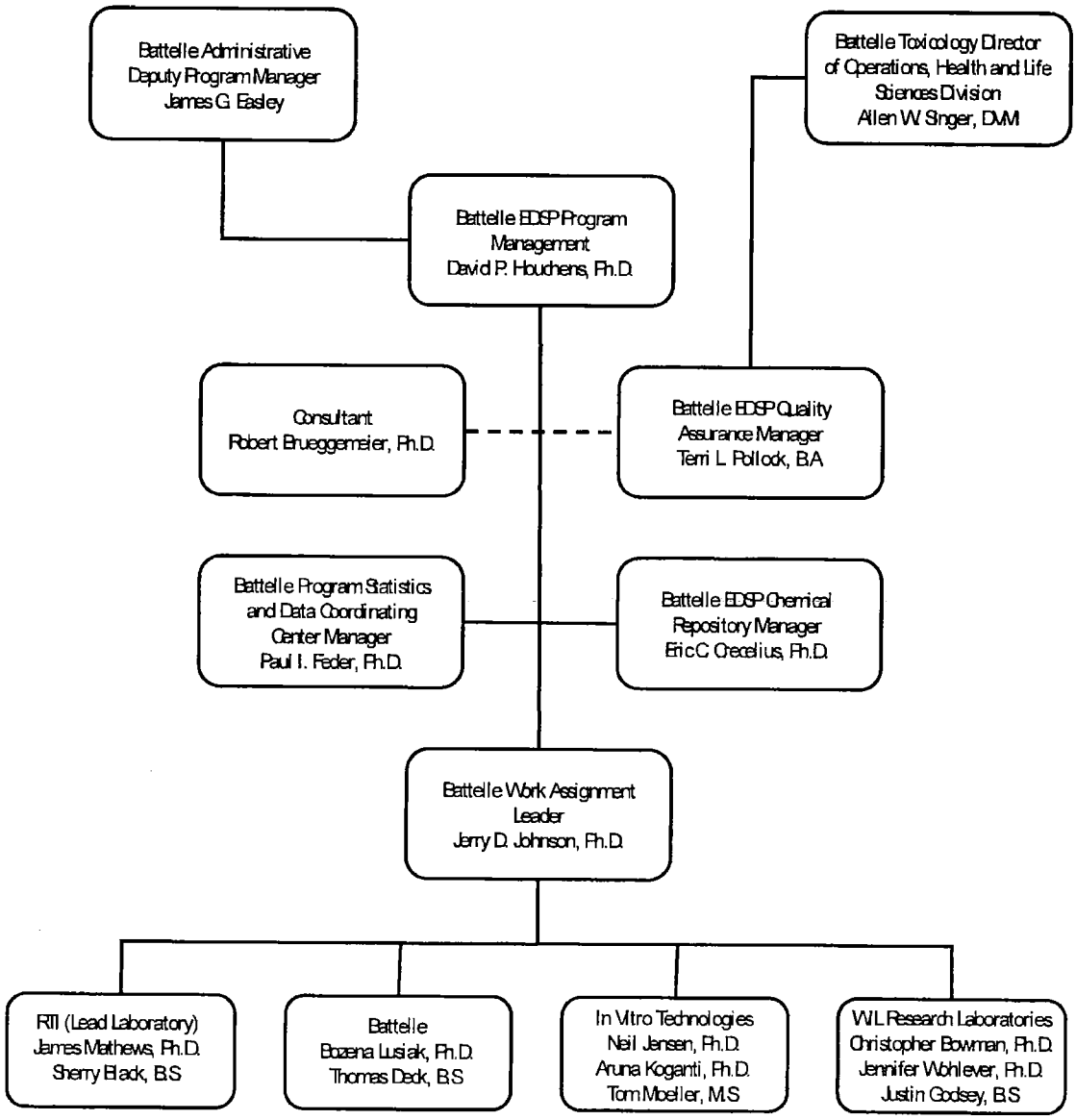

Figure 4-1. WA 4-16 Project Organization Overview

Portions of this work assignment will be managed at RTI, Battelle, WIL, and In Vitro. At each of these laboratories, there will be a person responsible for preparing the protocol, assigning appropriate staff to complete specified tasks within the protocol, and monitoring the progress of both technical and fiscal milestones as outlined in the technical work plan. A Study Director from each laboratory will report on the progress of the work assignment to Drs. David Houchens and Jerry D. Johnson at Battelle through a series of planned conference calls and through the use of written monthly reports.

General scientific direction and supervision of the work performed under this work assignment will be provided by Dr. Jerry D. Johnson, Battelle, and Dr. James Mathews, RTI International. Dr. Johnson will serve as the Work Assignment Leader (WAL) for the participating laboratories and Dr. Mathews for the lead laboratory (RTI).

Each laboratory will have a Study Director in charge of overseeing the daily operation and conduct of the study. The individual laboratory teams will execute the necessary tasks required in the study protocols and ensure the data are collected and handled appropriately. All of these tasks will be clearly defined in the study protocol.

The QAU representative for each laboratory will administer the QAPP for the EDSP facility QA team members. The specific responsibilities will include:

- Interact with the Study Director to ensure that QA and QC procedures are understood by WA personnel.
- Conduct technical systems audits (TSAs) and audits of data quality (ADQs) to evaluate the implementation of the program WAs with respect to the EDSP QMP, the WA QAPPs and/or GLP protocol, and applicable program and facility SOPs.
- Prepare and track reports of deficiencies and submit them to both line and program management.
- Consult with the Study Director and, as necessary, the EDSP Battelle QA Manager and Program Manager on actions required to correct deficiencies noted during the conduct of the WA.
- Ensure that all data produced as part of the EDSP WAs are maintained in a secure, environmentally-protected archive.
- Ensure, during the conduct of TSAs, that all staff participating on the EDSP are adequately trained.
- Maintain complete facility-specific QA records related to the program.

- Submit copies of resolved audits to the EDSP Battelle QA Manager.

- Submit a QA Statement to the EDSP Battelle QA Manager and Program Manager with each written deliverable that describes the audit and review activities completed and any outstanding issues that could affect data quality or interpretation of the results discussed in the report.

- Maintain effective communication with the EDSP QA Manager.

- Act as the facility's EDSP SOP Custodian for all SOPs received from the SOP Administrator.

As EDSP Manager, Dr. David Houchens will have ultimate responsibility for quality, timeliness, and budget adherence for all activities on the contract. He also will serve as the principal interface with the EPA's Project Officer on all contract-level administrative and technical issues. Because of the high level of subcontracting and purchases required by the program, such as test laboratory subcontracts and purchases of chemical supplies, Dr. Houchens will be assisted by an Administrative Deputy Manager, Mr. James Easley. Mr. Easley will manage the procurement of all subcontracts, consultants, and purchased materials and services, and will facilitate schedule and cost control. He has played a similar role on ten other large, multi-year, level-of-effort task-order contracts for EPA. Thus, he will be able to assure that all purchases are compliant with government regulations and that EPA is provided timely, accurate accounting of these substantial costs in our monthly progress reports.

Ms. Terri Pollock, the EDSP QA Manager at Battelle, will direct a team of QA specialists to monitor the technical activities on the chemical repository program, and provide oversight to all associated QA functions. Ms. Pollock will be responsible for reporting her findings and any quality concerns to Dr. Houchens. Ms. Pollock will report, for the purposes of this program, to Dr. Allen W. Singer, Director of Operations in the Toxicology Product Line in Battelle's Health and Life Sciences Division. This reporting relationship will assure that the QA function is independent of the technical activities on the program.

5.0 PROBLEM DEFINITION/BACKGROUND

5.1 Problem Definition

Prevalidation studies on the placental aromatase assay (WA 2-24) were conducted to optimize the microsomal aromatase assay protocol for human placenta, demonstrate the utility of the microsomal assay to detect known aromatase inhibitors, and compare the performance of a recombinant assay system and the placental microsomal assays. Concerns with this initial work

involving high variability in some runs and partial inhibition curves were addressed in a supplemental prevalidation study (WA 4-10).

With the prevalidation studies successfully completed, this work assignment directs Battelle to conduct the interlaboratory studies to determine the performance of several laboratories in conducting the assay and should complete the validation of the placental aromatase assay. A companion work assignment (WA 4-17) has been issued for the conduct of the recombinant aromatase assay.

The work assignment is comprised of 9 tasks of which five tasks involve experimentation. Task 3 is a training task. The work in Tasks 4 through 7 is described in this QAPP. Table 5-1 summarizes the validation tasks and the laboratory(ies) involved for each experimental task.

Table 5-1. Validation Study Plan Experiments

Task Number	Description of Experimental Task	Experimental Task Assignment
1	Not applicable (develop work plan, study plan, and identify/select participating laboratories)	Not an experimental task
2	Not applicable (develop QAPP and protocols)	Not an experimental task
3	Training Participating Laboratories in the Conduct of the Assay	Lead Laboratory + 3 Participating Laboratories
4	Conduct Positive Control Studies in the Participating Laboratories	3 Participating Laboratories
5	Conduct Multiple Chemical Studies with Centrally Prepared Microsomes (RTI/Participating Laboratories)	Lead Laboratory + 3 Participating Laboratories
6	Two Labs: Procure Placenta/Prepare & Analyze Microsomes/Determine Protein Concentration and Aromatase Activity/Determine 4-OH ASDN Inhibition Response/Distribute Microsomes to Labs All Labs: Using Microsomes Rec'd, Determine the Protein Concentration and Aromatase Activity	Lead Laboratory + 3 Participating Laboratories
7	Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories (RTI/Participating Laboratories)	Lead Laboratory + 3 Participating Laboratories
8	Prepare Study Reports (RTI/Participating Laboratories)	Not an experimental task
9	Prepare Presentation for EDMVAC*	Not an experimental task

*EDMVAC = Endocrine Disruptor Method Validation Committee

5.2 Background

The Food Quality Protection Act of 1996 was enacted by Congress to authorize the 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 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 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 P450arom 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 P450arom and consists of 10 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 mM to greater than 50 mM.

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. Concerns with this initial work involving high variability in some runs and partial inhibition curves were addressed in a supplemental prevalidation study (WA 4-10). The objective of the current work assignment is to use the now optimized assay to obtain intra- and interlaboratory assay variability estimates to complete the validation of the human placental microsome aromatase assay.

6.0 PROJECT/TASK DESCRIPTION

Only Task 7 is under the control of this QAPP. However, this QAPP also describes the other three experimental tasks in this work assignment; a separate QAPP was issued prior to the start of each new task together with a finalized task-specific template protocol included as an attachment. The Task 7 template protocol is attached to the present QAPP. The task numbering scheme for the original work assignment is employed in this document for ease of cross-referencing.

Task 4: Conduct Positive Control Studies in the Participating Laboratories

This task was completed by staff at Battelle, WIL and In Vitro. RTI staff did not conduct any experiments on this task but were involved in the review of the data produced by the other laboratories. RTI provided human placental microsomes to the other laboratories for use in this task. Battelle/RTI provided a boilerplate protocol for this task to the participating laboratories which they used to prepare their laboratory-specific protocols. These protocols contained all necessary technical detail for the conduct of this task. Briefly, the task required that each laboratory conduct three independent replicates of a Positive Control Study. In this Study, 4-OH androstenedione (4-OH ASDN, a known aromatase inhibitor) was tested in the aromatase assay at 6 concentrations to construct a dose/response curve from which an IC_{50} was calculated. Control runs also were included in the assay set to measure full aromatase activity (without any inhibitor added) and background activity (without NADPH co-factor). Battelle's Chemical Repository (CR) supplied 4-OH ASDN to each laboratory as a stock solution and conducted all necessary pre-assay chemistry activities for 4-OH ASDN.

Each laboratory presented their results in a separate spreadsheet for each of the three replicates and the results were compared both within and between laboratories.

The results of this experiment required technical review and approval prior to proceeding to Task 5.

Task 5: Conduct Studies with Centrally Prepared Microsomes

This task was completed by staff at RTI, Battelle, WIL and In Vitro. RTI provided human placental microsomes to the other laboratories for use in this task. Battelle/RTI provided a boilerplate protocol for this task to the participating laboratories, which they used to prepare their laboratory-specific protocols. These protocols contained all necessary technical detail for the conduct of this task. Briefly, the task required that each laboratory conduct three independent replicate studies on each of four test chemicals. All three replicates for a given chemical were conducted by the same technician within a laboratory. Control runs were also included in each assay set to measure full aromatase activity (without any inhibitor added) and background activity (without NADPH co-factor). In addition, positive control samples (containing a known aromatase inhibitor) and negative control samples (containing a known aromatase non-inhibitor) were included in each assay set. Battelle's CR supplied the test and control chemicals to each laboratory as individual stock solutions and conducted all necessary pre-assay chemistry activities for the test and control chemicals.

Each laboratory presented their results in a separate spreadsheet for each of the three replicates and the results were compared both within and between laboratories.

The results of this experiment required technical review and approval prior to proceeding to Task 7.

Task 6: Prepare Microsomes in Two Participating Laboratories

There were two activities in this task. The first, conducted by Battelle and In Vitro, required those laboratories to obtain a human placenta, prepare microsomes and then to analyze their microsome preparations for protein content and (uninhibited) aromatase activity. In addition, those laboratories conducted two independent replicates of the Positive Control Study (as used in Task 4) using their microsomal preparations. RTI/Battelle supplied a template protocol that included all technical detail required for the conduct of those experiments. Battelle's CR supplied 4-OH ASDN to each laboratory as a stock solution. The laboratories submitted the results of these studies to Battelle and the data were reviewed by Battelle and RTI prior to submission to EPA. After EPA approved the results, the second portion of the task was initiated.

For the second activity in this task, Battelle and In Vitro each shipped portions of their placental microsome preparations to the other three participating laboratories. All laboratories measured the protein content and (uninhibited) aromatase activity of the microsomal preparations received from both laboratories.

Each laboratory presented their results in a separate spreadsheet for each replicate and the results were compared both within and between laboratories.

Task 7: Conduct Studies with Microsomes Prepared in Participating Laboratories

Battelle and In Vitro will conduct the studies in this task with microsomes prepared in their laboratory in Task 6. RTI and WIL will receive microsomes from Battelle and In Vitro, respectively, for use on this task.

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

For each chemical (1 to 10) laboratory 2 (Battelle) will supply two of laboratories 1, 2, 3, or 4 (RTI, Battelle, In Vitro and WIL, respectively) and laboratory 4 (WIL) will supply the other two. The distribution of microsomal preparations will differ for different chemicals. Let M_2 denote the microsomal preparation from laboratory 2 and let $\mathcal{M}_4$ denote the microsomal preparation from laboratory 4. The four laboratories will use microsomal preparations from the laboratories as shown below.

Table 6-1. Testing Matrix for Microsomes and Chemicals

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

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

RTI/Battelle will supply a template protocol describing all technical details for this task to the participating laboratories from which they will prepare their laboratory-specific protocols. Each laboratory will conduct three independent replicate studies with each of 10 chemicals. All three replicates for a given chemical will be conducted by the same technician within a laboratory. Control runs will also be included in each assay set to measure full aromatase activity (without any inhibitor added) and background activity (without NADPH co-factor). In addition, positive control samples (containing a known aromatase inhibitor) and negative control samples (containing a known aromatase non-inhibitor) will be included in each assay set. Battelle's CR will supply the control and coded test chemicals to each laboratory as individual stock solutions and will conduct all necessary pre-assay chemistry activities for the test and control chemicals.

7.0 QUALITY OBJECTIVES AND CRITERIA

There are several critical components to the aromatase assay. Criteria for acceptance of each of these components are described below.

7.1 Incubation Temperature

The water bath for incubation of aromatase assay tubes will be held at 37 ± 1°C.

7.2 Minimum Full Aromatase Activity

Full aromatase activity should be at least 0.03 nmol product/mg protein/min.

7.3 Percent of Control Values for Controls

The mean percent of control value for the positive control tubes should be 55 ± 10%. The mean percent of control value for the negative control tubes should be 94 ± 10%. Background control percent of control values are defined as 0%.

7.4 Data Quality Indicators

7.4.1 Precision

The activities of replicate tubes will be within the mean activity ± 15%. Each control activity for each assay/laboratory will be within the overall mean ± 15% activity for that control type for that laboratory.

Variance between laboratories and within laboratories will be assessed for an appropriate level of precision as part of this WA. It is anticipated that full aromatase control activity between and within laboratories will be statistically equivalent at the $p > 0.1$ level. Any modifications to this criterion will be discussed with the sponsor and added to the QAPP by amendment.

IC_{50} and slope values calculated for each inhibitor will be statistically equivalent at the $p > 0.1$ level both between and within laboratories. If data from an assay are statistical outliers, the assay will be repeated.

7.4.2 Bias

The control samples that are run with each assay will be used to control for bias. If the control samples for any assay do not meet the precision criteria described above, the assay will be rerun. Assays will be conducted blind at the technician level for test chemical identity.

7.4.3 Accuracy

Accuracy of the liquid scintillation spectrometry (LSS) data (from which is derived the aromatase activity) will be assessed by analysis of a sealed standard of known radioactive content. If the radioactivity in the sealed standard is more than 5% different from the known value, the data will not be used. Samples will be recounted on another LSS or on the same LSS after any problems with the instrument are corrected.

8.0 SPECIAL TRAINING/CERTIFICATION

All personnel involved in handling radiolabeled materials will have completed a Radiation Safety Training course. Training documentation will be maintained in the individual training files. Each laboratory will be licensed to receive radiolabeled materials.

All personnel involved in handling human placental and human placental microsomes will have appropriate training in the handling and disposition of biohazards. Training documentation will be maintained in the individual training files.

Staff from the participating laboratories have been trained on the performance of the aromatase assay at RTI International as part of Task 3 of this Work Assignment. Personnel participating in this training conducted the aromatase assay including full aromatase control and background control samples and a series of samples containing varying amounts of a known aromatase inhibitor (4-OH ASDN). The resultant data were evaluated by Battelle and RTI International and then submitted to EPA for review.

9.0 DOCUMENTS AND RECORDS

9.1 Retention of Specimens and Records

Archiving procedures will be specified in the individual protocols.

9.2 Quality Assurance Project Plan

This QAPP will be distributed to project participants initially, and whenever revised. Previous versions will be marked as "obsolete" when newer versions are distributed, or collected and destroyed so that there is no confusion regarding the version in effect. The right-justified document control header example shown here

Version 1
Month, Year
Page 1 of 1

will be used to ensure that revision numbers and dates are obvious to document users. The QAPP will be reviewed annually and a determination made to either modify the document based on new or modified project requirements, or leave as is.

Controlled copies of the QAPP will be maintained, tracked, and managed by the laboratories' QAU through the use of a master distribution list.

9.3 Data Forms

All data forms will include a title identifying the type of data to be recorded, a unique study code or protocol number, and the initials and date of the data recorder(s) to authenticate the records.

Corrections to data entries will be made by drawing a single line through the error, recording the correct entry, initials, date, and error code that explains the reason for the correction.

9.4 Microsome Storage Conditions

Microsomes will be stored at -70 to -80°C and the freezer temperature records will be maintained.

9.5 Reports

9.5.1 Interim Data Summary, and Draft and Final Reports

An interim data summary from each laboratory will be submitted to the EPA after completion of each task. These data summaries will not be audited by Quality Assurance but will be checked for accuracy by technical staff. This procedure is necessary to provide a rapid turn around of the data so that approval to proceed can be given by EPA.

Each laboratory will prepare an individual report for each task to be based on a template provided by Battelle and will submit these reports to Battelle. The purpose of these reports is to provide a complete description about how the experiments were performed, present the results that were obtained (including tables and graphs), and state the conclusions that were made for each applicable WA task. RTI/Battelle will prepare a report for each task that summarizes all work on the particular task and incorporates the reports from the participating laboratories as Appendices for submission to EPA. After EPA comments have been received on each task report and, if applicable, incorporated into a new version of the draft task report, then it will be issued as a final report.

Each final task report will include:

- Abstract
- Objectives
- Materials and Methods
- Results
- Discussion
- Conclusions
- References
- Summary data with statistical analyses
- Appendices which will include final reports with compliance statements for each participating laboratory
- Protocol, any amendments, or any deviations from the protocol
- QAPP, any amendments, or any deviations from the QAPP.

RTI/Battelle will prepare a final Work Assignment report that summarizes the results of the entire Work Assignment. This report will consist of a statement of the objectives of the work assignment, a summary of the results and a statement of conclusions for the Work Assignment. The individual task reports will be referenced within this final report.

9.5.2 QA Assessment Reports

QA assessment reports will be maintained as confidential files in the QAU.

9.5.3 Status Reports

Status/progress reports will be submitted to the EPA Project Officer by Battelle on a monthly basis as stipulated in the contract.

10.0 SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)

The details of the experimental design for the task subject to this QAPP will be contained in a GLP compliant protocol. A template protocol for this task is attached as an Appendix to this document.

11.0 SAMPLING METHODS

The entire aqueous portion of the incubation mixtures remaining after extraction with methylene chloride (CH_2Cl_2) will be placed in appropriate containers. The samples will be mixed well prior to the removal of aliquots for liquid scintillation counting (LSC). If there is insufficient time for preparing LSC samples on the day the assay is run, the samples will be refrigerated overnight. Samples remaining after preparation of LSC aliquots will be frozen and stored at about -20°C. These samples will be thawed, mixed and realiquoted, if necessary, due to problems with LSC samples.

Each test and standard chemical will be supplied to the participating laboratories by Battelle as a stock solution at the highest concentration necessary for use in the assay. These solutions will be well-mixed prior to the preparation of dilutions of these stock solutions by the individual participating laboratories.

12.0 SAMPLE HANDLING AND CUSTODY

12.1 Test and Reference Chemical Solutions

The test and standard chemical stock solutions will be transferred to the Laboratories' Material Handling Facility with a study specific transfer of material form. The samples will be

processed according to the SOPs for packing, shipment and documentation of shipment and receipt.

12.2 Sample Collection Documentation

All samples (or sample sets) will be labeled with enough information to allow for unequivocal identification of each sample along with suitable storage conditions in accordance with applicable regulations.

13.0 ANALYTICAL METHODS

Analytical methods are described in the study protocol (Appendix). Failures of analytical systems are addressed in the relevant SOPs.

14.0 QUALITY CONTROL

14.1 Methods

Control samples will be run with each assay. These include: (1) full aromatase enzyme activity controls, (2) background controls, (3) positive controls and (4) negative controls. Acceptance criteria and corrective actions where acceptance criteria are not met are described in Section 7. Replicates will be used as a means to monitor variability of the assay. Replicates will be assessed for variance and those that are outside the acceptable range (mean ± 15%) will be flagged as statistical outliers.

14.2 Data Collection

Data collection documentation will be as described in applicable SOPs or protocols.

Assay data, including weights and/or volumes of chemicals, solvents or other materials used to prepare necessary solutions or samples, will be recorded manually on data sheets. Protein assay absorbance data will also be recorded manually on data sheets. All data sheets will include a title identifying the type of data to be recorded, the unique study code or protocol number, and the initials and date of the data recorder(s) to authenticate the records.

Scintillation counter data will be automatically saved to a data file that will automatically be assigned a unique filename. The data will be annotated to identify samples with the sequential vial number. Procedures for converting CPM data to DPM data will be documented.

Relevant data from the data sheets and scintillation counter output (as DPM) will be typed into a validated MS Excel spreadsheet for calculation of (1) substrate specific activity (2) protein content and/or (3) aromatase activity. All transcribed data will be verified (100% QC) before they are reported and this QC check will be documented on the spreadsheet printouts by technician initials and date.

Aromatase activity data will be entered automatically (through linked validated spreadsheets) or manually into Prism data files for calculation of IC_{50}. Data will be entered automatically (through linked validated spreadsheets) or manually into spreadsheets for import into SAS data files for statistical analysis. All manually entered data will undergo a 100% QC check.

15.0 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

The following types of equipment will be required for this WA: temperature controlled shaking water bath, pH meter, analytical balances, centrifuges (low and high speed and ultracentrifuges), pipettors, scintillation counters, spectrophotometer, and high performance liquid chromatography (HPLC) equipment (injector, pumps, detectors [radiochemical and ultraviolet {UV}], data collection system). The equipment will be tested, inspected and maintained according to schedules contained in the relevant SOPs.

16.0 INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

Balances used to obtain weight measurements, as well as the check weights that are used to verify a balance's calibration status, will be calibrated and maintained according to the schedule specified in relevant SOPs. Balances that do not meet the criteria specified in the SOP will not be used for this work assignment.

Scintillation Counters will be calibrated using procedures described in the relevant SOPs. Calibration of pH meters will occur as specified in relevant SOPs. The water bath, pipettes, spectrophotometer, and HPLC equipment will be calibrated using the procedures and schedule in applicable SOPs. Any equipment or instrument that does not meet acceptance criteria as described in the relevant SOP will not be used for this work assignment.

17.0 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

Upon receipt, purchased items will be inspected for conformance to quality requirements prior to use. All use of the product will be prior to the expiration dates, if applicable. Chemicals will be received and stored in accordance with applicable SOPs.

18.0 NON-DIRECT MEASUREMENTS

No collection of any samples or sample data will be obtained from non-direct measures such as computer databases or programs.

19.0 DATA MANAGEMENT

19.1 Data Management Overview

Data will be maintained in notebooks and/or files according to applicable facility SOPs. The records will be kept in the appropriate rooms until there is a signed final report at which time they will be inventoried and placed in the facility archives according to applicable facility SOPs, unless the sponsor requests that they be transferred to another archive location.

19.2 Data Transfer

Information will be sent to the Data Coordination Center in electronic format as specified in SOP EDSP.D-003-01. Specifically all raw data, all tables, graphs summarizing results of statistical analyses as presented in study reports, statistical analysis data files, statistical analysis programs, and all study documents will be sent to the EDSP Data Coordination Center in electronic format.

20.0 ASSESSMENTS AND RESPONSE ACTIONS

EDSP QA team members will perform assessments on WA activities and operations affecting data quality and the raw data and final report. They will report any findings to the Study Director and management to ensure that the requirements in relevant SOPs, study protocols and WA QAPP, the QMP, and the FIFRA GLPs are met. The assessments for this study will include TSAs and ADQs. Performance Evaluations will not apply to this QAPP.

20.1 Technical Systems Audits

A TSA is a process by which the quality of a study is assessed through evaluating a study activity's conformance with the protocols, applicable facility or program SOPs, QAPP, QMP, and GLPs. The acceptance criteria will be that WA activities and operations must meet the requirements of these planning documents and the GLPs or be explained and evaluated in a deviation report. Deviations from the GLPs, QAPP, protocol, or SOPs will be properly documented and assessed by management and the Study Director as to their impact on the study.

20.2 Type, Scheduling, and Performance of Technical Systems Audits

The following paragraphs provide an example of how the laboratories will perform technical system audits.

Prior to the experimental start, the facility QA Team Member will convey a list of inspections targeted for the study to the Study Director. Whenever possible, TSAs will be done at the commencement of the WA critical phase to ensure WA integrity based on compliance with the protocol, QAPP, SOPs, and GLPs. Critical phases targeted for TSAs will include, but are not limited to:

- Protocol review
- Aromatase assay sample preparation and analysis.

During the TSA, EDSP QA team members will record observations to be used later in preparing the audit report. EDSP QA team members will observe the procedure, data recording, and any equipment maintenance and calibration procedures and/or documentation, noting whether or not the activities adhered to the study protocols and QAPP, applicable SOPs, QMP, and the GLPs. Any findings will be communicated to the technical personnel at the completion of the procedure unless an error could compromise the study (e.g., misdiluting the stock solution). EDSP QA team members will immediately notify the Study Director by telephone and/or e-mail of any adverse findings that could impact the conduct of the study. This direct communication will also be documented in the audit report.

20.3 Audits of Data Quality

An ADQ is a process by which the accuracy of data calculations and reporting will be assessed to ensure that the reported results are of high quality and accurately reflect the raw data and accurately describe the materials used in the study. The acceptance criteria for the ADQ will be that data collection, analysis, and reporting must meet the requirements of the applicable

facility and program SOPs, the WA protocols and QAPP, QMP, and the FIFRA GLPs, or be explained and evaluated in a deviation report, as previously described.

20.4 Scheduling and Performance of Audits of Data Quality

Direct and frequent communication between the WA Leader/Study Director, laboratory supervisor, and the QA Manager will provide for sufficient time to perform an ADQ so that the submission date of the draft final report meets that specified in the study protocol. The scheduling process will also allow for a reasonable amount of time for corrections and subsequent verification of the corrections by QA.

EDSP QA team members will audit the study records at a frequency adequate to ensure that approved protocol requirements are met. The frequency required is specified by the type of data in the QMP, Section 2.4.1. Findings will be reported and corrective actions undertaken as described earlier. EDSP QA team members will review the final report using the audited data and corrected tables. The report text will be reviewed to ensure that every statement is supported by the data and any discussions or conclusions drawn from the study are supported by the data. Findings will then be reported and corrective actions undertaken as described earlier.

20.5 Audit Report Format

The following paragraphs provide an example of how the laboratories will format an audit report.

The audit report will consist of a cover page for study information and additional page(s) with the audit findings. All pages will have header information containing the study protocol number, audit report date, and audit type. The audit report date will be the date on which the EDSP QA team member signs the audit report and sends it to the Study Director and management.

The cover page will contain the study protocol title, number, and code; Sponsor; Study Director; audit type; audit date(s); EDSP QA team member; distribution list; the dated signature of the auditor; the date that the Study Director received the audit report; and the dated signatures of the Study Director and management. The distribution list will include additional names for individuals who have findings pertaining to their area of responsibility (e.g., the ARF Manager would address a finding pertaining to the ARF) and is used to ensure that the report is sent to all who need to respond. Subsequent page(s) will contain the audit finding(s), any recommended remedial actions, and space for the Study Director to respond to the findings and document remedial actions taken or to be taken.

20.6 Response Actions and Resolution of Issues

The Study Director will respond to the TSA report within a specified number of working days of receipt of the report as required by the laboratory's SOPs. There will be no deadline for the Study Director's response to an ADQ report except for the time constraint deriving from the submission date of the final WA report. The Study Director will forward the audit report to management for review. Management will add comments as necessary, sign and date the report and return it to the EDSP QA team member. The EDSP QA team member will assess the responses and verify the corrective actions. If a disagreement between the Study Director and EDSP QA team member arises over a finding, it will be discussed among the other EDSP QA team members. The EDSP QA team member will then present the majority opinion to the Study Director for further consideration. If the disagreement remains, the issue will be reported to the Study Director's management. The action decided on by management will be documented in the QA files.

During an assessment, if the auditor determines that adverse health effects could result or WA objectives of acceptable quality cannot be achieved, the auditor will follow the Stop Work Procedure specified in the EDSP QMP (Section 3.3).

20.7 Independent Assessments

The EDSP Battelle QA Manager (QAM), or designee, will conduct an independent TSA and ADQ during the conduct of this work assignment. Typically one independent audit will be conducted during the work assignment. If major deficiencies are uncovered, additional independent audits will be scheduled. The conduct and reporting of the audits will be consistent with the procedures described in the EDSP QMP (Section 3.3).

In addition, the EDSP EPA QAM, or designee, will have the option of conducting external TSAs/ADQs.

21.0 REPORTS TO MANAGEMENT

The QA Manager will send periodic reports to the Study Director and management, which detail significant regulatory, protocol, and SOP issues. Also, the participating laboratories will report to the EDSP Program Manager and WAL.

22.0 DATA REVIEW, VERIFICATION, AND VALIDATION

The data produced under this work assignment will be reviewed by the technical personnel for the validation process and by EDSP QA team members for the verification process (see section 23). The criteria used for validation will depend on the type of data. For dose solution sample data, information regarding the condition of the containers and whether or not samples were compromised will be recorded in the sample chain-of-custody records. Compromised samples will not be analyzed. The criteria for validating data are those found in Section 7 (Quality Objectives and Criteria).

23.0 VERIFICATION AND VALIDATION METHODS

23.1 Chain of Custody for Data

Study data, records, and specimens will be maintained in a secure and designated location, e.g., in the respective laboratory offices until study completion. Chain-of-custody procedures will be implemented according to facility SOPs. Chain-of-custody information, including the date, study record(s) removed or returned, and the name of the person removing or returning the data will be documented. At study completion, the Study Director will follow the procedures specified in the facility SOP for archiving study materials.

23.2 Data Validation

Data validation is a process by which the WA Leader/Study Director and/or other technical personnel evaluate the data for conformance to the stated requirements for methodology and quality. These personnel will be responsible for reviewing the data, evaluating any technical deviations or non-conformances, and then determining the degree to which the data meet the quality criteria stated in Section 7.

23.3 Data Verification

Data verification will constitute part of the ADQ process performed by EDSP QA team members and described earlier. Verification will ensure that (1) the data are of high quality and were collected according to the planning documents' requirements, and (2) the reported results accurately reflect the raw data. Each data type will be evaluated against its collection and reduction requirements specified in the planning documents. Errors discovered during the data evaluation will be corrected. The reported conclusions drawn from the data will be verified by

EDSP QA team members during the report audit to confirm that they are true and accurate. The procedure for resolving issues of data verification has been detailed in prior sections of this document.

24.0 RECONCILIATION AND USER REQUIREMENTS

Proposed methods for data analysis, including a test for statistical outliers, will be specified in the Study Plan and/or protocols.

25.0 REFERENCES

The following references were used to prepare the QAPP. Not all references are cited in the text.

Battelle (2003). Endocrine Disruptor Screening Program Quality Management Plan, Version 2. May 12, 2003.

Battelle (2004). Technical Work Plan on Microsomal Aromatase Validation Study, EPA Contract Number 68-W-01-023, Work Assignment 4-16. September 8, 2004.

FQPA (1996). Food Quality Protection Act of 1996, U.S. Public Law 104-170, 21 U.S.C. 46a(p), Section 408(p), 110 STAT.1489. August 3, 1996.

APPENDIX A:

TEMPLATE PROTOCOL FOR TASK 7

EPA Contract No.: 68-W-01-023
EPA Work Assignment No.: 4-16
Task Number: 7
RTI Study Code: An05-928

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

SPONSOR: Battelle
505 King Ave.
Columbus, OH 43201

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

PROPOSED EXPERIMENTAL START DATE: May 20, 2005
PROPOSED EXPERIMENTAL END DATE: August 30, 2005

AMENDMENTS:

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

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1.0 OBJECTIVES

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

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

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

1.1 Justification for Test System

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

1.2 Test Method

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

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

2.0 MATERIALS RECEIPT AND/OR PREPARATION

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

2.1 Substrate

2.1.1 Substrate Name/Supplier

The substrate for the aromatase assay is androstenedione (ASDN). Nonradiolabeled and radiolabeled ASDN will be used. The nonradiolabeled ASDN and the radiolabeled androstenedione ([1β-^{3}H]-androstenedione, [^{3}H]ASDN) will be provided to the laboratories by Battelle's Chemical Repository (CR). The CR will forward all applicable information regarding supplier, lot numbers, and reported/measured purity for the substrate to the laboratories and this information will be included in study reports. The radiochemical purity of the [^{3}H]ASDN was assessed by the lead laboratory in a previous Task and was found to be 97%.

2.1.2 Preparation of Substrate Solution for use in Aromatase Assay

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

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

2.2 Reference Chemicals

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

Table 1. Reference Chemicals for Aromatase Assay Validation

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

2.2.1 Reference Chemical Formulation and Analysis

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

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

2.3 Control Substances

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

Table 2. Control Substances

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

2.3.1 Control Substance Formulation and Analysis

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

2.4 Microsomes

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

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

If the human placental microsomes are supplied in aliquots in excess of what is required to conduct a single experiment, they will be thawed, pooled, homogenized, divided into appropriate aliquots for conduct of a single experiment and refrozen as described below in order

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

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

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

2.5 Other Assay Components

2.5.1 Buffer

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

2.5.2 Propylene Glycol

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

2.5.3 NADPH

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

each day and will be kept on ice.

3.0 PROTEIN ASSAY

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

4.0 AROMATASE ASSAY METHOD

The assays will be performed in 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 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 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 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 minutes at a setting of 1000 rpm. The methylene chloride layer will be removed and discarded; the aqueous layers will be extracted again with methylene chloride (2 mL). This extraction procedure will be performed one additional time, each time discarding the methylene chloride layer. The aqueous layers will be transferred to vials and duplicate aliquots (0.5 mL) will be transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Packard, 10 mL) will be added to each counting vial and shaken to mix the solution. The radiochemical content of each aliquot will be determined as described below.

Table 3. Optimized Aromatase Assay Conditions

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

[a] Final concentrations

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

5.0 DETERMINATION OF THE RESPONSE OF AROMATASE ACTIVITY TO REFERENCE CHEMICALS

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

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

Table 4. Microsome Distribution and Testing Matrix

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

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

Each replicate will test the response of aromatase activity to the presence of eight concentrations of a reference chemical. The reference chemicals must be coded prior to distribution to the assaying technicians in order that the replicates are conducted blind for reference chemical identity. This task will be conducted in three independent replicates. All three replicates for a given reference chemical must be conducted by the same technician. However, the same technician is not required to perform the three replicates for all 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. Each reference chemical will be tested at eight concentrations and there will be three (triplicate) repetitions for each concentration of a given replicate. A single replicate study of a given reference chemical is described in Table 5.

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

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

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

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

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

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

Table 5. Reference Chemical Study Design

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

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

6.0 DATA ANALYSIS

6.1 Aromatase Activity and Percent of Control Calculations

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

6.2 *Statistical Analyses*

6.2.1 Concentration Response Fits for the Reference Chemicals

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

superimposed on the plots. On a separate plot the average percent of control values for each replicate will be plotted versus logarithm of reference chemical concentration. The average concentration response curve across replicates will be superimposed on the same plot. The average concentration response curve will be the unweighted average of the response curves within each replicate.

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

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

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

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

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

- reference chemical
- portion (beginning or end)
- 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 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

ENDOCRINE DISRUPTOR SCREENING PROGRAM
AMENDMENT REPORT

STUDY NUMBERS: WA 4-16 and WA 4-17	WAL/STUDY DIRECTOR: Jerry D. Johnson
NOTEBOOK NUMBER: Not Applicable (NA)	AMENDMENT NUMBER: 1

TITLE OF STUDIES:

WA 4-16; Placental Aromatase Assay Validation Studies

- Conduct Multiple Chemical Studies with Centrally Prepared Microsomes (Task 5)
- Prepare/Analyze Microsomes and Conduct Positive Control Study At Two Participating Laboratories; Analyze Microsomes at Each Laboratory (Task 6)
- Conduct Multiple Chemical Studies with Microsomes Prepared in Participating Laboratories (Task 7)

WA 4-17; Recombinant Aromatase Assay Validation Studies

- Conduct Multiple Chemical Studies with Recombinant Microsomes (Task 4)

QAPP/PROTOCOL ID(e.g. number and/or date): WA 4-16: Dates range from 1/24/05 (Task 5) to 6/7/05 (Task 7). WA 4-17: Date was 6/29/05 (Task 4)

AMENDMENT RELATING TO:

[x]	QAPP	[ ]	QMP	[ ]	Protocol
[ ]	SOP	[ ]	Method		

ORIGINAL DOCUMENT SPECIFICATIONS:

Background section, 5th paragraph, last sentence reads, "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 mM to greater than 50 mM."

AMENDMENT:

The above sentence is changed to: "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."

REASON FOR CHANGE:

Correction of the IC_{50} value units.

APPROVAL:

WAL	Jerry D. Johnson	Date	5-26-06
~~Study Director~~	not applicable for QAPPs TMP 5-30-06	Date	
Program Management	Dd P Rountree	Date	5/30/06
EDSP Battelle QAM	Terri L Pollock	Date	5-30-06