DRAFT FINAL REPORT

RECOMBINANT AROMATASE VALIDATION STUDY: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES (Volume II-Appendices C through G)

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

June 2006

Prepared for

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

Battelle
505 King Avenue
Columbus, Ohio 43201

APPENDIX C

IN VITRO TECHNOLOGIES REPORT

DRAFT FINAL TASK REPORT

RECOMBINANT AROMATASE VALIDATION STUDY

WA 4-17 Task 4: Evaluation of the Potential of Reference Chemicals to Inhibit Activity of Recombinant Aromatase

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

Sponsor:

Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2639

Performing Laboratory:

In Vitro Technologies, Inc.
1450 South Rolling Road
Baltimore, MD 21227

DRAFT FINAL REPORT

Title:	RECOMBINANT AROMATASE VALIDATION STUDY WA 4-17 Task 4: Evaluation of the Potential of Reference Chemicals to Inhibit Activity of Recombinant Aromatase
Author:	Aruna Koganti, Ph.D.
Performing Laboratory:	In Vitro Technologies, Inc. 1450 South Rolling Road Baltimore, MD 21227
Sponsor:	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2639
Sponsor Representatives:	David P. Houchens, Ph.D. EDSP Program Manager Battelle Jerry D. Johnson, Ph.D. Diplomate, A.B.T. Work Assignment Leader Battelle
Experimental Dates:	27 July–21 September 2005
Draft Task Report Date	15 June 2006

Author:	Approved:
Signature. Date	Signature. Date

Table of Contents

This report contains 368 pages, including seven appendices.

List of Appendices

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:
15 July 2005	Protocol Review	19 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 counting	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	10 August 2005
27, 28 February and 02 March 2006	Data and report	06 March 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.
	Steve Sorrow, B.Sc.

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 recombinant aromatase microsomes. This study was part of a multi-laboratory effort for the evaluation of the recombinant 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 using human recombinant aromatase microsomes. 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 Disruptor Screening Program (EDSP). In this program, comprehensive toxicological and ecotoxicological screens and tests are being developed for identifying and characterizing the endocrine effects of various environmental contaminants, industrial chemicals, and pesticides. The program's aim is to develop a two-tiered approach, e.g., a combination of *in vitro* and *in vivo* mammalian and ecotoxicological screens (Tier 1) and a set of *in vivo* tests (Tier 2) for identifying and characterizing endocrine effects of pesticides, industrial chemicals, and environmental contaminants. Validation of the individual screens and tests is required, and the Endocrine Disruptor Method Validation Committee (EDMVAC) will provide advice and counsel on the validation assays.

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

Aromatase is a cytochrome P450 enzyme complex responsible for estrogen biosynthesis and converts androgens, such as testosterone and androstenedione, into the estrogens estradiol and estrone. Aromatase is present in the ovary, placenta, uterus, testis, brain, and extraglandular adipose tissues. Two proteins, cytochrome 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 recombinant 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 recombinant microsomal aromatase assay was recommended as the *in vitro* aromatase screening assay to be included in the Tier 1 Screening Battery. This assay will detect environmental toxicants that possess the ability to inhibit aromatase activity. Prevalidation studies on recombinant aromatase (WA 2-24) were conducted to optimize the 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 recombinant 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 recombinant microsomes. This study is part of a multi-laboratory effort for the evaluation of the recombinant aromatase assay. This protocol is specific to the work conducted by In Vitro Technologies.

The test system for this study was human recombinant 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 by the lead laboratory. The results of this analysis are presented in the overall report.

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, formulated in DMSO (lot 3-Lin-1). 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), high-performance liquid chromatography (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 Chemical Repository.

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 was 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 IC50 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 10 chemicals with human recombinant aromatase microsomes. 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, 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 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}

Sample type	Repetitions (test tubes)	Description	Reference Chemical concentration (M final)
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 recombinant microsomes (lot 5) were supplied to In Vitro Technologies by RTI. 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 0.008 mg/mL. The addition of 1 mL of that microsome dilution resulted in a final approximate protein concentration of 0.004 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.

3.4 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. The two solutions were combined 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 standard) 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 extrapolation 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 Recombinant
Microsomal Protein (mg/mL)[a]	0.004
NADPH (mM)[a]	0.3
[^{3}H]ASDN (nM)[a]	100
Incubation Time (min)	15

[a] Final concentrations

3.7 Data Analysis

The data analysis described in the following paragraphs addresses all of the experiments of this task.

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%. The radiochemical purity report is included in the Battelle Chemistry Report.

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 (Rep 1, 2, 3) and Ketoconazole (Rep 1, 2, 4)	5	1	27 JUL 2006	0.261	3.252 (± 1.687)	51.9
	5	2	28 JUL 2006	4.870		
	5	3	29 JUL 2006	NA		
	5	4	18 AUG 2006	4.624		
Incubations for Reference Chemicals Dicofol and Atrazine	5	1	08 SEP 2006	5.420		
	5	2	12 SEP 2006	3.378		
	5	3	13 SEP 2006	3.808		
Incubations for Reference Chemicals 4-nonylphenol and Dibenz[a,h]anthracene	5	1	20 SEP 2006	5.021		
	5	2	21 SEP 2006	4.871		
	5	3	22 SEP 2006	3.908		
Incubations for Reference Chemicals Prochloraz (Rep 2, 3, 4), Fenarimol (Rep 1, 2, 3) and Chrysin (Rep 4)	5	1	03 AUG 2006	0.570		
	5	2	05 AUG 2006	3.066		
	5	3	08 AUG 2006	0.142		
	5	4	15 AUG 2006	3.771		
Incubations for Reference Chemicals Econazole (Rep 1, 2 and 3) and Chrysin (Rep 1 and 3)	5	1	29 AUG 2006	3.009		
	5	2	05 SEP 2006	2.460		
	5	3	06 SEP 2006	2.846		

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 (Rep 1, 2, 3) and Ketoconazole (Rep 1, 2, 4)	5	1	5.7164	5.4272	5.3247	5.4681	5.484 (± 0.166)	3.0	1.275 (± 1.875)	147.1
	5	2	0.3039	0.3375	0.3247	0.3407	0.327 (± 0.0167)	5.1		
	5	3	0.3123	0.3024	0.2924	0.2929	0.300(± 0.00941)	3.1		
	5	4	0.2898	0.2811	0.2962	0.3083	0.294(± 0.0115)	3.9		
Incubations for Reference Chemicals Dicofol and Atrazine	5	1	0.5909	0.5877	0.5707	0.6040	0.588 (± 0.0137)	2.3		
	5	2	0.5864	0.5577	0.5837	0.5623	0.573 (± 0.0146)	2.6		
	5	3	0.6895	0.6997	0.6571	0.6555	0.675 (± 0.0225)	3.3		
Incubations for Reference Chemical 4-nonylphenol and Dibenz[a,h]anthrazene	5	1	0.3882	0.3921	0.3524	0.3573	0.373 (± 0.0205)	5.5		
	5	2	0.4008	0.4072	0.4031	0.4007	0.403 (± 0.0030)	0.8		
	5	3	0.5716	0.5532	0.4696	0.4713	0.516 (± 0.0536)	10.4		
Incubations for Reference Chemicals Prochloraz (Rep 2, 3,and 4), Fenarimol (Rep 1, 2, 3) and Chrysin (Rep 4)	5	1	2.8429	3.1532	2.6852	2.7206	2.850 (± 0.213)	7.5		
	5	2	0.4168	0.4143	0.3950	0.3923	0.405 (± 0.0128)	3.2		
	5	3	7.9478	7.8646	3.2804	6.8585	6.488 (± 2.19)	33.8		
	5	4	0.5131	0.5177	0.4902	0.5033	0.506 (± 0.0120)	2.4		
Incubations for Reference Chemicals Econazole (Rep 1, 2 and 3) and Chrysin (Rep 1 and 3)	5	1	0.5719	0.6145	0.5184	0.4980	0.551 (± 0.0527)	9.6		
	5	2	0.6115	0.5812	0.5443	0.5734	0.578 (± 0.0276)	4.8		
	5	3	0.7155	0.7851	0.7848	0.7873	0.771 (± 0.0372)	4.8		

4.5 Percent of Control – Inhibition Experiments

4.5.1 Aminoglutethimide

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Aminoglutethimide	1	-3.00	4.99	4.89	4.03	4.63 (± 0.53)	11.3
		-3.52	17.02	18.89	17.72	17.88 (± 0.94)	5.3
		-4.00	34.75	35.10	32.66	34.17 (± 1.32)	3.9
		-5.00	94.59	95.94	95.70	95.41 (± 0.72)	0.8
		-6.00	111.51	118.84	109.98	113.44 (± 4.74)	4.2
		-7.00	106.21	107.24	104.88	106.11 (± 1.18)	1.1
		-8.00	109.34	118.16	115.10	114.20 (± 4.48)	3.9
		-9.00	114.01	114.84	110.09	112.98 (± 2.54)	2.2
	2	-3.00	3.09	3.26	3.14	3.17 (± 0.09)	2.7
		-4.00	31.94	30.22	29.33	30.49 (± 1.33)	4.3
		-4.48	46.33	41.16	38.72	42.07 (± 3.88)	9.2
		-5.00	89.49	91.63	92.99	91.37 (± 1.77)	1.9
		-6.00	104.88	103.64	98.01	102.18 (± 3.66)	3.6
		-7.00	99.48	96.95	91.33	95.92 (± 4.17)	4.4
		-8.00	103.80	104.12	107.60	105.18 (± 2.11)	2.0
		-9.00	103.74	102.47	105.70	103.97 (± 1.63)	1.6
	3	-3.00	3.07	2.83	2.99	2.97 (± 0.12)	4.1
		-4.00	24.11	23.31	23.91	23.78 (± 0.42)	1.8
		-4.48	33.91	31.87	32.47	32.75 (± 1.05)	3.2
		-5.00	76.38	77.99	76.17	76.85 (± 0.99)	1.3
		-6.00	92.33	85.08	84.12	87.18 (± 4.49)	5.1
		-7.00	85.88	83.69	79.28	82.95 (± 3.36)	4.1
		-8.00	93.66	96.15	97.56	95.79 (± 1.97)	2.1
		-9.00	91.94	93.89	89.77	91.87 (± 2.06)	2.2

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	% CV
		Repl 1	Repl 2	Repl 3		
Aminoglutethimide	-3.00	4.63	3.17	2.97	3.59 (± 0.91)	25.4
	-3.52	17.88			17.88 (± NA)	NA
	-4.00	34.17	30.49	23.78	29.48 (± 5.27)	17.9
	-4.48		42.07	32.75	37.41 (± NA)	NA
	-5.00	95.41	91.37	76.85	87.88 (± 9.76)	11.1
	-6.00	113.44	102.18	87.18	100.93 (± 13.18)	13.1
	-7.00	106.11	95.92	82.95	94.99 (± 11.61)	12.2
	-8.00	114.20	105.18	95.79	105.05 (± 9.2)	8.8
	-9.00	112.98	103.97	91.87	102.94 (± 10.59)	10.3

4.5.2 Ketoconazole

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Ketoconazole	1	-4.00	0.03	0.48	0.15	0.22 (± 0.23)	105.9
		-5.00	4.81	4.55	4.43	4.60 (± 0.19)	4.2
		-6.00	33.81	33.78	32.95	33.51 (± 0.49)	1.5
		-7.00	90.64	88.43	87.89	88.99 (± 1.46)	1.6
		-8.00	102.12	105.46	105.08	104.22 (± 1.83)	1.8
		-9.00	109.06	116.33	116.14	113.85 (± 4.14)	3.6
		-10.00	113.10	117.32	108.20	112.87 (± 4.57)	4.0
		-11.00	109.87	107.22	110.26	109.12 (± 1.65)	1.5
	2	-4.00	0.35	0.31	0.45	0.37 (± 0.07)	18.7
		-5.00	3.96	4.25	4.29	4.17 (± 0.18)	4.3
		-6.00	33.41	31.75	31.45	32.20 (± 1.06)	3.3
		-7.00	87.43	83.89	77.48	82.93 (± 5.04)	6.1
		-8.00	109.90	108.79	109.02	109.24 (± 0.59)	0.5
		-9.00	114.63	112.90	112.86	113.47 (± 1.01)	0.9
		-10.00	114.64	109.66	109.40	111.23 (± 2.95)	2.7
		-11.00	108.04	106.94	134.08	116.36 (± 15.36)	13.2
	4	-4.00	0.40	0.15	0.73	0.43 (± 0.29)	69.0
		-5.00	4.46	4.55	4.34	4.45 (± 0.10)	2.3
		-6.00	32.84	31.13	28.38	30.78 (± 2.25)	7.3
		-7.00	85.83	84.44	85.65	85.31 (± 0.75)	0.9
		-8.00	102.97	105.90	105.18	104.68 (± 1.53)	1.5
		-9.00	102.50	103.63	102.44	102.86 (± 0.67)	0.6
		-10.00	102.98	114.38	110.72	109.36 (± 5.82)	5.3
		-11.00	103.78	110.83	98.80	104.47 (± 6.05)	5.8

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	% CV
		Repl 1	Repl 2	Repl 4		
Ketoconazole	-4.00	0.22	0.37	0.44	0.34 (± 0.11)	31.4
	-5.00	4.60	4.17	4.45	4.40 (± 0.22)	5.0
	-6.00	33.51	32.20	30.78	32.17 (± 1.36)	4.2
	-7.00	88.99	82.93	85.31	85.74 (± 3.05)	3.6
	-8.00	104.22	109.24	104.68	106.05 (± 2.77)	2.6
	-9.00	113.85	113.47	102.86	110.06 (± 6.24)	5.7
	-10.00	112.87	111.23	109.36	111.16 (± 1.76)	1.6
	-11.00	109.12	116.36	104.47	109.98 (± 5.99)	5.4

4.5.3 Dicofol

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Dicofol	1	-4.00	45.82	44.00	42.63	44.15 (± 1.60)	3.6
		-4.48	65.30	52.54	58.21	58.68 (± 6.39)	10.9
		-5.00	62.91	54.36	53.19	56.82 (± 5.31)	9.3
		-6.00	91.10	85.34	90.92	89.12 (± 3.27)	3.7
		-7.00	88.06	93.55	91.05	90.89 (± 2.75)	3.0
		-8.00	94.03	87.53	83.50	88.35 (± 5.31)	6.0
		-9.00	77.24	93.75	91.31	87.43 (± 8.91)	10.2
		-10.00	91.80	95.34	93.06	93.40 (± 1.79)	1.9
	2	-4.00	37.23	45.84	41.64	41.57 (± 4.30)	10.4
		-4.48	57.63	44.46	52.04	51.38 (± 6.61)	12.9
		-5.00	53.01	52.68	49.24	51.64 (± 2.09)	4.0
		-6.00	83.37	86.17	86.66	85.40 (± 1.77)	2.1
		-7.00	91.96	81.38	85.33	86.22 (± 5.35)	6.2
		-8.00	79.19	75.13	78.88	77.73 (± 2.26)	2.9
		-9.00	89.97	91.44	88.46	89.96 (± 1.49)	1.7
		-10.00	89.61	90.26	82.67	87.51 (± 4.21)	4.8
	3	-4.00	31.11	28.47	26.20	28.59 (± 2.46)	8.6
		-4.48	32.66	31.72	29.34	31.24 (± 1.71)	5.5
		-5.00	63.22	64.54	59.00	62.25 (± 2.89)	4.6
		-6.00	89.15	87.75	91.36	89.42 (± 1.82)	2.0
		-7.00	94.84	96.38	87.20	92.81 (± 4.91)	5.3
		-8.00	91.40	89.96	89.51	90.29 (± 0.99)	1.1
		-9.00	92.00	89.41	91.52	90.97 (± 1.38)	1.5
		-10.00	94.53	93.09	93.84	93.82 (± 0.72)	0.8

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Dicofol	-4.00	44.15	41.57	28.59	38.10 (± 8.34)	21.9
	-4.48	58.68	51.38	31.24	47.10 (± 14.21)	30.2
	-5.00	56.82	51.64	62.25	56.91 (± 5.31)	9.3
	-6.00	89.12	85.40	89.42	87.98 (± 2.24)	2.5
	-7.00	90.89	86.22	92.81	89.97 (± 3.39)	3.8
	-8.00	88.35	77.73	90.29	85.46 (± 6.76)	7.9
	-9.00	87.43	89.96	90.97	89.45 (± 1.82)	2.0
	-10.00	93.40	87.51	93.82	91.58 (± 3.53)	3.9

4.5.4 Atrazine

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Atrazine	1	-4.00	88.12	87.61	91.95	89.23 (± 2.37)	2.7
		-4.48	93.20	93.49	95.41	94.03 (± 1.20)	1.3
		-5.00	95.32	92.05	91.67	93.01 (± 2.01)	2.2
		-6.00	87.66	91.00	90.53	89.73 (± 1.81)	2.0
		-7.00	92.80	91.32	92.48	92.20 (± 0.78)	0.8
		-8.00	87.88	86.99	84.93	86.60 (± 1.51)	1.7
		-9.00	94.95	91.99	94.95	93.96 (± 1.71)	1.8
		-10.00	95.73	96.62	96.94	96.43 (± 0.62)	0.6
	2	-4.00	86.05	81.35	85.12	84.17 (± 2.49)	3.0
		-4.48	90.90	93.48	93.25	92.54 (± 1.43)	1.5
		-5.00	90.99	90.91	87.95	89.95 (± 1.73)	1.9
		-6.00	80.76	85.13	86.86	84.25 (± 3.14)	3.7
		-7.00	92.05	93.17	91.10	92.11 (± 1.04)	1.1
		-8.00	88.36	90.78	90.35	89.83 (± 1.29)	1.4
		-9.00	88.96	87.90	86.95	87.94 (± 1.00)	1.1
		-10.00	84.60	89.79	92.06	88.81 (± 3.83)	4.3
	3	-4.00	89.49	90.83	90.22	90.18 (± 0.67)	0.7
		-4.48	92.56	86.61	90.32	89.83 (± 3.00)	3.3
		-5.00	94.60	94.27	96.56	95.14 (± 1.24)	1.3
		-6.00	96.07	91.20	91.12	92.80 (± 2.83)	3.1
		-7.00	93.43	90.99	94.38	92.94 (± 1.75)	1.9
		-8.00	91.77	94.01	93.80	93.19 (± 1.24)	1.3
		-9.00	96.10	95.75	89.43	93.76 (± 3.75)	4.0
		-10.00	88.25	90.50	87.78	88.84 (± 1.45)	1.6

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Atrazine	-4.00	89.23	84.17	90.18	87.86 (± 3.23)	3.7
	-4.48	94.03	92.54	89.83	92.14 (± 2.13)	2.3
	-5.00	93.01	89.95	95.14	92.70 (± 2.61)	2.8
	-6.00	89.73	84.25	92.80	88.93 (± 4.33)	4.9
	-7.00	92.20	92.11	92.94	92.41 (± 0.45)	0.5
	-8.00	86.60	89.83	93.19	89.87 (± 3.30)	3.7
	-9.00	93.96	87.94	93.76	91.89 (± 3.42)	3.7
	-10.00	96.43	88.81	88.84	91.36 (± 4.39)	4.8

4.5.5 4-Nonylphenol

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
4-Nonylphenol	1	-4.00	8.35	6.91	5.96	7.07 (± 1.20)	17.0
		-4.48	37.14	38.45	33.02	36.20 (± 2.83)	7.8
		-5.00	70.56	36.18	57.73	54.82 (± 17.37)	31.7
		-6.00	93.39	96.47	96.94	95.60 (± 1.93)	2.0
		-7.00	97.33	96.78	90.66	94.92 (± 3.70)	3.9
		-8.00	93.24	86.27	83.12	87.54 (± 5.17)	5.9
		-9.00	97.63	79.94	94.00	90.52 (± 9.34)	10.3
		-10.00	95.87	83.09	72.09	83.68 (± 11.90)	14.2
	2	-4.00	2.82	3.43	3.35	3.20 (± 0.33)	10.4
		-4.48	20.61	20.27	21.28	20.72 (± 0.52)	2.5
		-5.00	63.86	61.64	62.55	62.68 (± 1.12)	1.8
		-6.00	96.25	95.81	93.95	95.34 (± 1.22)	1.3
		-7.00	97.80	97.93	94.50	96.75 (± 1.95)	2.0
		-8.00	95.29	94.43	94.69	94.80 (± 0.44)	0.5
		-9.00	90.41	94.06	92.01	92.16 (± 1.83)	2.0
		-10.00	95.68	93.13	93.46	94.09 (± 1.38)	1.5
	3	-4.00	2.48	3.38	2.77	2.88 (± 0.46)	15.9
		-4.48	24.96	27.43	20.80	24.40 (± 3.35)	13.7
		-5.00	64.57	62.27	66.72	64.52 (± 2.23)	3.4
		-6.00	95.36	94.43	92.46	94.08 (± 1.48)	1.6
		-7.00	96.50	96.73	89.19	94.14 (± 4.29)	4.6
		-8.00	93.12	93.80	86.88	91.27 (± 3.81)	4.2
		-9.00	90.16	90.48	91.81	90.81 (± 0.87)	1.0
		-10.00	92.95	94.54	89.18	92.22 (± 2.75)	3.0

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
4-nonylphenol	-4.00	7.07	3.20	2.88	4.38 (± 2.34)	53.3
	-4.48	36.20	20.72	24.40	27.11 (± 8.09)	29.8
	-5.00	54.82	62.68	64.52	60.67 (± 5.15)	8.5
	-6.00	95.60	95.34	94.08	95.01 (± 0.81)	0.9
	-7.00	94.92	96.75	94.14	95.27 (± 1.34)	1.4
	-8.00	87.54	94.80	91.27	91.21 (± 3.63)	4.0
	-9.00	90.52	92.16	90.81	91.17 (± 0.87)	1.0
	-10.00	83.68	94.09	92.22	90.00 (± 5.55)	6.2

4.5.6 Prochloraz

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Prochloraz	2	-4.00	0.12	-0.19	-0.27	-0.12 (± 0.21)	-177.3
		-5.00	0.36	0.32	0.25	0.31 (± 0.06)	18.4
		-6.00	1.46	1.78	1.99	1.74 (± 0.27)	15.4
		-7.00	15.32	16.64	16.84	16.26 (± 0.83)	5.1
		-8.00	61.21	61.54	58.83	60.52 (± 1.48)	2.4
		-8.48	78.28	74.58	73.35	75.40 (± 2.57)	3.4
		-9.00	89.51	86.32	87.47	87.77 (± 1.62)	1.8
		-10.00	95.65	93.07	88.18	92.30 (± 3.80)	4.1
	3	-4.00	0.80	0.13	0.56	0.50 (± 0.34)	67.7
		-5.00	0.41	0.80	0.42	0.54 (± 0.22)	40.3
		-6.00	2.95	2.00	2.41	2.45 (± 0.48)	19.5
		-7.00	19.27	20.64	20.20	20.04 (± 0.70)	3.5
		-8.00	80.68	72.58	77.34	76.87 (± 4.07)	5.3
		-8.48	104.51	99.28	96.52	100.10 (± 4.06)	4.1
		-9.00	112.16	110.28	106.73	109.72 (± 2.76)	2.5
		-10.00	111.94	114.05	115.57	113.86 (± 1.82)	1.6
	4	-4.00	0.06	0.59	0.34	0.33 (± 0.27)	80.9
		-5.00	0.75	0.29	0.12	0.39 (± 0.33)	84.5
		-6.00	2.79	2.66	2.76	2.74 (± 0.06)	2.4
		-7.00	20.47	21.66	18.22	20.11 (± 1.74)	8.7
		-7.48	86.46	83.19	78.41	82.69 (± 4.05)	4.9
		-8.00	64.55	64.87	65.42	64.95 (± 0.44)	0.7
		-9.00	85.83	87.91	85.42	86.39 (± 1.34)	1.5
		-10.00	91.24	91.20	84.41	88.95 (± 3.93)	4.4

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 2	Repl 3	Repl 4		
Prochloraz	-4.00	-0.12	0.50	0.33	0.24 (± 0.32)	133.6
	-5.00	0.31	0.54	0.39	0.41 (± 0.12)	29.4
	-6.00	1.74	2.45	2.74	2.31 (± 0.51)	22.1
	-7.00	16.26	20.04	20.11	18.81 (± 2.20)	11.7
	-7.48			82.69	82.69 (± NA)	NA
	-8.00	60.52	76.87	64.95	67.45 (± 8.45)	12.5
	-8.48	75.40	100.10		87.75 (± NA)	NA
	-9.00	87.77	109.72	86.39	94.62 (± 13.09)	13.8
	-10.00	92.30	113.86	88.95	98.37(±13.52)	1.37

4.5.7 Fenarimol

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Fenarimol	1	-4.48	3.51	49.67	48.24	33.81 (± 26.25)	77.6
		-5.00	29.29	29.55	29.57	29.47 (± 0.16)	0.5
		-5.48	42.73	41.83	42.80	42.45 (± 0.54)	1.3
		-6.00	71.24	73.00	74.71	72.98 (± 1.73)	2.4
		-7.00	86.47	87.24	90.34	88.02 (± 2.05)	2.3
		-8.00	82.06	82.80	78.94	81.26 (± 2.05)	2.5
		-9.00	80.36	85.39	87.61	84.45 (± 3.71)	4.4
		-10.00	86.83	85.50	84.20	85.51 (± 1.31)	1.5
	2	-4.48	13.47	11.39	11.16	12.01 (± 1.27)	10.6
		-5.00	28.79	30.45	31.48	30.24 (± 1.36)	4.5
		-5.48	51.79	54.66	55.05	53.83 (± 1.78)	3.3
		-6.00	74.52	74.80	74.20	74.51 (± 0.30)	0.4
		-7.00	90.11	91.75	89.99	90.62 (± 0.98)	1.1
		-8.00	92.69	90.32	91.94	91.65 (± 1.21)	1.3
		-9.00	92.22	93.15	90.51	91.96 (± 1.34)	1.5
		-10.00	90.04	93.33	93.23	92.20 (± 1.87)	2.0
	3	-4.48	16.61	15.59	14.44	15.55 (± 1.09)	7.0
		-5.00	36.89	35.98	38.04	36.97 (± 1.04)	2.8
		-5.48	61.26	66.97	67.67	65.30 (± 3.52)	5.4
		-6.00	90.94	86.35	91.86	89.72 (± 2.95)	3.3
		-7.00	106.78	107.73	112.31	108.94 (± 2.95)	2.7
		-8.00	110.41	107.26	110.49	109.39 (± 1.84)	1.7
		-9.00	109.16	112.73	108.51	110.13 (± 2.27)	2.1
		-10.00	107.75	109.30	100.64	105.90 (± 4.62)	4.4

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Fenarimol	-4.48	33.81	12.01	15.55	20.45 (± 11.7)	57.2
	-5.00	29.47	30.24	36.97	32.23 (± 4.13)	12.8
	-5.48	42.45	53.83	65.30	53.86 (± 11.42)	21.2
	-6.00	72.98	74.51	89.72	79.07 (± 9.25)	11.7
	-7.00	88.02	90.62	108.94	95.86 (± 11.40)	11.9
	-8.00	81.26	91.65	109.39	94.10 (± 14.22)	15.1
	-9.00	84.45	91.96	110.13	95.51 (± 13.20)	13.8
	-10.00	85.51	92.20	105.90	94.54 (± 10.39)	11.0

4.5.8 Econazole

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Econazole	1	-4.00	-0.05	0.00	0.02	-0.01 (± 0.04)	-324.7
		-4.48	-0.03	-0.09	-0.06	-0.06 (± 0.03)	-50.6
		-5.00	0.07	0.02	-0.01	0.02 (± 0.04)	178.2
		-6.00	0.06	0.32	0.15	0.18 (± 0.13)	73.5
		-7.00	1.31	1.47	1.72	1.50 (± 0.21)	13.8
		-8.00	12.80	12.75	13.11	12.88 (± 0.19)	1.5
		-9.00	54.45	54.28	58.64	55.79 (± 2.47)	4.4
		-10.00	86.36	81.71	85.55	84.54 (± 2.48)	2.9
	2	-5.00	0.34	0.82	0.65	0.60 (± 0.24)	40.6
		-6.00	0.45	0.13	0.74	0.44 (± 0.31)	70.3
		-7.00	1.27	1.39	1.11	1.26 (± 0.14)	11.2
		-8.00	6.81	12.18	12.26	10.42 (± 3.12)	30.0
		-8.48	29.20	28.88	27.61	28.56 (± 0.84)	2.9
		-9.00	60.96	57.42	55.24	57.87 (± 2.89)	5.0
		-9.48	65.84	81.28	80.73	75.95 (± 8.76)	11.5
		-10.00	90.59	87.77	86.79	88.38 (± 1.97)	2.2
	3	-5.00	0.01	-0.10	-0.01	-0.03 (± 0.06)	-178.7
		-6.00	0.14	0.25	-0.03	0.12 (± 0.14)	117.5
		-7.00	1.03	1.20	1.19	1.14 (± 0.09)	8.2
		-8.00	15.18	15.94	15.25	15.46 (± 0.42)	2.7
		-8.48	37.32	38.20	36.43	37.31 (± 0.88)	2.4
		-9.00	66.39	63.55	57.53	62.49 (± 4.53)	7.2
		-9.48	89.48	90.64	96.34	92.15 (± 3.67)	4.0
		-10.00	99.76	98.47	95.35	97.86 (± 2.27)	2.3

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Econazole	-4.00	-0.01			-0.01 (± NA)	NA
	-4.48	-0.06			-0.06 (± NA)	NA
	-5.00	0.02	0.60	-0.03	0.20 (± 0.35)	178.0
	-6.00	0.18	0.44	0.12	0.25 (± 0.17)	69.0
	-7.00	1.50	1.26	1.14	1.30 (± 0.18)	14.2
	-8.00	12.88	10.42	15.46	12.92 (± 2.52)	19.5
	-8.48		28.56	37.31	32.94 (± NA)	NA
	-9.00	55.79	57.87	62.49	58.72 (± 3.43)	5.8
	-9.48		75.95	92.15	84.05 (± NA)	NA
	-10.00	84.54	88.38	97.86	90.26 (± 6.85)	7.6

4.5.9 Chrysin

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Chrysin	1	-4.00	15.94	17.17	24.36	19.16 (± 4.55)	23.7
		-4.48	8.73	8.16	6.86	7.92 (± 0.96)	12.1
		-5.00	23.27	22.10	14.47	19.95 (± 4.78)	24.0
		-6.00	68.65	67.03	68.07	67.92 (± 0.82)	1.2
		-7.00	83.08	83.01	81.96	82.69 (± 0.63)	0.8
		-8.00	87.12	89.11	85.15	87.13 (± 1.98)	2.3
		-9.00	77.23	75.74	74.01	75.66 (± 1.61)	2.1
		-10.00	84.12	84.64	84.75	84.50 (± 0.34)	0.4
	3	-4.48	18.09	19.76	11.35	16.40 (± 4.45)	27.1
		-5.00	30.06	28.99	23.75	27.60 (± 3.37)	12.2
		-5.48	56.58	53.28	54.73	54.86 (± 1.65)	3.0
		-6.00	78.77	59.47	73.50	70.58 (± 9.98)	14.1
		-7.00	100.75	97.25	98.17	98.72 (± 1.82)	1.8
		-8.00	102.06	100.22	92.30	98.19 (± 5.18)	5.3
		-9.00	98.48	90.12	95.56	94.72 (± 4.24)	4.5
		-10.00	102.67	103.25	101.77	102.56 (± 0.74)	0.7
	4	-4.48	30.18	30.15	29.90	30.07 (± 0.15)	0.5
		-5.00	27.10	28.64	28.29	28.01 (± 0.81)	2.9
		-5.48	79.67	82.39	79.26	80.44 (± 1.70)	2.1
		-6.00	72.51	77.17	78.16	75.95 (± 3.02)	4.0
		-7.00	90.70	87.59	88.36	88.89 (± 1.62)	1.8
		-8.00	90.68	93.31	89.51	91.17 (± 1.94)	2.1
		-9.00	93.02	91.43	86.32	90.26 (± 3.50)	3.9
		-10.00	87.11	85.78	86.09	86.33 (± 0.69)	0.8

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 1	Repl 3	Repl 4		
Chrysin	-4.00	19.16			19.16 (± NA)	NA
	-4.48	7.92	16.40	30.07	18.13 (± 11.18)	61.7
	-5.00	19.95	27.60	28.01	25.19 (± 4.54)	18.0
	-5.48		54.86	80.44	67.65 (± 18.08)	26.7
	-6.00	67.92	70.58	75.95	71.48 (± 4.09)	5.7
	-7.00	82.69	98.72	88.89	90.10 (± 8.09)	9.0
	-8.00	87.13	98.19	91.17	92.16 (± 5.60)	6.1
	-9.00	75.66	94.72	90.26	86.88 (± 9.97)	11.5
	-10.00	84.50	102.56	86.33	91.13 (± 9.94)	10.9

4.5.10 Dibenz[*a,h*]anthrazine

Reference Chemical	Replicate	Log [Reference Chemical]	Percent of Control			Mean (± sd)	% CV
			Tube 1	Tube 2	Tube 3		
Diben[*a,h*]anthrazine	1	-4.48	92.26	91.91	87.77	90.65 (± 2.49)	2.8
		-5.00	92.68	92.25	92.59	92.51 (± 0.23)	0.2
		-5.48	96.48	94.07	92.96	94.50 (± 1.80)	1.9
		-6.00	91.40	89.02	90.69	90.37 (± 1.22)	1.4
		-7.00	98.16	95.76	94.23	96.05 (± 1.98)	2.1
		-8.00	96.64	91.80	93.44	93.96 (± 2.46)	2.6
		-9.00	79.83	59.44	92.53	77.27 (± 16.69)	21.6
		-10.00	85.94	88.03	91.74	88.57 (± 2.94)	3.3
	2	-4.00	92.82	90.59	89.66	91.02 (± 1.62)	1.8
		-4.48	90.75	93.31	86.57	90.21 (± 3.40)	3.8
		-5.00	91.11	90.89	91.17	91.06 (± 0.15)	0.2
		-6.00	91.80	88.68	91.57	90.68 (± 1.74)	1.9
		-7.00	85.56	90.84	91.27	89.22 (± 3.18)	3.6
		-8.00	86.51	90.02	88.20	88.24 (± 1.76)	2.0
		-9.00	89.59	88.66	87.49	88.58 (± 1.05)	1.2
		-10.00	83.20	86.48	87.13	85.60 (± 2.10)	2.5
	3	-4.00	86.85	86.92	82.60	85.46 (± 2.47)	2.9
		-4.48	85.07	90.59	87.80	87.82 (± 2.76)	3.1
		-5.00	89.24	92.33	88.56	90.04 (± 2.01)	2.2
		-6.00	89.65	85.28	90.14	88.35 (± 2.67)	3.0
		-7.00	85.72	90.31	85.34	87.13 (± 2.77)	3.2
		-8.00	87.16	87.53	88.03	87.57 (± 0.43)	0.5
		-9.00	86.64	87.39	87.58	87.20 (± 0.50)	0.6
		-10.00	83.13	82.64	79.03	81.60 (± 2.24)	2.7

Reference Chemical	Log [Reference Chemical]	Mean Percent of Control			Overall Mean (± sd)	Overall % CV
		Repl 1	Repl 2	Repl 3		
Diben[*a,h*]anthrazine	-4.00		91.02	85.46	88.24 (± NA)	NA
	-4.48	90.65	90.21	87.82	89.56 (± 1.52)	1.7
	-5.00	92.51	91.06	90.04	91.20 (± 1.24)	1.4
	-5.48	94.50			94.5 (± NA)	NA
	-6.00	90.37	90.68	88.35	89.80 (± 1.26)	1.4
	-7.00	96.05	89.22	87.13	90.80 (± 4.67)	5.1
	-8.00	93.96	88.24	87.57	89.93 (± 3.51)	3.9
	-9.00	77.27	88.58	87.20	84.35 (± 6.17)	7.3
	-10.00	88.57	85.60	81.60	85.26 (±3.50)	4.1

4.6 IC_{50}

Reference Chemical	Replicate	Log [IC_{50}]	SE Log[IC_{50}]	IC_{50} (M)	Slope	SE Slope	Status	Overall IC_{50} (M) Mean (± sd)	Overall % CV
Aminoglutethimide	1	-4.333	0.04537	4.64E-05	-1.108	0.09202	Inhibitor	3.34E-05 (± 1.164E-05)	34.9
	2	-4.531	0.05620	2.95E-05	-1.364	0.2097	Inhibitor		
	3	-4.617	0.05532	2.42E-05	-1.491	0.2419	Inhibitor		
Ketoconazole	1	-6.375	0.03463	4.22E-07	-0.9324	0.05623	Inhibitor	3.89E-07 (± 4.77E-08)	12.3
	2	-6.476	0.06510	3.34E-07	-0.8409	0.08796	Inhibitor		
	4	-6.387	0.03836	4.10E-07	-1.010	0.07004	Inhibitor		
Prochloraz	2	-7.724	0.02522	1.89E-08	-0.9053	0.03810	Inhibitor	4.36E-08 (± 4.133E-08)	94.8
	3	-7.687	0.02271	2.06E-08	-1.012	0.04046	Inhibitor		
	4	-7.040	65.49	9.13E-08	-12.77	20702	Inhibitor		
4-nonylphenol	1	-4.649	0.2626	2.25E-05	-1.039	0.4239	Inhibitor	1.87E-05 (± 3.56E-06)	19.1
	2	-4.813	0.01801	1.54E-05	-1.622	0.09771	Inhibitor		
	3	-4.740	0.03257	1.82E-05	-1.520	0.1414	Inhibitor		
Dibenz[*a,h*]anthracine	1	NA	NA	NA	NA	NA	No Inhibition	3.27E-11 (± NA)	NA
	2	-12.86	56.12	1.38E-13	0.2058	0.7724	No Inhibition		
	3	-10.19	48.49	6.53E-11	1.229	20.94	No Inhibition		
Fenarimol	1	-5.751	0.09697	1.77E-06	-2.269	0.7764	Inhibitor	3.79E-06 (± 1.743E-06)	46.0
	2	-5.316	0.03070	4.83E-06	-0.9373	0.04263	Inhibitor		
	3	-5.323	0.05467	4.76E-06	-0.9985	0.08885	Inhibitor		
Econazole	1	-8.783	0.01843	1.65E-09	-0.9904	0.03422	Inhibitor	1.63E-09 (± 5.915E-11)	3.6
	2	-8.807	0.04251	1.56E-09	-1.080	0.09289	Inhibitor		
	3	-8.777	0.03918	1.67E-09	-0.9838	0.07148	Inhibitor		
Chrysin	1	-5.660	0.09504	2.19E-06	-1.691	0.3911	Inhibitor	3.02E-06 (± 8.036E-07)	26.6
	3	-5.510	0.1147	3.09E-06	-0.8879	0.1666	Inhibitor		
	4	-5.421	876.5	3.79E-06	-15.72	228727	Inhibitor		
Dicofol	1	-5.325	0.1668	4.74E-06	-1.664	0.6983	Presumed Inhibitor	6.70E-06 (± 3.367E-06)	50.2
	2	-5.320	0.2135	4.78E-06	-2.236	1.393	Presumed Inhibitor		
	3	-4.975	0.02051	1.06E-05	-2.570	0.6095	Presumed Inhibitor		
Atrazine	1	-8.993	2919000	1.02E-09	-14.92	6.01E+09	No Inhibition	1.02E-09 (± NA)	NA
	2	NA	NA	NA	NA	NA	No Inhibition		
	3	NA	NA	NA	NA	NA	No Inhibition		

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

5.0 Discussion

Recombinant microsomes supplied by Battelle (Lot 5) 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. 1158

In Vitro Technologies, Inc.
Protocol No. 1158
Version: Final (15 July 2005)

Evaluation of the Potential of Reference Chemicals to Inhibit Activity of Recombinant Aromatase (WA 4-17, Task 4)

Sponsor:	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2639
Sponsor Representative:	David P. Houchens, Ph.D. E-mail: houchensd@battelle.org Tel.: (614) 424-3564 Fax: (614) 458-3564
Testing Facility:	In Vitro Technologies, Inc. 1450 South Rolling Road Baltimore, Maryland 21227 Tel.: (410) 455-1242 Fax: (410) 455-1245 E-mail: contractservices@invitrotech.com
Study Director:	Aruna Koganti, Ph.D.
EPA Contract Number:	68-W-01-023 (Battelle Prime Contractor)
In Vitro Technologies Study Number:	270-1158-13
Proposed Experimental Start Date:	18 July 2005
Proposed Experimental End Date:	19 September 2005

Objectives

The objective of this study is to evaluate the inhibition of the activity of recombinant aromatase by 10 reference chemicals. 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

Test System Identification

The test system for this study is recombinant human aromatase. The lot or batch number of the recombinant aromatase provided by Battelle 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 recombinant microsomes. This test system was selected because it provides a commercially available source of the aromatase enzyme and, since the assay is being evaluated for its potential to serve as a screening assay, the use of recombinant human enzyme enhances its predictive potential.

Description of Study

The study will evaluate the inhibition of aromatase activity by 10 reference chemicals in recombinant microsomes. 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

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.

Experimental Methods

Materials

Battelle will provide the following materials:

- Recombinant microsomes
- 4-Androstene-3, 17-dione (ASDN; CAS no. 63-05-8)
- [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 number 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:

- 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.

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 androstenedione (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.

Preparation of Microsomes

Recombinant microsomes will be supplied to each laboratory by RTI. 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 recombinant microsomes are supplied in aliquots in excess of what is required to conduct a single experiment, they will be thawed, pooled, homogenized, divided into appropriate aliquots for conduct of a single experiment and refrozen as described below in order to minimize and standardize the number of freeze/thaw cycles each preparation undergoes. Microsomes will be thawed quickly in a 37 ± 1°C water bath and 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 divided into aliquots appropriate for use in a single experiments (approximatley 160 µL, depending on the protein concentration of the preparation). 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 will be immediately transferred to an ice bath. The microsomes will be rehomogenized using a Potter-Elvejhem homogenizer (about 5 to 10 passes) or by vortexing about 5 seconds prior to use. The microsomes will be diluted in buffer (serial dilutions may be necessary) to an approximate protein concentration of 0.008 mg/mL. The addition of 1 mL of that microsome dilution will result in a final approximate protein concentration of 0.004 mg/mL in the assay tubes. All microsome samples must be kept on ice until they are placed in the water bath just prior to their addition to the aromatase assay. Microsomes are not to be left on ice for longer than approximately 1 hour 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.

Aromatase Assay Reagent Preparation

The assay buffer will be 0.1 M sodium phosphate buffer, pH 7.4. Sodium phosphate monobasic (JT Baker, catalog number 4011-01, 137.99 g/mol) and sodium phosphate dibasic (JT Baker, catalog number 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. 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), prepared by RTI will be run in duplicate with each assay. To a 25 µL aliquot of unknown or standard, 125 µL of BioRad DC Protein Kit Reagent A will be added and mixed. Next, 1 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 extrapolation of the absorbance value using the curve developed using the protein standards.

Aromatase Assay

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 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 approximately 5 seconds and placed on ice. The tubes will then be vortex-mixed an additional 20 to 25 seconds. The tubes will be spun using a Beckman GS-6R centrifuge with GH-3.8 rotor 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 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) 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 liquid scintillation spectrometry (LSS). Radioactivity found in the aqueous fractions represents ^3H_2O formed.

Optimized Aromatase Assay Conditions

Assay factor (units)	Recombinant
Microsomal Protein (mg/mL)[a]	0.004
NADPH (mM)[a]	0.3
[^{3}H]ASDN (nM)[a]	100
Incubation Time (min)	15

[a] Final concentrations

Determination of the Inhibition of Aromatase Activity by Reference Chemicals

Ten reference chemicals will be tested. The reference chemicals must be coded prior to distribution to the assaying technicians in order that the replicates are conducted blindly for reference chemical identity. Each reference chemical will be tested at eight concentrations and there will be three (triplicate) repetitions for each concentration of a given replicate. All three replicates for a given reference chemical must be conducted by the same technician. However, the same technician is not required to perform the three replicates for all 10 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, then those replicates should use different reference chemicals. A single replicate study of a given reference chemical is described in Table "Reference Chemical Study Design".

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 control 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 "Aromatase Assay" 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 Reference	1×10^{-4}

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

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 (DPM) per tube and total aromatase activity per tube. The DPM and aromatase activity values are corrected for the background DPM, 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) DPM 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 DPM 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:

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

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 GraphPad 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}.

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.

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

Within each replicate of each reference chemical quadruplicate repetitions will be made of the full enzyme activity control, background activity control, and positive and negative 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, then 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 DPM, 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 symbols 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, then 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, then 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, then 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.

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.

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

Protocol Approval

This protocol has been reviewed and approved by the following:

Sponsor Representatives

David P. Houchens, Ph.D.
Program Manager
Endocrine Disruptor
Screening Program
Battelle Memorial Institute

Signature: David P. Houchens Date: 7/15/05

Jerry D. Johnson, Ph.D.
Work Assignment Leader
Endocrine Disruptor
Screening Program
Battelle Memorial Institute

Signature: Jerry D. Johnson Date: 7-15-05

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.
Study Director
In Vitro Technologies

Signature: K. Aruna Prabha Date: 20 July 2005

Review

Terri L. Pollock, B.A.
Quality Assurance Manager
Battelle Memorial Institute

Signature: Terri L Pollock Date: 7-15-05

Sharon Isbell
Director, Quality Systems
In Vitro Technologies

Signature: Sharon Isbell Date: 21 July 2005

APPENDIX A

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

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 identification, Sample identifications, absorbance data (in triplicate) for standards and unknowns, and appropriate dilution factors.

Absorbance values will be corrected for blank absorbance. A calibration curve will be prepared by linear regression of the standards data (corrected absorbance vs. mg protein measured). The concentration of protein in the unknowns will be 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.

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

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]

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.

Appendix 2: Copy of Protocol Amendment PAM05-044

PROTOCOL AMENDMENT FORM

IVT Study Number: 270-1158-13 Document Number: PAM05-044

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. Alvina Patlewa 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 PDV06-020, PDV06-029, PDV06-035, and PDV06-038

PROTOCOL DEVIATION FORM

IVT Study Number: 270-1158-14 Document Number: PDV06-020

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.

Describe the corrective action:

1. IVT does not possess a Beckman GS-6R centrifuge and the Juoan 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: _____ 05APR06
Signature Date

Approved by: K. Anna Pallera Date: 05 Apr 2006
Study Director

PROTOCOL DEVIATION FORM

IVT Study Number: 270-1158-13 Document Number: PDV06-029

Date of Deviation: n/a

Scientist (if applicable): AK/TM

Date Sponsor Notified (if applicable): n/a

Describe the deviation:
The protocol in the 'Protein Assay' section states that a 25 µL aliquot of unknown or standard, will be mixed with125 µL of Reagent A and 1 mL of Reagent B from BioRad DC Protein Kit. A deviation occurred in that the actual procedure used was to mix a 200 µL aliquot of unknown or standard, with 100 µL of Reagent A and 0.8 mL of Reagent B from BioRad DC Protein Kit.

Describe the corrective action:
None.

Assessment:
Changes to the 'Protein Assay' were made to improve the accuracy of the protein determination during WA 4-16, Task 6. These changes were not captured in the protocol for WA 4-17, Task 4 (protocol 1158). The procedure used in the conduct of the study was the improved method and thus increased the accuracy of the data obtained.

Form Completed by: K. Anne Pattee 14 April 2006
Signature Date

Approved by: K. Anne Pattee Date: 14 April 2006
Study Director

PROTOCOL DEVIATION FORM

IVT Study Number: 270-1158-13 Document Number: PDV06-035

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: _____ 25APR06
Signature Date

Approved by: K. Aruna Prabhala Date: 25 April 2006
Study Director

PROTOCOL DEVIATION FORM

IVT Study Number: 270-1158-13 Document Number: PDV06-038

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: K. Anuna Prakasa (Signature) 10 May 2006 (Date)

Approved by: K. Anuna Prakasa (Study Director) Date: 10 May 2006

Appendix 4: Excel Spreadsheets for Task 4

Assay Date	7/27/2005	Test Chemical ID	RC#1 AG	# Concentrations tested	8		
Technician ID	EJB	Replicate #	1	Microsome type	Recombina	Microsome ID	5

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	EJB	Replicate #	1	Microsome type	Recombinant	Microsome ID	5				

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.191	0.101	0.055	0.044	0.026	0.022	0.018	2	1	
	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
	0.021	0.025	0.096
	0.021	0.026	0.096
	0.021	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	62.35	1000	0.00012	200	0.0249	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	1880	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	0.021	0.002	0.000	200	120	72000	0.000	0.000	0.261
Microsomes	0.021	0.002	0.000	200	120	72000	0.000		
Microsomes	0.021	0.002	0.000	200	120	72000	0.000		
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	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	1

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	0.26054
Protein Concentration (dilution added to assay, mg/mL):	0.000434

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	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume dilutec microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmo estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	8240.063	16480.126	16277.157	32554.314	1	1728563	1.88	32127	0.0372	1	0.000	15	5.7164
			0.5	2	8037.094	16074.188			1					1		15	
	2	2	0.5	1	7959.487	15918.974	15464.589	30929.178	1	1728563	1.79	30502	0.0354	1	0.000	15	5.4272
			0.5	2	7505.102	15010.204			1					1		15	
	3	2	0.5	1	7709.119	15418.238	15176.698	30353.396	1	1728563	1.76	29926	0.0347	1	0.000	15	5.3247
			0.5	2	7467.579	14935.158			1					1		15	
	4	2	0.5	1	7698.211	15396.422	15579.585	31159.17	1	1728563	1.80	30732	0.0356	1	0.000	15	5.4681
			0.5	2	7881.374	15762.748			1					1		15	
Background control	1	2	0.5	1	143.3589	286.7178	325.1904	650.3808	1	1728563	0.04	223	0.0003	1	0.000	15	0.0396
			0.5	2	181.8315	363.663			1					1		15	
	2	2	0.5	1	111.5013	223.0026	216.9404	433.8808	1	1728563	0.03	6	0.0000	1	0.000	15	0.0011
			0.5	2	105.4391	210.8782			1					1		15	
	3	2	0.5	1	80.80698	161.61396	158.92039	317.84078	1	1728563	0.02	-110	-0.0001	1	0.000	15	-0.0195
			0.5	2	78.11341	156.22682			1					1		15	
	4	2	0.5	1	67.33915	134.6783	154.17191	308.34382	1	1728563	0.02	-119	-0.0001	1	0.000	15	-0.0212
			0.5	2	86.83276	173.66552			1					1		15	
Positive control	1	2	0.5	1	4686.63	9373.66	9378.513	18757.026	1	1728563	1.09	18329	0.0212	1	0.000	15	3.2614
			0.5	2	4691.683	9383.366			1					1		15	
	2	2	0.5	1	3948.789	7897.578	7783.314	15566.628	1	1728563	0.90	15139	0.0175	1	0.000	15	2.6937
			0.5	2	3834.525	7669.05			1					1		15	
	3	2	0.5	1	3967.166	7934.332	7775.888	15551.776	1	1728563	0.90	15124	0.0175	1	0.000	15	2.6911
			0.5	2	3808.722	7617.444			1					1		15	
	4	2	0.5	1	4053.793	8107.586	8197.344	16394.688	1	1728563	0.95	15967	0.0185	1	0.000	15	2.8411
			0.5	2	4143.551	8287.102			1					1		15	
Negative Control	1	2	0.5	1	5930.945	11861.89	11920.192	23840.384	1	1728563	1.38	23413	0.0271	1	0.000	15	4.1659
			0.5	2	5989.247	11978.494			1					1		15	
	2	2	0.5	1	5239.815	10479.63	10301.052	20602.104	1	1728563	1.19	20174	0.0234	1	0.000	15	3.5897
			0.5	2	5061.237	10122.474			1					1		15	
	3	2	0.5	1	7975.691	15951.382	15980.587	31961.174	1	1728563	1.85	31534	0.0365	1	0.000	15	5.6108
			0.5	2	8004.896	16009.792			1					1		15	
	4	2	0.5	1	7818.794	15637.589	15714.192	31428.384	1	1728563	1.82	31001	0.0359	1	0.000	15	5.5160
			0.5	2	7895.398	15790.796			1					1		15	
RC#1 AG	1-1	2	0.5	1	498.3123	996.6246	982.3074	1964.6148	1	1728563	0.11	1537	0.0018	1	0.000	15	0.2735
			0.5	2	483.9951	967.9902			1					1		15	
	1-2	2	0.5	1	478.4204	956.8408	966.8547	1933.7094	1	1728563	0.11	1506	0.0017	1	0.000	15	0.2680
			0.5	2	488.4343	976.8686			1					1		15	
	1-3	2	0.5	1	436.36	872.72	835.0098	1670.0196	1	1728563	0.10	1242	0.0014	1	0.000	15	0.2211
			0.5	2	398.6498	797.2996			1					1		15	
	2-1	2	0.5	1	1465.308	2930.616	2837.073	5674.146	1	1728563	0.33	5247	0.0061	1	0.000	15	0.9335
			0.5	2	1371.765	2743.53			1					1		15	
	2-2	2	0.5	1	1502.791	3005.582	3124.326	6248.652	1	1728563	0.36	5821	0.0067	1	0.000	15	1.0357
			0.5	2	1621.535	3243.07			1					1		15	
	2-3	2	0.5	1	1430.593	2861.186	2944.081	5888.162	1	1728563	0.34	5461	0.0063	1	0.000	15	0.9716
			0.5	2	1513.488	3026.976			1					1		15	
	3-1	2	0.5	1	2716.79	5433.58	5569.291	11138.582	1	1728563	0.64	10711	0.0124	1	0.000	15	1.9058
			0.5	2	2852.501	5705.002			1					1		15	
	3-2	2	0.5	1	2889.659	5779.318	5623.643	11247.286	1	1728563	0.65	10820	0.0125	1	0.000	15	1.9252
			0.5	2	2733.984	5467.968			1					1		15	
	3-3	2	0.5	1	2495.532	4991.064	5247.06	10494.12	1	1728563	0.61	10067	0.0117	1	0.000	15	1.7912
			0.5	2	2751.528	5503.056			1					1		15	
	4-1	2	0.5	1	7426.24	14852.48	14790.492	29580.984	1	1728563	1.71	29153	0.0338	1	0.000	15	5.1873
			0.5	2	7364.252	14728.504			1					1		15	
	4-2	2	0.5	1	7530.042	15060.084	14998.907	29997.814	1	1728563	1.74	29570	0.0343	1	0.000	15	5.2615
			0.5	2	7468.865	14937.73			1					1		15	
	4-3	2	0.5	1	7509.978	15019.956	14962.063	29924.126	1	1728563	1.73	29497	0.0342	1	0.000	15	5.2484
			0.5	2	7452.085	14904.17			1					1		15	
	5-1	2	0.5	1	8748.795	17497.59	17397.927	34795.854	1	1728563	2.01	34368	0.0398	1	0.000	15	6.1152
			0.5	2	8649.132	17298.264			1					1		15	
	5-2	2	0.5	1	9265.329	18530.658	18528.6	37057.2	1	1728563	2.14	36630	0.0425	1	0.000	15	6.5176
			0.5	2	9263.271	18526.542			1					1		15	
	5-3	2	0.5	1	8340.27	16680.54	17161.984	34323.968	1	1728563	1.99	33896	0.0393	1	0.000	15	6.0312
			0.5	2	8821.714	17643.428			1					1		15	
	6-1	2	0.5	1	8522.533	17045.066	16581.767	33163.534	1	1728563	1.92	32736	0.0379	1	0.000	15	5.8248
			0.5	2	8059.234	16118.468			1					1		15	
	6-2	2	0.5	1	8250.48	16500.96	16740.69	33481.38	1	1728563	1.94	33054	0.0383	1	0.000	15	5.8813
			0.5	2	8490.21	16980.42			1					1		15	
	6-3	2	0.5	1	8118.326	16236.652	16376.887	32753.774	1	1728563	1.89	32326	0.0375	1	0.000	15	5.7519
			0.5	2	8258.561	16517.122			1					1		15	
	7-1	2	0.5	1	8498.085	16996.17	17063.715	34127.43	1	1728563	1.97	33700	0.0391	1	0.000	15	5.9963
			0.5	2	8565.63	17131.26			1					1		15	
	7-2	2	0.5	1	9137.896	18275.792	18422.716	36845.432	1	1728563	2.13	36418	0.0422	1	0.000	15	6.4799
			0.5	2	9284.82	18569.64			1					1		15	
	7-3	2	0.5	1	8760.091	17520.182	17950.846	35901.692	1	1728563	2.08	35474	0.0411	1	0.000	15	6.3120
			0.5	2	9190.755	18381.51			1					1		15	
	8-1	2	0.5	1	8911.261	17822.522	17783.961	35567.922	1	1728563	2.06	35140	0.0407	1	0.000	15	6.2526
			0.5	2	8872.7	17745.4			1					1		15	
	8-2	2	0.5	1	9136.483	18272.966	17912.066	35824.132	1	1728563	2.07	35397	0.0410	1	0.000	15	6.2982
			0.5	2	8775.583	17551.166			1					1		15	
	8-3	2	0.5	1	8783.812	17567.624	17179.746	34359.492	1	1728563	1.99	33932	0.0393	1	0.000	15	6.0376
			0.5	2	8395.934	16791.868			1					1		15	

Assay Date	7/27/2005	Test Chemical ID	RC#1 AG	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	5.5718	0.2045
Full activity	End	5.3964	0.1014
Full activity	Overall	5.4841	0.1662
Background	Beginning	0.0204	0.027239329
Background	End	-0.0204	0.001194877
Background	Overall	0.0000	0.028309211
Positive	Beginning	2.9775	0.4014
Positive	End	2.7661	0.1061
Positive	Overall	2.8718	0.2690
Negative	Beginning	3.8778	0.4074
Negative	End	5.5634	0.0670
Negative	Overall	4.7206	1.0020

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#1 AG	1	1	1.00E-03	-3.00	0.2735
RC#1 AG	1	2	1.00E-03	-3.00	0.2680
RC#1 AG	1	3	1.00E-03	-3.00	0.2211
RC#1 AG	2	1	3.00E-04	-3.52	0.9335
RC#1 AG	2	2	3.00E-04	-3.52	1.0357
RC#1 AG	2	3	3.00E-04	-3.52	0.9716
RC#1 AG	3	1	1.00E-04	-4.00	1.9058
RC#1 AG	3	2	1.00E-04	-4.00	1.9252
RC#1 AG	3	3	1.00E-04	-4.00	1.7912
RC#1 AG	4	1	1.00E-05	-5.00	5.1873
RC#1 AG	4	2	1.00E-05	-5.00	5.2615
RC#1 AG	4	3	1.00E-05	-5.00	5.2484
RC#1 AG	5	1	1.00E-06	-6.00	6.1152
RC#1 AG	5	2	1.00E-06	-6.00	6.5176
RC#1 AG	5	3	1.00E-06	-6.00	6.0312
RC#1 AG	6	1	1.00E-07	-7.00	5.8248
RC#1 AG	6	2	1.00E-07	-7.00	5.8813
RC#1 AG	6	3	1.00E-07	-7.00	5.7519
RC#1 AG	7	1	1.00E-08	-8.00	5.9963
RC#1 AG	7	2	1.00E-08	-8.00	6.4799
RC#1 AG	7	3	1.00E-08	-8.00	6.3120
RC#1 AG	8	1	1.00E-09	-9.00	6.2526
RC#1 AG	8	2	1.00E-09	-9.00	6.2982
RC#1 AG	8	3	1.00E-09	-9.00	6.0376

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	4.99	4.89	4.03
2	-3.52	17.02	18.89	17.72
3	-4.00	34.75	35.10	32.66
4	-5.00	94.59	95.94	95.70
5	-6.00	111.51	118.84	109.98
6	-7.00	106.21	107.24	104.88
7	-8.00	109.34	118.16	115.10
8	-9.00	114.01	114.84	110.09

Assay Date	7/28/2005	Test Chemical ID	RC#1 AG	# Concentrations tested	8		
Technician ID	EJB	Replicate #	2	Microsome type	Recombina	Microsome ID	5

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	EJB	Replicate #	2	Microsome type	Recombinant	Microsome ID	5		

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.206	0.131	0.060	0.040	0.028	0.023	0.019	2	1	
	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:	Recombinan	0.01 QC	0.1 QC
	0.022	0.026	0.102
	0.031	0.028	0.101
	0.028	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
Recombinan	0.022	0.003	0.000	200	120	72000	0.001	0.005	4.870
Recombinan	0.031	0.012	0.003	200	120	72000	0.008		
Recombinan	0.028	0.009	0.002	200	120	72000	0.006		
0.01 QC	0.026	0.007	0.001	200	100	100	0.000	0.000	0.011
0.01 QC	0.028	0.009	0.002	200	100	100	0.000		
0.01 QC	0.033	0.015	0.003	200	100	100	0.000		
0.1 QC	0.102	0.083	0.021	200	100	100	0.000	0.000	0.107
0.1 QC	0.101	0.082	0.021	200	100	100	0.000		
0.1 QC	0.109	0.091	0.023	200	100	100	0.000		

		Test Chemical ID		# Concentrations tested		Microsome type				Replicate #	
Assay Date	7/28/2005		RC#1 AG		8		Recombin: Microsome ID	5 Technician ID	EJB		2

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.87042
Protein Concentration (dilution added to assay, mg/mL):	0.008117

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	7681.946	15363.892	15244.497	30488.994	0.1	162890	18.72	30100	0.0370	1	0.004	15	0.3039
			0.5	2	7562.551	15125.102			0.1					1		15	
	2	2	0.5	1	8723.607	17447.214	16906.66	33813.32	0.1	162890	20.76	33424	0.0411	1	0.004	15	0.3375
			0.5	2	8183.053	16366.106			0.1					1		15	
	3	2	0.5	1	8245.005	16490.01	16271.831	32543.662	0.1	162890	19.98	32155	0.0395	1	0.004	15	0.3247
			0.5	2	8026.826	16053.652			0.1					1		15	
	4	2	0.5	1	8727.91	17455.82	17061.803	34123.606	0.1	162890	20.95	33735	0.0415	1	0.004	15	0.3407
			0.5	2	8333.893	16667.786			0.1					1		15	
Background control	1	2	0.5	1	97.68635	195.3727	179.09164	358.18328	0.1	162890	0.22	-31	0.0000	1	0.004	15	-0.0003
			0.5	2	81.40529	162.81058			0.1					1		15	
	2	2	0.5	1	92.25933	184.51866	181.80515	363.6103	0.1	162890	0.22	-25	0.0000	1	0.004	15	-0.0003
			0.5	2	89.54582	179.09164			0.1					1		15	
	3	2	0.5	1	126.5976	253.1952	254.1328	508.2652	0.1	162890	0.31	119	0.0001	1	0.004	15	0.0012
			0.5	2	127.535	255.07			0.1					1		15	
	4	2	0.5	1	79.87989	159.75978	162.77409	325.54818	0.1	162890	0.20	-63	-0.0001	1	0.004	15	-0.0006
			0.5	2	82.8942	165.7884			0.1					1		15	
Positive control	1	2	0.5	1	4698.928	9397.856	9070.323	18140.646	0.1	162890	11.14	17752	0.0218	1	0.004	15	0.1793
			0.5	2	4371.395	8742.79			0.1					1		15	
	2	2	0.5	1	3865.288	7730.576	7412.111	14824.222	0.1	162890	9.10	14435	0.0177	1	0.004	15	0.1458
			0.5	2	3546.823	7093.646			0.1					1		15	
	3	2	0.5	1	4385.37	8770.74	8509.242	17018.484	0.1	162890	10.45	16630	0.0204	1	0.004	15	0.1679
			0.5	2	4123.872	8247.744			0.1					1		15	
	4	2	0.5	1	4007.875	8015.75	7917.05	15834.1	0.1	162890	9.72	15445	0.0190	1	0.004	15	0.1560
			0.5	2	3909.175	7818.35			0.1					1		15	
Negative Control	1	2	0.5	1	7139.166	14278.332	13984.353	27968.706	0.1	162890	17.17	27580	0.0339	1	0.004	15	0.2785
			0.5	2	6795.187	13590.374			0.1					1		15	
	2	2	0.5	1	5302.226	10604.452	10702.139	21404.278	0.1	162890	13.14	21015	0.0258	1	0.004	15	0.2122
			0.5	2	5399.913	10799.826			0.1					1		15	
	3	2	0.5	1	7628.219	15256.438	15375.33	30750.65	0.1	162890	18.88	30362	0.0373	1	0.004	15	0.3066
			0.5	2	7747.111	15494.222			0.1					1		15	
	4	2	0.5	1	8387.809	16775.618	16653.204	33306.408	0.1	162890	20.45	32918	0.0405	1	0.004	15	0.3324
			0.5	2	8265.395	16530.79			0.1					1		15	
RC#1 AG	1-1	2	0.5	1	336.477	672.954	694.7232	1389.4464	0.1	162890	0.85	1001	0.0012	1	0.004	15	0.0101
			0.5	2	358.2462	716.4924			0.1					1		15	
	1-2	2	0.5	1	382.3829	764.7658	721.774	1443.548	0.1	162890	0.89	1055	0.0013	1	0.004	15	0.0106
			0.5	2	339.3911	678.7822			0.1					1		15	
	1-3	2	0.5	1	344.7783	689.5566	702.9635	1405.927	0.1	162890	0.86	1017	0.0013	1	0.004	15	0.0103
			0.5	2	358.1852	716.3704			0.1					1		15	
	2-1	2	0.5	1	2634.832	5269.664	5360.735	10721.47	0.1	162890	6.58	10333	0.0127	1	0.004	15	0.1043
			0.5	2	2725.903	5451.806			0.1					1		15	
	2-2	2	0.5	1	2531.965	5063.93	5082.967	10165.934	0.1	162890	6.24	9777	0.0120	1	0.004	15	0.0987
			0.5	2	2551.002	5102.004			0.1					1		15	
	2-3	2	0.5	1	2313.785	4627.57	4938.817	9877.634	0.1	162890	6.06	9489	0.0117	1	0.004	15	0.0958
			0.5	2	2625.032	5250.064			0.1					1		15	
	3-1	2	0.5	1	3999.734	7999.468	7688.379	15376.758	0.1	162890	9.44	14988	0.0184	1	0.004	15	0.1513
			0.5	2	3688.645	7377.29			0.1					1		15	
	3-2	2	0.5	1	3400.046	6800.092	6853.216	13706.432	0.1	162890	8.41	13318	0.0164	1	0.004	15	0.1345
			0.5	2	3453.17	6906.34			0.1					1		15	
	3-3	2	0.5	1	3161.893	6323.786	6458.852	12917.704	0.1	162890	7.93	12529	0.0154	1	0.004	15	0.1265
			0.5	2	3296.959	6593.918			0.1					1		15	
	4-1	2	0.5	1	7306.07	14612.14	14670.357	29340.714	0.1	162890	18.01	28952	0.0356	1	0.004	15	0.2924
			0.5	2	7364.287	14728.574			0.1					1		15	
	4-2	2	0.5	1	7623.632	15247.264	15017.299	30034.598	0.1	162890	18.44	29646	0.0365	1	0.004	15	0.2994
			0.5	2	7393.667	14787.334			0.1					1		15	
	4-3	2	0.5	1	7426.24	14852.48	15237.664	30475.328	0.1	162890	18.71	30086	0.0370	1	0.004	15	0.3038
			0.5	2	7811.424	15622.848			0.1					1		15	
	5-1	2	0.5	1	8692.229	17384.458	17161.111	34322.222	0.1	162890	21.07	33933	0.0417	1	0.004	15	0.3427
			0.5	2	8468.882	16937.764			0.1					1		15	
	5-2	2	0.5	1	8699.604	17399.208	16959.616	33919.232	0.1	162890	20.82	33530	0.0412	1	0.004	15	0.3386
			0.5	2	8260.012	16520.024			0.1					1		15	
	5-3	2	0.5	1	8270.745	16541.49	16049.47	32098.94	0.1	162890	19.71	31710	0.0390	1	0.004	15	0.3202
			0.5	2	7778.725	15557.45			0.1					1		15	
	6-1	2	0.5	1	8357.698	16715.396	16287.64	32575.28	0.1	162890	20.00	32186	0.0396	1	0.004	15	0.3250
			0.5	2	7929.942	15859.884			0.1					1		15	
	6-2	2	0.5	1	7913.761	15827.562	15878.016	31756.032	0.1	162890	19.50	31367	0.0386	1	0.004	15	0.3167
			0.5	2	7964.235	15928.47			0.1					1		15	
	6-3	2	0.5	1	7377.755	14755.51	14968.304	29936.608	0.1	162890	18.38	29548	0.0363	1	0.004	15	0.2984
			0.5	2	7590.549	15181.098			0.1					1		15	
	7-1	2	0.5	1	8280.109	16560.218	16986.719	33973.438	0.1	162890	20.86	33585	0.0413	1	0.004	15	0.3391
			0.5	2	8706.61	17413.22			0.1					1		15	
	7-2	2	0.5	1	8541.389	17082.778	17037.478	34074.956	0.1	162890	20.92	33686	0.0414	1	0.004	15	0.3402
			0.5	2	8496.089	16992.178			0.1					1		15	
	7-3	2	0.5	1	8718.6	17437.2	17600.863	35201.726	0.1	162890	21.61	34813	0.0428	1	0.004	15	0.3515
			0.5	2	8882.263	17764.526			0.1					1		15	
	8-1	2	0.5	1	8698.589	17397.178	16976.005	33952.01	0.1	162890	20.84	33563	0.0413	1	0.004	15	0.3389
			0.5	2	8277.416	16554.832			0.1					1		15	
	8-2	2	0.5	1	8140.042	16280.084	16770.319	33540.638	0.1	162890	20.59	33152	0.0408	1	0.004	15	0.3348
			0.5	2	8630.277	17260.554			0.1					1		15	
	8-3	2	0.5	1	8563.928	17127.856	17293.381	34586.762	0.1	162890	21.23	34198	0.0420	1	0.004	15	0.3453
			0.5	2	8729.453	17458.906			0.1					1		15	

Assay Date	7/28/2005	Test Chemical ID	RC#1 AG	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3207	0.0237
Full activity	End	0.3327	0.0113
Full activity	Overall	0.3267	0.0167
Background	Beginning	-0.0003	3.87507E-05
Background	End	0.0003	0.001304661
Background	Overall	0.0000	0.000821285
Positive	Beginning	0.1625	0.0237
Positive	End	0.1619	0.0085
Positive	Overall	0.1622	0.0145
Negative	Beginning	0.2454	0.0469
Negative	End	0.3195	0.0182
Negative	Overall	0.2824	0.0517

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#1 AG	1	1	1.00E-03	-3.00	0.0101
RC#1 AG	1	2	1.00E-03	-3.00	0.0106
RC#1 AG	1	3	1.00E-03	-3.00	0.0103
RC#1 AG	2	1	1.00E-04	-4.00	0.1043
RC#1 AG	2	2	1.00E-04	-4.00	0.0987
RC#1 AG	2	3	1.00E-04	-4.00	0.0958
RC#1 AG	3	1	3.30E-05	-4.48	0.1513
RC#1 AG	3	2	3.30E-05	-4.48	0.1345
RC#1 AG	3	3	3.30E-05	-4.48	0.1265
RC#1 AG	4	1	1.00E-05	-5.00	0.2924
RC#1 AG	4	2	1.00E-05	-5.00	0.2994
RC#1 AG	4	3	1.00E-05	-5.00	0.3038
RC#1 AG	5	1	1.00E-06	-6.00	0.3427
RC#1 AG	5	2	1.00E-06	-6.00	0.3386
RC#1 AG	5	3	1.00E-06	-6.00	0.3202
RC#1 AG	6	1	1.00E-07	-7.00	0.3250
RC#1 AG	6	2	1.00E-07	-7.00	0.3167
RC#1 AG	6	3	1.00E-07	-7.00	0.2984
RC#1 AG	7	1	1.00E-08	-8.00	0.3391
RC#1 AG	7	2	1.00E-08	-8.00	0.3402
RC#1 AG	7	3	1.00E-08	-8.00	0.3515
RC#1 AG	8	1	1.00E-09	-9.00	0.3389
RC#1 AG	8	2	1.00E-09	-9.00	0.3348
RC#1 AG	8	3	1.00E-09	-9.00	0.3453

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	3.09	3.26	3.14
2	-4.00	31.94	30.22	29.33
3	-4.48	46.33	41.16	38.72
4	-5.00	89.49	91.63	92.99
5	-6.00	104.88	103.64	98.01
6	-7.00	99.48	96.95	91.33
7	-8.00	103.80	104.12	107.60
8	-9.00	103.74	102.47	105.70

Assay Date	7/29/2005	Test Chemical ID	RC#1 AG	# Concentrations tested	8		
Technician ID	EJB	Replicate #	3	Microsome type	Recombina	Microsome ID	5

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	EJB	Replicate #	3	Microsome type	Recombinant	Microsome ID	5

								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	
	0.496	0.309	0.161	0.112	0.056	0.044	0.027			
	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:	Recombinan	0.01 QC	0.1 QC
	0.039	0.048	0.246
	0.041	0.047	0.277
	0.044	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
Recombinan	0.039	0.010	-0.001	200	120	72000	-0.004	-0.003	-3.076
Recombinan	0.041	0.012	-0.001	200	120	72000	-0.003		
Recombinan	0.044	0.015	-0.001	200	120	72000	-0.002		
0.01 QC	0.048	0.019	0.000	200	100	100	0.000	0.000	-0.002
0.01 QC	0.047	0.019	0.000	200	100	100	0.000		
0.01 QC	0.046	0.017	-0.001	200	100	100	0.000		
0.1 QC	0.246	0.217	0.020	200	100	100	0.000	0.000	0.110
0.1 QC	0.277	0.248	0.023	200	100	100	0.000		
0.1 QC	0.276	0.248	0.023	200	100	100	0.000		

Assay Date	7/29/2005	Test Chemical ID	RC#1 AG	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	3

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.87
Protein Concentration (dilution added to assay, mg/mL):	0.008117

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	8204.602	16409.204	16314.929	32629.858	0.1	169659	19.23	32269	0.0380	1	0.004	15	0.3123
			0.5	2	8110.327	16220.654			0.1					1		15	
	2	2	0.5	1	7900.666	15801.332	15799.692	31599.384	0.1	169659	18.63	31238	0.0368	1	0.004	15	0.3024
			0.5	2	7898.026	15796.052			0.1					1		15	
	3	2	0.5	1	7703.771	15407.542	15286.159	30572.318	0.1	169659	18.02	30211	0.0356	1	0.004	15	0.2924
			0.5	2	7582.388	15164.776			0.1					1		15	
	4	2	0.5	1	7665.621	15331.242	15309.961	30619.922	0.1	169659	18.05	30259	0.0357	1	0.004	15	0.2929
			0.5	2	7644.34	15288.68			0.1					1		15	
Background control	1	2	0.5	1	78.11341	156.22682	171.70313	343.40626	0.1	169659	0.20	-18	0.0000	1	0.004	15	-0.0002
			0.5	2	93.58972	187.17944			0.1					1		15	
	2	2	0.5	1	92.25933	184.51866	166.59356	333.18712	0.1	169659	0.20	-28	0.0000	1	0.004	15	-0.0003
			0.5	2	74.33423	148.66846			0.1					1		15	
	3	2	0.5	1	94.2748	188.5496	203.9085	407.817	0.1	169659	0.24	47	0.0001	1	0.004	15	0.0005
			0.5	2	109.6337	219.2674			0.1					1		15	
	4	2	0.5	1	107.7426	215.4852	179.94077	359.88154	0.1	169659	0.21	-1	0.0000	1	0.004	15	0.0000
			0.5	2	72.19817	144.39634			0.1					1		15	
Positive control	1	2	0.5	1	3036.433	6072.866	6051.158	12102.316	0.1	169659	7.13	11741	0.0138	1	0.004	15	0.1136
			0.5	2	3014.725	6029.45			0.1					1		15	
	2	2	0.5	1	2604.482	5208.964	5346.899	10693.798	0.1	169659	6.30	10333	0.0122	1	0.004	15	0.1000
			0.5	2	2742.417	5484.834			0.1					1		15	
	3	2	0.5	1	3922.767	7845.534	8269.832	16539.664	0.1	169659	9.75	16179	0.0191	1	0.004	15	0.1566
			0.5	2	4347.065	8694.13			0.1					1		15	
	4	2	0.5	1	4150.807	8301.614	8126.531	16253.062	0.1	169659	9.58	15892	0.0187	1	0.004	15	0.1538
			0.5	2	3975.724	7951.448			0.1					1		15	
Negative Control	1	2	0.5	1	3071.709	6143.418	6130.048	12260.096	0.1	169659	7.23	11899	0.0140	1	0.004	15	0.1152
			0.5	2	3058.339	6116.678			0.1					1		15	
	2	2	0.5	1	3422.047	6844.094	6751.541	13503.082	0.1	169659	7.96	13142	0.0155	1	0.004	15	0.1272
			0.5	2	3329.494	6658.988			0.1					1		15	
	3	2	0.5	1	6684.806	13369.612	13383.74	26767.48	0.1	169659	15.78	26406	0.0311	1	0.004	15	0.2556
			0.5	2	6698.934	13397.868			0.1					1		15	
	4	2	0.5	1	5741.817	11483.634	11624.871	23249.742	0.1	169659	13.70	22889	0.0270	1	0.004	15	0.2215
			0.5	2	5883.054	11766.108			0.1					1		15	
RC#1 AG	1-1	2	0.5	1	308.9229	617.8458	656.5437	1313.0874	0.1	169659	0.77	952	0.0011	1	0.004	15	0.0092
			0.5	2	347.6208	695.2416			0.1					1		15	
	1-2	2	0.5	1	320.536	641.072	619.5234	1239.0468	0.1	169659	0.73	878	0.0010	1	0.004	15	0.0085
			0.5	2	298.9674	597.9748			0.1					1		15	
	1-3	2	0.5	1	312.8587	625.7174	644.1691	1288.3382	0.1	169659	0.76	927	0.0011	1	0.004	15	0.0090
			0.5	2	331.3104	662.6208			0.1					1		15	
	2-1	2	0.5	1	1952.846	3905.692	3917.437	7834.874	0.1	169659	4.62	7474	0.0088	1	0.004	15	0.0723
			0.5	2	1964.591	3929.182			0.1					1		15	
	2-2	2	0.5	1	1907.055	3814.11	3792.229	7584.458	0.1	169659	4.47	7223	0.0085	1	0.004	15	0.0699
			0.5	2	1885.174	3770.348			0.1					1		15	
	2-3	2	0.5	1	1929.316	3858.632	3885.767	7771.534	0.1	169659	4.58	7410	0.0087	1	0.004	15	0.0717
			0.5	2	1956.451	3912.902			0.1					1		15	
	3-1	2	0.5	1	2628.934	5257.868	5435.644	10871.288	0.1	169659	6.41	10510	0.0124	1	0.004	15	0.1017
			0.5	2	2806.71	5613.42			0.1					1		15	
	3-2	2	0.5	1	2612.504	5225.008	5120.227	10240.454	0.1	169659	6.04	9879	0.0116	1	0.004	15	0.0956
			0.5	2	2507.723	5015.446			0.1					1		15	
	3-3	2	0.5	1	2599.57	5199.14	5212.708	10425.416	0.1	169659	6.14	10064	0.0119	1	0.004	15	0.0974
			0.5	2	2613.138	5226.276			0.1					1		15	
	4-1	2	0.5	1	5810.646	11621.292	12016.688	24033.376	0.1	169659	14.17	23672	0.0279	1	0.004	15	0.2291
			0.5	2	6206.042	12412.084			0.1					1		15	
	4-2	2	0.5	1	6092.816	12185.632	12266.116	24532.232	0.1	169659	14.46	24171	0.0285	1	0.004	15	0.2340
			0.5	2	6173.3	12346.6			0.1					1		15	
	4-3	2	0.5	1	5915.134	11830.268	11985.32	23970.64	0.1	169659	14.13	23610	0.0278	1	0.004	15	0.2285
			0.5	2	6070.186	12140.372			0.1					1		15	
	5-1	2	0.5	1	7209.872	14419.744	14488.555	28977.11	0.1	169659	17.08	28616	0.0337	1	0.004	15	0.2770
			0.5	2	7278.683	14557.366			0.1					1		15	
	5-2	2	0.5	1	6690.889	13381.778	13366.194	26732.388	0.1	169659	15.76	26371	0.0311	1	0.004	15	0.2553
			0.5	2	6675.305	13350.61			0.1					1		15	
	5-3	2	0.5	1	6547.768	13095.536	13216.775	26433.55	0.1	169659	15.58	26072	0.0307	1	0.004	15	0.2524
			0.5	2	6669.007	13338.014			0.1					1		15	
	6-1	2	0.5	1	6816.409	13632.818	13489	26978	0.1	169659	15.90	26617	0.0314	1	0.004	15	0.2576
			0.5	2	6672.591	13345.182			0.1					1		15	
	6-2	2	0.5	1	6767.565	13535.13	13150.452	26300.904	0.1	169659	15.50	25940	0.0306	1	0.004	15	0.2511
			0.5	2	6382.887	12765.774			0.1					1		15	
	6-3	2	0.5	1	6187.186	12374.372	12466.314	24932.628	0.1	169659	14.70	24572	0.0290	1	0.004	15	0.2378
			0.5	2	6279.128	12558.256			0.1					1		15	
	7-1	2	0.5	1	7043.75	14087.5	14695.928	29391.856	0.1	169659	17.32	29031	0.0342	1	0.004	15	0.2810
			0.5	2	7652.178	15304.356			0.1					1		15	
	7-2	2	0.5	1	7445.95	14891.9	15080.278	30160.556	0.1	169659	17.78	29799	0.0351	1	0.004	15	0.2884
			0.5	2	7634.328	15268.656			0.1					1		15	
	7-3	2	0.5	1	7655.195	15310.39	15300.219	30600.438	0.1	169659	18.04	30239	0.0356	1	0.004	15	0.2927
			0.5	2	7645.024	15290.048			0.1					1		15	
	8-1	2	0.5	1	7215.299	14430.598	14428.745	28857.49	0.1	169659	17.01	28496	0.0336	1	0.004	15	0.2758
			0.5	2	7213.446	14426.892			0.1					1		15	
	8-2	2	0.5	1	7507.048	15014.096	14731.268	29462.536	0.1	169659	17.37	29101	0.0343	1	0.004	15	0.2817
			0.5	2	7224.22	14448.44			0.1					1		15	
	8-3	2	0.5	1	7191.897	14383.794	14092.123	28184.246	0.1	169659	16.61	27823	0.0328	1	0.004	15	0.2693
			0.5	2	6900.226	13800.452			0.1					1		15	

Assay Date	7/29/2005	Test Chemical ID	RC#1 AG	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3073	0.0071
Full activity	End	0.2926	0.0003
Full activity	Overall	0.3000	0.0094
Background	Beginning	-0.0002	6.99417E-05
Background	End	0.0002	0.000328079
Background	Overall	0.0000	0.000319859
Positive	Beginning	0.1068	0.0096
Positive	End	0.1552	0.0020
Positive	Overall	0.1310	0.0285
Negative	Beginning	0.1212	0.0085
Negative	End	0.2386	0.0241
Negative	Overall	0.1799	0.0694

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#1 AG	1	1	1.00E-03	-3.00	0.0092
RC#1 AG	1	2	1.00E-03	-3.00	0.0085
RC#1 AG	1	3	1.00E-03	-3.00	0.0090
RC#1 AG	2	1	1.00E-04	-4.00	0.0723
RC#1 AG	2	2	1.00E-04	-4.00	0.0699
RC#1 AG	2	3	1.00E-04	-4.00	0.0717
RC#1 AG	3	1	3.30E-05	-4.48	0.1017
RC#1 AG	3	2	3.30E-05	-4.48	0.0956
RC#1 AG	3	3	3.30E-05	-4.48	0.0974
RC#1 AG	4	1	1.00E-05	-5.00	0.2291
RC#1 AG	4	2	1.00E-05	-5.00	0.2340
RC#1 AG	4	3	1.00E-05	-5.00	0.2285
RC#1 AG	5	1	1.00E-06	-6.00	0.2770
RC#1 AG	5	2	1.00E-06	-6.00	0.2553
RC#1 AG	5	3	1.00E-06	-6.00	0.2524
RC#1 AG	6	1	1.00E-07	-7.00	0.2576
RC#1 AG	6	2	1.00E-07	-7.00	0.2511
RC#1 AG	6	3	1.00E-07	-7.00	0.2378
RC#1 AG	7	1	1.00E-08	-8.00	0.2810
RC#1 AG	7	2	1.00E-08	-8.00	0.2884
RC#1 AG	7	3	1.00E-08	-8.00	0.2927
RC#1 AG	8	1	1.00E-09	-9.00	0.2758
RC#1 AG	8	2	1.00E-09	-9.00	0.2817
RC#1 AG	8	3	1.00E-09	-9.00	0.2693

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-3.00	3.07	2.83	2.99
2	-4.00	24.11	23.31	23.91
3	-4.48	33.91	31.87	32.47
4	-5.00	76.38	77.99	76.17
5	-6.00	92.33	85.08	84.12
6	-7.00	85.88	83.69	79.28
7	-8.00	93.66	96.15	97.56
8	-9.00	91.94	93.89	89.77

Assay Date	7/27/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	1	Microsome type	Recombina	Microsome ID	5

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	EJB	Replicate #	1	Microsome type	Recombinant	Microsome ID	5			

								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:	Microsomes	QC 10	QC100
	0.021	0.025	0.096
	0.021	0.026	0.096
	0.021	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	62.35	1000	0.00012	200	0.0249	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		1880	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	0.021	0.002	0.000	200	120	72000	0.000	0.000	0.261
Microsomes	0.021	0.002	0.000	200	120	72000	0.000		
Microsomes	0.021	0.002	0.000	200	120	72000	0.000		
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	Recombinz	Microsome ID	5	Technician ID	EJB	Replicate #	1

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	0.26054
Protein Concentration (dilution added to assay, mg/mL):	0.000434

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	Recombinan	Microsome ID	5	Technician ID	EJB	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	8240.063	16480.126	16277.157	32554.314	1	1728563	1.88	32127	0.0372	1	0.000	15	5.7164
			0.5	2	8037.094	16074.188			1					1		15	
	2	2	0.5	1	7959.487	15918.974	15464.589	30929.178	1	1728563	1.79	30502	0.0354	1	0.000	15	5.4272
			0.5	2	7505.102	15010.204			1					1		15	
	3	2	0.5	1	7709.119	15418.238	15176.698	30353.396	1	1728563	1.76	29926	0.0347	1	0.000	15	5.3247
			0.5	2	7467.579	14935.158			1					1		15	
	4	2	0.5	1	7698.211	15396.422	15579.585	31159.17	1	1728563	1.80	30732	0.0356	1	0.000	15	5.4681
			0.5	2	7881.374	15762.748			1					1		15	
Background control	1	2	0.5	1	143.3589	286.7178	325.1904	650.3808	1	1728563	0.04	223	0.0003	1	0.000	15	0.0396
			0.5	2	181.8315	363.663			1					1		15	
	2	2	0.5	1	111.5013	223.0026	216.9404	433.8808	1	1728563	0.03	6	0.0000	1	0.000	15	0.0011
			0.5	2	105.4391	210.8782			1					1		15	
	3	2	0.5	1	80.80698	161.61396	158.92039	317.84078	1	1728563	0.02	-110	-0.0001	1	0.000	15	-0.0195
			0.5	2	78.11341	156.22682			1					1		15	
	4	2	0.5	1	67.33915	134.6783	154.17191	308.34382	1	1728563	0.02	-119	-0.0001	1	0.000	15	-0.0212
			0.5	2	86.83276	173.66552			1					1		15	
Positive control	1	2	0.5	1	4686.83	9373.66	9378.513	18757.026	1	1728563	1.09	18329	0.0212	1	0.000	15	3.2614
			0.5	2	4691.663	9383.366			1					1		15	
	2	2	0.5	1	3948.789	7897.578	7783.314	15566.628	1	1728563	0.90	15139	0.0175	1	0.000	15	2.6937
			0.5	2	3834.525	7669.05			1					1		15	
	3	2	0.5	1	3967.166	7934.332	7775.888	15551.776	1	1728563	0.90	15124	0.0175	1	0.000	15	2.6911
			0.5	2	3808.722	7617.444			1					1		15	
	4	2	0.5	1	4053.793	8107.586	8197.344	16394.688	1	1728563	0.95	15967	0.0185	1	0.000	15	2.8411
			0.5	2	4143.551	8287.102			1					1		15	
Negative Control	1	2	0.5	1	5930.945	11861.89	11920.192	23840.384	1	1728563	1.38	23413	0.0271	1	0.000	15	4.1659
			0.5	2	5989.247	11978.494			1					1		15	
	2	2	0.5	1	5239.815	10479.63	10301.052	20602.104	1	1728563	1.19	20174	0.0234	1	0.000	15	3.5897
			0.5	2	5061.237	10122.474			1					1		15	
	3	2	0.5	1	7975.691	15951.382	15980.587	31961.174	1	1728563	1.85	31534	0.0365	1	0.000	15	5.6108
			0.5	2	8004.896	16009.792			1					1		15	
	4	2	0.5	1	7818.794	15637.588	15714.192	31428.384	1	1728563	1.82	31001	0.0359	1	0.000	15	5.5160
			0.5	2	7895.398	15790.796			1					1		15	
RC#2 KCZ	1-1	2	0.5	1	91.58221	183.16442	219.01431	438.02862	1	1728563	0.03	10	0.0000	1	0.000	15	0.0019
			0.5	2	127.4321	254.8642			1					1		15	
	1-2	2	0.5	1	144.3979	288.7958	288.2162	576.4324	1	1728563	0.03	149	0.0002	1	0.000	15	0.0265
			0.5	2	143.8183	287.6366			1					1		15	
	1-3	2	0.5	1	123.906	247.812	236.2154	472.4308	1	1728563	0.03	45	0.0001	1	0.000	15	0.0080
			0.5	2	112.3094	224.6188			1					1		15	
	2-1	2	0.5	1	434.1684	868.3368	955.1705	1910.341	1	1728563	0.11	1483	0.0017	1	0.000	15	0.2638
			0.5	2	521.0021	1042.0042			1					1		15	
	2-2	2	0.5	1	422.4974	844.9948	915.4278	1830.8556	1	1728563	0.11	1403	0.0016	1	0.000	15	0.2497
			0.5	2	492.9304	985.8608			1					1		15	
	2-3	2	0.5	1	495.624	991.248	896.9717	1793.9434	1	1728563	0.10	1386	0.0016	1	0.000	15	0.2431
			0.5	2	401.3477	802.6954			1					1		15	
	3-1	2	0.5	1	2711.471	5422.942	5423.935	10847.87	1	1728563	0.63	10420	0.0121	1	0.000	15	1.8541
			0.5	2	2712.464	5424.928			1					1		15	
	3-2	2	0.5	1	2653.854	5307.708	5418.806	10837.612	1	1728563	0.63	10410	0.0121	1	0.000	15	1.8523
			0.5	2	2764.952	5529.904			1					1		15	
	3-3	2	0.5	1	2721.693	5443.386	5291.44	10582.88	1	1728563	0.61	10155	0.0118	1	0.000	15	1.8069
			0.5	2	2569.747	5139.494			1					1		15	
	4-1	2	0.5	1	7296.743	14593.486	14182.382	28364.764	1	1728563	1.64	27937	0.0324	1	0.000	15	4.9709
			0.5	2	6885.639	13771.278			1					1		15	
	4-2	2	0.5	1	6911.799	13823.598	13840.921	27681.842	1	1728563	1.60	27254	0.0316	1	0.000	15	4.8494
			0.5	2	6929.122	13858.244			1					1		15	
	4-3	2	0.5	1	7006.882	14013.764	13758.819	27517.638	1	1728563	1.59	27090	0.0314	1	0.000	15	4.8202
			0.5	2	6751.937	13503.874			1					1		15	
	5-1	2	0.5	1	7899.097	15798.194	15950.614	31901.228	1	1728563	1.85	31474	0.0365	1	0.000	15	5.6002
			0.5	2	8051.517	16103.034			1					1		15	
	5-2	2	0.5	1	8172.408	16344.816	16466.116	32932.232	1	1728563	1.91	32505	0.0377	1	0.000	15	5.7836
			0.5	2	8293.708	16587.416			1					1		15	
	5-3	2	0.5	1	8184.859	16369.718	16406.885	32813.77	1	1728563	1.90	32386	0.0375	1	0.000	15	5.7625
			0.5	2	8222.026	16444.052			1					1		15	
	6-1	2	0.5	1	8320.557	16641.114	17020.912	34041.824	1	1728563	1.97	33614	0.0390	1	0.000	15	5.9810
			0.5	2	8700.355	17400.71			1					1		15	
	6-2	2	0.5	1	8999.347	17998.694	18141.503	36283.006	1	1728563	2.10	35855	0.0416	1	0.000	15	6.3798
			0.5	2	9142.156	18284.312			1					1		15	
	6-3	2	0.5	1	9070.373	18140.746	18111.978	36223.956	1	1728563	2.10	35796	0.0415	1	0.000	15	6.3693
			0.5	2	9041.605	18083.21			1					1		15	
	7-1	2	0.5	1	8738.79	17477.58	17643.907	35287.814	1	1728563	2.04	34860	0.0404	1	0.000	15	6.2027
			0.5	2	8905.117	17810.234			1					1		15	
	7-2	2	0.5	1	9348.475	18696.95	18293.996	36587.992	1	1728563	2.12	36160	0.0419	1	0.000	15	6.4341
			0.5	2	8945.521	17891.042			1					1		15	
	7-3	2	0.5	1	8596.58	17193.16	16887.552	33775.104	1	1728563	1.95	33347	0.0386	1	0.000	15	5.9336
			0.5	2	8290.972	16581.944			1					1		15	
	8-1	2	0.5	1	8501.073	17002.146	17144.909	34289.818	1	1728563	1.98	33862	0.0392	1	0.000	15	6.0252
			0.5	2	8643.836	17287.672			1					1		15	
	8-2	2	0.5	1	8067.604	16135.208	16737.451	33474.902	1	1728563	1.94	33047	0.0383	1	0.000	15	5.8802
			0.5	2	8669.847	17339.694			1					1		15	
	8-3	2	0.5	1	8596.58	17193.16	17205.399	34410.798	1	1728563	1.99	33983	0.0394	1	0.000	15	6.0467
			0.5	2	8609.819	17217.636			1					1		15	

Assay Date	7/27/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	5.5718	0.2045
Full activity	End	5.3964	0.1014
Full activity	Overall	5.4841	0.1662
Background	Beginning	0.0204	0.027239329
Background	End	-0.0204	0.001194877
Background	Overall	0.0000	0.028309211
Positive	Beginning	2.9775	0.4014
Positive	End	2.7661	0.1061
Positive	Overall	2.8718	0.2690
Negative	Beginning	3.8778	0.4074
Negative	End	5.5634	0.0670
Negative	Overall	4.7206	1.0020

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#2 KCZ	1	1	1.00E-04	-4.00	0.0019
RC#2 KCZ	1	2	1.00E-04	-4.00	0.0265
RC#2 KCZ	1	3	1.00E-04	-4.00	0.0080
RC#2 KCZ	2	1	1.00E-05	-5.00	0.2638
RC#2 KCZ	2	2	1.00E-05	-5.00	0.2497
RC#2 KCZ	2	3	1.00E-05	-5.00	0.2431
RC#2 KCZ	3	1	1.00E-06	-6.00	1.8541
RC#2 KCZ	3	2	1.00E-06	-6.00	1.8523
RC#2 KCZ	3	3	1.00E-06	-6.00	1.8069
RC#2 KCZ	4	1	1.00E-07	-7.00	4.9709
RC#2 KCZ	4	2	1.00E-07	-7.00	4.8494
RC#2 KCZ	4	3	1.00E-07	-7.00	4.8202
RC#2 KCZ	5	1	1.00E-08	-8.00	5.6002
RC#2 KCZ	5	2	1.00E-08	-8.00	5.7836
RC#2 KCZ	5	3	1.00E-08	-8.00	5.7625
RC#2 KCZ	6	1	1.00E-09	-9.00	5.9810
RC#2 KCZ	6	2	1.00E-09	-9.00	6.3798
RC#2 KCZ	6	3	1.00E-09	-9.00	6.3693
RC#2 KCZ	7	1	1.00E-10	-10.00	6.2027
RC#2 KCZ	7	2	1.00E-10	-10.00	6.4341
RC#2 KCZ	7	3	1.00E-10	-10.00	5.9336
RC#2 KCZ	8	1	1.00E-11	-11.00	6.0252
RC#2 KCZ	8	2	1.00E-11	-11.00	5.8802
RC#2 KCZ	8	3	1.00E-11	-11.00	6.0467

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.03	0.48	0.15
2	-5.00	4.81	4.55	4.43
3	-6.00	33.81	33.78	32.95
4	-7.00	90.64	88.43	87.89
5	-8.00	102.12	105.46	105.08
6	-9.00	109.06	116.33	116.14
7	-10.00	113.10	117.32	108.20
8	-11.00	109.87	107.22	110.26

Assay Date	7/28/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	2	Microsome type	Recombina	Microsome ID	5

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	EJB	Replicate #	2		Microsome type		Recombinant	Microsome ID	5	

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.206	0.131	0.060	0.040	0.028	0.023	0.019	2	1	
	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:	Recombinan	0.01 QC	0.1 QC
	0.022	0.026	0.102
	0.031	0.028	0.101
	0.028	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

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
Recombinan	0.022	0.003	0.000	200	120	72000	0.001	0.005	4.870
Recombinan	0.031	0.012	0.003	200	120	72000	0.008		
Recombinan	0.028	0.009	0.002	200	120	72000	0.006		
0.01 QC	0.026	0.007	0.001	200	100	100	0.000	0.000	0.011
0.01 QC	0.028	0.009	0.002	200	100	100	0.000		
0.01 QC	0.033	0.015	0.003	200	100	100	0.000		
0.1 QC	0.102	0.083	0.021	200	100	100	0.000	0.000	0.107
0.1 QC	0.101	0.082	0.021	200	100	100	0.000		
0.1 QC	0.109	0.091	0.023	200	100	100	0.000		

Assay Date	7/28/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	2

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.87042
Protein Concentration (dilution added to assay, mg/mL):	0.008117

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	7681.946	15363.892	15244.497	30488.994	0.1	162890	18.72	30100	0.0370	1	0.004	15	0.3039
			0.5	2	7562.551	15125.102			0.1					1		15	
	2	2	0.5	1	8723.607	17447.214	16906.66	33813.32	0.1	162890	20.76	33424	0.0411	1	0.004	15	0.3375
			0.5	2	8183.053	16366.106			0.1					1		15	
	3	2	0.5	1	8245.005	16490.01	16271.831	32543.662	0.1	162890	19.98	32155	0.0395	1	0.004	15	0.3247
			0.5	2	8026.826	16053.652			0.1					1		15	
	4	2	0.5	1	8727.91	17455.82	17061.803	34123.606	0.1	162890	20.95	33735	0.0415	1	0.004	15	0.3407
			0.5	2	8333.893	16667.786			0.1					1		15	
Background control	1	2	0.5	1	97.68635	195.3727	179.09164	358.18328	0.1	162890	0.22	-31	0.0000	1	0.004	15	-0.0003
			0.5	2	81.40529	162.81058			0.1					1		15	
	2	2	0.5	1	92.25933	184.51866	181.80515	363.6103	0.1	162890	0.22	-25	0.0000	1	0.004	15	-0.0003
			0.5	2	89.54582	179.09164			0.1					1		15	
	3	2	0.5	1	126.5976	253.1952	254.1326	508.2652	0.1	162890	0.31	119	0.0001	1	0.004	15	0.0012
			0.5	2	127.535	255.07			0.1					1		15	
	4	2	0.5	1	79.87989	159.75978	162.77409	325.54818	0.1	162890	0.20	-63	-0.0001	1	0.004	15	-0.0006
			0.5	2	82.8942	165.7884			0.1					1		15	
Positive control	1	2	0.5	1	4698.928	9397.856	9070.323	18140.646	0.1	162890	11.14	17752	0.0218	1	0.004	15	0.1793
			0.5	2	4371.395	8742.79			0.1					1		15	
	2	2	0.5	1	3865.288	7730.576	7412.111	14824.222	0.1	162890	9.10	14435	0.0177	1	0.004	15	0.1458
			0.5	2	3546.823	7093.646			0.1					1		15	
	3	2	0.5	1	4385.37	8770.74	8509.242	17018.484	0.1	162890	10.45	16630	0.0204	1	0.004	15	0.1679
			0.5	2	4123.872	8247.744			0.1					1		15	
	4	2	0.5	1	4007.875	8015.75	7917.05	15834.1	0.1	162890	9.72	15445	0.0190	1	0.004	15	0.1560
			0.5	2	3909.175	7818.35			0.1					1		15	
Negative Control	1	2	0.5	1	7189.166	14378.332	13984.353	27968.706	0.1	162890	17.17	27580	0.0339	1	0.004	15	0.2785
			0.5	2	6795.187	13590.374			0.1					1		15	
	2	2	0.5	1	5302.226	10604.452	10702.139	21404.278	0.1	162890	13.14	21015	0.0258	1	0.004	15	0.2122
			0.5	2	5399.913	10799.826			0.1					1		15	
	3	2	0.5	1	7528.219	15256.438	15375.33	30750.66	0.1	162890	18.88	30362	0.0373	1	0.004	15	0.3066
			0.5	2	7747.111	15494.222			0.1					1		15	
	4	2	0.5	1	8387.809	16775.619	16653.204	33306.408	0.1	162890	20.45	32918	0.0405	1	0.004	15	0.3324
			0.5	2	8265.395	16530.79			0.1					1		15	
RC#2 KCZ	1-1	2	0.5	1	110.438	220.876	250.5057	501.0114	0.1	162890	0.31	112	0.0001	1	0.004	15	0.0011
			0.5	2	140.0677	280.1354			0.1					1		15	
	1-2	2	0.5	1	117.6575	235.315	245.1945	490.389	0.1	162890	0.30	101	0.0001	1	0.004	15	0.0010
			0.5	2	127.537	255.074			0.1					1		15	
	1-3	2	0.5	1	121.2124	242.4248	266.6673	533.3346	0.1	162890	0.33	144	0.0002	1	0.004	15	0.0015
			0.5	2	145.4549	290.9098			0.1					1		15	
	2-1	2	0.5	1	382.7404	765.4808	835.2666	1670.5332	0.1	162890	1.03	1282	0.0016	1	0.004	15	0.0129
			0.5	2	452.5262	905.0524			0.1					1		15	
	2-2	2	0.5	1	451.0868	902.1736	881.6063	1763.2126	0.1	162890	1.08	1374	0.0017	1	0.004	15	0.0139
			0.5	2	430.5195	861.039			0.1					1		15	
	2-3	2	0.5	1	492.0947	984.1894	888.0552	1776.1104	0.1	162890	1.09	1387	0.0017	1	0.004	15	0.0140
			0.5	2	395.9605	791.921			0.1					1		15	
	3-1	2	0.5	1	2851.944	5703.888	5599.425	11198.85	0.1	162890	6.88	10810	0.0133	1	0.004	15	0.1092
			0.5	2	2747.481	5494.962			0.1					1		15	
	3-2	2	0.5	1	2712.464	5424.928	5330.652	10661.304	0.1	162890	6.55	10272	0.0126	1	0.004	15	0.1037
			0.5	2	2618.188	5236.376			0.1					1		15	
	3-3	2	0.5	1	2698.996	5397.992	5282.298	10564.596	0.1	162890	6.49	10176	0.0125	1	0.004	15	0.1028
			0.5	2	2583.302	5166.604			0.1					1		15	
	4-1	2	0.5	1	7237.726	14475.452	14338.079	28676.158	0.1	162890	17.60	28287	0.0348	1	0.004	15	0.2856
			0.5	2	7100.353	14200.705			0.1					1		15	
	4-2	2	0.5	1	6968.608	13937.216	13764.583	27529.166	0.1	162890	16.90	27140	0.0334	1	0.004	15	0.2741
			0.5	2	6795.975	13591.95			0.1					1		15	
	4-3	2	0.5	1	6322.578	12645.156	12728.697	25457.394	0.1	162890	15.63	25068	0.0308	1	0.004	15	0.2531
			0.5	2	6406.119	12812.238			0.1					1		15	
	5-1	2	0.5	1	8977.995	17955.99	17973.422	35946.844	0.1	162890	22.07	35558	0.0437	1	0.004	15	0.3591
			0.5	2	8995.427	17990.854			0.1					1		15	
	5-2	2	0.5	1	8791.057	17582.114	17793.871	35587.742	0.1	162890	21.85	35199	0.0433	1	0.004	15	0.3554
			0.5	2	9012.814	18025.628			0.1					1		15	
	5-3	2	0.5	1	8963.363	17926.726	17830.461	35660.922	0.1	162890	21.89	35272	0.0434	1	0.004	15	0.3562
			0.5	2	8867.098	17734.196			0.1					1		15	
	6-1	2	0.5	1	9345.822	18693.644	18738.427	37476.854	0.1	162890	23.01	37088	0.0456	1	0.004	15	0.3745
			0.5	2	9391.605	18783.21			0.1					1		15	
	6-2	2	0.5	1	9276.226	18552.452	18456.787	36917.574	0.1	162890	22.66	36529	0.0449	1	0.004	15	0.3689
			0.5	2	9182.561	18365.122			0.1					1		15	
	6-3	2	0.5	1	9093.671	18187.342	18451.317	36902.634	0.1	162890	22.65	36514	0.0449	1	0.004	15	0.3687
			0.5	2	9357.646	18715.292			0.1					1		15	
	7-1	2	0.5	1	9077.51	18155.02	18739.535	37479.07	0.1	162890	23.01	37090	0.0456	1	0.004	15	0.3745
			0.5	2	9662.025	19324.05			0.1					1		15	
	7-2	2	0.5	1	8829.947	17659.894	17933.965	35867.93	0.1	162890	22.02	35479	0.0436	1	0.004	15	0.3583
			0.5	2	9104.018	18208.036			0.1					1		15	
	7-3	2	0.5	1	8597.065	17194.13	17891.032	35782.064	0.1	162890	21.97	35393	0.0435	1	0.004	15	0.3574
			0.5	2	9293.967	18587.934			0.1					1		15	
	8-1	2	0.5	1	8686.669	17373.338	17672.595	35345.19	0.1	162890	21.70	34956	0.0430	1	0.004	15	0.3530
			0.5	2	8985.926	17971.852			0.1					1		15	
	8-2	2	0.5	1	8687.963	17375.966	17493.625	34987.25	0.1	162890	21.48	34598	0.0425	1	0.004	15	0.3494
			0.5	2	8805.642	17611.284			0.1					1		15	
	8-3	2	0.5	1	10805.42	21610.84	21884.91	43769.82	0.1	162890	26.87	43381	0.0533	1	0.004	15	0.4381
			0.5	2	11079.49	22158.98			0.1					1		15	

Assay Date	7/28/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3207	0.0237
Full activity	End	0.3327	0.0113
Full activity	Overall	0.3267	0.0167
Background	Beginning	-0.0003	3.87507E-05
Background	End	0.0003	0.001304661
Background	Overall	0.0000	0.000821285
Positive	Beginning	0.1625	0.0237
Positive	End	0.1619	0.0085
Positive	Overall	0.1622	0.0145
Negative	Beginning	0.2454	0.0469
Negative	End	0.3195	0.0182
Negative	Overall	0.2824	0.0517

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#2 KCZ	1	1	1.00E-04	-4.00	0.0011
RC#2 KCZ	1	2	1.00E-04	-4.00	0.0010
RC#2 KCZ	1	3	1.00E-04	-4.00	0.0015
RC#2 KCZ	2	1	1.00E-05	-5.00	0.0129
RC#2 KCZ	2	2	1.00E-05	-5.00	0.0139
RC#2 KCZ	2	3	1.00E-05	-5.00	0.0140
RC#2 KCZ	3	1	1.00E-06	-6.00	0.1092
RC#2 KCZ	3	2	1.00E-06	-6.00	0.1037
RC#2 KCZ	3	3	1.00E-06	-6.00	0.1028
RC#2 KCZ	4	1	1.00E-07	-7.00	0.2856
RC#2 KCZ	4	2	1.00E-07	-7.00	0.2741
RC#2 KCZ	4	3	1.00E-07	-7.00	0.2531
RC#2 KCZ	5	1	1.00E-08	-8.00	0.3591
RC#2 KCZ	5	2	1.00E-08	-8.00	0.3554
RC#2 KCZ	5	3	1.00E-08	-8.00	0.3562
RC#2 KCZ	6	1	1.00E-09	-9.00	0.3745
RC#2 KCZ	6	2	1.00E-09	-9.00	0.3689
RC#2 KCZ	6	3	1.00E-09	-9.00	0.3687
RC#2 KCZ	7	1	1.00E-10	-10.00	0.3745
RC#2 KCZ	7	2	1.00E-10	-10.00	0.3583
RC#2 KCZ	7	3	1.00E-10	-10.00	0.3574
RC#2 KCZ	8	1	1.00E-11	-11.00	0.3530
RC#2 KCZ	8	2	1.00E-11	-11.00	0.3494
RC#2 KCZ	8	3	1.00E-11	-11.00	0.4381

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.35	0.31	0.45
2	-5.00	3.96	4.25	4.29
3	-6.00	33.41	31.75	31.45
4	-7.00	87.43	83.89	77.48
5	-8.00	109.90	108.79	109.02
6	-9.00	114.63	112.90	112.86
7	-10.00	114.64	109.66	109.40
8	-11.00	108.04	106.94	134.08

Assay Date	8/18/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	4	Microsome type	Recombina	Microsome ID	5

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0206	28614.04	1389031		
2	0.0205	32009.13	1561421		
3	0.0212	31960.89	1507589		
4	0.0207	32894.49	1589106		
5	0.0207	34755.95	1679031		
			Average DPM/g soln	1545236	
			SD	107127	
			CV	6.93	
			µCi/g soln	0.696	

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

ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)
Stock	10.6	10.6		1000.00
Dilution A			100	10.00
Dilution B			10	1.00

Calculation of concentration nonradiolabeled ASDN in substrate solution

Total g substrate solution	8.0813 g
Mass of dilution B used in substrate prep	4.5482 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562805 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00788 µg/g soln.
µg/g soln.

a. µCi/g soln	0.696
b. Specific activity of [^{3}H]ASDN (µCi/mmol)	25300000
c. Molecular wt of ASDN (mg/mmol)	286.4

Formula=a/b*c

2) Calculate total µg ASDN/g soln.

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

= 0.562805 + 0.00788
= 0.570685 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.220 µCi/µg ASDN

775481 dpm/nmol

Assay Date	8/18/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8					
Technician ID	EJB	Replicate #	4	Microsome type	Recombinant	Microsome ID	5			

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks
	0.559	0.331	0.155	0.101	0.057	0.044	0.035
	0.573	0.342	0.167	0.107	0.062	0.046	0.035
	0.587	0.355	0.176	0.112	0.064	0.051	0.035

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

Samples:	Microsomes	0.01 QC	0.1 QC
	0.057	0.065	0.276
	0.061	0.068	0.273
	0.057		

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	0.573	0.538	0.0436
0.125	62.5	1000	0.00013	200	0.0250	0.342	0.308	0.0248
0.05	25	1000	0.00005	200	0.0100	0.166	0.132	0.0104
0.025	25	2000	0.00003	200	0.0050	0.107	0.072	0.0055
0.01	5	1000	0.00001	200	0.0020	0.061	0.026	0.0018
0.005	5	2000	0.00001	200	0.0010	0.047	0.012	0.0006
			Blank	0.035				

Regression results		
Variables		
m, b	0.082	0.000
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.000
F, df	1709	3
ss_{reg}, ss_{resid}	0.000	0.000

r^2= 0.998
m= 0.082
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
Microsomes	0.057	0.023	0.001	200	70	42000	0.004	0.005	4.624
Microsomes	0.061	0.026	0.002	200	70	42000	0.005		
Microsomes	0.057	0.022	0.001	200	70	42000	0.004		
0.01 QC	0.065	0.030	0.002	200	200	200	0.000	0.000	0.011
0.01 QC	0.068	0.033	0.002	200	200	200	0.000		
0.01 QC									
0.1 QC	0.276	0.242	0.019	200	200	200	0.000	0.000	0.096
0.1 QC	0.273	0.238	0.019	200	200	200	0.000		
0.1 QC									

Assay Date	8/18/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	4

Microsome Dilution Details		
Dilution A	0.07	mL microsome Stock used
	42	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.624413
Protein Concentration (dilution added to assay, mg/mL):	0.007707

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	1.00E-07
5	1.00E-08
6	1.00E-09
7	1.00E-10
8	1.00E-11

Assay Date	8/18/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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 (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	6601.93	13203.86	13174.231	26348.462	1	1545236	1.71	25978	0.0335	1	0.004	15	0.2698
			0.5	2	6572.301	13144.602			1					1		15	
	2	2	0.5	1	6382.175	12764.35	12784.773	25569.546	1	1545236	1.65	25199	0.0325	1	0.004	15	0.2811
			0.5	2	6402.598	12805.196			1					1		15	
	3	2	0.5	1	6604.624	13209.248	13462.443	26924.886	1	1545236	1.74	26555	0.0342	1	0.004	15	0.2962
			0.5	2	6857.819	13715.638			1					1		15	
	4	2	0.5	1	7041.558	14083.116	14007.322	28014.644	1	1545236	1.81	27644	0.0356	1	0.004	15	0.3083
			0.5	2	6965.764	13931.528			1					1		15	
Background control	1	2	0.5	1	74.87177	149.74354	170.55534	341.11068	1	1545236	0.02	-29	0.0000	1	0.004	15	-0.0003
			0.5	2	95.68357	191.36714			1					1		15	
	2	2	0.5	1	94.97284	189.94568	178.47338	356.94676	1	1545236	0.02	-13	0.0000	1	0.004	15	-0.0001
			0.5	2	83.50054	167.00108			1					1		15	
	3	2	0.5	1	84.9534	169.9068	182.63975	365.2795	1	1545236	0.02	-5	0.0000	1	0.004	15	-0.0001
			0.5	2	97.68635	195.3727			1					1		15	
	4	2	0.5	1	97.68635	195.3727	208.94085	417.8817	1	1545236	0.03	48	0.0001	1	0.004	15	0.0005
			0.5	2	111.2545	222.509			1					1		15	
Positive control	1	2	0.5	1	3641.549	7283.098	7370.494	14740.988	1	1545236	0.95	14371	0.0185	1	0.004	15	0.1603
			0.5	2	3728.945	7457.89			1					1		15	
	2	2	0.5	1	3633.261	7266.522	7390.39	14780.78	1	1545236	0.96	14410	0.0186	1	0.004	15	0.1607
			0.5	2	3757.129	7514.258			1					1		15	
	3	2	0.5	1	3573.117	7146.234	6996.657	13993.314	1	1545236	0.91	13623	0.0176	1	0.004	15	0.1520
			0.5	2	3423.54	6847.08			1					1		15	
	4	2	0.5	1	3509.735	7019.47	6999.327	13998.654	1	1545236	0.91	13628	0.0176	1	0.004	15	0.1520
			0.5	2	3489.592	6979.184			1					1		15	
Negative Control	1	2	0.5	1	5508.372	11016.744	10816.274	21632.548	1	1545236	1.40	21262	0.0274	1	0.004	15	0.2372
			0.5	2	5307.902	10615.804			1					1		15	
	2	2	0.5	1	6010.456	12020.912	12243.59	24487.18	1	1545236	1.58	24117	0.0311	1	0.004	15	0.2690
			0.5	2	6233.134	12466.268			1					1		15	
	3	2	0.5	1	6787.822	13575.644	13676.81	27353.62	1	1545236	1.77	26983	0.0348	1	0.004	15	0.3010
			0.5	2	6888.988	13777.976			1					1		15	
	4	2	0.5	1	6243.819	12487.638	12829.542	25659.084	1	1545236	1.66	25289	0.0326	1	0.004	15	0.2821
			0.5	2	6585.723	13171.446			1					1		15	
RC#2 KCZ	1-1	2	0.5	1	123.0244	246.0488	237.8453	475.6906	1	1545236	0.03	105	0.0001	1	0.004	15	0.0012
			0.5	2	114.8209	229.6418			1					1		15	
	1-2	2	0.5	1	92.95025	185.9005	204.45215	408.9043	1	1545236	0.03	39	0.0000	1	0.004	15	0.0004
			0.5	2	111.5019	223.0038			1					1		15	
	1-3	2	0.5	1	143.8168	287.6336	281.8668	563.7336	1	1545236	0.04	193	0.0002	1	0.004	15	0.0022
			0.5	2	138.05	276.1			1					1		15	
	2-1	2	0.5	1	427.841	855.682	772.3037	1544.6074	1	1545236	0.10	1174	0.0015	1	0.004	15	0.0131
			0.5	2	344.4627	688.9254			1					1		15	
	2-2	2	0.5	1	398.6498	797.2996	783.8318	1567.6636	1	1545236	0.10	1197	0.0015	1	0.004	15	0.0134
			0.5	2	385.182	770.364			1					1		15	
	2-3	2	0.5	1	393.6716	787.3432	757.2711	1514.5422	1	1545236	0.10	1144	0.0015	1	0.004	15	0.0126
			0.5	2	363.5995	727.199			1					1		15	
	3-1	2	0.5	1	2290.95	4581.9	4510.46	9020.92	1	1545236	0.58	8651	0.0112	1	0.004	15	0.0965
			0.5	2	2219.51	4439.02			1					1		15	
	3-2	2	0.5	1	2197.955	4395.91	4285.872	8571.744	1	1545236	0.55	8201	0.0106	1	0.004	15	0.0915
			0.5	2	2087.917	4175.834			1					1		15	
	3-3	2	0.5	1	2135.555	4271.11	3923.777	7847.554	1	1545236	0.51	7477	0.0096	1	0.004	15	0.0834
			0.5	2	1788.222	3576.444			1					1		15	
	4-1	2	0.5	1	5560.041	11120.082	11490.497	22980.994	1	1545236	1.49	22611	0.0292	1	0.004	15	0.2522
			0.5	2	5930.456	11860.912			1					1		15	
	4-2	2	0.5	1	5752.702	11505.404	11307.876	22615.752	1	1545236	1.46	22245	0.0287	1	0.004	15	0.2481
			0.5	2	5555.174	11110.348			1					1		15	
	4-3	2	0.5	1	5702.339	11404.678	11466.631	22933.262	1	1545236	1.48	22563	0.0291	1	0.004	15	0.2517
			0.5	2	5764.292	11528.584			1					1		15	
	5-1	2	0.5	1	6607.504	13215.008	13748.308	27496.616	1	1545236	1.78	27126	0.0350	1	0.004	15	0.3026
			0.5	2	7140.804	14281.608			1					1		15	
	5-2	2	0.5	1	6908.669	13817.338	14134.822	28269.644	1	1545236	1.83	27899	0.0360	1	0.004	15	0.3112
			0.5	2	7226.153	14452.306			1					1		15	
	5-3	2	0.5	1	6952.453	13904.906	14039.3	28078.6	1	1545236	1.82	27708	0.0357	1	0.004	15	0.3091
			0.5	2	7086.847	14173.694			1					1		15	
	6-1	2	0.5	1	7116.477	14232.954	13686.545	27373.09	1	1545236	1.77	27003	0.0348	1	0.004	15	0.3012
			0.5	2	6570.068	13140.136			1					1		15	
	6-2	2	0.5	1	7091.595	14183.19	13834.738	27669.476	1	1545236	1.79	27299	0.0352	1	0.004	15	0.3045
			0.5	2	6743.143	13486.286			1					1		15	
	6-3	2	0.5	1	6821.426	13642.852	13678.538	27357.076	1	1545236	1.77	26987	0.0348	1	0.004	15	0.3010
			0.5	2	6857.112	13714.224			1					1		15	
	7-1	2	0.5	1	6876.106	13752.212	13750.159	27500.318	1	1545236	1.78	27130	0.0350	1	0.004	15	0.3026
			0.5	2	6874.053	13748.106			1					1		15	
	7-2	2	0.5	1	7771.574	15543.148	15251.375	30502.75	1	1545236	1.97	30132	0.0389	1	0.004	15	0.3361
			0.5	2	7479.801	14959.602			1					1		15	
	7-3	2	0.5	1	7536.677	15073.354	14768.978	29537.956	1	1545236	1.91	29168	0.0376	1	0.004	15	0.3253
			0.5	2	7232.301	14464.602			1					1		15	
	8-1	2	0.5	1	7053.321	14106.642	13854.975	27709.95	1	1545236	1.79	27340	0.0353	1	0.004	15	0.3049
			0.5	2	6801.654	13603.308			1					1		15	
	8-2	2	0.5	1	7405.987	14811.974	14783.742	29567.484	1	1545236	1.91	29197	0.0377	1	0.004	15	0.3257
			0.5	2	7377.755	14755.51			1					1		15	
	8-3	2	0.5	1	6404.279	12808.558	13199.001	26398.002	1	1545236	1.71	26028	0.0336	1	0.004	15	0.2903
			0.5	2	6794.722	13589.444			1					1		15	

Assay Date	8/18/2005	Test Chemical ID	RC#2 KCZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	4

Control Type	Portion	Average	SD
Full activity	Beginning	0.2854	0.0061
Full activity	End	0.3023	0.0086
Full activity	Overall	0.2938	0.0115
Background	Beginning	-0.0002	0.000124901
Background	End	0.0002	0.000414878
Background	Overall	0.0000	0.000371032
Positive	Beginning	0.1605	0.0003
Positive	End	0.1520	0.0000
Positive	Overall	0.1562	0.0049
Negative	Beginning	0.2531	0.0225
Negative	End	0.2915	0.0134
Negative	Overall	0.2723	0.0269

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#2 KCZ	1	1	1.00E-04	-4.00	0.0012
RC#2 KCZ	1	2	1.00E-04	-4.00	0.0004
RC#2 KCZ	1	3	1.00E-04	-4.00	0.0022
RC#2 KCZ	2	1	1.00E-05	-5.00	0.0131
RC#2 KCZ	2	2	1.00E-05	-5.00	0.0134
RC#2 KCZ	2	3	1.00E-05	-5.00	0.0128
RC#2 KCZ	3	1	1.00E-06	-6.00	0.0965
RC#2 KCZ	3	2	1.00E-06	-6.00	0.0915
RC#2 KCZ	3	3	1.00E-06	-6.00	0.0834
RC#2 KCZ	4	1	1.00E-07	-7.00	0.2522
RC#2 KCZ	4	2	1.00E-07	-7.00	0.2481
RC#2 KCZ	4	3	1.00E-07	-7.00	0.2517
RC#2 KCZ	5	1	1.00E-08	-8.00	0.3026
RC#2 KCZ	5	2	1.00E-08	-8.00	0.3112
RC#2 KCZ	5	3	1.00E-08	-8.00	0.3091
RC#2 KCZ	6	1	1.00E-09	-9.00	0.3012
RC#2 KCZ	6	2	1.00E-09	-9.00	0.3045
RC#2 KCZ	6	3	1.00E-09	-9.00	0.3010
RC#2 KCZ	7	1	1.00E-10	-10.00	0.3026
RC#2 KCZ	7	2	1.00E-10	-10.00	0.3361
RC#2 KCZ	7	3	1.00E-10	-10.00	0.3253
RC#2 KCZ	8	1	1.00E-11	-11.00	0.3049
RC#2 KCZ	8	2	1.00E-11	-11.00	0.3257
RC#2 KCZ	8	3	1.00E-11	-11.00	0.2903

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.40	0.15	0.73
2	-5.00	4.46	4.55	4.34
3	-6.00	32.84	31.13	28.38
4	-7.00	85.83	84.44	85.65
5	-8.00	102.97	105.90	105.18
6	-9.00	102.50	103.63	102.44
7	-10.00	102.98	114.38	110.72
8	-11.00	103.78	110.83	98.80

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

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	EJB	Replicate #	2	Microsome type	Recombinant	Microsome ID	5				

								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	
	0.491	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:	Microsomes	QC 10	QC 100
	0.048	0.061	0.285
	0.053	0.064	0.276
	0.050	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
0.25	125	1000	0.00025	200	0.0500	0.509	0.473	0.0392
0.125	62.5	1000	0.00013	200	0.0250	0.334	0.299	0.0247
0.05	25	1000	0.00005	200	0.0100	0.165	0.130	0.0106
0.025	25	2000	0.00003	200	0.0050	0.104	0.068	0.0055
0.01	5	1000	0.00001	200	0.0020	0.058	0.022	0.0016
0.005	5	2000	0.00001	200	0.0010	0.046	0.010	0.0006
			Blank	0.035				

Variables	Regression results	
m, b	0.083	0.000
se_m, se_b	0.002	0.000
r^2, se_y	0.998	0.001
F, df	1273	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

								r^2=	0.998		
								m=	0.083		
								b=	0.000		

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
Microsomes	0.048	0.013	0.001	200	120	72000	0.002	0.003	3.066
Microsomes	0.053	0.018	0.001	200	120	72000	0.004		
Microsomes	0.050	0.014	0.001	200	120	72000	0.003		
QC 10	0.061	0.026	0.002	200	200	200	0.000	0.000	0.011
QC 10	0.064	0.029	0.002	200	200	200	0.000		
QC 10	0.065	0.030	0.002	200	200	200	0.000		
QC 100	0.285	0.250	0.021	200	200	200	0.000	0.000	0.102
QC 100	0.276	0.241	0.020	200	200	200	0.000		
QC 100	0.290	0.255	0.021	200	200	200	0.000		

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

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.065756
Protein Concentration (dilution added to assay, mg/mL):	0.00511

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	6	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJS	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	6930.284	13860.568	13959.349	27918.698	1	1717129	1.63	27494	0.0319	1	0.003	15	0.4168
			0.5	2	7029.065	14058.13			1					1		15	
	2	2	0.5	1	6990.611	13981.222	13876.703	27753.406	1	1717129	1.62	27329	0.0318	1	0.003	15	0.4143
			0.5	2	6886.092	13772.184			1					1		15	
	3	2	0.5	1	6529.315	13058.63	13237.876	26475.752	1	1717129	1.54	26051	0.0303	1	0.003	15	0.3950
			0.5	2	6708.561	13417.122			1					1		15	
	4	2	0.5	1	6654.736	13309.472	13148.303	26296.606	1	1717129	1.53	25872	0.0301	1	0.003	15	0.3923
			0.5	2	6493.567	12987.134			1					1		15	
Background control	1	2	0.5	1	105.8563	211.7126	209.8366	419.6732	1	1717129	0.02	-5	0.0000	1	0.003	15	-0.0001
			0.5	2	103.9803	207.9606			1					1		15	
	2	2	0.5	1	95.52938	191.05876	191.71163	383.42326	1	1717129	0.02	-41	0.0000	1	0.003	15	-0.0006
			0.5	2	96.18225	192.3645			1					1		15	
	3	2	0.5	1	115.1727	230.3454	200.95687	401.91374	1	1717129	0.02	-23	0.0000	1	0.003	15	-0.0003
			0.5	2	85.78417	171.56834			1					1		15	
	4	2	0.5	1	133.4956	266.9912	247.0981	494.1962	1	1717129	0.03	69	0.0001	1	0.003	15	0.0011
			0.5	2	113.6025	227.205			1					1		15	
Positive control	1	2	0.5	1	3641.026	7282.052	7204.968	14409.936	1	1717129	0.84	13985	0.0163	1	0.003	15	0.2120
			0.5	2	3563.942	7127.884			1					1		15	
	2	2	0.5	1	3652.326	7304.652	7026.837	14053.674	1	1717129	0.82	13629	0.0158	1	0.003	15	0.2066
			0.5	2	3374.511	6749.022			1					1		15	
	3	2	0.5	1	3121.487	6242.974	6137.117	12274.234	1	1717129	0.71	11849	0.0138	1	0.003	15	0.1797
			0.5	2	3015.63	6031.26			1					1		15	
	4	2	0.5	1	3352.98	6705.96	6633.574	13267.148	1	1717129	0.77	12842	0.0149	1	0.003	15	0.1947
			0.5	2	3280.594	6561.188			1					1		15	
Negative Control	1	2	0.5	1	5808.366	11616.732	11865.248	23730.496	1	1717129	1.38	23306	0.0271	1	0.003	15	0.3534
			0.5	2	6055.882	12113.764			1					1		15	
	2	2	0.5	1	5934.009	11868.018	11574.851	23149.702	1	1717129	1.35	22725	0.0264	1	0.003	15	0.3445
			0.5	2	5640.842	11281.684			1					1		15	
	3	2	0.5	1	5567.55	11135.1	11383.769	22767.538	1	1717129	1.33	22343	0.0260	1	0.003	15	0.3388
			0.5	2	5816.219	11632.438			1					1		15	
	4	2	0.5	1	5565.572	11131.144	11332.057	22664.114	1	1717129	1.32	22239	0.0258	1	0.003	15	0.3372
			0.5	2	5766.485	11532.97			1					1		15	
RC#3 PCZ	1-1	2	0.5	1	103.275	206.55	228.052	456.104	1	1717129	0.03	31	0.0000	1	0.003	15	0.0005
			0.5	2	124.777	249.554			1					1		15	
	1-2	2	0.5	1	78.52681	157.05362	186.60241	373.20482	1	1717129	0.02	-52	-0.0001	1	0.003	15	-0.0008
			0.5	2	108.0756	216.1512			1					1		15	
	1-3	2	0.5	1	83.76192	167.52384	176.0216	352.0432	1	1717129	0.02	-73	-0.0001	1	0.003	15	-0.0011
			0.5	2	92.25968	184.51936			1					1		15	
	2-1	2	0.5	1	135.175	270.35	259.952	519.904	1	1717129	0.03	95	0.0001	1	0.003	15	0.0014
			0.5	2	124.777	249.554			1					1		15	
	2-2	2	0.5	1	135.175	270.35	254.7529	509.5058	1	1717129	0.03	85	0.0001	1	0.003	15	0.0013
			0.5	2	119.5779	239.1558			1					1		15	
	2-3	2	0.5	1	112.5557	225.1114	245.1319	490.2638	1	1717129	0.03	65	0.0001	1	0.003	15	0.0010
			0.5	2	132.5762	265.1524			1					1		15	
	3-1	2	0.5	1	217.2587	434.5174	407.0247	814.0494	1	1717129	0.05	389	0.0005	1	0.003	15	0.0059
			0.5	2	189.786	379.532			1					1		15	
	3-2	2	0.5	1	229.3324	458.6648	450.2928	900.5856	1	1717129	0.05	476	0.0006	1	0.003	15	0.0072
			0.5	2	220.9604	441.9208			1					1		15	
	3-3	2	0.5	1	222.4938	444.9876	478.1863	956.3726	1	1717129	0.06	532	0.0006	1	0.003	15	0.0081
			0.5	2	255.8925	511.385			1					1		15	
	4-1	2	0.5	1	1151.594	2303.188	2256.396	4512.792	1	1717129	0.26	4088	0.0048	1	0.003	15	0.0620
			0.5	2	1104.802	2209.604			1					1		15	
	4-2	2	0.5	1	1246.416	2492.832	2432.177	4864.354	1	1717129	0.28	4440	0.0052	1	0.003	15	0.0673
			0.5	2	1186.761	2371.522			1					1		15	
	4-3	2	0.5	1	1149.298	2298.596	2459.392	4918.784	1	1717129	0.29	4494	0.0052	1	0.003	15	0.0681
			0.5	2	1310.094	2620.188			1					1		15	
	5-1	2	0.5	1	4146.257	8292.514	8379.681	16759.362	1	1717129	0.98	16335	0.0190	1	0.003	15	0.2477
			0.5	2	4233.424	8466.848			1					1		15	
	5-2	2	0.5	1	4245.705	8491.41	8423.202	16846.404	1	1717129	0.98	16422	0.0191	1	0.003	15	0.2490
			0.5	2	4177.497	8354.994			1					1		15	
	5-3	2	0.5	1	4010.124	8020.248	8062.129	16124.258	1	1717129	0.94	15699	0.0182	1	0.003	15	0.2380
			0.5	2	4052.005	8104.01			1					1		15	
	6-1	2	0.5	1	5324.146	10648.292	10657.683	21315.366	1	1717129	1.24	20891	0.0243	1	0.003	15	0.3167
			0.5	2	5333.537	10667.074			1					1		15	
	6-2	2	0.5	1	5067.624	10135.248	10164.041	20328.082	1	1717129	1.18	19903	0.0231	1	0.003	15	0.3018
			0.5	2	5096.417	10192.834			1					1		15	
	6-3	2	0.5	1	5083.329	10166.658	9999.133	19998.266	1	1717129	1.16	19573	0.0227	1	0.003	15	0.2968
			0.5	2	4915.804	9831.608			1					1		15	
	7-1	2	0.5	1	5873.836	11747.672	12156.014	24312.028	1	1717129	1.42	23887	0.0278	1	0.003	15	0.3622
			0.5	2	6282.178	12564.356			1					1		15	
	7-2	2	0.5	1	5848.573	11697.146	11730.262	23460.524	1	1717129	1.37	23036	0.0268	1	0.003	15	0.3493
			0.5	2	5881.689	11763.378			1					1		15	
	7-3	2	0.5	1	5797.276	11594.552	11883.136	23766.272	1	1717129	1.38	23341	0.0271	1	0.003	15	0.3539
			0.5	2	6085.86	12171.72			1					1		15	
	8-1	2	0.5	1	6518.875	13037.75	12975.436	25950.872	1	1717129	1.51	25526	0.0297	1	0.003	15	0.3870
			0.5	2	6456.561	12913.122			1					1		15	
	8-2	2	0.5	1	6221.004	12442.008	12631.477	25262.954	1	1717129	1.47	24838	0.0289	1	0.003	15	0.3766
			0.5	2	6410.473	12820.946			1					1		15	
	8-3	2	0.5	1	5861.877	11723.754	11978.404	23956.808	1	1717129	1.40	23532	0.0273	1	0.003	15	0.3568
			0.5	2	6116.527	12233.054			1					1		15	

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

Control Type	Portion	Average	SD
Full activity	Beginning	0.4156	0.0018
Full activity	End	0.3936	0.0019
Full activity	Overall	0.4046	0.0128
Background	Beginning	-0.0004	0.000388629
Background	End	0.0004	0.000989344
Background	Overall	0.0000	0.000736437
Positive	Beginning	0.2093	0.0038
Positive	End	0.1872	0.0106
Positive	Overall	0.1983	0.0144
Negative	Beginning	0.3489	0.0062
Negative	End	0.3380	0.0011
Negative	Overall	0.3435	0.0073

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	-0.0008
RC#3 PCZ	1	3	1.00E-04	-4.00	-0.0011
RC#3 PCZ	2	1	1.00E-05	-5.00	0.0014
RC#3 PCZ	2	2	1.00E-05	-5.00	0.0013
RC#3 PCZ	2	3	1.00E-05	-5.00	0.0010
RC#3 PCZ	3	1	1.00E-06	-6.00	0.0059
RC#3 PCZ	3	2	1.00E-06	-6.00	0.0072
RC#3 PCZ	3	3	1.00E-06	-6.00	0.0081
RC#3 PCZ	4	1	1.00E-07	-7.00	0.0620
RC#3 PCZ	4	2	1.00E-07	-7.00	0.0673
RC#3 PCZ	4	3	1.00E-07	-7.00	0.0681
RC#3 PCZ	5	1	1.00E-08	-8.00	0.2477
RC#3 PCZ	5	2	1.00E-08	-8.00	0.2490
RC#3 PCZ	5	3	1.00E-08	-8.00	0.2380
RC#3 PCZ	6	1	3.30E-09	-8.48	0.3167
RC#3 PCZ	6	2	3.30E-09	-8.48	0.3018
RC#3 PCZ	6	3	3.30E-09	-8.48	0.2968
RC#3 PCZ	7	1	1.00E-09	-9.00	0.3622
RC#3 PCZ	7	2	1.00E-09	-9.00	0.3493
RC#3 PCZ	7	3	1.00E-09	-9.00	0.3539
RC#3 PCZ	8	1	1.00E-10	-10.00	0.3870
RC#3 PCZ	8	2	1.00E-10	-10.00	0.3766
RC#3 PCZ	8	3	1.00E-10	-10.00	0.3568

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.12	-0.19	-0.27
2	-5.00	0.36	0.32	0.25
3	-6.00	1.46	1.78	1.99
4	-7.00	15.32	16.64	16.84
5	-8.00	61.21	61.54	58.83
6	-8.48	78.28	74.58	73.35
7	-9.00	89.51	86.32	87.47
8	-10.00	95.65	93.07	88.18

Assay Date	8/8/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	3	Microsome type	Recombina	Microsome ID	5

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	EJB	Replicate #	3	Microsome type	Recombinant	Microsome ID	5		

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
	0.448	0.301	0.163	0.110	0.068	0.061	0.039	2	1	
	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:	Microsomes	QC 10	QC 100
	0.055	0.067	0.256
	0.057	0.070	0.245
	0.054	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	0.0410
0.125	62.5	1000	0.00013	200	0.0250	0.291	0.251	0.0240
0.05	25	1000	0.00005	200	0.0100	0.171	0.132	0.0119
0.025	25	2000	0.00003	200	0.0050	0.110	0.070	0.0056
0.01	5	1000	0.00001	200	0.0020	0.069	0.029	0.0015
0.005	5	2000	0.00001	200	0.0010	0.056	0.016	0.0001
			Blank	0.040				

Variables	Regression results	
m, b	0.102	-0.002
se_m, se_b	0.007	0.001
r^2, se_y	0.984	0.001
F, df	190	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

r^2= 0.984
m= 0.102
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	0.055	0.016	0.000	200	120	72000	0.000	0.000	0.142
Microsomes	0.057	0.017	0.000	200	120	72000	0.001		
Microsomes	0.054	0.014	0.000	200	120	72000	0.000		
QC 10	0.067	0.027	0.001	200	200	200	0.000	0.000	0.006
QC 10	0.070	0.030	0.002	200	200	200	0.000		
QC 10	0.063	0.023	0.001	200	200	200	0.000		
QC 100	0.256	0.216	0.020	200	200	200	0.000	0.000	0.097
QC 100	0.245	0.205	0.019	200	200	200	0.000		
QC 100	0.239	0.199	0.019	200	200	200	0.000		

Assay Date	8/8/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	3

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	0.14221
Protein Concentration (dilution added to assay, mg/mL):	0.000237

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	6126.116	12252.232	12260.034	24520.068	1	1716832	1.43	24205	0.0283	1	0.000	15	7.9478
			0.5	2	6133.918	12267.836			1					1		15	
	2	2	0.5	1	6207.726	12415.452	12133.402	24266.804	1	1716832	1.41	23952	0.0280	1	0.000	15	7.8646
			0.5	2	5925.676	11851.352			1					1		15	
	3	2	0.5	1	2628.017	5256.034	5152.726	10305.452	1	1716832	0.60	9991	0.0117	1	0.000	15	3.2804
			0.5	2	2524.709	5049.418			1					1		15	
	4	2	0.5	1	5177.507	10355.014	10601.251	21202.502	1	1716832	1.23	20888	0.0244	1	0.000	15	6.8585
			0.5	2	5423.744	10847.488			1					1		15	
Background control	1	2	0.5	1	78.52639	157.05278	150.20582	300.41164	1	1716832	0.02	-14	0.0000	1	0.000	15	-0.0047
			0.5	2	71.67943	143.35886			1					1		15	
	2	2	0.5	1	69.02464	138.04928	142.83199	285.66398	1	1716832	0.02	-29	0.0000	1	0.000	15	-0.0096
			0.5	2	73.80735	147.6147			1					1		15	
	3	2	0.5	1	92.91779	185.83558	166.20909	332.41818	1	1716832	0.02	18	0.0000	1	0.000	15	0.0058
			0.5	2	73.2913	146.5826			1					1		15	
	4	2	0.5	1	73.2913	146.5826	170.26018	340.52036	1	1716832	0.02	26	0.0000	1	0.000	15	0.0085
			0.5	2	96.96888	193.93776			1					1		15	
Positive control	1	2	0.5	1	3514.966	7029.932	6611.54	13223.08	1	1716832	0.77	12908	0.0151	1	0.000	15	4.2384
			0.5	2	3096.574	6193.148			1					1		15	
	2	2	0.5	1	3269.333	6538.666	6624.949	13249.898	1	1716832	0.77	12935	0.0151	1	0.000	15	4.2472
			0.5	2	3355.616	6711.232			1					1		15	
	3	2	0.5	1	3143.69	6287.38	6061.031	12122.062	1	1716832	0.71	11807	0.0138	1	0.000	15	3.8769
			0.5	2	2917.341	5834.682			1					1		15	
	4	2	0.5	1	3163.189	6326.378	6291.776	12583.552	1	1716832	0.73	12269	0.0143	1	0.000	15	4.0284
			0.5	2	3128.587	6257.174			1					1		15	
Negative Control	1	2	0.5	1	6039.055	12078.11	11786.714	23573.428	1	1716832	1.37	23259	0.0272	1	0.000	15	7.6370
			0.5	2	5747.659	11495.318			1					1		15	
	2	2	0.5	1	5513.583	11027.166	10970.084	21940.168	1	1716832	1.28	21625	0.0252	1	0.000	15	7.1007
			0.5	2	5456.501	10913.002			1					1		15	
	3	2	0.5	1	5094.576	10189.152	10419.784	20839.568	1	1716832	1.21	20525	0.0240	1	0.000	15	6.7393
			0.5	2	5325.208	10650.416			1					1		15	
	4	2	0.5	1	4576.889	9153.778	9177.671	18355.342	1	1716832	1.07	18041	0.0211	1	0.000	15	5.9236
			0.5	2	4600.782	9201.564			1					1		15	
RC#3 PCZ	1-1	2	0.5	1	130.0856	260.1712	236.2779	472.5558	1	1716832	0.03	158	0.0002	1	0.000	15	0.0518
			0.5	2	106.1923	212.3846			1					1		15	
	1-2	2	0.5	1	106.9602	213.9204	170.67559	341.35118	1	1716832	0.02	27	0.0000	1	0.000	15	0.0087
			0.5	2	63.71539	127.43078			1					1		15	
	1-3	2	0.5	1	92.91828	185.83656	212.38468	424.76936	1	1716832	0.02	110	0.0001	1	0.000	15	0.0361
			0.5	2	119.4664	238.9328			1					1		15	
	2-1	2	0.5	1	76.98943	153.97886	198.24503	396.49006	1	1716832	0.02	82	0.0001	1	0.000	15	0.0268
			0.5	2	121.2556	242.5112			1					1		15	
	2-2	2	0.5	1	122.1086	244.2172	236.2653	472.5306	1	1716832	0.03	158	0.0002	1	0.000	15	0.0518
			0.5	2	114.1567	228.3134			1					1		15	
	2-3	2	0.5	1	100.8827	201.7654	199.11059	398.22118	1	1716832	0.02	83	0.0001	1	0.000	15	0.0274
			0.5	2	98.22789	196.45578			1					1		15	
	3-1	2	0.5	1	256.7046	513.4092	449.0691	898.1382	1	1716832	0.05	583	0.0007	1	0.000	15	0.1916
			0.5	2	192.3645	384.729			1					1		15	
	3-2	2	0.5	1	191.1462	382.2924	354.916	709.832	1	1716832	0.04	395	0.0005	1	0.000	15	0.1297
			0.5	2	163.7698	327.5396			1					1		15	
	3-3	2	0.5	1	192.4264	384.8528	395.4008	790.8016	1	1716832	0.05	476	0.0006	1	0.000	15	0.1563
			0.5	2	202.9724	405.9448			1					1		15	
	4-1	2	0.5	1	972.2257	1944.4514	2060.7027	4121.4054	1	1716832	0.24	3807	0.0044	1	0.000	15	1.2499
			0.5	2	1088.477	2176.954			1					1		15	
	4-2	2	0.5	1	1097.146	2194.292	2196.168	4392.336	1	1716832	0.26	4078	0.0048	1	0.000	15	1.3389
			0.5	2	1099.022	2198.044			1					1		15	
	4-3	2	0.5	1	1101.751	2203.502	2153.517	4307.034	1	1716832	0.25	3992	0.0047	1	0.000	15	1.3109
			0.5	2	1051.766	2103.532			1					1		15	
	5-1	2	0.5	1	4176.82	8353.64	8128.191	16256.382	1	1716832	0.95	15942	0.0186	1	0.000	15	5.2344
			0.5	2	3951.371	7902.742			1					1		15	
	5-2	2	0.5	1	3653.503	7307.006	7327.777	14655.554	1	1716832	0.85	14341	0.0167	1	0.000	15	4.7088
			0.5	2	3674.274	7348.548			1					1		15	
	5-3	2	0.5	1	3701.736	7403.472	7798.126	15596.252	1	1716832	0.91	15281	0.0178	1	0.000	15	5.0177
			0.5	2	4096.39	8192.78			1					1		15	
	6-1	2	0.5	1	5373.36	10746.72	10482.865	20965.73	1	1716832	1.22	20651	0.0241	1	0.000	15	6.7807
			0.5	2	5109.505	10219.01			1					1		15	
	6-2	2	0.5	1	5000.418	10000.836	9965.528	19931.056	1	1716832	1.16	19616	0.0229	1	0.000	15	6.4410
			0.5	2	4965.11	9930.22			1					1		15	
	6-3	2	0.5	1	4816.336	9632.672	9693.244	19386.488	1	1716832	1.13	19072	0.0223	1	0.000	15	6.2622
			0.5	2	4876.908	9753.816			1					1		15	
	7-1	2	0.5	1	5709.586	11419.172	11237.902	22475.804	1	1716832	1.31	22161	0.0259	1	0.000	15	7.2765
			0.5	2	5528.316	11056.632			1					1		15	
	7-2	2	0.5	1	5540.613	11081.226	11052.024	22104.048	1	1716832	1.29	21789	0.0254	1	0.000	15	7.1545
			0.5	2	5511.411	11022.822			1					1		15	
	7-3	2	0.5	1	5377.45	10754.9	10701.296	21402.592	1	1716832	1.25	21088	0.0246	1	0.000	15	6.9242
			0.5	2	5323.846	10647.692			1					1		15	
	8-1	2	0.5	1	5596.365	11192.73	11216.623	22433.246	1	1716832	1.31	22118	0.0258	1	0.000	15	7.2626
			0.5	2	5620.258	11240.516			1					1		15	
	8-2	2	0.5	1	5923.103	11846.206	11425.244	22850.488	1	1716832	1.33	22536	0.0263	1	0.000	15	7.3996
			0.5	2	5502.141	11004.282			1					1		15	
	8-3	2	0.5	1	5910.482	11820.964	11575.256	23150.512	1	1716832	1.35	22836	0.0267	1	0.000	15	7.4981
			0.5	2	5664.774	11329.548			1					1		15	

Assay Date	8/8/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	7.9062	0.0588
Full activity	End	5.0694	2.5300
Full activity	Overall	6.4878	2.1948
Background	Beginning	-0.0071	0.003424075
Background	End	0.0071	0.001881144
Background	Overall	0.0000	0.00853677
Positive	Beginning	4.2428	0.0062
Positive	End	3.9527	0.1071
Positive	Overall	4.0978	0.1786
Negative	Beginning	7.3688	0.3792
Negative	End	6.3314	0.5768
Negative	Overall	6.8501	0.7194

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#3 PCZ	1	1	1.00E-04	-4.00	0.0518
RC#3 PCZ	1	2	1.00E-04	-4.00	0.0087
RC#3 PCZ	1	3	1.00E-04	-4.00	0.0361
RC#3 PCZ	2	1	1.00E-05	-5.00	0.0268
RC#3 PCZ	2	2	1.00E-05	-5.00	0.0518
RC#3 PCZ	2	3	1.00E-05	-5.00	0.0274
RC#3 PCZ	3	1	1.00E-06	-6.00	0.1916
RC#3 PCZ	3	2	1.00E-06	-6.00	0.1297
RC#3 PCZ	3	3	1.00E-06	-6.00	0.1563
RC#3 PCZ	4	1	1.00E-07	-7.00	1.2499
RC#3 PCZ	4	2	1.00E-07	-7.00	1.3389
RC#3 PCZ	4	3	1.00E-07	-7.00	1.3109
RC#3 PCZ	5	1	1.00E-08	-8.00	5.2344
RC#3 PCZ	5	2	1.00E-08	-8.00	4.7088
RC#3 PCZ	5	3	1.00E-08	-8.00	5.0177
RC#3 PCZ	6	1	3.30E-09	-8.48	6.7807
RC#3 PCZ	6	2	3.30E-09	-8.48	6.4410
RC#3 PCZ	6	3	3.30E-09	-8.48	6.2622
RC#3 PCZ	7	1	1.00E-09	-9.00	7.2765
RC#3 PCZ	7	2	1.00E-09	-9.00	7.1545
RC#3 PCZ	7	3	1.00E-09	-9.00	6.9242
RC#3 PCZ	8	1	1.00E-10	-10.00	7.2626
RC#3 PCZ	8	2	1.00E-10	-10.00	7.3996
RC#3 PCZ	8	3	1.00E-10	-10.00	7.4981

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.80	0.13	0.56
2	-5.00	0.41	0.80	0.42
3	-6.00	2.95	2.00	2.41
4	-7.00	19.27	20.64	20.20
5	-8.00	80.68	72.58	77.34
6	-8.48	104.51	99.28	96.52
7	-9.00	112.16	110.28	106.73
8	-10.00	111.94	114.05	115.57

Assay Date	8/15/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	4	Microsome type	Recombina	Microsome ID	5

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	EJB	Replicate #	4	Microsome type	Recombinant	Microsome ID	5		

								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:	Mic 0.008	QC 10	QC 100	Mic 0.08
	0.069	0.086	0.306	0.229
	0.067	0.082	0.319	0.214
	0.066	0.080	0.312	0.230

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

r^2= 0.981
m= 0.087
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
Mic 0.008	0.069	0.011	-0.001	200	120	72000	-0.003	-0.004	-3.643
Mic 0.008	0.067	0.008	-0.001	200	120	72000	-0.004		
Mic 0.008	0.066	0.008	-0.001	200	120	72000	-0.004		
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		
Mic 0.08	0.229	0.170	0.013	200	140	8540	0.004	0.004	3.771
Mic 0.08	0.214	0.155	0.011	200	140	8540	0.003		
Mic 0.08	0.230	0.171	0.013	200	140	8540	0.004		

Assay Date	8/15/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	Recombina Microsome ID	5	Technician ID	EJB	Replicate #	4

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.771282
Protein Concentration (dilution added to assay, mg/mL):	0.006182

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	9388.386	18776.772	18882.956	37765.912	1	1563166	2.42	37365	0.0476	1	0.003	15	0.5131
			0.5	2	9494.57	18989.14			1					1		15	
	2	2	0.5	1	9432.159	18864.318	19050.883	38101.766	1	1563166	2.44	37701	0.0480	1	0.003	15	0.5177
			0.5	2	9618.724	19237.448			1					1		15	
	3	2	0.5	1	8924.733	17849.466	18047.84	36095.68	1	1563166	2.31	35695	0.0455	1	0.003	15	0.4902
			0.5	2	9123.107	18246.214			1					1		15	
	4	2	0.5	1	9204.225	18408.45	18523.805	37047.61	1	1563166	2.37	36647	0.0467	1	0.003	15	0.5033
			0.5	2	9319.58	18639.16			1					1		15	
Background control	1	2	0.5	1	113.9674	227.9348	222.5078	445.0156	1	1563166	0.03	45	0.0001	1	0.003	15	0.0006
			0.5	2	108.5404	217.0808			1					1		15	
	2	2	0.5	1	105.0491	210.0982	220.8724	441.7448	1	1563166	0.03	41	0.0001	1	0.003	15	0.0006
			0.5	2	115.8233	231.6466			1					1		15	
	3	2	0.5	1	78.11341	156.22682	179.26461	358.52922	1	1563166	0.02	-42	-0.0001	1	0.003	15	-0.0006
			0.5	2	101.1512	202.3024			1					1		15	
	4	2	0.5	1	81.40529	162.81058	178.25451	356.50902	1	1563166	0.02	-44	-0.0001	1	0.003	15	-0.0006
			0.5	2	96.84922	193.69844			1					1		15	
Positive control	1	2	0.5	1	4571.006	9142.012	9213.05	18426.1	1	1563166	1.18	18026	0.0230	1	0.003	15	0.2475
			0.5	2	4642.044	9284.088			1					1		15	
	2	2	0.5	1	4218.49	8436.98	8746.398	17492.796	1	1563166	1.12	17092	0.0218	1	0.003	15	0.2347
			0.5	2	4527.908	9055.816			1					1		15	
	3	2	0.5	1	4495.585	8991.17	9170.987	18341.974	1	1563166	1.17	17942	0.0228	1	0.003	15	0.2464
			0.5	2	4675.402	9350.804			1					1		15	
	4	2	0.5	1	4471.343	8942.686	9099.718	18199.436	1	1563166	1.16	17799	0.0227	1	0.003	15	0.2444
			0.5	2	4628.373	9256.75			1					1		15	
Negative Control	1	2	0.5	1	8127.004	16254.008	16242.761	32485.522	1	1563166	2.08	32085	0.0409	1	0.003	15	0.4406
			0.5	2	8115.757	16231.514			1					1		15	
	2	2	0.5	1	7942.485	15884.97	15796.78	31593.56	1	1563166	2.02	31193	0.0397	1	0.003	15	0.4284
			0.5	2	7854.295	15708.59			1					1		15	
	3	2	0.5	1	7614.148	15228.296	15401.962	30803.924	1	1563166	1.97	30403	0.0387	1	0.003	15	0.4175
			0.5	2	7787.814	15575.628			1					1		15	
	4	2	0.5	1	7627.716	15255.432	15345.32	30690.64	1	1563166	1.96	30290	0.0386	1	0.003	15	0.4160
			0.5	2	7717.604	15435.208			1					1		15	
RC#3 PCZ	1-1	2	0.5	1	104.2862	208.5724	210.9056	421.8112	1	1563166	0.03	21	0.0000	1	0.003	15	0.0003
			0.5	2	106.8194	213.2388			1					1		15	
	1-2	2	0.5	1	131.9854	263.9708	309.6844	619.3688	1	1563166	0.04	219	0.0003	1	0.003	15	0.0030
			0.5	2	177.699	355.398			1					1		15	
	1-3	2	0.5	1	130.4487	260.8974	263.4114	526.8228	1	1563166	0.03	126	0.0002	1	0.003	15	0.0017
			0.5	2	132.9627	265.9254			1					1		15	
	2-1	2	0.5	1	199.3249	398.6498	338.7503	677.5006	1	1563166	0.04	277	0.0004	1	0.003	15	0.0038
			0.5	2	139.4254	278.8508			1					1		15	
	2-2	2	0.5	1	117.5547	235.1094	252.9499	505.8998	1	1563166	0.03	105	0.0001	1	0.003	15	0.0014
			0.5	2	135.3952	270.7904			1					1		15	
	2-3	2	0.5	1	99.66246	199.32492	222.68486	445.36972	1	1563166	0.03	45	0.0001	1	0.003	15	0.0006
			0.5	2	123.0224	246.0448			1					1		15	
	3-1	2	0.5	1	382.6069	765.2138	713.6569	1427.3138	1	1563166	0.09	1027	0.0013	1	0.003	15	0.0141
			0.5	2	331.05	662.1			1					1		15	
	3-2	2	0.5	1	297.9875	595.975	690.8991	1381.7982	1	1563166	0.09	981	0.0012	1	0.003	15	0.0135
			0.5	2	392.9116	785.8232			1					1		15	
	3-3	2	0.5	1	377.1819	754.3638	708.494	1416.988	1	1563166	0.09	1017	0.0013	1	0.003	15	0.0140
			0.5	2	331.3121	662.6242			1					1		15	
	4-1	2	0.5	1	2020.196	4040.392	3971.23	7942.46	1	1563166	0.51	7542	0.0096	1	0.003	15	0.1036
			0.5	2	1951.034	3902.068			1					1		15	
	4-2	2	0.5	1	2118.217	4236.434	4190.472	8380.944	1	1563166	0.54	7980	0.0102	1	0.003	15	0.1096
			0.5	2	2072.255	4144.51			1					1		15	
	4-3	2	0.5	1	1774.654	3549.308	3558.226	7116.452	1	1563166	0.46	6716	0.0086	1	0.003	15	0.0922
			0.5	2	1783.572	3567.144			1					1		15	
	5-1	2	0.5	1	8267.148	16534.296	16131.442	32262.884	1	1563166	2.06	31862	0.0406	1	0.003	15	0.4376
			0.5	2	7864.294	15728.588			1					1		15	
	5-2	2	0.5	1	7698.309	15396.618	15528.588	31057.176	1	1563166	1.99	30657	0.0390	1	0.003	15	0.4210
			0.5	2	7830.279	15660.558			1					1		15	
	5-3	2	0.5	1	7126.264	14252.528	14648.283	29296.566	1	1563166	1.87	28896	0.0368	1	0.003	15	0.3968
			0.5	2	7522.019	15044.038			1					1		15	
	6-1	2	0.5	1	6108.175	12216.35	12094.241	24188.482	1	1563166	1.55	23788	0.0303	1	0.003	15	0.3267
			0.5	2	5980.066	11972.132			1					1		15	
	6-2	2	0.5	1	6059.332	12118.664	12153.939	24307.678	1	1563166	1.56	23907	0.0304	1	0.003	15	0.3283
			0.5	2	6094.607	12189.214			1					1		15	
	6-3	2	0.5	1	6224.896	12449.792	12254.812	24509.624	1	1563166	1.57	24109	0.0307	1	0.003	15	0.3311
			0.5	2	6029.916	12059.832			1					1		15	
	7-1	2	0.5	1	8064.622	16129.244	16015.29	32030.58	1	1563166	2.05	31630	0.0403	1	0.003	15	0.4344
			0.5	2	7950.668	15901.336			1					1		15	
	7-2	2	0.5	1	8127.048	16254.096	16399.486	32798.972	1	1563166	2.10	32399	0.0413	1	0.003	15	0.4449
			0.5	2	8272.438	16544.876			1					1		15	
	7-3	2	0.5	1	7954.185	15908.37	15940.693	31881.386	1	1563166	2.04	31481	0.0401	1	0.003	15	0.4323
			0.5	2	7986.508	15973.016			1					1		15	
	8-1	2	0.5	1	8429.251	16858.502	17012.173	34024.346	1	1563166	2.18	33624	0.0428	1	0.003	15	0.4617
			0.5	2	8582.922	17165.844			1					1		15	
	8-2	2	0.5	1	8450.315	16900.63	17005.171	34010.342	1	1563166	2.16	33610	0.0428	1	0.003	15	0.4616
			0.5	2	8554.856	17109.712			1					1		15	
	8-3	2	0.5	1	7986.508	15973.016	15754.537	31509.074	1	1563166	2.02	31109	0.0396	1	0.003	15	0.4272
			0.5	2	7768.029	15536.058			1					1		15	

Assay Date	8/15/2005	Test Chemical ID	RC#3 PCZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	4

Control Type	Portion	Average	SD
Full activity	Beginning	0.5154	0.0033
Full activity	End	0.4967	0.0092
Full activity	Overall	0.5061	0.0122
Background	Beginning	0.0006	3.17611E-05
Background	End	-0.0006	1.96172E-05
Background	Overall	0.0000	0.000681099
Positive	Beginning	0.2411	0.0091
Positive	End	0.2454	0.0014
Positive	Overall	0.2433	0.0058
Negative	Beginning	0.4345	0.0087
Negative	End	0.4167	0.0011
Negative	Overall	0.4256	0.0114

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	0.0030
RC#3 PCZ	1	3	1.00E-04	-4.00	0.0017
RC#3 PCZ	2	1	1.00E-05	-5.00	0.0038
RC#3 PCZ	2	2	1.00E-05	-5.00	0.0014
RC#3 PCZ	2	3	1.00E-05	-5.00	0.0006
RC#3 PCZ	3	1	1.00E-06	-6.00	0.0141
RC#3 PCZ	3	2	1.00E-06	-6.00	0.0135
RC#3 PCZ	3	3	1.00E-06	-6.00	0.0140
RC#3 PCZ	4	1	1.00E-07	-7.00	0.1036
RC#3 PCZ	4	2	1.00E-07	-7.00	0.1096
RC#3 PCZ	4	3	1.00E-07	-7.00	0.0922
RC#3 PCZ	5	1	3.30E-08	-7.48	0.4376
RC#3 PCZ	5	2	3.30E-08	-7.48	0.4210
RC#3 PCZ	5	3	3.30E-08	-7.48	0.3968
RC#3 PCZ	6	1	1.00E-08	-8.00	0.3267
RC#3 PCZ	6	2	1.00E-08	-8.00	0.3283
RC#3 PCZ	6	3	1.00E-08	-8.00	0.3311
RC#3 PCZ	7	1	1.00E-09	-9.00	0.4344
RC#3 PCZ	7	2	1.00E-09	-9.00	0.4449
RC#3 PCZ	7	3	1.00E-09	-9.00	0.4323
RC#3 PCZ	8	1	1.00E-10	-10.00	0.4617
RC#3 PCZ	8	2	1.00E-10	-10.00	0.4616
RC#3 PCZ	8	3	1.00E-10	-10.00	0.4272

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	0.06	0.59	0.34
2	-5.00	0.75	0.29	0.12
3	-6.00	2.79	2.66	2.76
4	-7.00	20.47	21.66	18.22
5	-7.48	86.46	83.19	78.41
6	-8.00	64.55	64.87	65.42
7	-9.00	85.83	87.91	85.42
8	-10.00	91.24	91.20	84.41

Assay Date	9/19/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8
Technician ID	EJB	Replicate #	1	Microsome type	Recombina Microsome ID 5

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#4 NYP		# Concentrations tested	8				
Technician ID	EJB	Replicate #	1		Microsome type	Recombinant	Microsome ID	5		

								Protein stock (mg	Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks	BSA)	stock (mL)	Protein stock ID
	0.597	0.368	0.182	0.103	0.055	0.041	0.030	2	1	
	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:	Mics 0.008	QC 10	QC100	Mics 0.08
	0.121	0.063	0.304	0.238
	0.110	0.052	0.312	0.239
	0.113	0.055	0.316	0.236

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	0.598	0.567	0.0476
0.125	62.35	1000	0.00012	200	0.0249	0.373	0.342	0.0282
0.05	25	1000	0.00005	200	0.0100	0.183	0.151	0.0117
0.025	25	2000	0.00003	200	0.0050	0.109	0.078	0.0053
0.01	5	1000	0.00001	200	0.0020	0.053	0.022	0.0005
0.005	5	2000	0.00001	200	0.0010	0.042	0.011	-0.0004

Variables	Regression results	
m, b	0.087	-0.001
se_m, se_b	0.005	0.001
r^2, se_y	0.987	0.002
F, df	306	4
ss_{reg}, ss_{resid}	0.002	0.000

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
Mics 0.008	0.121	0.090	0.006	200	120	72000	0.019	0.017	17.464
Mics 0.008	0.110	0.079	0.005	200	120	72000	0.016		
Mics 0.008	0.113	0.082	0.006	200	120	72000	0.017		
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		
Mics 0.08	0.238	0.206	0.016	200	140	8540	0.005	0.005	5.021
Mics 0.08	0.239	0.208	0.017	200	140	8540	0.005		
Mics 0.08	0.236	0.205	0.016	200	140	8540	0.005		

Assay Date	9/19/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Recombine	Microsome ID	5	Technician ID EJB	Replicate # 1

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.021487
Protein Concentration (dilution added to assay, mg/mL):	0.008232

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	9/19/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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	10211.31	20422.62	20289.95	40579.9	1	1669334	2.43	40241	0.0479	1	0.004	15	0.3882
			0.5	2	10078.64	20157.28			1					1		15	
	2	2	0.5	1	10373.75	20747.5	20495.14	40990.28	1	1669334	2.46	40652	0.0484	1	0.004	15	0.3921
			0.5	2	10121.39	20242.78			1					1		15	
	3	2	0.5	1	9304.625	18609.25	18435.813	36871.626	1	1669334	2.21	36533	0.0435	1	0.004	15	0.3524
			0.5	2	9131.188	18262.376			1					1		15	
	4	2	0.5	1	9081.737	18163.474	18691.101	37382.202	1	1669334	2.24	37043	0.0441	1	0.004	15	0.3573
			0.5	2	9609.364	19218.728			1					1		15	
Background control	1	2	0.5	1	82.01449	164.02898	176.28929	352.57858	1	1669334	0.02	14	0.0000	1	0.004	15	0.0001
			0.5	2	94.2748	188.5496			1					1		15	
	2	2	0.5	1	97.68635	195.3727	182.43466	364.86932	1	1669334	0.02	26	0.0000	1	0.004	15	0.0003
			0.5	2	84.74831	169.49662			1					1		15	
	3	2	0.5	1	56.9837	113.9674	138.38899	276.77798	1	1669334	0.02	-62	-0.0001	1	0.004	15	-0.0006
			0.5	2	81.40529	162.81058			1					1		15	
	4	2	0.5	1	87.48212	174.96424	180.43237	360.86474	1	1669334	0.02	22	0.0000	1	0.004	15	0.0002
			0.5	2	92.95025	185.9005			1					1		15	
Positive control	1	2	0.5	1	5451.258	10902.516	10734.489	21468.978	1	1669334	1.29	21130	0.0252	1	0.004	15	0.2038
			0.5	2	5283.231	10566.462			1					1		15	
	2	2	0.5	1	4673.949	9347.898	9357.001	18714.002	1	1669334	1.12	18375	0.0219	1	0.004	15	0.1772
			0.5	2	4683.052	9366.104			1					1		15	
	3	2	0.5	1	5024.781	10049.562	9966.109	19932.218	1	1669334	1.19	19593	0.0233	1	0.004	15	0.1890
			0.5	2	4941.328	9882.656			1					1		15	
	4	2	0.5	1	4889.226	9778.452	9903.071	19806.142	1	1669334	1.19	19467	0.0232	1	0.004	15	0.1878
			0.5	2	5013.845	10027.69			1					1		15	
Negative Control	1	2	0.5	1	7934.344	15868.688	15988.209	31976.418	1	1669334	1.92	31638	0.0377	1	0.004	15	0.3052
			0.5	2	8053.865	16107.73			1					1		15	
	2	2	0.5	1	7326.953	14653.906	14823.117	29646.234	1	1669334	1.78	29307	0.0349	1	0.004	15	0.2827
			0.5	2	7496.164	14992.328			1					1		15	
	3	2	0.5	1	8444.487	16888.974	17121.141	34242.282	1	1669334	2.05	33904	0.0404	1	0.004	15	0.3270
			0.5	2	8676.654	17353.308			1					1		15	
	4	2	0.5	1	7932.941	15865.882	16320.335	32640.67	1	1669334	1.96	32302	0.0385	1	0.004	15	0.3116
			0.5	2	8387.394	16774.788			1					1		15	
RC#4 NYP	1-1	2	0.5	1	838.4789	1676.9578	1781.6505	3563.301	1	1669334	0.21	3225	0.0038	1	0.004	15	0.0311
			0.5	2	943.1716	1886.3432			1					1		15	
	1-2	2	0.5	1	779.5217	1559.0434	1503.9868	3007.9736	1	1669334	0.18	2669	0.0032	1	0.004	15	0.0257
			0.5	2	724.4651	1448.9302			1					1		15	
	1-3	2	0.5	1	645.1841	1290.3682	1320.4403	2640.8806	1	1669334	0.16	2302	0.0027	1	0.004	15	0.0222
			0.5	2	675.2562	1350.5124			1					1		15	
	2-1	2	0.5	1	3682.758	7365.516	7340.623	14681.246	1	1669334	0.88	14342	0.0171	1	0.004	15	0.1383
			0.5	2	3657.865	7315.73			1					1		15	
	2-2	2	0.5	1	3563.606	7127.212	7593.189	15186.378	1	1669334	0.91	14848	0.0177	1	0.004	15	0.1432
			0.5	2	4029.583	8059.166			1					1		15	
	2-3	2	0.5	1	3264.369	6528.738	6544.971	13089.942	1	1669334	0.78	12751	0.0152	1	0.004	15	0.1230
			0.5	2	3280.602	6561.204			1					1		15	
	3-1	2	0.5	1	6900.189	13800.378	13793.061	27586.122	1	1669334	1.65	27247	0.0325	1	0.004	15	0.2628
			0.5	2	6892.872	13785.744			1					1		15	
	3-2	2	0.5	1	3584.052	7168.104	7155.758	14311.516	1	1669334	0.86	13973	0.0166	1	0.004	15	0.1348
			0.5	2	3571.706	7143.412			1					1		15	
	3-3	2	0.5	1	5584.462	11168.924	11315.456	22630.912	1	1669334	1.36	22292	0.0266	1	0.004	15	0.2150
			0.5	2	5730.994	11461.988			1					1		15	
	4-1	2	0.5	1	9155.479	18310.958	18201.773	36403.546	1	1669334	2.18	36065	0.0430	1	0.004	15	0.3479
			0.5	2	9046.294	18092.588			1					1		15	
	4-2	2	0.5	1	9341.549	18683.098	18795.517	37591.034	1	1669334	2.25	37252	0.0444	1	0.004	15	0.3593
			0.5	2	9453.968	18907.936			1					1		15	
	4-3	2	0.5	1	9574.67	19149.34	18886.535	37773.07	1	1669334	2.26	37434	0.0446	1	0.004	15	0.3611
			0.5	2	9311.885	18623.73			1					1		15	
	5-1	2	0.5	1	9521.806	19043.612	18961.774	37923.548	1	1669334	2.27	37585	0.0448	1	0.004	15	0.3625
			0.5	2	9439.968	18879.936			1					1		15	
	5-2	2	0.5	1	9497.544	18995.088	18856.594	37713.188	1	1669334	2.26	37374	0.0445	1	0.004	15	0.3605
			0.5	2	9359.05	18718.1			1					1		15	
	5-3	2	0.5	1	8808.146	17616.292	17675.458	35350.916	1	1669334	2.12	35012	0.0417	1	0.004	15	0.3377
			0.5	2	8867.312	17734.624			1					1		15	
	6-1	2	0.5	1	9163.85	18327.7	18171.871	36343.742	1	1669334	2.18	36005	0.0429	1	0.004	15	0.3473
			0.5	2	9008.021	18016.042			1					1		15	
	6-2	2	0.5	1	8365.566	16731.132	16827.415	33654.83	1	1669334	2.02	33316	0.0397	1	0.004	15	0.3214
			0.5	2	8461.849	16923.698			1					1		15	
	6-3	2	0.5	1	7951.491	15902.982	16219.642	32439.264	1	1669334	1.94	32101	0.0382	1	0.004	15	0.3096
			0.5	2	8268.151	16536.302			1					1		15	
	7-1	2	0.5	1	9525.089	19050.178	19020.009	38040.018	1	1669334	2.28	37701	0.0449	1	0.004	15	0.3637
			0.5	2	9494.92	18989.84			1					1		15	
	7-2	2	0.5	1	7836.699	15673.398	15605.376	31210.752	1	1669334	1.87	30872	0.0368	1	0.004	15	0.2978
			0.5	2	7768.677	15537.354			1					1		15	
	7-3	2	0.5	1	9128.592	18257.184	18319.347	36638.694	1	1669334	2.19	36300	0.0432	1	0.004	15	0.3502
			0.5	2	9190.755	18381.51			1					1		15	
	8-1	2	0.5	1	9134.453	18268.906	18681.041	37362.082	1	1669334	2.24	37023	0.0441	1	0.004	15	0.3571
			0.5	2	9546.588	19093.176			1					1		15	
	8-2	2	0.5	1	8309.903	16619.806	16213.45	32426.9	1	1669334	1.94	32088	0.0382	1	0.004	15	0.3095
			0.5	2	7903.547	15807.094			1					1		15	
	8-3	2	0.5	1	7044.346	14088.692	14088.729	28177.458	1	1669334	1.69	27839	0.0332	1	0.004	15	0.2685
			0.5	2	7044.383	14088.766			1					1		15	

Assay Date	9/19/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.3902	0.0028
Full activity	End	0.3549	0.0035
Full activity	Overall	0.3725	0.0205
Background	Beginning	0.0002	8.38333E-05
Background	End	-0.0002	0.000573543
Background	Overall	0.0000	0.000401718
Positive	Beginning	0.1905	0.0188
Positive	End	0.1884	0.0009
Positive	Overall	0.1895	0.0109
Negative	Beginning	0.2939	0.0159
Negative	End	0.3193	0.0109
Negative	Overall	0.3066	0.0184

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#4 NYP	1	1	1.00E-04	-4.00	0.0311
RC#4 NYP	1	2	1.00E-04	-4.00	0.0257
RC#4 NYP	1	3	1.00E-04	-4.00	0.0222
RC#4 NYP	2	1	3.30E-05	-4.48	0.1383
RC#4 NYP	2	2	3.30E-05	-4.48	0.1432
RC#4 NYP	2	3	3.30E-05	-4.48	0.1230
RC#4 NYP	3	1	1.00E-05	-5.00	0.2628
RC#4 NYP	3	2	1.00E-05	-5.00	0.1348
RC#4 NYP	3	3	1.00E-05	-5.00	0.2150
RC#4 NYP	4	1	1.00E-06	-6.00	0.3479
RC#4 NYP	4	2	1.00E-06	-6.00	0.3593
RC#4 NYP	4	3	1.00E-06	-6.00	0.3611
RC#4 NYP	5	1	1.00E-07	-7.00	0.3625
RC#4 NYP	5	2	1.00E-07	-7.00	0.3605
RC#4 NYP	5	3	1.00E-07	-7.00	0.3377
RC#4 NYP	6	1	1.00E-08	-8.00	0.3473
RC#4 NYP	6	2	1.00E-08	-8.00	0.3214
RC#4 NYP	6	3	1.00E-08	-8.00	0.3096
RC#4 NYP	7	1	1.00E-09	-9.00	0.3637
RC#4 NYP	7	2	1.00E-09	-9.00	0.2978
RC#4 NYP	7	3	1.00E-09	-9.00	0.3502
RC#4 NYP	8	1	1.00E-10	-10.00	0.3571
RC#4 NYP	8	2	1.00E-10	-10.00	0.3095
RC#4 NYP	8	3	1.00E-10	-10.00	0.2685

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	8.35	6.91	5.96
2	-4.48	37.14	38.45	33.02
3	-5.00	70.56	36.18	57.73
4	-6.00	93.39	96.47	96.94
5	-7.00	97.33	96.78	90.66
6	-8.00	93.24	86.27	83.12
7	-9.00	97.63	79.94	94.00
8	-10.00	95.87	83.09	72.09

Assay Date	9/20/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8		
Technician ID	EJB	Replicate #	2	Microsome type	Recombina	Microsome ID	5

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#4 NYP	# Concentrations tested	8				
Technician ID	EJB	Replicate #	2	Microsome type	Recombinant	Microsome ID	5		

								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:	Mic 0.008	QC 10	QC100	Mic 0.08
	0.098	0.104	0.347	0.252
	0.098	0.107	0.343	0.309
	0.099	0.106	0.353	0.309

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
Mic 0.008	0.098	0.024	0.000	200	120	72000	0.000	0.000	0.072
Mic 0.008	0.098	0.025	0.000	200	120	72000	0.000		
Mic 0.008	0.099	0.025	0.000	200	120	72000	0.000		
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		
Mic 0.08	0.252	0.178	0.013	200	140	8540	0.004	0.005	4.871
Mic 0.08	0.309	0.236	0.018	200	140	8540	0.005		
Mic 0.08	0.309	0.235	0.018	200	140	8540	0.005		

Assay Date	9/20/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Recombin	Microsome ID	5	Technician ID	EJB	Replicate #	2

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.871465
Protein Concentration (dilution added to assay, mg/mL):	0.007986

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	10126.82	20253.64	20164.67	40329.34	1	1659502	2.43	39918	0.0480	1	0.004	15	0.4008
			0.5	2	10037.85	20075.7			1					1		15	
	2	2	0.5	1	10075.6	20151.2	20482.64	40965.28	1	1659502	2.47	40554	0.0488	1	0.004	15	0.4072
			0.5	2	10407.04	20814.08			1					1		15	
	3	2	0.5	1	10178	20356	20282.18	40564.36	1	1659502	2.44	40153	0.0483	1	0.004	15	0.4031
			0.5	2	10104.18	20208.36			1					1		15	
	4	2	0.5	1	10299	20598	20162.609	40325.218	1	1659502	2.43	39914	0.0480	1	0.004	15	0.4007
			0.5	2	9863.609	19727.218			1					1		15	
Background control	1	2	0.5	1	97.68635	195.3727	201.97205	403.9441	1	1659502	0.02	-8	0.0000	1	0.004	15	-0.0001
			0.5	2	104.2857	208.5714			1					1		15	
	2	2	0.5	1	120.2879	240.5758	225.337	450.674	1	1659502	0.03	39	0.0000	1	0.004	15	0.0004
			0.5	2	105.0491	210.0982			1					1		15	
	3	2	0.5	1	94.2748	188.5496	206.5825	413.165	1	1659502	0.02	2	0.0000	1	0.004	15	0.0000
			0.5	2	112.3077	224.6154			1					1		15	
	4	2	0.5	1	80.80698	161.61396	189.34798	378.69596	1	1659502	0.02	-33	0.0000	1	0.004	15	-0.0003
			0.5	2	108.541	217.082			1					1		15	
Positive control	1	2	0.5	1	5907.342	11814.684	11915.084	23830.168	1	1659502	1.44	23419	0.0282	1	0.004	15	0.2351
			0.5	2	6007.742	12015.484			1					1		15	
	2	2	0.5	1	6172.99	12345.98	12172.277	24344.554	1	1659502	1.47	23933	0.0288	1	0.004	15	0.2403
			0.5	2	5999.287	11998.574			1					1		15	
	3	2	0.5	1	5564.937	11129.874	11200.926	22401.852	1	1659502	1.35	21990	0.0264	1	0.004	15	0.2208
			0.5	2	5635.989	11271.978			1					1		15	
	4	2	0.5	1	5424.334	10848.668	10967.549	21935.098	1	1659502	1.32	21523	0.0259	1	0.004	15	0.2161
			0.5	2	5543.215	11086.43			1					1		15	
Negative Control	1	2	0.5	1	9416.756	18833.512	18935.868	37871.736	1	1659502	2.28	37460	0.0451	1	0.004	15	0.3761
			0.5	2	9519.112	19038.224			1					1		15	
	2	2	0.5	1	9104.99	18209.98	18372.676	36745.352	1	1659502	2.21	36334	0.0437	1	0.004	15	0.3648
			0.5	2	9267.686	18535.372			1					1		15	
	3	2	0.5	1	7942.485	15884.97	16193.186	32386.372	1	1659502	1.95	31975	0.0385	1	0.004	15	0.3210
			0.5	2	8250.701	16501.402			1					1		15	
	4	2	0.5	1	8040.171	16080.342	16375.62	32751.24	1	1659502	1.97	32340	0.0389	1	0.004	15	0.3247
			0.5	2	8335.449	16670.898			1					1		15	
RC#4 NYP	1-1	2	0.5	1	402.156	804.312	771.1953	1542.3906	1	1659502	0.09	1131	0.0014	1	0.004	15	0.0114
			0.5	2	369.0393	738.0786			1					1		15	
	1-2	2	0.5	1	431.4503	862.9006	893.4677	1786.9354	1	1659502	0.11	1375	0.0017	1	0.004	15	0.0138
			0.5	2	462.0174	924.0348			1					1		15	
	1-3	2	0.5	1	433.6654	867.3328	878.1071	1756.2142	1	1659502	0.11	1345	0.0016	1	0.004	15	0.0135
			0.5	2	444.4407	888.8814			1					1		15	
	2-1	2	0.5	1	2206.095	4412.19	4342.626	8685.252	1	1659502	0.52	8274	0.0100	1	0.004	15	0.0831
			0.5	2	2136.531	4273.062			1					1		15	
	2-2	2	0.5	1	2088.399	4176.798	4272.73	8545.46	1	1659502	0.51	8134	0.0098	1	0.004	15	0.0817
			0.5	2	2184.331	4368.662			1					1		15	
	2-3	2	0.5	1	2136.009	4272.018	4476.73	8953.46	1	1659502	0.54	8542	0.0103	1	0.004	15	0.0858
			0.5	2	2340.721	4681.442			1					1		15	
	3-1	2	0.5	1	6620.999	13241.998	13020.946	26041.892	1	1659502	1.57	25630	0.0308	1	0.004	15	0.2573
			0.5	2	6399.947	12799.894			1					1		15	
	3-2	2	0.5	1	6276.875	12553.75	12575.621	25151.242	1	1659502	1.52	24740	0.0298	1	0.004	15	0.2484
			0.5	2	6298.746	12597.492			1					1		15	
	3-3	2	0.5	1	6386.513	12773.026	12756.864	25513.728	1	1659502	1.54	25102	0.0302	1	0.004	15	0.2520
			0.5	2	6370.351	12740.702			1					1		15	
	4-1	2	0.5	1	9877.393	19754.786	19520.461	39040.922	1	1659502	2.35	38629	0.0465	1	0.004	15	0.3879
			0.5	2	9643.068	19286.136			1					1		15	
	4-2	2	0.5	1	9634.987	19269.974	19433.088	38866.176	1	1659502	2.34	38455	0.0463	1	0.004	15	0.3861
			0.5	2	9798.101	19596.202			1					1		15	
	4-3	2	0.5	1	9209.399	18418.798	19059.444	38118.888	1	1659502	2.30	37707	0.0454	1	0.004	15	0.3786
			0.5	2	9850.045	19700.09			1					1		15	
	5-1	2	0.5	1	10025.01	20050.02	19832.388	39664.776	1	1659502	2.39	39253	0.0472	1	0.004	15	0.3941
			0.5	2	9807.378	19614.756			1					1		15	
	5-2	2	0.5	1	9958.686	19917.372	19857.91	39715.82	1	1659502	2.39	39304	0.0473	1	0.004	15	0.3946
			0.5	2	9899.224	19798.448			1					1		15	
	5-3	2	0.5	1	9482.076	18964.152	19169.408	38338.816	1	1659502	2.31	37927	0.0456	1	0.004	15	0.3808
			0.5	2	9687.332	19374.664			1					1		15	
	6-1	2	0.5	1	9744.316	19488.632	19328.125	38656.25	1	1659502	2.33	38245	0.0460	1	0.004	15	0.3840
			0.5	2	9583.809	19167.618			1					1		15	
	6-2	2	0.5	1	9437.688	18875.376	19154.78	38309.56	1	1659502	2.31	37898	0.0456	1	0.004	15	0.3805
			0.5	2	9717.092	19434.184			1					1		15	
	6-3	2	0.5	1	9691.481	19382.962	19207.86	38415.72	1	1659502	2.31	38004	0.0457	1	0.004	15	0.3816
			0.5	2	9516.379	19032.758			1					1		15	
	7-1	2	0.5	1	9185.328	18370.656	18349.577	36699.154	1	1659502	2.21	36288	0.0436	1	0.004	15	0.3643
			0.5	2	9164.249	18328.498			1					1		15	
	7-2	2	0.5	1	9631.337	19262.674	19080.467	38160.934	1	1659502	2.30	37749	0.0454	1	0.004	15	0.3790
			0.5	2	9449.13	18898.26			1					1		15	
	7-3	2	0.5	1	9295.074	18590.148	18668.783	37337.566	1	1659502	2.25	36926	0.0444	1	0.004	15	0.3707
			0.5	2	9373.709	18747.418			1					1		15	
	8-1	2	0.5	1	9792.275	19584.55	19405.378	38810.756	1	1659502	2.34	38399	0.0462	1	0.004	15	0.3855
			0.5	2	9613.103	19226.206			1					1		15	
	8-2	2	0.5	1	9391.159	18782.318	18894.16	37788.32	1	1659502	2.28	37377	0.0450	1	0.004	15	0.3753
			0.5	2	9503.001	19006.002			1					1		15	
	8-3	2	0.5	1	9255.191	18510.382	18961.517	37923.034	1	1659502	2.29	37511	0.0451	1	0.004	15	0.3766
			0.5	2	9706.326	19412.652			1					1		15	

Assay Date	9/20/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.4040	0.0045
Full activity	End	0.4019	0.0017
Full activity	Overall	0.4030	0.0030
Background	Beginning	0.0002	0.000331763
Background	End	-0.0002	0.000244716
Background	Overall	0.0000	0.000299561
Positive	Beginning	0.2377	0.0037
Positive	End	0.2184	0.0033
Positive	Overall	0.2281	0.0115
Negative	Beginning	0.3705	0.0080
Negative	End	0.3229	0.0026
Negative	Overall	0.3467	0.0279

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#4 NYP	1	1	1.00E-04	-4.00	0.0114
RC#4 NYP	1	2	1.00E-04	-4.00	0.0138
RC#4 NYP	1	3	1.00E-04	-4.00	0.0135
RC#4 NYP	2	1	3.30E-05	-4.48	0.0831
RC#4 NYP	2	2	3.30E-05	-4.48	0.0817
RC#4 NYP	2	3	3.30E-05	-4.48	0.0858
RC#4 NYP	3	1	1.00E-05	-5.00	0.2573
RC#4 NYP	3	2	1.00E-05	-5.00	0.2484
RC#4 NYP	3	3	1.00E-05	-5.00	0.2520
RC#4 NYP	4	1	1.00E-06	-6.00	0.3879
RC#4 NYP	4	2	1.00E-06	-6.00	0.3861
RC#4 NYP	4	3	1.00E-06	-6.00	0.3786
RC#4 NYP	5	1	1.00E-07	-7.00	0.3941
RC#4 NYP	5	2	1.00E-07	-7.00	0.3946
RC#4 NYP	5	3	1.00E-07	-7.00	0.3808
RC#4 NYP	6	1	1.00E-08	-8.00	0.3840
RC#4 NYP	6	2	1.00E-08	-8.00	0.3805
RC#4 NYP	6	3	1.00E-08	-8.00	0.3816
RC#4 NYP	7	1	1.00E-09	-9.00	0.3643
RC#4 NYP	7	2	1.00E-09	-9.00	0.3790
RC#4 NYP	7	3	1.00E-09	-9.00	0.3707
RC#4 NYP	8	1	1.00E-10	-10.00	0.3855
RC#4 NYP	8	2	1.00E-10	-10.00	0.3753
RC#4 NYP	8	3	1.00E-10	-10.00	0.3766

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	2.82	3.43	3.35
2	-4.48	20.61	20.27	21.28
3	-5.00	63.86	61.64	62.55
4	-6.00	96.25	95.81	93.95
5	-7.00	97.80	97.93	94.50
6	-8.00	95.29	94.43	94.69
7	-9.00	90.41	94.06	92.01
8	-10.00	95.68	93.13	93.46

Assay Date	9/21/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8		
Technician ID	EJB	Replicate #	3	Microsome type	Recombina	Microsome ID	5

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	EJB	Replicate #	3	Microsome type	Recombinant	Microsome ID	5			

								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	0.185	0.112	0.031	0.042	0.032			
	0.628	0.396	0.199	0.118	0.036	0.042	0.027			

Samples:	Mic 0.008	QC 10	QC100	Mic 0.08
	0.049	0.056	0.283	0.199
	0.046	0.059	0.280	0.205
	0.042	0.059	0.312	0.200

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	0.0470	m, b	0.082	-0.001
0.125	62.5	1000	0.00013	200	0.0250	0.396	0.364	0.0287	se_m, se_b	0.006	0.002
0.05	25	1000	0.00005	200	0.0100	0.195	0.163	0.0123	r^2, se_y	0.979	0.003
0.025	25	2000	0.00003	200	0.0050	0.117	0.085	0.0059	F, df	184	4
0.01	5	1000	0.00001	200	0.0020	0.033	0.002	-0.0009	ss_{regr} ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.046	0.015	0.0001			

Regression results are calculated using the function LINEST

Blank	0.031	r^2=	0.979	
		m=	0.082	
		b=	-0.001	

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
Mic 0.008	0.049	0.017	0.000	200	120	72000	0.001	0.000	0.168
Mic 0.008	0.046	0.015	0.000	200	120	72000	0.000		
Mic 0.008	0.042	0.010	0.000	200	120	72000	-0.001		
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		
Mic 0.08	0.199	0.167	0.013	200	140	8540	0.004	0.004	3.908
Mic 0.08	0.205	0.174	0.013	200	140	8540	0.004		
Mic 0.08	0.200	0.169	0.013	200	140	8540	0.004		

Assay Date	9/21/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Recombin:	Microsome ID	5	Technician ID	EJB	Replicate #	3

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.907664
Protein Concentration (dilution added to assay, mg/mL):	0.006406

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	9/21/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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	10618.14	21236.28	21383.91	42767.82	1	1537563	2.78	42405	0.0549	1	0.003	15	0.5716
			0.5	2	10765.77	21531.54			1					1		15	
	2	2	0.5	1	10208.22	20416.44	20700.61	41401.22	1	1537563	2.69	41038	0.0532	1	0.003	15	0.5532
			0.5	2	10492.39	20984.78			1					1		15	
	3	2	0.5	1	8538.057	17076.114	17599.212	35198.424	1	1537563	2.29	34835	0.0451	1	0.003	15	0.4696
			0.5	2	9061.155	18122.31			1					1		15	
	4	2	0.5	1	8546.685	17093.37	17661.712	35323.424	1	1537563	2.30	34960	0.0453	1	0.003	15	0.4713
			0.5	2	9115.027	18230.054			1					1		15	
Background control	1	2	0.5	1	95.68557	191.36714	199.96927	399.93854	1	1537563	0.03	37	0.0000	1	0.003	15	0.0005
			0.5	2	104.2857	208.5714			1					1		15	
	2	2	0.5	1	92.94975	185.8995	182.49557	364.99114	1	1537563	0.02	2	0.0000	1	0.003	15	0.0000
			0.5	2	89.54582	179.09164			1					1		15	
	3	2	0.5	1	58.82782	117.65564	165.01952	330.03904	1	1537563	0.02	-33	0.0000	1	0.003	15	-0.0004
			0.5	2	106.1917	212.3834			1					1		15	
	4	2	0.5	1	78.11341	156.22682	178.51381	357.02762	1	1537563	0.02	-5	0.0000	1	0.003	15	-0.0001
			0.5	2	100.4004	200.8008			1					1		15	
Positive control	1	2	0.5	1	5231.674	10463.348	10772.368	21544.736	1	1537563	1.40	21182	0.0274	1	0.003	15	0.2855
			0.5	2	5540.694	11081.388			1					1		15	
	2	2	0.5	1	5527.448	11054.896	10707.202	21414.404	1	1537563	1.39	21051	0.0273	1	0.003	15	0.2838
			0.5	2	5179.754	10359.508			1					1		15	
	3	2	0.5	1	4390.718	8781.436	9008.726	18017.452	1	1537563	1.17	17654	0.0229	1	0.003	15	0.2380
			0.5	2	4618.008	9236.016			1					1		15	
	4	2	0.5	1	4891.542	9783.084	9772.312	19544.624	1	1537563	1.27	19182	0.0248	1	0.003	15	0.2586
			0.5	2	4880.77	9761.54			1					1		15	
Negative Control	1	2	0.5	1	7923.08	15846.16	16477.891	32955.782	1	1537563	2.14	32593	0.0422	1	0.003	15	0.4394
			0.5	2	8554.811	17109.622			1					1		15	
	2	2	0.5	1	8070.021	16140.042	16067.261	32134.522	1	1537563	2.09	31772	0.0412	1	0.003	15	0.4283
			0.5	2	7997.24	15994.48			1					1		15	
	3	2	0.5	1	7625.002	15250.004	15372.678	30745.356	1	1537563	2.00	30382	0.0394	1	0.003	15	0.4096
			0.5	2	7747.676	15495.352			1					1		15	
	4	2	0.5	1	6987.325	13974.65	13939.741	27879.482	1	1537563	1.81	27516	0.0356	1	0.003	15	0.3709
			0.5	2	6952.416	13904.832			1					1		15	
RC#4 NYP	1-1	2	0.5	1	336.477	672.954	656.6729	1313.3458	1	1537563	0.09	950	0.0012	1	0.003	15	0.0128
			0.5	2	320.1959	640.3918			1					1		15	
	1-2	2	0.5	1	429.2114	858.4228	828.0994	1656.1988	1	1537563	0.11	1293	0.0017	1	0.003	15	0.0174
			0.5	2	398.888	797.776			1					1		15	
	1-3	2	0.5	1	410.0746	820.1492	712.2373	1424.4746	1	1537563	0.09	1061	0.0014	1	0.003	15	0.0143
			0.5	2	302.1627	604.3254			1					1		15	
	2-1	2	0.5	1	2512.723	5025.446	4963.035	9926.07	1	1537563	0.65	9563	0.0124	1	0.003	15	0.1289
			0.5	2	2450.312	4900.624			1					1		15	
	2-2	2	0.5	1	2742.065	5484.13	5434.888	10869.776	1	1537563	0.71	10507	0.0136	1	0.003	15	0.1416
			0.5	2	2692.823	5385.646			1					1		15	
	2-3	2	0.5	1	2090.218	4180.436	4166.064	8332.128	1	1537563	0.54	7969	0.0103	1	0.003	15	0.1074
			0.5	2	2075.846	4151.692			1					1		15	
	3-1	2	0.5	1	6381.158	12762.316	12548.998	25097.996	1	1537563	1.63	24735	0.0320	1	0.003	15	0.3334
			0.5	2	6167.84	12335.68			1					1		15	
	3-2	2	0.5	1	6022.529	12045.258	12109.096	24218.192	1	1537563	1.58	23855	0.0309	1	0.003	15	0.3216
			0.5	2	6086.467	12172.934			1					1		15	
	3-3	2	0.5	1	6518.499	13036.998	12961.577	25923.154	1	1537563	1.69	25560	0.0331	1	0.003	15	0.3446
			0.5	2	6443.078	12886.156			1					1		15	
	4-1	2	0.5	1	9142.06	18284.12	18446.784	36893.568	1	1537563	2.40	36531	0.0473	1	0.003	15	0.4924
			0.5	2	9304.724	18609.448			1					1		15	
	4-2	2	0.5	1	9257.885	18515.77	18269.546	36539.092	1	1537563	2.38	36176	0.0469	1	0.003	15	0.4877
			0.5	2	9011.661	18023.322			1					1		15	
	4-3	2	0.5	1	8941.109	17882.218	17891.923	35783.846	1	1537563	2.33	35421	0.0459	1	0.003	15	0.4775
			0.5	2	8950.814	17901.628			1					1		15	
	5-1	2	0.5	1	9187.851	18375.702	18666.241	37332.482	1	1537563	2.43	36969	0.0479	1	0.003	15	0.4984
			0.5	2	9478.39	18956.78			1					1		15	
	5-2	2	0.5	1	9456.682	18913.364	18709.179	37418.358	1	1537563	2.43	37055	0.0480	1	0.003	15	0.4995
			0.5	2	9252.497	18504.994			1					1		15	
	5-3	2	0.5	1	8804.63	17209.26	17265.781	34531.562	1	1537563	2.25	34169	0.0443	1	0.003	15	0.4606
			0.5	2	8661.151	17322.302			1					1		15	
	6-1	2	0.5	1	8938.94	17873.88	18019.154	36038.308	1	1537563	2.34	35675	0.0462	1	0.003	15	0.4809
			0.5	2	9082.214	18164.428			1					1		15	
	6-2	2	0.5	1	8781.11	17562.22	18149.192	36298.384	1	1537563	2.36	35935	0.0465	1	0.003	15	0.4844
			0.5	2	9368.082	18736.164			1					1		15	
	6-3	2	0.5	1	8527.92	17055.84	16824.191	33648.382	1	1537563	2.19	33285	0.0431	1	0.003	15	0.4487
			0.5	2	8296.271	16592.542			1					1		15	
	7-1	2	0.5	1	8631.766	17263.532	17450.685	34901.37	1	1537563	2.27	34538	0.0447	1	0.003	15	0.4656
			0.5	2	8818.919	17637.838			1					1		15	
	7-2	2	0.5	1	8805.433	17610.866	17513.178	35026.356	1	1537563	2.28	34663	0.0449	1	0.003	15	0.4673
			0.5	2	8707.745	17415.49			1					1		15	
	7-3	2	0.5	1	8837.684	17675.368	17766.95	35533.9	1	1537563	2.31	35171	0.0456	1	0.003	15	0.4741
			0.5	2	8929.266	17858.532			1					1		15	
	8-1	2	0.5	1	8824.216	17648.432	17985.332	35970.664	1	1537563	2.34	35608	0.0461	1	0.003	15	0.4800
			0.5	2	9161.116	18322.232			1					1		15	
	8-2	2	0.5	1	9255.191	18510.382	18290.549	36581.098	1	1537563	2.38	36218	0.0469	1	0.003	15	0.4882
			0.5	2	9035.358	18070.716			1					1		15	
	8-3	2	0.5	1	8557.55	17115.1	17263.292	34526.584	1	1537563	2.25	34164	0.0443	1	0.003	15	0.4605
			0.5	2	8705.742	17411.484			1					1		15	

Assay Date	9/21/2005	Test Chemical ID	RC#4 NYP	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.5624	0.0130
Full activity	End	0.4704	0.0012
Full activity	Overall	0.5164	0.0536
Background	Beginning	0.0003	0.000333117
Background	End	-0.0003	0.000257254
Background	Overall	0.0000	0.000388402
Positive	Beginning	0.2847	0.0012
Positive	End	0.2483	0.0146
Positive	Overall	0.2665	0.0226
Negative	Beginning	0.4338	0.0078
Negative	End	0.3902	0.0273
Negative	Overall	0.4120	0.0300

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#4 NYP	1	1	1.00E-04	-4.00	0.0128
RC#4 NYP	1	2	1.00E-04	-4.00	0.0174
RC#4 NYP	1	3	1.00E-04	-4.00	0.0143
RC#4 NYP	2	1	3.30E-05	-4.48	0.1289
RC#4 NYP	2	2	3.30E-05	-4.48	0.1416
RC#4 NYP	2	3	3.30E-05	-4.48	0.1074
RC#4 NYP	3	1	1.00E-05	-5.00	0.3334
RC#4 NYP	3	2	1.00E-05	-5.00	0.3216
RC#4 NYP	3	3	1.00E-05	-5.00	0.3446
RC#4 NYP	4	1	1.00E-06	-6.00	0.4924
RC#4 NYP	4	2	1.00E-06	-6.00	0.4877
RC#4 NYP	4	3	1.00E-06	-6.00	0.4775
RC#4 NYP	5	1	1.00E-07	-7.00	0.4984
RC#4 NYP	5	2	1.00E-07	-7.00	0.4995
RC#4 NYP	5	3	1.00E-07	-7.00	0.4606
RC#4 NYP	6	1	1.00E-08	-8.00	0.4809
RC#4 NYP	6	2	1.00E-08	-8.00	0.4844
RC#4 NYP	6	3	1.00E-08	-8.00	0.4487
RC#4 NYP	7	1	1.00E-09	-9.00	0.4656
RC#4 NYP	7	2	1.00E-09	-9.00	0.4673
RC#4 NYP	7	3	1.00E-09	-9.00	0.4741
RC#4 NYP	8	1	1.00E-10	-10.00	0.4800
RC#4 NYP	8	2	1.00E-10	-10.00	0.4882
RC#4 NYP	8	3	1.00E-10	-10.00	0.4605

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	2.48	3.38	2.77
2	-4.48	24.96	27.43	20.80
3	-5.00	64.57	62.27	66.72
4	-6.00	95.36	94.43	92.46
5	-7.00	96.50	96.73	89.19
6	-8.00	93.12	93.80	86.88
7	-9.00	90.16	90.48	91.81
8	-10.00	92.95	94.54	89.18

Assay Date	9/19/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8		
Technician ID	EJB	Replicate #	1	Microsome type	Recombina	Microsome ID	5

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	EJB	Replicate #	1	Microsome type	Recombinant	Microsome ID	5				

								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:	Mics 0.008	QC 10	QC100	Mics 0.08	
	0.121	0.063	0.304	0.238	
	0.110	0.052	0.312	0.239	
	0.113	0.055	0.316	0.236	

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	0.0476	m, b	0.087	-0.001
0.125	62.35	1000	0.00012	200	0.0249	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	306	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
Mics 0.008	0.121	0.090	0.006	200	120	72000	0.019	0.017	17.464
Mics 0.008	0.110	0.079	0.005	200	120	72000	0.016		
Mics 0.008	0.113	0.082	0.006	200	120	72000	0.017		
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		
Mics 0.08	0.238	0.206	0.016	200	140	8540	0.005	0.005	5.021
Mics 0.08	0.239	0.208	0.017	200	140	8540	0.005		
Mics 0.08	0.236	0.205	0.016	200	140	8540	0.005		

Assay Date	9/19/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	1

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.021487
Protein Concentration (dilution added to assay, mg/mL):	0.008232

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	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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	10211.31	20422.62	20289.95	40579.9	1	1669334	2.43	40241	0.0479	1	0.004	15	0.3882
			0.5	2	10078.64	20157.26			1					1		15	
	2	2	0.5	1	10373.75	20747.5	20495.14	40990.28	1	1669334	2.46	40652	0.0484	1	0.004	15	0.3921
			0.5	2	10121.39	20242.78			1					1		15	
	3	2	0.5	1	9304.625	18609.25	18435.813	36871.626	1	1669334	2.21	36533	0.0435	1	0.004	15	0.3524
			0.5	2	9131.188	18262.376			1					1		15	
	4	2	0.5	1	9081.737	18163.474	18691.101	37382.202	1	1669334	2.24	37043	0.0441	1	0.004	15	0.3573
			0.5	2	9609.364	19218.728			1					1		15	
Background control	1	2	0.5	1	82.01449	164.02898	176.26929	352.57858	1	1669334	0.02	14	0.0000	1	0.004	15	0.0001
			0.5	2	94.2748	188.5496			1					1		15	
	2	2	0.5	1	97.69835	195.3727	182.43466	364.86932	1	1669334	0.02	26	0.0000	1	0.004	15	0.0003
			0.5	2	84.74831	169.49662			1					1		15	
	3	2	0.5	1	56.9837	113.9674	138.38899	276.77798	1	1669334	0.02	-62	-0.0001	1	0.004	15	-0.0006
			0.5	2	81.40529	162.81058			1					1		15	
	4	2	0.5	1	87.48212	174.96424	180.43237	360.86474	1	1669334	0.02	22	0.0000	1	0.004	15	0.0002
			0.5	2	92.98025	185.9605			1					1		15	
Positive control	1	2	0.5	1	5451.258	10902.516	10734.489	21468.978	1	1669334	1.29	21130	0.0252	1	0.004	15	0.2038
			0.5	2	5283.231	10566.462			1					1		15	
	2	2	0.5	1	4673.949	9347.898	9357.001	18714.002	1	1669334	1.12	18375	0.0219	1	0.004	15	0.1772
			0.5	2	4683.052	9366.104			1					1		15	
	3	2	0.5	1	5024.781	10049.562	9966.109	19932.218	1	1669334	1.19	19593	0.0233	1	0.004	15	0.1890
			0.5	2	4941.328	9882.656			1					1		15	
	4	2	0.5	1	4889.226	9778.452	9903.071	19806.142	1	1669334	1.19	19467	0.0232	1	0.004	15	0.1878
			0.5	2	5013.845	10027.69			1					1		15	
Negative Control	1	2	0.5	1	7934.344	15868.688	15988.209	31976.418	1	1669334	1.92	31638	0.0377	1	0.004	15	0.3052
			0.5	2	8053.865	16107.73			1					1		15	
	2	2	0.5	1	7326.953	14653.906	14823.117	29646.234	1	1669334	1.78	29307	0.0349	1	0.004	15	0.2827
			0.5	2	7496.164	14992.328			1					1		15	
	3	2	0.5	1	8444.487	16888.974	17121.141	34242.282	1	1669334	2.05	33904	0.0404	1	0.004	15	0.3270
			0.5	2	8676.654	17353.308			1					1		15	
	4	2	0.5	1	7932.941	15865.882	16320.335	32640.67	1	1669334	1.96	32302	0.0385	1	0.004	15	0.3116
			0.5	2	8387.394	16774.788			1					1		15	
RC#5 DBA	1-1	2	0.5	1	9190.803	18381.606	17962.714	35965.428	1	1669334	2.15	35627	0.0424	1	0.004	15	0.3437
			0.5	2	8791.911	17583.822			1					1		15	
	1-2	2	0.5	1	9010.12	18020.24	17915.492	35830.984	1	1669334	2.15	35492	0.0423	1	0.004	15	0.3424
			0.5	2	8905.372	17810.744			1					1		15	
	1-3	2	0.5	1	8504.274	17008.548	17117.09	34234.18	1	1669334	2.05	33895	0.0404	1	0.004	15	0.3270
			0.5	2	8612.816	17225.632			1					1		15	
	2-1	2	0.5	1	8971.386	17942.772	18065.009	36130.018	1	1669334	2.16	35791	0.0426	1	0.004	15	0.3452
			0.5	2	9093.623	18187.246			1					1		15	
	2-2	2	0.5	1	9003.567	18007.134	17981.562	35963.124	1	1669334	2.15	35624	0.0424	1	0.004	15	0.3436
			0.5	2	8977.995	17955.99			1					1		15	
	2-3	2	0.5	1	8961.591	17923.182	18046.565	36093.13	1	1669334	2.16	35754	0.0426	1	0.004	15	0.3449
			0.5	2	9084.974	18169.948			1					1		15	
	3-1	2	0.5	1	9554.839	19109.678	18798.976	37597.952	1	1669334	2.25	37259	0.0444	1	0.004	15	0.3594
			0.5	2	9244.137	18488.274			1					1		15	
	3-2	2	0.5	1	9171.737	18343.474	18332.691	36665.382	1	1669334	2.20	36327	0.0433	1	0.004	15	0.3504
			0.5	2	9160.964	18321.908			1					1		15	
	3-3	2	0.5	1	9131.104	18262.208	18118.534	36237.068	1	1669334	2.17	35898	0.0428	1	0.004	15	0.3463
			0.5	2	8987.43	17974.86			1					1		15	
	4-1	2	0.5	1	8835.327	17670.654	17816.637	35633.274	1	1669334	2.13	35295	0.0420	1	0.004	15	0.3405
			0.5	2	8981.31	17962.62			1					1		15	
	4-2	2	0.5	1	8540.866	17081.732	17357.198	34714.396	1	1669334	2.08	34376	0.0409	1	0.004	15	0.3316
			0.5	2	8816.332	17632.664			1					1		15	
	4-3	2	0.5	1	8848.505	17697.01	17680.84	35361.68	1	1669334	2.12	35023	0.0417	1	0.004	15	0.3378
			0.5	2	8832.335	17664.67			1					1		15	
	5-1	2	0.5	1	9494.721	18989.442	19123.374	38246.748	1	1669334	2.29	37908	0.0452	1	0.004	15	0.3657
			0.5	2	9628.653	19257.306			1					1		15	
	5-2	2	0.5	1	9333.354	18666.708	18656.626	37317.252	1	1669334	2.24	36978	0.0440	1	0.004	15	0.3567
			0.5	2	9325.272	18650.544			1					1		15	
	5-3	2	0.5	1	9303.324	18606.648	18363.877	36727.754	1	1669334	2.20	36389	0.0433	1	0.004	15	0.3510
			0.5	2	9060.553	18121.106			1					1		15	
	6-1	2	0.5	1	9150.276	18300.552	18829.719	37659.438	1	1669334	2.26	37321	0.0445	1	0.004	15	0.3600
			0.5	2	9679.443	19358.886			1					1		15	
	6-2	2	0.5	1	9004.781	18009.562	17895.34	35790.68	1	1669334	2.14	35452	0.0422	1	0.004	15	0.3420
			0.5	2	8890.559	17781.118			1					1		15	
	6-3	2	0.5	1	9400.781	18801.562	18211.771	36423.542	1	1669334	2.18	36085	0.0430	1	0.004	15	0.3481
			0.5	2	8810.99	17621.98			1					1		15	
	7-1	2	0.5	1	7933.703	15867.406	15584.156	31168.312	1	1669334	1.87	30830	0.0367	1	0.004	15	0.2974
			0.5	2	7650.453	15300.906			1					1		15	
	7-2	2	0.5	1	5907.115	11814.23	11646.309	23292.618	1	1669334	1.40	22954	0.0273	1	0.004	15	0.2214
			0.5	2	5739.194	11478.388			1					1		15	
	7-3	2	0.5	1	8941.204	17882.408	18035.643	36071.286	1	1669334	2.16	35733	0.0426	1	0.004	15	0.3447
			0.5	2	9094.439	18188.878			1					1		15	
	8-1	2	0.5	1	8588.439	17176.878	16763.585	33527.17	1	1669334	2.01	33188	0.0395	1	0.004	15	0.3201
			0.5	2	8175.146	16350.292			1					1		15	
	8-2	2	0.5	1	8423.066	16846.132	17165.995	34331.99	1	1669334	2.06	33993	0.0405	1	0.004	15	0.3279
			0.5	2	8742.929	17485.858			1					1		15	
	8-3	2	0.5	1	8857.083	17714.166	17883.414	35766.828	1	1669334	2.14	35428	0.0422	1	0.004	15	0.3417
			0.5	2	9026.331	18052.662			1					1		15	

Assay Date	9/19/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.3902	0.0028
Full activity	End	0.3549	0.0035
Full activity	Overall	0.3725	0.0205
Background	Beginning	0.0002	8.38333E-05
Background	End	-0.0002	0.000573543
Background	Overall	0.0000	0.000401718
Positive	Beginning	0.1905	0.0188
Positive	End	0.1884	0.0009
Positive	Overall	0.1895	0.0109
Negative	Beginning	0.2939	0.0159
Negative	End	0.3193	0.0109
Negative	Overall	0.3066	0.0184

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#5 DBA	1	1	3.30E-05	-4.48	0.3437
RC#5 DBA	1	2	3.30E-05	-4.48	0.3424
RC#5 DBA	1	3	3.30E-05	-4.48	0.3270
RC#5 DBA	2	1	1.00E-05	-5.00	0.3452
RC#5 DBA	2	2	1.00E-05	-5.00	0.3436
RC#5 DBA	2	3	1.00E-05	-5.00	0.3449
RC#5 DBA	3	1	3.30E-06	-5.48	0.3594
RC#5 DBA	3	2	3.30E-06	-5.48	0.3504
RC#5 DBA	3	3	3.30E-06	-5.48	0.3463
RC#5 DBA	4	1	1.00E-06	-6.00	0.3405
RC#5 DBA	4	2	1.00E-06	-6.00	0.3316
RC#5 DBA	4	3	1.00E-06	-6.00	0.3378
RC#5 DBA	5	1	1.00E-07	-7.00	0.3657
RC#5 DBA	5	2	1.00E-07	-7.00	0.3567
RC#5 DBA	5	3	1.00E-07	-7.00	0.3510
RC#5 DBA	6	1	1.00E-08	-8.00	0.3600
RC#5 DBA	6	2	1.00E-08	-8.00	0.3420
RC#5 DBA	6	3	1.00E-08	-8.00	0.3481
RC#5 DBA	7	1	1.00E-09	-9.00	0.2974
RC#5 DBA	7	2	1.00E-09	-9.00	0.2214
RC#5 DBA	7	3	1.00E-09	-9.00	0.3447
RC#5 DBA	8	1	1.00E-10	-10.00	0.3201
RC#5 DBA	8	2	1.00E-10	-10.00	0.3279
RC#5 DBA	8	3	1.00E-10	-10.00	0.3417

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	92.26	91.91	87.77
2	-5.00	92.68	92.25	92.59
3	-5.48	96.48	94.07	92.96
4	-6.00	91.40	89.02	90.69
5	-7.00	98.16	95.76	94.23
6	-8.00	96.64	91.80	93.44
7	-9.00	79.83	59.44	92.53
8	-10.00	85.94	88.03	91.74

Assay Date	9/20/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8		
Technician ID	EJB	Replicate #	2	Microsome type	Recombina	Microsome ID	5

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	EJB	Replicate #	2	Microsome type	Recombinant	Microsome ID	5			

								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:	Mic 0.008	QC 10	QC100	Mic 0.08
	0.098	0.104	0.347	0.252
	0.098	0.107	0.343	0.309
	0.099	0.106	0.353	0.309

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
Mic 0.008	0.098	0.024	0.000	200	120	72000	0.000	0.000	0.072
Mic 0.008	0.098	0.025	0.000	200	120	72000	0.000		
Mic 0.008	0.099	0.025	0.000	200	120	72000	0.000		
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		
Mic 0.08	0.252	0.178	0.013	200	140	8540	0.004	0.005	4.871
Mic 0.08	0.309	0.236	0.018	200	140	8540	0.005		
Mic 0.08	0.309	0.235	0.018	200	140	8540	0.005		

Assay Date	9/20/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Recombin	Microsome ID	5	Technician ID	EJB	Replicate #	2

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.871465
Protein Concentration (dilution added to assay, mg/mL):	0.007986

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#5 DBA	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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	10126.82	20253.64	20164.67	40329.34	1	1659502	2.43	39918	0.0480	1	0.004	15	0.4008
			0.5	2	10037.85	20075.7			1					1		15	
	2	2	0.5	1	10075.6	20151.2	20482.64	40965.28	1	1659502	2.47	40554	0.0488	1	0.004	15	0.4072
			0.5	2	10407.04	20814.08			1					1		15	
	3	2	0.5	1	10178	20356	20282.18	40564.36	1	1659502	2.44	40153	0.0483	1	0.004	15	0.4031
			0.5	2	10104.18	20208.36			1					1		15	
	4	2	0.5	1	10299	20598	20162.609	40325.218	1	1659502	2.43	39914	0.0480	1	0.004	15	0.4007
			0.5	2	9863.609	19727.218			1					1		15	
Background control	1	2	0.5	1	97.68635	195.3727	201.97205	403.9441	1	1659502	0.02	-8	0.0000	1	0.004	15	-0.0001
			0.5	2	104.2857	208.5714			1					1		15	
	2	2	0.5	1	120.2879	240.5758	225.337	450.674	1	1659502	0.03	39	0.0000	1	0.004	15	0.0004
			0.5	2	105.0491	210.0982			1					1		15	
	3	2	0.5	1	94.2748	188.5496	206.5825	413.165	1	1659502	0.02	2	0.0000	1	0.004	15	0.0000
			0.5	2	112.3077	224.6154			1					1		15	
	4	2	0.5	1	80.80698	161.61396	189.34798	378.69596	1	1659502	0.02	-33	0.0000	1	0.004	15	-0.0003
			0.5	2	108.541	217.082			1					1		15	
Positive control	1	2	0.5	1	5907.342	11814.684	11915.084	23830.168	1	1659502	1.44	23419	0.0282	1	0.004	15	0.2351
			0.5	2	6007.742	12015.484			1					1		15	
	2	2	0.5	1	6172.99	12345.98	12172.277	24344.554	1	1659502	1.47	23933	0.0288	1	0.004	15	0.2403
			0.5	2	5999.287	11998.574			1					1		15	
	3	2	0.5	1	5564.937	11129.874	11200.926	22401.852	1	1659502	1.35	21990	0.0264	1	0.004	15	0.2208
			0.5	2	5635.989	11271.978			1					1		15	
	4	2	0.5	1	5424.334	10848.668	10967.549	21935.098	1	1659502	1.32	21523	0.0259	1	0.004	15	0.2161
			0.5	2	5543.215	11086.43			1					1		15	
Negative Control	1	2	0.5	1	9416.756	18833.512	18935.868	37871.736	1	1659502	2.28	37460	0.0451	1	0.004	15	0.3761
			0.5	2	9519.112	19038.224			1					1		15	
	2	2	0.5	1	9104.99	18209.98	18372.676	36745.352	1	1659502	2.21	36334	0.0437	1	0.004	15	0.3648
			0.5	2	9267.686	18535.372			1					1		15	
	3	2	0.5	1	7942.485	15884.97	16193.186	32386.372	1	1659502	1.95	31975	0.0385	1	0.004	15	0.3210
			0.5	2	8250.701	16501.402			1					1		15	
	4	2	0.5	1	8040.171	16080.342	16375.62	32751.24	1	1659502	1.97	32340	0.0389	1	0.004	15	0.3247
			0.5	2	8335.449	16670.898			1					1		15	
RC#5 DBA	1-1	2	0.5	1	9345.427	18690.854	18831.92	37663.84	1	1659502	2.27	37252	0.0448	1	0.004	15	0.3740
			0.5	2	9486.493	18972.986			1					1		15	
	1-2	2	0.5	1	8958.015	17916.03	18384.897	36769.794	1	1659502	2.22	36358	0.0437	1	0.004	15	0.3650
			0.5	2	9426.882	18853.764			1					1		15	
	1-3	2	0.5	1	8829.661	17659.322	18198.598	36397.196	1	1659502	2.19	35986	0.0433	1	0.004	15	0.3613
			0.5	2	9368.937	18737.874			1					1		15	
	2-1	2	0.5	1	9393.542	18787.084	18417.131	36834.262	1	1659502	2.22	36423	0.0438	1	0.004	15	0.3657
			0.5	2	9023.589	18047.178			1					1		15	
	2-2	2	0.5	1	9385.34	18770.68	18931.488	37862.976	1	1659502	2.28	37451	0.0450	1	0.004	15	0.3760
			0.5	2	9546.148	19092.296			1					1		15	
	2-3	2	0.5	1	8727.292	17454.584	17578.901	35157.802	1	1659502	2.12	34746	0.0418	1	0.004	15	0.3489
			0.5	2	8851.609	17703.218			1					1		15	
	3-1	2	0.5	1	9260.704	18521.408	18489.497	36978.994	1	1659502	2.23	36567	0.0440	1	0.004	15	0.3671
			0.5	2	9228.793	18457.586			1					1		15	
	3-2	2	0.5	1	9106.683	18213.366	18445.548	36891.096	1	1659502	2.22	36479	0.0439	1	0.004	15	0.3663
			0.5	2	9338.865	18677.73			1					1		15	
	3-3	2	0.5	1	9267.433	18534.866	18500.873	37001.746	1	1659502	2.23	36590	0.0440	1	0.004	15	0.3674
			0.5	2	9233.44	18466.88			1					1		15	
	4-1	2	0.5	1	9254.833	18509.666	18627.443	37254.886	1	1659502	2.24	36843	0.0443	1	0.004	15	0.3699
			0.5	2	9372.61	18745.22			1					1		15	
	4-2	2	0.5	1	8949.297	17898.594	18001.709	36003.418	1	1659502	2.17	35592	0.0428	1	0.004	15	0.3574
			0.5	2	9052.412	18104.824			1					1		15	
	4-3	2	0.5	1	9321.304	18642.608	18581.93	37163.86	1	1659502	2.24	36752	0.0442	1	0.004	15	0.3690
			0.5	2	9260.626	18521.252			1					1		15	
	5-1	2	0.5	1	8615.53	17231.06	17375.667	34751.334	1	1659502	2.09	34340	0.0413	1	0.004	15	0.3448
			0.5	2	8760.137	17520.274			1					1		15	
	5-2	2	0.5	1	8964.33	17928.66	18434.629	36869.258	1	1659502	2.22	36458	0.0438	1	0.004	15	0.3660
			0.5	2	9470.299	18940.598			1					1		15	
	5-3	2	0.5	1	8969.717	17939.434	18521.422	37042.844	1	1659502	2.23	36631	0.0441	1	0.004	15	0.3676
			0.5	2	9551.705	19103.41			1					1		15	
	6-1	2	0.5	1	8768.158	17536.316	17565.719	35131.438	1	1659502	2.12	34720	0.0418	1	0.004	15	0.3486
			0.5	2	8797.561	17595.122			1					1		15	
	6-2	2	0.5	1	9392.404	18784.808	18269.945	36539.89	1	1659502	2.20	36128	0.0435	1	0.004	15	0.3627
			0.5	2	8877.541	17755.082			1					1		15	
	6-3	2	0.5	1	9050.573	18101.146	17905.592	35811.184	1	1659502	2.16	35400	0.0426	1	0.004	15	0.3554
			0.5	2	8855.019	17710.038			1					1		15	
	7-1	2	0.5	1	8958.905	17917.81	18184.563	36369.126	1	1659502	2.19	35958	0.0432	1	0.004	15	0.3610
			0.5	2	9225.658	18451.316			1					1		15	
	7-2	2	0.5	1	8983.232	17966.464	17997.702	35995.404	1	1659502	2.17	35584	0.0428	1	0.004	15	0.3573
			0.5	2	9014.47	18028.94			1					1		15	
	7-3	2	0.5	1	8767.742	17535.484	17763.217	35526.434	1	1659502	2.14	35115	0.0422	1	0.004	15	0.3526
			0.5	2	8995.475	17990.95			1					1		15	
	8-1	2	0.5	1	8519.93	17039.86	16902.139	33804.278	1	1659502	2.04	33393	0.0402	1	0.004	15	0.3353
			0.5	2	8382.209	16764.418			1					1		15	
	8-2	2	0.5	1	8770.249	17540.498	17559.846	35119.692	1	1659502	2.12	34708	0.0417	1	0.004	15	0.3485
			0.5	2	8789.597	17579.194			1					1		15	
	8-3	2	0.5	1	8778.39	17556.78	17689.744	35379.488	1	1659502	2.13	34968	0.0421	1	0.004	15	0.3511
			0.5	2	8911.354	17822.708			1					1		15	

Assay Date	9/20/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.4040	0.0045
Full activity	End	0.4019	0.0017
Full activity	Overall	0.4030	0.0030
Background	Beginning	0.0002	0.000331763
Background	End	-0.0002	0.000244716
Background	Overall	0.0000	0.000299561
Positive	Beginning	0.2377	0.0037
Positive	End	0.2184	0.0033
Positive	Overall	0.2281	0.0115
Negative	Beginning	0.3705	0.0080
Negative	End	0.3229	0.0026
Negative	Overall	0.3467	0.0279

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#5 DBA	1	1	1.00E-04	-4.00	0.3740
RC#5 DBA	1	2	1.00E-04	-4.00	0.3650
RC#5 DBA	1	3	1.00E-04	-4.00	0.3613
RC#5 DBA	2	1	3.30E-05	-4.48	0.3657
RC#5 DBA	2	2	3.30E-05	-4.48	0.3760
RC#5 DBA	2	3	3.30E-05	-4.48	0.3489
RC#5 DBA	3	1	1.00E-05	-5.00	0.3671
RC#5 DBA	3	2	1.00E-05	-5.00	0.3663
RC#5 DBA	3	3	1.00E-05	-5.00	0.3674
RC#5 DBA	4	1	1.00E-06	-6.00	0.3699
RC#5 DBA	4	2	1.00E-06	-6.00	0.3574
RC#5 DBA	4	3	1.00E-06	-6.00	0.3690
RC#5 DBA	5	1	1.00E-07	-7.00	0.3448
RC#5 DBA	5	2	1.00E-07	-7.00	0.3660
RC#5 DBA	5	3	1.00E-07	-7.00	0.3678
RC#5 DBA	6	1	1.00E-08	-8.00	0.3486
RC#5 DBA	6	2	1.00E-08	-8.00	0.3627
RC#5 DBA	6	3	1.00E-08	-8.00	0.3554
RC#5 DBA	7	1	1.00E-09	-9.00	0.3610
RC#5 DBA	7	2	1.00E-09	-9.00	0.3573
RC#5 DBA	7	3	1.00E-09	-9.00	0.3526
RC#5 DBA	8	1	1.00E-10	-10.00	0.3353
RC#5 DBA	8	2	1.00E-10	-10.00	0.3485
RC#5 DBA	8	3	1.00E-10	-10.00	0.3511

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	92.82	90.59	89.66
2	-4.48	90.75	93.31	86.57
3	-5.00	91.11	90.89	91.17
4	-6.00	91.80	88.68	91.57
5	-7.00	85.56	90.84	91.27
6	-8.00	86.51	90.02	88.20
7	-9.00	89.59	88.66	87.49
8	-10.00	83.20	86.48	87.13

Assay Date	9/21/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8		
Technician ID	EJB	Replicate #	3	Microsome type	Recombina	Microsome ID	5

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#5 DBA		# Concentrations tested		8				
Technician ID	EJB	Replicate #	3	Microsome type	Recombinant	Microsome ID	5				

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blanks
	0.602	0.390	0.200	0.120	0.032	0.054	0.035
	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:	Mic 0.008	QC 10	QC100	Mic 0.08
	0.049	0.056	0.283	0.199
	0.046	0.059	0.280	0.205
	0.042	0.059	0.312	0.200

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

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

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

Blank 0.031

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
Mic 0.008	0.049	0.017	0.000	200	120	72000	0.001	0.000	0.168
Mic 0.008	0.046	0.015	0.000	200	120	72000	0.000		
Mic 0.008	0.042	0.010	0.000	200	120	72000	-0.001		
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		
Mic 0.08	0.199	0.167	0.013	200	140	8540	0.004	0.004	3.908
Mic 0.08	0.205	0.174	0.013	200	140	8540	0.004		
Mic 0.08	0.200	0.169	0.013	200	140	8540	0.004		

Assay Date	9/21/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	3

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.907664
Protein Concentration (dilution added to assay, mg/mL):	0.006406

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	9/21/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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 (Backgrround 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	10618.14	21236.28	21383.91	42767.82	1	1537563	2.78	42405	0.0549	1	0.003	15	0.5716
			0.5	2	10765.77	21531.54			1					1		15	
	2	2	0.5	1	10208.22	20416.44	20700.61	41401.22	1	1537563	2.69	41038	0.0532	1	0.003	15	0.5532
			0.5	2	10492.39	20984.78			1					1		15	
	3	2	0.5	1	8538.057	17076.114	17599.212	35198.424	1	1537563	2.29	34835	0.0451	1	0.003	15	0.4696
			0.5	2	9061.155	18122.31			1					1		15	
	4	2	0.5	1	8546.685	17093.37	17661.712	35323.424	1	1537563	2.30	34960	0.0453	1	0.003	15	0.4713
			0.5	2	9115.027	18230.054			1					1		15	
Background control	1	2	0.5	1	95.68357	191.36714	199.96927	399.93854	1	1537563	0.03	37	0.0000	1	0.003	15	0.0005
			0.5	2	104.2557	208.5714			1					1		15	
	2	2	0.5	1	92.94975	185.8995	182.49557	364.99114	1	1537563	0.02	2	0.0000	1	0.003	15	0.0000
			0.5	2	89.54582	179.09164			1					1		15	
	3	2	0.5	1	58.82782	117.65564	165.01952	330.03904	1	1537563	0.02	-33	0.0000	1	0.003	15	-0.0004
			0.5	2	106.1917	212.3834			1					1		15	
	4	2	0.5	1	78.11341	156.22682	178.51381	357.02762	1	1537563	0.02	-6	0.0000	1	0.003	15	-0.0001
			0.5	2	100.4004	200.8008			1					1		15	
Positive control	1	2	0.5	1	5231.674	10463.348	10772.368	21544.736	1	1537563	1.40	21182	0.0274	1	0.003	15	0.2655
			0.5	2	5540.694	11081.388			1					1		15	
	2	2	0.5	1	5527.448	11054.896	10707.202	21414.404	1	1537563	1.39	21051	0.0273	1	0.003	15	0.2638
			0.5	2	5179.754	10359.508			1					1		15	
	3	2	0.5	1	4390.718	8781.436	9008.726	18017.452	1	1537563	1.17	17654	0.0229	1	0.003	15	0.2380
			0.5	2	4618.008	9236.016			1					1		15	
	4	2	0.5	1	4891.542	9783.084	9772.312	19544.624	1	1537563	1.27	19182	0.0248	1	0.003	15	0.2586
			0.5	2	4880.77	9761.54			1					1		15	
Negative Control	1	2	0.5	1	7923.08	15846.16	16477.891	32955.782	1	1537563	2.14	32593	0.0422	1	0.003	15	0.4394
			0.5	2	8554.811	17109.622			1					1		15	
	2	2	0.5	1	8070.021	16140.042	16067.261	32134.522	1	1537563	2.09	31772	0.0412	1	0.003	15	0.4283
			0.5	2	7997.24	15994.48			1					1		15	
	3	2	0.5	1	7625.002	15250.004	15372.678	30745.356	1	1537563	2.00	30382	0.0394	1	0.003	15	0.4096
			0.5	2	7747.676	15495.352			1					1		15	
	4	2	0.5	1	6987.325	13974.65	13939.741	27879.482	1	1537563	1.81	27516	0.0356	1	0.003	15	0.3709
			0.5	2	6952.416	13904.832			1					1		15	
RC#5 DBA	1-1	2	0.5	1	8355.029	16710.058	16818.346	33636.692	1	1537563	2.19	33274	0.0431	1	0.003	15	0.4485
			0.5	2	8463.317	16926.634			1					1		15	
	1-2	2	0.5	1	8479.48	16958.96	16830.49	33660.98	1	1537563	2.19	33298	0.0431	1	0.003	15	0.4489
			0.5	2	8351.01	16702.02			1					1		15	
	1-3	2	0.5	1	7963.325	15926.65	16003.606	32007.212	1	1537563	2.08	31644	0.0410	1	0.003	15	0.4266
			0.5	2	8040.281	16080.562			1					1		15	
	2-1	2	0.5	1	8131.738	16263.476	16476.537	32953.074	1	1537563	2.14	32590	0.0422	1	0.003	15	0.4393
			0.5	2	8344.799	16689.598			1					1		15	
	2-2	2	0.5	1	8685.472	17370.944	17534.368	35068.736	1	1537563	2.28	34706	0.0450	1	0.003	15	0.4678
			0.5	2	8848.896	17697.792			1					1		15	
	2-3	2	0.5	1	8501.561	17003.122	17000.408	34000.816	1	1537563	2.21	33638	0.0436	1	0.003	15	0.4534
			0.5	2	8498.847	16997.694			1					1		15	
	3-1	2	0.5	1	8392.948	16785.896	17274.406	34548.812	1	1537563	2.25	34186	0.0443	1	0.003	15	0.4608
			0.5	2	8881.458	17762.916			1					1		15	
	3-2	2	0.5	1	8863.173	17726.346	17867.906	35735.812	1	1537563	2.32	35373	0.0456	1	0.003	15	0.4768
			0.5	2	9004.733	18009.466			1					1		15	
	3-3	2	0.5	1	8551.562	17103.124	17145.384	34290.768	1	1537563	2.23	33928	0.0439	1	0.003	15	0.4574
			0.5	2	8593.822	17187.644			1					1		15	
	4-1	2	0.5	1	8675.229	17350.458	17353.17	34706.34	1	1537563	2.26	34343	0.0445	1	0.003	15	0.4630
			0.5	2	8677.941	17355.882			1					1		15	
	4-2	2	0.5	1	8342.105	16684.21	16517.207	33034.414	1	1537563	2.15	32671	0.0423	1	0.003	15	0.4404
			0.5	2	8175.102	16350.204			1					1		15	
	4-3	2	0.5	1	8784.173	17568.346	17446.819	34893.638	1	1537563	2.27	34531	0.0447	1	0.003	15	0.4655
			0.5	2	8662.646	17325.292			1					1		15	
	5-1	2	0.5	1	8329.617	16659.234	16601.688	33203.376	1	1537563	2.16	32840	0.0425	1	0.003	15	0.4427
			0.5	2	8272.071	16544.142			1					1		15	
	5-2	2	0.5	1	8683.368	17366.736	17480.929	34961.858	1	1537563	2.27	34599	0.0448	1	0.003	15	0.4664
			0.5	2	8797.561	17595.122			1					1		15	
	5-3	2	0.5	1	8011.407	16022.814	16528.597	33057.194	1	1537563	2.15	32694	0.0423	1	0.003	15	0.4407
			0.5	2	8517.19	17034.38			1					1		15	
	6-1	2	0.5	1	8514.496	17028.992	16877.71	33755.42	1	1537563	2.20	33392	0.0433	1	0.003	15	0.4501
			0.5	2	8363.214	16726.428			1					1		15	
	6-2	2	0.5	1	8514.41	17028.82	16948.177	33896.354	1	1537563	2.20	33533	0.0434	1	0.003	15	0.4520
			0.5	2	8433.767	16867.534			1					1		15	
	6-3	2	0.5	1	8455.475	16910.95	17042.745	34085.49	1	1537563	2.22	33722	0.0437	1	0.003	15	0.4546
			0.5	2	8587.27	17174.54			1					1		15	
	7-1	2	0.5	1	8526.028	17052.056	16776.595	33553.19	1	1537563	2.18	33190	0.0430	1	0.003	15	0.4474
			0.5	2	8250.567	16501.134			1					1		15	
	7-2	2	0.5	1	8466.209	16932.418	16921.491	33842.982	1	1537563	2.20	33480	0.0434	1	0.003	15	0.4513
			0.5	2	8455.282	16910.564			1					1		15	
	7-3	2	0.5	1	8528.01	17056.02	16956.894	33913.788	1	1537563	2.21	33551	0.0435	1	0.003	15	0.4523
			0.5	2	8428.884	16857.768			1					1		15	
	8-1	2	0.5	1	8260.099	16520.198	16105.021	32210.042	1	1537563	2.09	31847	0.0413	1	0.003	15	0.4293
			0.5	2	7844.922	15689.844			1					1		15	
	8-2	2	0.5	1	7892.315	15784.63	16011.308	32022.616	1	1537563	2.08	31660	0.0410	1	0.003	15	0.4266
			0.5	2	8118.993	16237.986			1					1		15	
	8-3	2	0.5	1	7768.943	15537.886	15319.129	30638.258	1	1537563	1.99	30275	0.0392	1	0.003	15	0.4081
			0.5	2	7550.186	15100.372			1					1		15	

Assay Date	9/21/2005	Test Chemical ID	RC#5 DBA	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.5624	0.0130
Full activity	End	0.4704	0.0012
Full activity	Overall	0.5164	0.0536
Background	Beginning	0.0003	0.000333117
Background	End	-0.0003	0.000257254
Background	Overall	0.0000	0.000388402
Positive	Beginning	0.2847	0.0012
Positive	End	0.2483	0.0146
Positive	Overall	0.2665	0.0226
Negative	Beginning	0.4338	0.0078
Negative	End	0.3902	0.0273
Negative	Overall	0.4120	0.0300

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#5 DBA	1	1	1.00E-04	-4.00	0.4485
RC#5 DBA	1	2	1.00E-04	-4.00	0.4489
RC#5 DBA	1	3	1.00E-04	-4.00	0.4266
RC#5 DBA	2	1	3.30E-05	-4.48	0.4393
RC#5 DBA	2	2	3.30E-05	-4.48	0.4678
RC#5 DBA	2	3	3.30E-05	-4.48	0.4534
RC#5 DBA	3	1	1.00E-05	-5.00	0.4608
RC#5 DBA	3	2	1.00E-05	-5.00	0.4768
RC#5 DBA	3	3	1.00E-05	-5.00	0.4574
RC#5 DBA	4	1	1.00E-06	-6.00	0.4630
RC#5 DBA	4	2	1.00E-06	-6.00	0.4404
RC#5 DBA	4	3	1.00E-06	-6.00	0.4655
RC#5 DBA	5	1	1.00E-07	-7.00	0.4427
RC#5 DBA	5	2	1.00E-07	-7.00	0.4664
RC#5 DBA	5	3	1.00E-07	-7.00	0.4407
RC#5 DBA	6	1	1.00E-08	-8.00	0.4501
RC#5 DBA	6	2	1.00E-08	-8.00	0.4520
RC#5 DBA	6	3	1.00E-08	-8.00	0.4546
RC#5 DBA	7	1	1.00E-09	-9.00	0.4474
RC#5 DBA	7	2	1.00E-09	-9.00	0.4513
RC#5 DBA	7	3	1.00E-09	-9.00	0.4523
RC#5 DBA	8	1	1.00E-10	-10.00	0.4293
RC#5 DBA	8	2	1.00E-10	-10.00	0.4268
RC#5 DBA	8	3	1.00E-10	-10.00	0.4081

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	86.85	86.92	82.60
2	-4.48	85.07	90.59	87.80
3	-5.00	89.24	92.33	88.56
4	-6.00	89.65	85.28	90.14
5	-7.00	85.72	90.31	85.34
6	-8.00	87.16	87.53	88.03
7	-9.00	86.64	87.39	87.58
8	-10.00	83.13	82.64	79.03

Assay Date	8/3/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8		
Technician ID	EJB	Replicate #	1	Microsome type	Recombina	Microsome ID	5

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/b*c

2) Calculate total µg ASDN/g soln.

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

= 0.563831 + 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	EJB	Replicate #	1	Microsome type	Recombinant	Microsome ID	5		

								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	
	0.505	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:	Microsomes	QC 10	QC 100
	0.038	0.059	0.288
	0.039	0.060	0.288
	0.040	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	Variables	Regression results	
0.25	125	1000	0.00025	200	0.0500	0.506	0.477	0.0424	m, b	0.090	-0.001
0.125	62.5	1000	0.00013	200	0.0250	0.306	0.277	0.0243	se_m, se_b	0.005	0.001
0.05	25	1000	0.00005	200	0.0100	0.161	0.131	0.0112	r^2, se_y	0.991	0.001
0.025	25	2000	0.00003	200	0.0050	0.100	0.071	0.0057	F, df	348	3
0.01	5	1000	0.00001	200	0.0020	0.049	0.019	0.0010	ss_{reg}, ss_{resid}	0.000	0.000
0.005	5	2000	0.00001	200	0.0010	0.045	0.015	0.0007			

Regression results are calculated using the function LINEST

Blank	0.030	r^2=	0.991		
		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
Microsomes	0.038	0.009	0.000	200	120	72000	0.000	0.001	0.570
Microsomes	0.039	0.010	0.000	200	120	72000	0.001		
Microsomes	0.040	0.010	0.000	200	120	72000	0.001		
QC 10	0.059	0.029	0.002	200	200	200	0.000	0.000	0.010
QC 10	0.060	0.031	0.002	200	200	200	0.000		
QC 10	0.060	0.031	0.002	200	200	200	0.000		
QC 100	0.288	0.258	0.023	200	200	200	0.000	0.000	0.113
QC 100	0.288	0.258	0.023	200	200	200	0.000		
QC 100	0.282	0.253	0.022	200	200	200	0.000		

Assay Date	8/3/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	1

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	0.570003
Protein Concentration (dilution added to assay, mg/mL):	0.00095

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	7484.817	14969.634	15266.221	30532.442	1	1483915	2.06	30132	0.0405	1	0.000	15	2.8429
			0.5	2	7781.404	15562.808			1					1		15	
	2	2	0.5	1	8508.185	17016.37	16910.509	33821.018	1	1483915	2.28	33420	0.0449	1	0.000	15	3.1532
			0.5	2	8402.324	16804.648			1					1		15	
	3	2	0.5	1	7232.281	14464.562	14430.314	28860.628	1	1483915	1.94	28460	0.0383	1	0.000	15	2.6852
			0.5	2	7198.033	14396.066			1					1		15	
	4	2	0.5	1	7218.829	14437.658	14818.016	29236.032	1	1483915	1.97	28835	0.0388	1	0.000	15	2.7206
			0.5	2	7399.187	14798.374			1					1		15	
Background control	1	2	0.5	1	154.4352	308.8704	263.6145	527.229	1	1483915	0.04	127	0.0002	1	0.000	15	0.0120
			0.5	2	109.1793	218.3586			1					1		15	
	2	2	0.5	1	113.347	226.694	238.9892	477.9784	1	1483915	0.03	77	0.0001	1	0.000	15	0.0073
			0.5	2	125.6422	251.2844			1					1		15	
	3	2	0.5	1	61.96468	123.92936	136.83867	273.67734	1	1483915	0.02	-127	-0.0002	1	0.000	15	-0.0120
			0.5	2	74.87399	149.74798			1					1		15	
	4	2	0.5	1	88.38322	176.76644	161.67491	323.34982	1	1483915	0.02	-77	-0.0001	1	0.000	15	-0.0073
			0.5	2	73.29169	146.58338			1					1		15	
Positive control	1	2	0.5	1	2824.347	5648.694	5955.904	11911.808	1	1483915	0.80	11511	0.0155	1	0.000	15	1.0861
			0.5	2	3131.557	6263.114			1					1		15	
	2	2	0.5	1	2844.234	5688.468	5617.922	11235.844	1	1483915	0.76	10835	0.0146	1	0.000	15	1.0223
			0.5	2	2773.688	5547.376			1					1		15	
	3	2	0.5	1	3722.171	7444.342	7317.662	14635.324	1	1483915	0.99	14235	0.0191	1	0.000	15	1.3430
			0.5	2	3595.491	7190.982			1					1		15	
	4	2	0.5	1	3574.404	7148.808	7172.531	14345.062	1	1483915	0.97	13945	0.0187	1	0.000	15	1.3157
			0.5	2	3598.127	7196.254			1					1		15	
Negative Control	1	2	0.5	1	4536.163	9072.326	9258.242	18516.484	1	1483915	1.25	18116	0.0244	1	0.000	15	1.7092
			0.5	2	4722.079	9444.158			1					1		15	
	2	2	0.5	1	4377.592	8755.184	8973.544	17947.088	1	1483915	1.21	17547	0.0236	1	0.000	15	1.6555
			0.5	2	4595.952	9191.904			1					1		15	
	3	2	0.5	1	6568.987	13137.974	12900.865	25801.73	1	1483915	1.74	25401	0.0342	1	0.000	15	2.3966
			0.5	2	6331.878	12663.756			1					1		15	
	4	2	0.5	1	7127.719	14255.438	14107.431	28214.862	1	1483915	1.90	27814	0.0374	1	0.000	15	2.6243
			0.5	2	6979.712	13959.424			1					1		15	
RC#6 FRM	1-1	2	0.5	1	322.3422	644.6844	730.6859	1461.3718	1	1483915	0.10	1061	0.0014	1	0.000	15	0.1001
			0.5	2	408.3437	816.6874			1					1		15	
	1-2	2	0.5	1	3934.234	7868.468	7703.741	15407.482	1	1483915	1.04	15007	0.0202	1	0.000	15	1.4159
			0.5	2	3769.507	7539.014			1					1		15	
	1-3	2	0.5	1	3680.959	7361.918	7486.932	14973.864	1	1483915	1.01	14573	0.0196	1	0.000	15	1.3750
			0.5	2	3805.973	7611.946			1					1		15	
	2-1	2	0.5	1	2240.655	4481.31	4624.44	9248.88	1	1483915	0.62	8848	0.0119	1	0.000	15	0.8348
			0.5	2	2383.785	4767.57			1					1		15	
	2-2	2	0.5	1	2282.537	4565.074	4664.542	9329.084	1	1483915	0.63	8929	0.0120	1	0.000	15	0.8424
			0.5	2	2382.005	4764.01			1					1		15	
	2-3	2	0.5	1	2331.794	4663.588	4666.682	9333.364	1	1483915	0.63	8933	0.0120	1	0.000	15	0.8428
			0.5	2	2334.888	4669.776			1					1		15	
	3-1	2	0.5	1	3472.659	6945.318	6655.397	13310.794	1	1483915	0.90	12910	0.0174	1	0.000	15	1.2181
			0.5	2	3182.738	6365.476			1					1		15	
	3-2	2	0.5	1	3462.285	6924.57	6519.353	13038.706	1	1483915	0.88	12638	0.0170	1	0.000	15	1.1924
			0.5	2	3057.068	6114.136			1					1		15	
	3-3	2	0.5	1	3408.111	6816.222	6665.344	13330.688	1	1483915	0.90	12930	0.0174	1	0.000	15	1.2200
			0.5	2	3257.233	6514.466			1					1		15	
	4-1	2	0.5	1	5659.225	11318.45	10962.453	21924.906	1	1483915	1.48	21524	0.0289	1	0.000	15	2.0308
			0.5	2	5303.228	10606.456			1					1		15	
	4-2	2	0.5	1	5654.26	11308.52	11227.105	22454.21	1	1483915	1.51	22054	0.0297	1	0.000	15	2.0808
			0.5	2	5572.845	11145.69			1					1		15	
	4-3	2	0.5	1	5698.489	11396.978	11485.976	22971.952	1	1483915	1.55	22571	0.0303	1	0.000	15	2.1296
			0.5	2	5787.487	11574.974			1					1		15	
	5-1	2	0.5	1	6680.084	13360.168	13262.139	26524.278	1	1483915	1.79	26124	0.0351	1	0.000	15	2.4648
			0.5	2	6582.055	13164.11			1					1		15	
	5-2	2	0.5	1	6694.179	13188.358	13378.998	26757.996	1	1483915	1.80	26357	0.0354	1	0.000	15	2.4868
			0.5	2	6784.819	13569.638			1					1		15	
	5-3	2	0.5	1	6848.516	13697.032	13846.498	27692.996	1	1483915	1.87	27292	0.0367	1	0.000	15	2.5750
			0.5	2	6997.982	13995.964			1					1		15	
	6-1	2	0.5	1	6351.48	12702.96	12595.593	25191.186	1	1483915	1.70	24791	0.0333	1	0.000	15	2.3390
			0.5	2	6244.113	12488.226			1					1		15	
	6-2	2	0.5	1	6493.67	12987.34	12709.342	25416.684	1	1483915	1.71	25016	0.0336	1	0.000	15	2.3603
			0.5	2	6214.672	12429.344			1					1		15	
	6-3	2	0.5	1	5984.19	11968.38	12124.354	24248.708	1	1483915	1.63	23848	0.0321	1	0.000	15	2.2501
			0.5	2	6140.164	12280.328			1					1		15	
	7-1	2	0.5	1	6329.361	12658.722	12339.547	24679.094	1	1483915	1.66	24279	0.0326	1	0.000	15	2.2907
			0.5	2	6010.186	12020.372			1					1		15	
	7-2	2	0.5	1	6496.888	12993.776	13099.774	26199.548	1	1483915	1.77	25799	0.0347	1	0.000	15	2.4341
			0.5	2	6602.886	13205.772			1					1		15	
	7-3	2	0.5	1	6788.68	13577.36	13434.118	26868.236	1	1483915	1.81	26468	0.0356	1	0.000	15	2.4972
			0.5	2	6645.438	13290.876			1					1		15	
	8-1	2	0.5	1	6670.475	13340.95	13316.566	26633.132	1	1483915	1.79	26233	0.0353	1	0.000	15	2.4750
			0.5	2	6646.091	13292.182			1					1		15	
	8-2	2	0.5	1	6645.058	13290.116	13115.769	26231.538	1	1483915	1.77	25831	0.0347	1	0.000	15	2.4371
			0.5	2	6470.711	12941.422			1					1		15	
	8-3	2	0.5	1	6553.494	13106.988	12919.82	25839.64	1	1483915	1.74	25439	0.0342	1	0.000	15	2.4002
			0.5	2	6366.326	12732.652			1					1		15	

Assay Date	8/3/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	2.9981	0.2194
Full activity	End	2.7029	0.0250
Full activity	Overall	2.8505	0.2128
Background	Beginning	0.0096	0.003285764
Background	End	-0.0096	0.00331391
Background	Overall	0.0000	0.01143918
Positive	Beginning	1.0542	0.0451
Positive	End	1.3294	0.0194
Positive	Overall	1.1918	0.1614
Negative	Beginning	1.6824	0.0380
Negative	End	2.5104	0.1610
Negative	Overall	2.0964	0.4875

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#6 FRM	1	1	3.33E-05	-4.48	0.1001
RC#6 FRM	1	2	3.33E-05	-4.48	1.4159
RC#6 FRM	1	3	3.33E-05	-4.48	1.3750
RC#6 FRM	2	1	1.00E-05	-5.00	0.8348
RC#6 FRM	2	2	1.00E-05	-5.00	0.8424
RC#6 FRM	2	3	1.00E-05	-5.00	0.8428
RC#6 FRM	3	1	3.30E-06	-5.48	1.2181
RC#6 FRM	3	2	3.30E-06	-5.48	1.1924
RC#6 FRM	3	3	3.30E-06	-5.48	1.2200
RC#6 FRM	4	1	1.00E-06	-6.00	2.0308
RC#6 FRM	4	2	1.00E-06	-6.00	2.0808
RC#6 FRM	4	3	1.00E-06	-6.00	2.1296
RC#6 FRM	5	1	1.00E-07	-7.00	2.4648
RC#6 FRM	5	2	1.00E-07	-7.00	2.4868
RC#6 FRM	5	3	1.00E-07	-7.00	2.5750
RC#6 FRM	6	1	1.00E-08	-8.00	2.3390
RC#6 FRM	6	2	1.00E-08	-8.00	2.3603
RC#6 FRM	6	3	1.00E-08	-8.00	2.2501
RC#6 FRM	7	1	1.00E-09	-9.00	2.2907
RC#6 FRM	7	2	1.00E-09	-9.00	2.4341
RC#6 FRM	7	3	1.00E-09	-9.00	2.4972
RC#6 FRM	8	1	1.00E-10	-10.00	2.4750
RC#6 FRM	8	2	1.00E-10	-10.00	2.4371
RC#6 FRM	8	3	1.00E-10	-10.00	2.4002

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	3.51	49.67	48.24
2	-5.00	29.29	29.55	29.57
3	-5.48	42.73	41.83	42.80
4	-6.00	71.24	73.00	74.71
5	-7.00	86.47	87.24	90.34
6	-8.00	82.06	82.80	78.94
7	-9.00	80.36	85.39	87.61
8	-10.00	86.83	85.50	84.20

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

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#6 FRM		# Concentrations tested		8				
Technician ID	EJB	Replicate #	2	Microsome type		Recombinant	Microsome ID	5			

								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:	Microsomes	QC 10	QC 100
	0.048	0.061	0.285
	0.053	0.064	0.276
	0.050	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	0.0392	m, b	0.083	0.000
0.125	62.5	1000	0.00013	200	0.0250	0.334	0.299	0.0247	se_m, se_b	0.002	0.000
0.05	25	1000	0.00005	200	0.0100	0.165	0.130	0.0106	r^2, se_y	0.998	0.001
0.025	25	2000	0.00003	200	0.0050	0.104	0.068	0.0055	F, df	1273	3
0.01	5	1000	0.00001	200	0.0020	0.058	0.022	0.0016	ss_{reg}, ss_{resid}	0.000	0.000
0.005	5	2000	0.00001	200	0.0010	0.046	0.010	0.0006			

Regression results are calculated using the function LINEST

Blank	0.035	r^2=	0.998	
		m=	0.083	
		b=	0.000	

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
Microsomes	0.048	0.013	0.001	200	120	72000	0.002	0.003	3.066
Microsomes	0.053	0.018	0.001	200	120	72000	0.004		
Microsomes	0.050	0.014	0.001	200	120	72000	0.003		
QC 10	0.061	0.026	0.002	200	200	200	0.000	0.000	0.011
QC 10	0.064	0.029	0.002	200	200	200	0.000		
QC 10	0.065	0.030	0.002	200	200	200	0.000		
QC 100	0.285	0.250	0.021	200	200	200	0.000	0.000	0.102
QC 100	0.276	0.241	0.020	200	200	200	0.000		
QC 100	0.290	0.255	0.021	200	200	200	0.000		

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

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.065756
Protein Concentration (dilution added to assay, mg/mL):	0.00511

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	6	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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	6930.284	13860.568	13959.349	27918.698	1	1717129	1.63	27494	0.0319	1	0.003	15	0.4168
			0.5	2	7029.065	14058.13			1					1		15	
	2	2	0.5	1	6990.611	13981.222	13876.703	27753.406	1	1717129	1.62	27329	0.0318	1	0.003	15	0.4143
			0.5	2	6886.092	13772.184			1					1		15	
	3	2	0.5	1	6529.315	13058.63	13237.876	26475.752	1	1717129	1.54	26051	0.0303	1	0.003	15	0.3950
			0.5	2	6708.561	13417.122			1					1		15	
	4	2	0.5	1	6654.736	13309.472	13146.303	26296.606	1	1717129	1.53	25872	0.0301	1	0.003	15	0.3923
			0.5	2	6493.567	12987.134			1					1		15	
Background control	1	2	0.5	1	105.8563	211.7126	209.8366	419.6732	1	1717129	0.02	-5	0.0000	1	0.003	15	-0.0001
			0.5	2	103.9803	207.9606			1					1		15	
	2	2	0.5	1	95.52938	191.0587	191.71163	383.42326	1	1717129	0.02	-41	0.0000	1	0.003	15	-0.0006
			0.5	2	96.18225	192.3645			1					1		15	
	3	2	0.5	1	115.1727	230.3454	200.96687	401.91374	1	1717129	0.02	-23	0.0000	1	0.003	15	-0.0003
			0.5	2	85.78417	171.56834			1					1		15	
	4	2	0.5	1	133.4956	266.9912	247.0981	494.1962	1	1717129	0.03	69	0.0001	1	0.003	15	0.0011
			0.5	2	113.6025	227.205			1					1		15	
Positive control	1	2	0.5	1	3641.026	7282.052	7204.968	14409.936	1	1717129	0.84	13985	0.0163	1	0.003	15	0.2120
			0.5	2	3563.942	7127.884			1					1		15	
	2	2	0.5	1	3652.326	7304.652	7026.837	14053.674	1	1717129	0.82	13629	0.0158	1	0.003	15	0.2066
			0.5	2	3374.511	6749.022			1					1		15	
	3	2	0.5	1	3121.487	6242.974	6137.117	12274.234	1	1717129	0.71	11849	0.0138	1	0.003	15	0.1797
			0.5	2	3015.63	6031.26			1					1		15	
	4	2	0.5	1	3352.98	6705.96	6633.574	13267.148	1	1717129	0.77	12842	0.0149	1	0.003	15	0.1947
			0.5	2	3280.594	6561.188			1					1		15	
Negative Control	1	2	0.5	1	5808.366	11616.732	11865.248	23730.496	1	1717129	1.38	23306	0.0271	1	0.003	15	0.3534
			0.5	2	6056.882	12113.764			1					1		15	
	2	2	0.5	1	5934.009	11868.018	11574.851	23149.702	1	1717129	1.35	22725	0.0264	1	0.003	15	0.3445
			0.5	2	5640.842	11281.684			1					1		15	
	3	2	0.5	1	5567.55	11135.1	11383.769	22767.538	1	1717129	1.33	22343	0.0260	1	0.003	15	0.3388
			0.5	2	5816.219	11632.438			1					1		15	
	4	2	0.5	1	5565.572	11131.144	11332.057	22664.114	1	1717129	1.32	22239	0.0258	1	0.003	15	0.3372
			0.5	2	5766.485	11532.97			1					1		15	
RC#6 FRM	1-1	2	0.5	1	980.0295	1960.059	2010.1055	4020.211	1	1717129	0.23	3595	0.0042	1	0.003	15	0.0545
			0.5	2	1030.076	2060.152			1					1		15	
	1-2	2	0.5	1	808.8347	1617.6694	1732.8432	3465.6864	1	1717129	0.20	3041	0.0035	1	0.003	15	0.0461
			0.5	2	924.0085	1848.017			1					1		15	
	1-3	2	0.5	1	841.583	1683.166	1700.9251	3401.8502	1	1717129	0.20	2977	0.0035	1	0.003	15	0.0451
			0.5	2	859.3421	1718.6842			1					1		15	
	2-1	2	0.5	1	1997.175	3994.35	4053.417	8106.834	1	1717129	0.47	7682	0.0089	1	0.003	15	0.1165
			0.5	2	2056.242	4112.484			1					1		15	
	2-2	2	0.5	1	2193.167	4386.334	4275.622	8551.244	1	1717129	0.50	8126	0.0094	1	0.003	15	0.1232
			0.5	2	2082.455	4164.91			1					1		15	
	2-3	2	0.5	1	2195.804	4391.608	4412.901	8825.802	1	1717129	0.51	8401	0.0098	1	0.003	15	0.1274
			0.5	2	2217.097	4434.194			1					1		15	
	3-1	2	0.5	1	3609.654	7219.308	7123.467	14246.934	1	1717129	0.83	13822	0.0161	1	0.003	15	0.2096
			0.5	2	3513.813	7027.626			1					1		15	
	3-2	2	0.5	1	3788.451	7576.902	7505.804	15011.608	1	1717129	0.87	14587	0.0170	1	0.003	15	0.2212
			0.5	2	3717.353	7434.706			1					1		15	
	3-3	2	0.5	1	3758.963	7517.926	7557.466	15114.932	1	1717129	0.88	14690	0.0171	1	0.003	15	0.2227
			0.5	2	3798.503	7597.006			1					1		15	
	4-1	2	0.5	1	5110.08	10220.16	10155.42	20310.84	1	1717129	1.18	19886	0.0231	1	0.003	15	0.3015
			0.5	2	5045.34	10090.68			1					1		15	
	4-2	2	0.5	1	5110.599	10221.198	10192.843	20385.686	1	1717129	1.19	19961	0.0232	1	0.003	15	0.3026
			0.5	2	5082.244	10164.488			1					1		15	
	4-3	2	0.5	1	5071.7	10143.4	10113.233	20226.466	1	1717129	1.18	19802	0.0230	1	0.003	15	0.3002
			0.5	2	5041.533	10083.066			1					1		15	
	5-1	2	0.5	1	5923.134	11846.268	12236.432	24472.864	1	1717129	1.43	24048	0.0279	1	0.003	15	0.3646
			0.5	2	6313.298	12626.596			1					1		15	
	5-2	2	0.5	1	6313.298	12626.596	12455.254	24910.508	1	1717129	1.45	24486	0.0285	1	0.003	15	0.3712
			0.5	2	6141.956	12283.912			1					1		15	
	5-3	2	0.5	1	6163.044	12326.088	12220.022	24440.044	1	1717129	1.42	24015	0.0279	1	0.003	15	0.3641
			0.5	2	6056.978	12113.956			1					1		15	
	6-1	2	0.5	1	6253.451	12506.902	12580.195	25160.39	1	1717129	1.47	24736	0.0287	1	0.003	15	0.3750
			0.5	2	6326.744	12653.488			1					1		15	
	6-2	2	0.5	1	6102.416	12204.832	12263.376	24526.752	1	1717129	1.43	24102	0.0280	1	0.003	15	0.3654
			0.5	2	6160.96	12321.92			1					1		15	
	6-3	2	0.5	1	6250.034	12500.068	12480.198	24960.396	1	1717129	1.45	24536	0.0285	1	0.003	15	0.3720
			0.5	2	6230.164	12460.328			1					1		15	
	7-1	2	0.5	1	6369.583	12739.166	12516.811	25033.622	1	1717129	1.46	24609	0.0286	1	0.003	15	0.3731
			0.5	2	6147.228	12294.456			1					1		15	
	7-2	2	0.5	1	6148.747	12297.494	12641.295	25282.59	1	1717129	1.47	24858	0.0289	1	0.003	15	0.3769
			0.5	2	6492.548	12985.096			1					1		15	
	7-3	2	0.5	1	5965.448	11930.896	12289.301	24578.602	1	1717129	1.43	24154	0.0281	1	0.003	15	0.3662
			0.5	2	6323.853	12647.706			1					1		15	
	8-1	2	0.5	1	5944.254	11888.508	12225.92	24451.84	1	1717129	1.42	24027	0.0279	1	0.003	15	0.3643
			0.5	2	6281.666	12563.332			1					1		15	
	8-2	2	0.5	1	6489.035	12978.07	12665.26	25330.52	1	1717129	1.48	24906	0.0289	1	0.003	15	0.3776
			0.5	2	6176.225	12352.45			1					1		15	
	8-3	2	0.5	1	6164.562	12329.124	12651.638	25303.676	1	1717129	1.47	24879	0.0289	1	0.003	15	0.3772
			0.5	2	6487.276	12974.552			1					1		15	

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

Control Type	Portion	Average	SD
Full activity	Beginning	0.4156	0.0018
Full activity	End	0.3936	0.0019
Full activity	Overall	0.4046	0.0128
Background	Beginning	-0.0004	0.000388629
Background	End	0.0004	0.000989344
Background	Overall	0.0000	0.000736437
Positive	Beginning	0.2093	0.0038
Positive	End	0.1872	0.0106
Positive	Overall	0.1983	0.0144
Negative	Beginning	0.3489	0.0062
Negative	End	0.3380	0.0011
Negative	Overall	0.3435	0.0073

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#6 FRM	1	1	3.30E-05	-4.48	0.0545
RC#6 FRM	1	2	3.30E-05	-4.48	0.0461
RC#6 FRM	1	3	3.30E-05	-4.48	0.0451
RC#6 FRM	2	1	1.00E-05	-5.00	0.1165
RC#6 FRM	2	2	1.00E-05	-5.00	0.1232
RC#6 FRM	2	3	1.00E-05	-5.00	0.1274
RC#6 FRM	3	1	3.30E-06	-5.48	0.2096
RC#6 FRM	3	2	3.30E-06	-5.48	0.2212
RC#6 FRM	3	3	3.30E-06	-5.48	0.2227
RC#6 FRM	4	1	1.00E-06	-6.00	0.3015
RC#6 FRM	4	2	1.00E-06	-6.00	0.3026
RC#6 FRM	4	3	1.00E-06	-6.00	0.3002
RC#6 FRM	5	1	1.00E-07	-7.00	0.3646
RC#6 FRM	5	2	1.00E-07	-7.00	0.3712
RC#6 FRM	5	3	1.00E-07	-7.00	0.3641
RC#6 FRM	6	1	1.00E-08	-8.00	0.3750
RC#6 FRM	6	2	1.00E-08	-8.00	0.3654
RC#6 FRM	6	3	1.00E-08	-8.00	0.3720
RC#6 FRM	7	1	1.00E-09	-9.00	0.3731
RC#6 FRM	7	2	1.00E-09	-9.00	0.3769
RC#6 FRM	7	3	1.00E-09	-9.00	0.3662
RC#6 FRM	8	1	1.00E-10	-10.00	0.3643
RC#6 FRM	8	2	1.00E-10	-10.00	0.3776
RC#6 FRM	8	3	1.00E-10	-10.00	0.3772

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	13.47	11.39	11.16
2	-5.00	28.79	30.45	31.48
3	-5.48	51.79	54.66	55.05
4	-6.00	74.52	74.80	74.20
5	-7.00	90.11	91.75	89.99
6	-8.00	92.69	90.32	91.94
7	-9.00	92.22	93.15	90.51
8	-10.00	90.04	93.33	93.23

Assay Date	8/8/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8		
Technician ID	EJB	Replicate #	3	Microsome type	Recombina	Microsome ID	5

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#6 FRM	# Concentrations tested	8
Technician ID	EJB	Replicate #	3	Microsome type	Recombinant
Microsome ID	5				

								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	
	0.448	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:	Microsomes	QC 10	QC 100
	0.055	0.067	0.256
	0.057	0.070	0.245
	0.054	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	0.0410	m, b	0.102	-0.002
0.125	62.5	1000	0.00013	200	0.0250	0.291	0.251	0.0240	se_m, se_b	0.007	0.001
0.05	25	1000	0.00005	200	0.0100	0.171	0.132	0.0119	r^2, se_y	0.984	0.001
0.025	25	2000	0.00003	200	0.0050	0.110	0.070	0.0056	F, df	190	3
0.01	5	1000	0.00001	200	0.0020	0.069	0.029	0.0015	ss_{reg}, ss_{resid}	0.000	0.000
0.005	5	2000	0.00001	200	0.0010	0.056	0.016	0.0001			

Regression results are calculated using the function LINEST

Blank	0.040	r^2=	0.984
		m=	0.102
		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	0.055	0.016	0.000	200	120	72000	0.000	0.000	0.142
Microsomes	0.057	0.017	0.000	200	120	72000	0.001		
Microsomes	0.054	0.014	0.000	200	120	72000	0.000		
QC 10	0.067	0.027	0.001	200	200	200	0.000	0.000	0.006
QC 10	0.070	0.030	0.002	200	200	200	0.000		
QC 10	0.063	0.023	0.001	200	200	200	0.000		
QC 100	0.256	0.216	0.020	200	200	200	0.000	0.000	0.097
QC 100	0.245	0.205	0.019	200	200	200	0.000		
QC 100	0.239	0.199	0.019	200	200	200	0.000		

Assay Date	8/8/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID EJB	Replicate # 3

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	0.14221
Protein Concentration (dilution added to assay, mg/mL):	0.000237

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	6126.116	12252.232	12260.034	24520.068	1	1716832	1.43	24205	0.0283	1	0.000	15	7.9478
			0.5	2	6133.918	12267.836			1					1		15	
	2	2	0.5	1	6207.726	12415.452	12133.402	24266.804	1	1716832	1.41	23952	0.0280	1	0.000	15	7.8646
			0.5	2	5925.676	11851.352			1					1		15	
	3	2	0.5	1	2628.017	5256.034	5152.726	10305.452	1	1716832	0.60	9991	0.0117	1	0.000	15	3.2804
			0.5	2	2524.709	5049.418			1					1		15	
	4	2	0.5	1	5177.507	10355.014	10601.251	21202.502	1	1716832	1.23	20888	0.0244	1	0.000	15	6.8585
			0.5	2	5423.744	10847.488			1					1		15	
Background control	1	2	0.5	1	78.52639	157.05278	150.20582	300.41164	1	1716832	0.02	-14	0.0000	1	0.000	15	-0.0047
			0.5	2	71.67943	143.35886			1					1		15	
	2	2	0.5	1	69.02464	138.04928	142.83199	285.66398	1	1716832	0.02	-29	0.0000	1	0.000	15	-0.0096
			0.5	2	73.80735	147.6147			1					1		15	
	3	2	0.5	1	92.91779	185.83558	166.20909	332.41818	1	1716832	0.02	18	0.0000	1	0.000	15	0.0058
			0.5	2	73.2913	146.5826			1					1		15	
	4	2	0.5	1	73.2913	146.5826	170.26018	340.52036	1	1716832	0.02	26	0.0000	1	0.000	15	0.0085
			0.5	2	96.96883	193.93776			1					1		15	
Positive control	1	2	0.5	1	3514.966	7029.932	6611.54	13223.08	1	1716832	0.77	12908	0.0151	1	0.000	15	4.2384
			0.5	2	3096.574	6193.148			1					1		15	
	2	2	0.5	1	3269.333	6538.666	6624.949	13249.898	1	1716832	0.77	12935	0.0151	1	0.000	15	4.2472
			0.5	2	3355.616	6711.232			1					1		15	
	3	2	0.5	1	3143.69	6287.38	6061.031	12122.062	1	1716832	0.71	11807	0.0138	1	0.000	15	3.8759
			0.5	2	2917.341	5834.682			1					1		15	
	4	2	0.5	1	3163.189	6326.378	6291.776	12583.552	1	1716832	0.73	12269	0.0143	1	0.000	15	4.0284
			0.5	2	3128.587	6257.174			1					1		15	
Negative Control	1	2	0.5	1	6039.056	12078.11	11786.714	23573.428	1	1716832	1.37	23259	0.0272	1	0.000	15	7.6370
			0.5	2	5747.659	11495.318			1					1		15	
	2	2	0.5	1	5513.583	11027.166	10970.084	21940.168	1	1716832	1.28	21625	0.0252	1	0.000	15	7.1007
			0.5	2	5456.501	10913.002			1					1		15	
	3	2	0.5	1	5094.576	10189.152	10419.784	20839.568	1	1716832	1.21	20525	0.0240	1	0.000	15	6.7393
			0.5	2	5325.208	10650.416			1					1		15	
	4	2	0.5	1	4576.889	9153.778	9177.671	18355.342	1	1716832	1.07	18041	0.0211	1	0.000	15	5.9236
			0.5	2	4600.782	9201.564			1					1		15	
RC#6 FRM	1-1	2	0.5	1	876.892	1753.784	1798.2829	3596.5658	1	1716832	0.21	3282	0.0038	1	0.000	15	1.0776
			0.5	2	921.3909	1842.7818			1					1		15	
	1-2	2	0.5	1	851.434	1702.868	1697.596	3395.192	1	1716832	0.20	3080	0.0036	1	0.000	15	1.0115
			0.5	2	846.162	1692.324			1					1		15	
	1-3	2	0.5	1	829.7754	1659.5508	1583.6408	3167.2816	1	1716832	0.18	2853	0.0033	1	0.000	15	0.9366
			0.5	2	753.8654	1507.7308			1					1		15	
	2-1	2	0.5	1	1934.838	3869.676	3801.553	7603.106	1	1716832	0.44	7288	0.0085	1	0.000	15	2.3931
			0.5	2	1866.715	3733.43			1					1		15	
	2-2	2	0.5	1	1863.665	3727.33	3711.682	7423.364	1	1716832	0.43	7109	0.0083	1	0.000	15	2.3341
			0.5	2	1848.017	3696.034			1					1		15	
	2-3	2	0.5	1	1962.658	3925.316	3915.948	7831.896	1	1716832	0.46	7517	0.0088	1	0.000	15	2.4682
			0.5	2	1953.29	3906.58			1					1		15	
	3-1	2	0.5	1	3172.529	6345.058	6209.661	12419.322	1	1716832	0.72	12105	0.0141	1	0.000	15	3.9745
			0.5	2	3037.132	6074.264			1					1		15	
	3-2	2	0.5	1	3316.484	6632.968	6774.011	13548.022	1	1716832	0.79	13233	0.0154	1	0.000	15	4.3451
			0.5	2	3457.527	6915.054			1					1		15	
	3-3	2	0.5	1	3403.5	6807	6843.01	13686.02	1	1716832	0.80	13371	0.0156	1	0.000	15	4.3904
			0.5	2	3439.51	6879.02			1					1		15	
	4-1	2	0.5	1	4584.901	9169.802	9142.122	18284.244	1	1716832	1.06	17969	0.0210	1	0.000	15	5.9003
			0.5	2	4557.221	9114.442			1					1		15	
	4-2	2	0.5	1	4299.652	8599.304	8688.623	17377.246	1	1716832	1.01	17062	0.0199	1	0.000	15	5.6024
			0.5	2	4388.971	8777.942			1					1		15	
	4-3	2	0.5	1	4510.566	9021.132	9232.911	18465.822	1	1716832	1.08	18151	0.0212	1	0.000	15	5.9599
			0.5	2	4722.345	9444.69			1					1		15	
	5-1	2	0.5	1	5471.617	10943.234	10706.793	21413.586	1	1716832	1.25	21099	0.0246	1	0.000	15	6.9278
			0.5	2	5235.176	10470.352			1					1		15	
	5-2	2	0.5	1	5188.059	10376.118	10800.857	21601.714	1	1716832	1.26	21287	0.0248	1	0.000	15	6.9895
			0.5	2	5612.798	11225.596			1					1		15	
	5-3	2	0.5	1	5481.229	10962.458	11252.842	22505.684	1	1716832	1.31	22191	0.0259	1	0.000	15	7.2864
			0.5	2	5771.613	11543.226			1					1		15	
	6-1	2	0.5	1	5527.731	11055.462	11065.791	22131.582	1	1716832	1.29	21817	0.0255	1	0.000	15	7.1635
			0.5	2	5538.06	11076.12			1					1		15	
	6-2	2	0.5	1	5503.504	11007.008	10754.481	21508.962	1	1716832	1.25	21194	0.0247	1	0.000	15	6.9591
			0.5	2	5250.977	10501.954			1					1		15	
	6-3	2	0.5	1	5572.53	11145.06	11073.048	22146.096	1	1716832	1.29	21831	0.0255	1	0.000	15	7.1683
			0.5	2	5500.518	11001.036			1					1		15	
	7-1	2	0.5	1	5670.106	11340.212	10942.171	21884.342	1	1716832	1.27	21570	0.0252	1	0.000	15	7.0823
			0.5	2	5272.065	10544.13			1					1		15	
	7-2	2	0.5	1	5572.875	11145.75	11294.077	22588.154	1	1716832	1.32	22273	0.0260	1	0.000	15	7.3134
			0.5	2	5721.202	11442.404			1					1		15	
	7-3	2	0.5	1	5387.024	10774.048	10877.253	21754.506	1	1716832	1.27	21440	0.0250	1	0.000	15	7.0397
			0.5	2	5490.229	10980.458			1					1		15	
	8-1	2	0.5	1	5219.498	10438.996	10802.647	21605.294	1	1716832	1.26	21291	0.0249	1	0.000	15	6.9907
			0.5	2	5583.149	11166.298			1					1		15	
	8-2	2	0.5	1	5434.141	10868.282	10956.051	21912.102	1	1716832	1.28	21597	0.0252	1	0.000	15	7.0915
			0.5	2	5521.91	11043.82			1					1		15	
	8-3	2	0.5	1	5052.199	10104.398	10100.201	20200.402	1	1716832	1.18	19886	0.0232	1	0.000	15	6.5294
			0.5	2	5048.002	10096.004			1					1		15	

Assay Date	8/8/2005	Test Chemical ID	RC#6 FRM	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	7.9062	0.0588
Full activity	End	5.0694	2.5300
Full activity	Overall	6.4878	2.1948
Background	Beginning	-0.0071	0.003424075
Background	End	0.0071	0.001881144
Background	Overall	0.0000	0.00853677
Positive	Beginning	4.2428	0.0062
Positive	End	3.9527	0.1071
Positive	Overall	4.0978	0.1786
Negative	Beginning	7.3688	0.3792
Negative	End	6.3314	0.5768
Negative	Overall	6.8501	0.7194

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#6 FRM	1	1	3.30E-05	-4.48	1.0776
RC#6 FRM	1	2	3.30E-05	-4.48	1.0115
RC#6 FRM	1	3	3.30E-05	-4.48	0.9366
RC#6 FRM	2	1	1.00E-05	-5.00	2.3931
RC#6 FRM	2	2	1.00E-05	-5.00	2.3341
RC#6 FRM	2	3	1.00E-05	-5.00	2.4682
RC#6 FRM	3	1	3.30E-06	-5.48	3.9745
RC#6 FRM	3	2	3.30E-06	-5.48	4.3451
RC#6 FRM	3	3	3.30E-06	-5.48	4.3904
RC#6 FRM	4	1	1.00E-06	-6.00	5.9003
RC#6 FRM	4	2	1.00E-06	-6.00	5.6024
RC#6 FRM	4	3	1.00E-06	-6.00	5.9599
RC#6 FRM	5	1	1.00E-07	-7.00	6.9278
RC#6 FRM	5	2	1.00E-07	-7.00	6.9895
RC#6 FRM	5	3	1.00E-07	-7.00	7.2864
RC#6 FRM	6	1	1.00E-08	-8.00	7.1635
RC#6 FRM	6	2	1.00E-08	-8.00	6.9591
RC#6 FRM	6	3	1.00E-08	-8.00	7.1683
RC#6 FRM	7	1	1.00E-09	-9.00	7.0823
RC#6 FRM	7	2	1.00E-09	-9.00	7.3134
RC#6 FRM	7	3	1.00E-09	-9.00	7.0397
RC#6 FRM	8	1	1.00E-10	-10.00	6.9907
RC#6 FRM	8	2	1.00E-10	-10.00	7.0915
RC#6 FRM	8	3	1.00E-10	-10.00	6.5294

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	16.61	15.59	14.44
2	-5.00	36.89	35.98	38.04
3	-5.48	61.26	66.97	67.67
4	-6.00	90.94	86.35	91.86
5	-7.00	106.78	107.73	112.31
6	-8.00	110.41	107.26	110.49
7	-9.00	109.16	112.73	108.51
8	-10.00	107.75	109.30	100.64

Assay Date	8/29/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	1	Microsome type	Recombina	Microsome ID	5

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/b*c

2) Calculate total µg ASDN/g soln.

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

= 0.559158 + 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	EJB	Replicate #	1	Microsome type	Recombinant	Microsome ID	5		

								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:	Recombinan	QC 10	QC 100
	0.058	0.071	0.306
	0.058	0.070	0.302
	0.058	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	Variables	Regression results	
0.25	125	1000	0.00025	200	0.0500	0.583	0.544	0.0414	m, b	0.077	0.000
0.125	62.5	1000	0.00013	200	0.0250	0.367	0.328	0.0248	se_m, se_b	0.002	0.000
0.05	25	1000	0.00005	200	0.0100	0.182	0.143	0.0105	r^2, se_y	0.999	0.000
0.025	25	2000	0.00003	200	0.0050	0.111	0.071	0.0051	F, df	2556	3
0.01	5	1000	0.00001	200	0.0020	0.069	0.030	0.0019	ss_{reg}, ss_{resid}	0.000	0.000
0.005	5	2000	0.00001	200	0.0010	0.054	0.015	0.0007			

Regression results are calculated using the function LINEST

Blank	0.040	r^2=	0.999	
		m=	0.077	
		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
Recombinan	0.058	0.019	0.001	200	120	72000	0.003	0.003	3.009
Recombinan	0.058	0.018	0.001	200	120	72000	0.003		
Recombinan	0.058	0.018	0.001	200	120	72000	0.003		
QC 10	0.071	0.031	0.002	200	200	200	0.000	0.000	0.009
QC 10	0.070	0.030	0.002	200	200	200	0.000		
QC 10	0.068	0.029	0.002	200	200	200	0.000		
QC 100	0.306	0.266	0.020	200	200	200	0.000	0.000	0.098
QC 100	0.302	0.262	0.020	200	200	200	0.000		
QC 100	0.295	0.255	0.019	200	200	200	0.000		

Assay Date	8/29/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	1

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.009059
Protein Concentration (dilution added to assay, mg/mL):	0.005015

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	9114.543	18229.086	18008.755	36017.51	1	1642378	2.19	35657	0.0430	1	0.003	15	0.5719
			0.5	2	8894.212	17788.424			1					1		15	
	2	2	0.5	1	9781.595	19563.19	19335.863	38671.726	1	1642378	2.35	38311	0.0462	1	0.003	15	0.6145
			0.5	2	9554.268	19108.536			1					1		15	
	3	2	0.5	1	8095.414	16190.828	16339.569	32679.138	1	1642378	1.99	32319	0.0390	1	0.003	15	0.5184
			0.5	2	8244.155	16488.31			1					1		15	
	4	2	0.5	1	7821.448	15642.896	15705.774	31411.548	1	1642378	1.91	31051	0.0375	1	0.003	15	0.4980
			0.5	2	7884.326	15768.652			1					1		15	
Background control	1	2	0.5	1	79.28067	158.56134	194.96877	389.93754	1	1642378	0.02	29	0.0000	1	0.003	15	0.0005
			0.5	2	115.6881	231.3762			1					1		15	
	2	2	0.5	1	87.48212	174.96424	172.87097	345.74194	1	1642378	0.02	-15	0.0000	1	0.003	15	-0.0002
			0.5	2	85.38885	170.7777			1					1		15	
	3	2	0.5	1	90.21593	180.43186	170.09582	340.19164	1	1642378	0.02	-20	0.0000	1	0.003	15	-0.0003
			0.5	2	79.87989	159.75978			1					1		15	
	4	2	0.5	1	88.14333	176.28666	183.11667	366.23334	1	1642378	0.02	6	0.0000	1	0.003	15	0.0001
			0.5	2	94.97334	189.94668			1					1		15	
Positive control	1	2	0.5	1	3821.895	7643.79	7449.877	14899.754	1	1642378	0.91	14539	0.0175	1	0.003	15	0.2332
			0.5	2	3627.982	7255.964			1					1		15	
	2	2	0.5	1	3930.662	7861.324	7855.815	15711.63	1	1642378	0.96	15351	0.0185	1	0.003	15	0.2462
			0.5	2	3925.153	7850.306			1					1		15	
	3	2	0.5	1	3608.656	7217.312	7192.708	14385.416	1	1642378	0.88	14025	0.0169	1	0.003	15	0.2249
			0.5	2	3584.052	7168.104			1					1		15	
	4	2	0.5	1	3512.972	7025.944	7143.394	14286.788	1	1642378	0.87	13926	0.0168	1	0.003	15	0.2234
			0.5	2	3630.422	7260.844			1					1		15	
Negative Control	1	2	0.5	1	7947.912	15895.824	15711.304	31422.608	1	1642378	1.91	31062	0.0375	1	0.003	15	0.4982
			0.5	2	7763.392	15526.784			1					1		15	
	2	2	0.5	1	8319.047	16638.094	16560.491	33120.982	1	1642378	2.02	32760	0.0395	1	0.003	15	0.5254
			0.5	2	8241.444	16482.888			1					1		15	
	3	2	0.5	1	6238.392	12476.784	12712.103	25424.206	1	1642378	1.55	25064	0.0302	1	0.003	15	0.4020
			0.5	2	6473.711	12947.422			1					1		15	
	4	2	0.5	1	6307.79	12615.58	12491.715	24983.43	1	1642378	1.52	24623	0.0297	1	0.003	15	0.3949
			0.5	2	6183.925	12367.85			1					1		15	
RC#7 ECZ	1-1	2	0.5	1	79.28109	158.56218	170.86282	341.72564	1	1642378	0.02	-19	0.0000	1	0.003	15	-0.0003
			0.5	2	91.58173	183.16346			1					1		15	
	1-2	2	0.5	1	87.48258	174.96516	179.7424	359.4848	1	1642378	0.02	-1	0.0000	1	0.003	15	0.0000
			0.5	2	92.25982	184.51964			1					1		15	
	1-3	2	0.5	1	110.1797	220.3594	183.99313	367.98626	1	1642378	0.02	7	0.0000	1	0.003	15	0.0001
			0.5	2	73.81343	147.62686			1					1		15	
	2-1	2	0.5	1	68.86234	137.72468	174.68974	349.37948	1	1642378	0.02	-11	0.0000	1	0.003	15	-0.0002
			0.5	2	105.8274	211.6548			1					1		15	
	2-2	2	0.5	1	71.0796	142.1592	164.02985	328.0597	1	1642378	0.02	-32	0.0000	1	0.003	15	-0.0005
			0.5	2	92.95025	185.9005			1					1		15	
	2-3	2	0.5	1	60.14428	120.28856	170.32398	340.64796	1	1642378	0.02	-20	0.0000	1	0.003	15	-0.0003
			0.5	2	110.1797	220.3594			1					1		15	
	3-1	2	0.5	1	92.95025	185.9005	192.11202	384.22404	1	1642378	0.02	24	0.0000	1	0.003	15	0.0004
			0.5	2	99.16177	198.32354			1					1		15	
	3-2	2	0.5	1	90.21642	180.43284	183.16716	366.33432	1	1642378	0.02	6	0.0000	1	0.003	15	0.0001
			0.5	2	92.95074	185.90148			1					1		15	
	3-3	2	0.5	1	86.83323	173.66646	177.732	355.464	1	1642378	0.02	-5	0.0000	1	0.003	15	-0.0001
			0.5	2	90.89677	181.79354			1					1		15	
	4-1	2	0.5	1	114.8215	229.643	190.80058	381.60116	1	1642378	0.02	21	0.0000	1	0.003	15	0.0003
			0.5	2	75.97908	151.95816			1					1		15	
	4-2	2	0.5	1	115.6893	231.3786	235.085	470.17	1	1642378	0.03	110	0.0001	1	0.003	15	0.0018
			0.5	2	119.3957	238.7914			1					1		15	
	4-3	2	0.5	1	83.50143	167.00286	206.52443	413.04886	1	1642378	0.03	53	0.0001	1	0.003	15	0.0008
			0.5	2	123.023	246.046			1					1		15	
	5-1	2	0.5	1	180.4338	360.8676	405.2508	810.5016	1	1642378	0.05	450	0.0005	1	0.003	15	0.0072
			0.5	2	224.817	449.634			1					1		15	
	5-2	2	0.5	1	217.6061	435.2122	433.0937	866.1874	1	1642378	0.05	506	0.0006	1	0.003	15	0.0081
			0.5	2	215.4876	430.9752			1					1		15	
	5-3	2	0.5	1	215.9738	431.9476	475.6891	951.3782	1	1642378	0.06	591	0.0007	1	0.003	15	0.0095
			0.5	2	259.7153	519.4306			1					1		15	
	6-1	2	0.5	1	1115.95	2231.9	2377.515	4755.03	1	1642378	0.29	4395	0.0053	1	0.003	15	0.0705
			0.5	2	1261.565	2523.13			1					1		15	
	6-2	2	0.5	1	1172.82	2345.64	2368.277	4736.554	1	1642378	0.29	4376	0.0053	1	0.003	15	0.0702
			0.5	2	1195.457	2390.914			1					1		15	
	6-3	2	0.5	1	1209.229	2418.458	2430.321	4860.642	1	1642378	0.30	4500	0.0054	1	0.003	15	0.0722
			0.5	2	1221.092	2442.184			1					1		15	
	7-1	2	0.5	1	4790.089	9580.178	9527.843	19055.686	1	1642378	1.16	18695	0.0226	1	0.003	15	0.2998
			0.5	2	4737.754	9475.508			1					1		15	
	7-2	2	0.5	1	4792.431	9584.862	9498.14	18996.28	1	1642378	1.16	18636	0.0225	1	0.003	15	0.2989
			0.5	2	4705.709	9411.418			1					1		15	
	7-3	2	0.5	1	4997.469	9994.938	10246.452	20492.904	1	1642378	1.25	20132	0.0243	1	0.003	15	0.3229
			0.5	2	5248.983	10497.966			1					1		15	
	8-1	2	0.5	1	7343.108	14686.216	15006.076	30012.152	1	1642378	1.83	29652	0.0358	1	0.003	15	0.4756
			0.5	2	7662.968	15325.936			1					1		15	
	8-2	2	0.5	1	7075.192	14150.384	14207.794	28415.588	1	1642378	1.73	28055	0.0338	1	0.003	15	0.4500
			0.5	2	7132.602	14265.204			1					1		15	
	8-3	2	0.5	1	7441.527	14883.054	14866.69	29733.38	1	1642378	1.81	29373	0.0354	1	0.003	15	0.4711
			0.5	2	7425.163	14850.326			1					1		15	

Assay Date	8/29/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.5932	0.0301
Full activity	End	0.5082	0.0144
Full activity	Overall	0.5507	0.0527
Background	Beginning	0.0001	0.000501231
Background	End	-0.0001	0.000295344
Background	Overall	0.0000	0.000362169
Positive	Beginning	0.2397	0.0092
Positive	End	0.2242	0.0011
Positive	Overall	0.2319	0.0105
Negative	Beginning	0.5118	0.0193
Negative	End	0.3985	0.0050
Negative	Overall	0.4551	0.0665

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#7 ECZ	1	1	1.00E-04	-4.00	-0.0003
RC#7 ECZ	1	2	1.00E-04	-4.00	0.0000
RC#7 ECZ	1	3	1.00E-04	-4.00	0.0001
RC#7 ECZ	2	1	3.30E-05	-4.48	-0.0002
RC#7 ECZ	2	2	3.30E-05	-4.48	-0.0005
RC#7 ECZ	2	3	3.30E-05	-4.48	-0.0003
RC#7 ECZ	3	1	1.00E-05	-5.00	0.0004
RC#7 ECZ	3	2	1.00E-05	-5.00	0.0001
RC#7 ECZ	3	3	1.00E-05	-5.00	-0.0001
RC#7 ECZ	4	1	1.00E-06	-6.00	0.0003
RC#7 ECZ	4	2	1.00E-06	-6.00	0.0018
RC#7 ECZ	4	3	1.00E-06	-6.00	0.0008
RC#7 ECZ	5	1	1.00E-07	-7.00	0.0072
RC#7 ECZ	5	2	1.00E-07	-7.00	0.0081
RC#7 ECZ	5	3	1.00E-07	-7.00	0.0095
RC#7 ECZ	6	1	1.00E-08	-8.00	0.0705
RC#7 ECZ	6	2	1.00E-08	-8.00	0.0702
RC#7 ECZ	6	3	1.00E-08	-8.00	0.0722
RC#7 ECZ	7	1	1.00E-09	-9.00	0.2998
RC#7 ECZ	7	2	1.00E-09	-9.00	0.2989
RC#7 ECZ	7	3	1.00E-09	-9.00	0.3229
RC#7 ECZ	8	1	1.00E-10	-10.00	0.4756
RC#7 ECZ	8	2	1.00E-10	-10.00	0.4500
RC#7 ECZ	8	3	1.00E-10	-10.00	0.4711

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	-0.05	0.00	0.02
2	-4.48	-0.03	-0.09	-0.06
3	-5.00	0.07	0.02	-0.01
4	-6.00	0.06	0.32	0.15
5	-7.00	1.31	1.47	1.72
6	-8.00	12.80	12.75	13.11
7	-9.00	54.45	54.28	58.64
8	-10.00	86.36	81.71	85.55

Assay Date	9/5/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8
Technician ID	EJB	Replicate #	2	Microsome type	Recombina Microsome ID 5

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/b*c

2) Calculate total µg ASDN/g soln.

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

= 0.563896 + 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 ECZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	2	Microsome type	Recombinant	Microsome ID	5

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.302	0.178	0.091	0.060	0.039	0.034	0.029	2	1	
	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:	Recombinan	QC 10	QC 100
	0.035	0.038	0.132
	0.033	0.039	0.131
	0.032	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	0.0447
0.125	62.5	1000	0.00013	200	0.0250	0.180	0.152	0.0249
0.05	25	1000	0.00005	200	0.0100	0.090	0.061	0.0101
0.025	25	2000	0.00003	200	0.0050	0.060	0.032	0.0053
0.01	5	1000	0.00001	200	0.0020	0.039	0.011	0.0018
0.005	5	2000	0.00001	200	0.0010	0.034	0.005	0.0009

Variables	Regression results	
m, b	0.164	0.000
se_m, se_b	0.002	0.000
r^2, se_y	1.000	0.000
F, df	7863	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

Blank	0.029	r^2=	1.000
		m=	0.164
		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
Recombinan	0.035	0.006	0.001	200	120	72000	0.003	0.002	2.460
Recombinan	0.033	0.004	0.001	200	120	72000	0.002		
Recombinan	0.032	0.004	0.001	200	120	72000	0.002		
QC 10	0.038	0.009	0.002	200	200	200	0.000	0.000	0.008
QC 10	0.039	0.010	0.002	200	200	200	0.000		
QC 10	0.036	0.007	0.001	200	200	200	0.000		
QC 100	0.132	0.103	0.017	200	200	200	0.000	0.000	0.085
QC 100	0.131	0.102	0.017	200	200	200	0.000		
QC 100	0.132	0.103	0.017	200	200	200	0.000		

Assay Date	9/5/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8	Microsome type	Recombina Microsome ID	5	Technician ID	EJB	Replicate #	2

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	2.460305
Protein Concentration (dilution added to assay, mg/mL):	0.004101

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 ECZ	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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	7861.283	15722.566	15578.424	31156.848	1	1639699	1.90	30867	0.0376	1	0.002	15	0.6115
			0.5	2	7717.141	15434.282			1					1		15	
	2	2	0.5	1	7401.285	14802.57	14812.661	29625.322	1	1639699	1.81	29335	0.0357	1	0.002	15	0.5812
			0.5	2	7411.376	14822.752			1					1		15	
	3	2	0.5	1	6900.152	13800.304	13882.319	27764.638	1	1639699	1.69	27474	0.0335	1	0.002	15	0.5443
			0.5	2	6982.167	13964.334			1					1		15	
	4	2	0.5	1	7220.649	14441.298	14616.425	29232.85	1	1639699	1.78	28943	0.0353	1	0.002	15	0.5734
			0.5	2	7395.776	14791.552			1					1		15	
Background control	1	2	0.5	1	90.21593	180.43186	150.35989	300.71978	1	1639699	0.02	11	0.0000	1	0.002	15	0.0002
			0.5	2	60.14396	120.28792			1					1		15	
	2	2	0.5	1	54.67833	109.35266	117.08705	234.1741	1	1639699	0.01	-56	-0.0001	1	0.002	15	-0.0011
			0.5	2	62.41072	124.82144			1					1		15	
	3	2	0.5	1	87.60819	175.21638	186.02557	372.05114	1	1639699	0.02	82	0.0001	1	0.002	15	0.0016
			0.5	2	98.41736	196.83476			1					1		15	
	4	2	0.5	1	48.84317	97.68634	126.95699	253.91398	1	1639699	0.02	-36	0.0000	1	0.002	15	-0.0007
			0.5	2	78.11382	156.22764			1					1		15	
Positive control	1	2	0.5	1	3531.261	7062.522	6917.091	13834.182	1	1639699	0.84	13544	0.0165	1	0.002	15	0.2683
			0.5	2	3385.83	6771.66			1					1		15	
	2	2	0.5	1	3001.157	6002.314	6013.838	12027.676	1	1639699	0.73	11737	0.0143	1	0.002	15	0.2325
			0.5	2	3012.681	6025.362			1					1		15	
	3	2	0.5	1	3098.844	6197.688	6156.332	12312.664	1	1639699	0.75	12022	0.0147	1	0.002	15	0.2382
			0.5	2	3057.488	6114.976			1					1		15	
	4	2	0.5	1	3052.714	6105.428	6101.938	12203.876	1	1639699	0.74	11914	0.0145	1	0.002	15	0.2360
			0.5	2	3049.224	6098.448			1					1		15	
Negative Control	1	2	0.5	1	5385.647	10771.294	10779.495	21558.99	1	1639699	1.31	21269	0.0259	1	0.002	15	0.4214
			0.5	2	5393.848	10787.696			1					1		15	
	2	2	0.5	1	4024.209	8048.418	8264.38	16528.76	1	1639699	1.01	16239	0.0198	1	0.002	15	0.3217
			0.5	2	4240.171	8480.342			1					1		15	
	3	2	0.5	1	6189.393	12378.786	12370.801	24741.602	1	1639699	1.51	24451	0.0298	1	0.002	15	0.4844
			0.5	2	6181.408	12362.816			1					1		15	
	4	2	0.5	1	5938.688	11877.376	12037.864	24075.728	1	1639699	1.47	23786	0.0290	1	0.002	15	0.4712
			0.5	2	6099.176	12198.352			1					1		15	
RC#7 ECZ	1-1	2	0.5	1	104.6708	209.3416	194.2171	388.4342	1	1639699	0.02	98	0.0001	1	0.002	15	0.0019
			0.5	2	89.5463	179.0926			1					1		15	
	1-2	2	0.5	1	124.8977	249.7954	264.3231	528.6462	1	1639699	0.03	238	0.0003	1	0.002	15	0.0047
			0.5	2	139.4254	278.8508			1					1		15	
	1-3	2	0.5	1	109.3532	218.7064	239.6023	479.2046	1	1639699	0.03	189	0.0002	1	0.002	15	0.0037
			0.5	2	130.2491	260.4982			1					1		15	
	2-1	2	0.5	1	103.1139	206.2278	210.5391	421.0782	1	1639699	0.03	131	0.0002	1	0.002	15	0.0026
			0.5	2	107.4252	214.8504			1					1		15	
	2-2	2	0.5	1	70.55163	141.10326	163.50188	327.00376	1	1639699	0.02	37	0.0000	1	0.002	15	0.0007
			0.5	2	92.95025	185.9005			1					1		15	
	2-3	2	0.5	1	141.5507	283.1014	253.6378	507.2756	1	1639699	0.03	217	0.0003	1	0.002	15	0.0043
			0.5	2	112.0871	224.1742			1					1		15	
	3-1	2	0.5	1	176.3791	352.7582	330.6307	661.2614	1	1639699	0.04	371	0.0005	1	0.002	15	0.0074
			0.5	2	154.2516	308.5032			1					1		15	
	3-2	2	0.5	1	184.5196	369.0392	347.3319	694.6638	1	1639699	0.04	404	0.0005	1	0.002	15	0.0080
			0.5	2	162.8123	325.6246			1					1		15	
	3-3	2	0.5	1	189.9477	379.8954	306.6299	613.2598	1	1639699	0.04	323	0.0004	1	0.002	15	0.0064
			0.5	2	116.6822	233.3644			1					1		15	
	4-1	2	0.5	1	611.5008	1223.0016	1137.9273	2275.8546	1	1639699	0.14	1986	0.0024	1	0.002	15	0.0393
			0.5	2	526.4265	1052.853			1					1		15	
	4-2	2	0.5	1	964.112	1928.224	1920.9443	3841.8886	1	1639699	0.23	3552	0.0043	1	0.002	15	0.0704
			0.5	2	966.8323	1933.6646			1					1		15	
	4-3	2	0.5	1	965.0474	1930.0948	1931.8797	3863.7594	1	1639699	0.24	3574	0.0044	1	0.002	15	0.0708
			0.5	2	966.8323	1933.6646			1					1		15	
	5-1	2	0.5	1	2189.892	4379.784	4401.762	8803.524	1	1639699	0.54	8513	0.0104	1	0.002	15	0.1687
			0.5	2	2211.87	4423.74			1					1		15	
	5-2	2	0.5	1	2057.617	4115.234	4354.877	8709.754	1	1639699	0.53	8420	0.0103	1	0.002	15	0.1668
			0.5	2	2297.28	4594.52			1					1		15	
	5-3	2	0.5	1	2025.779	4051.558	4169.88	8339.76	1	1639699	0.51	8050	0.0098	1	0.002	15	0.1595
			0.5	2	2144.101	4288.202			1					1		15	
	6-1	2	0.5	1	4533.92	9067.84	9031.096	18062.192	1	1639699	1.10	17772	0.0217	1	0.002	15	0.3521
			0.5	2	4497.176	8994.352			1					1		15	
	6-2	2	0.5	1	4319.476	8638.952	8515.928	17031.856	1	1639699	1.04	16742	0.0204	1	0.002	15	0.3317
			0.5	2	4196.452	8392.904			1					1		15	
	6-3	2	0.5	1	4016.037	8032.074	8197.6	16395.2	1	1639699	1.00	16105	0.0196	1	0.002	15	0.3191
			0.5	2	4181.563	8363.126			1					1		15	
	7-1	2	0.5	1	4781.826	9563.652	9742.174	19484.348	1	1639699	1.19	19194	0.0234	1	0.002	15	0.3803
			0.5	2	4960.348	9920.696			1					1		15	
	7-2	2	0.5	1	6079.199	12158.398	11993.128	23986.256	1	1639699	1.46	23696	0.0289	1	0.002	15	0.4695
			0.5	2	5913.929	11827.858			1					1		15	
	7-3	2	0.5	1	5963.51	11927.02	11913.66	23827.32	1	1639699	1.45	23537	0.0287	1	0.002	15	0.4663
			0.5	2	5950.15	11900.3			1					1		15	
	8-1	2	0.5	1	6751.284	13502.568	13350.609	26701.218	1	1639699	1.63	26411	0.0322	1	0.002	15	0.5233
			0.5	2	6599.325	13198.65			1					1		15	
	8-2	2	0.5	1	6454.608	12909.216	12939.289	25878.578	1	1639699	1.58	25588	0.0312	1	0.002	15	0.5070
			0.5	2	6484.681	12969.362			1					1		15	
	8-3	2	0.5	1	6408.167	12816.334	12797.197	25594.394	1	1639699	1.56	25304	0.0308	1	0.002	15	0.5013
			0.5	2	6389.03	12778.06			1					1		15	

Assay Date	9/5/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.5964	0.0215
Full activity	End	0.5589	0.0206
Full activity	Overall	0.5776	0.0276
Background	Beginning	-0.0005	0.000932246
Background	End	0.0005	0.001654996
Background	Overall	0.0000	0.001214079
Positive	Beginning	0.2504	0.0253
Positive	End	0.2371	0.0015
Positive	Overall	0.2438	0.0165
Negative	Beginning	0.3715	0.0705
Negative	End	0.4778	0.0093
Negative	Overall	0.4247	0.0738

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#7 ECZ	1	1	1.00E-05	-5.00	0.0019
RC#7 ECZ	1	2	1.00E-05	-5.00	0.0047
RC#7 ECZ	1	3	1.00E-05	-5.00	0.0037
RC#7 ECZ	2	1	1.00E-06	-6.00	0.0026
RC#7 ECZ	2	2	1.00E-06	-6.00	0.0007
RC#7 ECZ	2	3	1.00E-06	-6.00	0.0043
RC#7 ECZ	3	1	1.00E-07	-7.00	0.0074
RC#7 ECZ	3	2	1.00E-07	-7.00	0.0080
RC#7 ECZ	3	3	1.00E-07	-7.00	0.0064
RC#7 ECZ	4	1	1.00E-08	-8.00	0.0393
RC#7 ECZ	4	2	1.00E-08	-8.00	0.0704
RC#7 ECZ	4	3	1.00E-08	-8.00	0.0708
RC#7 ECZ	5	1	3.30E-09	-8.48	0.1687
RC#7 ECZ	5	2	3.30E-09	-8.48	0.1668
RC#7 ECZ	5	3	3.30E-09	-8.48	0.1595
RC#7 ECZ	6	1	1.00E-09	-9.00	0.3521
RC#7 ECZ	6	2	1.00E-09	-9.00	0.3317
RC#7 ECZ	6	3	1.00E-09	-9.00	0.3191
RC#7 ECZ	7	1	3.30E-10	-9.48	0.3803
RC#7 ECZ	7	2	3.30E-10	-9.48	0.4695
RC#7 ECZ	7	3	3.30E-10	-9.48	0.4663
RC#7 ECZ	8	1	1.00E-10	-10.00	0.5233
RC#7 ECZ	8	2	1.00E-10	-10.00	0.5070
RC#7 ECZ	8	3	1.00E-10	-10.00	0.5013

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	0.34	0.82	0.65
2	-6.00	0.45	0.13	0.74
3	-7.00	1.27	1.39	1.11
4	-8.00	6.81	12.18	12.26
5	-8.48	29.20	28.88	27.61
6	-9.00	60.96	57.42	55.24
7	-9.48	65.84	81.28	80.73
8	-10.00	90.59	87.77	86.79

Assay Date	9/6/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	3	Microsome type	Recombina	Microsome ID	5

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#7 ECZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	3	Microsome type	Recombinant	Microsome ID	5

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	Blank	Protein stock (mg BSA)	Total volume of stock (mL)	Protein stock ID
	0.267	0.154	0.082	0.052	0.035	0.031	0.031	2	1	
	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:	Recombinan	QC 10	QC 100
	0.032	0.034	0.115
	0.031	0.040	0.121
	0.031	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	0.0450	m, b	0.188	0.000
0.125	62.5	1000	0.00013		200	0.0250	0.158	0.130	0.0248	se_m, se_b	0.004	0.000
0.05	25	1000	0.00005		200	0.0100	0.082	0.054	0.0106	r^2, se_y	0.999	0.000
0.025	25	2000	0.00003		200	0.0050	0.053	0.026	0.0051	F, df	2142	3
0.01	5	1000	0.00001		200	0.0020	0.035	0.007	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
0.005	5	2000	0.00001		200	0.0010	0.031	0.003	0.0009			
				Blank	0.028							

r^2= 0.999
m= 0.188
b= 0.000

Regression results are calculated using the function LINEST

	A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
Recombinan	0.032	0.004	0.001	200	120	72000	0.003	0.003	2.846
Recombinan	0.031	0.003	0.001	200	120	72000	0.003		
Recombinan	0.031	0.003	0.001	200	120	72000	0.003		
QC 10	0.034	0.006	0.002	200	200	200	0.000	0.000	0.009
QC 10	0.040	0.012	0.003	200	200	200	0.000		
QC 10	0.033	0.005	0.001	200	200	200	0.000		
QC 100	0.115	0.087	0.017	200	200	200	0.000	0.000	0.087
QC 100	0.121	0.093	0.018	200	200	200	0.000		
QC 100	0.120	0.092	0.018	200	200	200	0.000		

Assay Date	9/6/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	3

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	2.845523
Protein Concentration (dilution added to assay, mg/mL):	0.004743

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 ECZ	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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	10518.38	21036.76	21603.07	43206.14	1	1680873	2.57	42835	0.0509	1	0.002	15	0.7155
			0.5	2	11084.69	22169.38			1					1		15	
	2	2	0.5	1	12210.6	24421.2	23687.16	47374.32	1	1680873	2.82	47003	0.0559	1	0.002	15	0.7851
			0.5	2	11476.56	22953.12			1					1		15	
	3	2	0.5	1	11831.96	23663.92	23676.22	47352.44	1	1680873	2.82	46981	0.0558	1	0.002	15	0.7848
			0.5	2	11844.26	23688.52			1					1		15	
	4	2	0.5	1	11928.59	23857.18	24052.33	48104.66	1	1680873	2.86	47733	0.0567	1	0.002	15	0.7973
			0.5	2	12123.74	24247.48			1					1		15	
Background control	1	2	0.5	1	86.19411	172.38822	165.47478	330.94956	1	1680873	0.02	-40	0.0000	1	0.002	15	-0.0007
			0.5	2	79.28067	158.56134			1					1		15	
	2	2	0.5	1	86.19411	172.38822	172.38822	344.77644	1	1680873	0.02	-27	0.0000	1	0.002	15	-0.0004
			0.5	2	86.19411	172.38822			1					1		15	
	3	2	0.5	1	100.3999	200.7998	207.3596	414.7192	1	1680873	0.02	43	0.0001	1	0.002	15	0.0007
			0.5	2	106.9597	213.9194			1					1		15	
	4	2	0.5	1	91.58124	183.16248	197.40864	394.81728	1	1680873	0.02	24	0.0000	1	0.002	15	0.0004
			0.5	2	105.8274	211.6548			1					1		15	
Positive control	1	2	0.5	1	5076.724	10153.448	9628.552	19257.104	1	1680873	1.15	18886	0.0224	1	0.002	15	0.3155
			0.5	2	4551.828	9103.656			1					1		15	
	2	2	0.5	1	3991.594	7983.189	8207.161	16414.322	1	1680873	0.98	16043	0.0191	1	0.002	15	0.2680
			0.5	2	4215.567	8431.134			1					1		15	
	3	2	0.5	1	5608.149	11216.298	10796.96	21593.92	1	1680873	1.28	21223	0.0252	1	0.002	15	0.3545
			0.5	2	5188.811	10377.622			1					1		15	
	4	2	0.5	1	5544.208	11088.416	10771.292	21542.584	1	1680873	1.28	21171	0.0252	1	0.002	15	0.3536
			0.5	2	5227.084	10454.168			1					1		15	
Negative Control	1	2	0.5	1	6712.402	13424.804	13273.703	26547.406	1	1680873	1.58	26176	0.0311	1	0.002	15	0.4372
			0.5	2	6561.301	13122.602			1					1		15	
	2	2	0.5	1	6080.69	12161.38	12131.849	24263.698	1	1680873	1.44	23892	0.0284	1	0.002	15	0.3991
			0.5	2	6051.159	12102.318			1					1		15	
	3	2	0.5	1	10486.11	20972.22	21296.18	42512.36	1	1680873	2.53	42141	0.0501	1	0.002	15	0.7039
			0.5	2	10770.07	21540.14			1					1		15	
	4	2	0.5	1	10227.26	20454.52	21154.38	42308.76	1	1680873	2.52	41937	0.0498	1	0.002	15	0.7005
			0.5	2	10927.12	21854.24			1					1		15	
RC#7 ECZ	1-1	2	0.5	1	105.8274	211.6548	187.23312	374.46624	1	1680873	0.02	3	0.0000	1	0.002	15	0.0001
			0.5	2	81.40572	162.81144			1					1		15	
	1-2	2	0.5	1	84.11924	168.23848	162.23306	324.46612	1	1680873	0.02	-47	-0.0001	1	0.002	15	-0.0008
			0.5	2	78.11382	156.22764			1					1		15	
	1-3	2	0.5	1	75.97868	151.95736	184.49268	368.98536	1	1680873	0.02	-2	0.0000	1	0.002	15	0.0000
			0.5	2	108.514	217.028			1					1		15	
	2-1	2	0.5	1	96.40727	192.81454	218.51587	437.03174	1	1680873	0.03	66	0.0001	1	0.002	15	0.0011
			0.5	2	122.1086	244.2172			1					1		15	
	2-2	2	0.5	1	106.9602	213.9204	243.6517	487.3034	1	1680873	0.03	116	0.0001	1	0.002	15	0.0019
			0.5	2	136.6915	273.383			1					1		15	
	2-3	2	0.5	1	82.8642	165.7284	178.57828	357.15656	1	1680873	0.02	-14	0.0000	1	0.002	15	-0.0002
			0.5	2	95.68408	191.36816			1					1		15	
	3-1	2	0.5	1	177.7783	355.5526	423.8211	847.6422	1	1680873	0.05	476	0.0006	1	0.002	15	0.0080
			0.5	2	246.0448	492.0896			1					1		15	
	3-2	2	0.5	1	203.5143	407.0286	462.4381	924.8762	1	1680873	0.06	554	0.0007	1	0.002	15	0.0092
			0.5	2	258.9238	517.8476			1					1		15	
	3-3	2	0.5	1	214.8516	429.7032	459.9667	919.9374	1	1680873	0.05	549	0.0007	1	0.002	15	0.0092
			0.5	2	245.1171	490.2342			1					1		15	
	4-1	2	0.5	1	1834.352	3668.704	3687.899	7375.798	1	1680873	0.44	7004	0.0083	1	0.002	15	0.1170
			0.5	2	1853.547	3707.094			1					1		15	
	4-2	2	0.5	1	1983.246	3966.492	3863.375	7726.75	1	1680873	0.46	7355	0.0087	1	0.002	15	0.1229
			0.5	2	1880.129	3760.258			1					1		15	
	4-3	2	0.5	1	1898.984	3797.968	3703.071	7406.142	1	1680873	0.44	7035	0.0084	1	0.002	15	0.1175
			0.5	2	1804.087	3608.174			1					1		15	
	5-1	2	0.5	1	4331.913	8663.826	8795.199	17590.398	1	1680873	1.05	17219	0.0205	1	0.002	15	0.2876
			0.5	2	4463.286	8926.572			1					1		15	
	5-2	2	0.5	1	4516.312	9032.624	8997.084	17994.168	1	1680873	1.07	17623	0.0209	1	0.002	15	0.2944
			0.5	2	4480.772	8961.544			1					1		15	
	5-3	2	0.5	1	4117.171	8234.342	8589.082	17178.164	1	1680873	1.02	16807	0.0200	1	0.002	15	0.2807
			0.5	2	4471.911	8943.822			1					1		15	
	6-1	2	0.5	1	7693.041	15386.082	15500.903	31001.806	1	1680873	1.84	30630	0.0364	1	0.002	15	0.5117
			0.5	2	7807.862	15615.724			1					1		15	
	6-2	2	0.5	1	7649.299	15298.598	14846.583	29693.166	1	1680873	1.77	29322	0.0348	1	0.002	15	0.4898
			0.5	2	7197.284	14394.568			1					1		15	
	6-3	2	0.5	1	7310.464	14620.928	13456.564	26913.128	1	1680873	1.60	26542	0.0315	1	0.002	15	0.4434
			0.5	2	6146.1	12292.2			1					1		15	
	7-1	2	0.5	1	10346.1	20692.2	20828.26	41656.52	1	1680873	2.48	41285	0.0491	1	0.002	15	0.6896
			0.5	2	10482.16	20964.32			1					1		15	
	7-2	2	0.5	1	10160.24	20320.48	21095.8	42191.6	1	1680873	2.51	41820	0.0497	1	0.002	15	0.6986
			0.5	2	10935.56	21871.12			1					1		15	
	7-3	2	0.5	1	11439.47	22878.94	22410.31	44820.62	1	1680873	2.67	44449	0.0528	1	0.002	15	0.7425
			0.5	2	10970.84	21941.68			1					1		15	
	8-1	2	0.5	1	11353.44	22706.88	23199.19	46398.38	1	1680873	2.76	46027	0.0547	1	0.002	15	0.7688
			0.5	2	11845.75	23691.5			1					1		15	
	8-2	2	0.5	1	11124.02	22248.04	22901.43	45802.86	1	1680873	2.72	45432	0.0540	1	0.002	15	0.7589
			0.5	2	11777.41	23554.82			1					1		15	
	8-3	2	0.5	1	11009.2	22018.4	22180.9	44361.8	1	1680873	2.64	43990	0.0523	1	0.002	15	0.7348
			0.5	2	11171.7	22343.4			1					1		15	

Assay Date	9/6/2005	Test Chemical ID	RC#7 ECZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.7503	0.0492
Full activity	End	0.7911	0.0089
Full activity	Overall	0.7707	0.0372
Background	Beginning	-0.0006	0.000163317
Background	End	0.0006	0.000235072
Background	Overall	0.0000	0.000666067
Positive	Beginning	0.2917	0.0336
Positive	End	0.3541	0.0006
Positive	Overall	0.3229	0.0409
Negative	Beginning	0.4182	0.0270
Negative	End	0.7022	0.0024
Negative	Overall	0.5602	0.1647

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#7 ECZ	1	1	1.00E-05	-5.00	0.0001
RC#7 ECZ	1	2	1.00E-05	-5.00	-0.0008
RC#7 ECZ	1	3	1.00E-05	-5.00	0.0000
RC#7 ECZ	2	1	1.00E-06	-6.00	0.0011
RC#7 ECZ	2	2	1.00E-06	-6.00	0.0019
RC#7 ECZ	2	3	1.00E-06	-6.00	-0.0002
RC#7 ECZ	3	1	1.00E-07	-7.00	0.0080
RC#7 ECZ	3	2	1.00E-07	-7.00	0.0092
RC#7 ECZ	3	3	1.00E-07	-7.00	0.0092
RC#7 ECZ	4	1	1.00E-08	-8.00	0.1170
RC#7 ECZ	4	2	1.00E-08	-8.00	0.1229
RC#7 ECZ	4	3	1.00E-08	-8.00	0.1175
RC#7 ECZ	5	1	3.30E-09	-8.48	0.2876
RC#7 ECZ	5	2	3.30E-09	-8.48	0.2944
RC#7 ECZ	5	3	3.30E-09	-8.48	0.2807
RC#7 ECZ	6	1	1.00E-09	-9.00	0.5117
RC#7 ECZ	6	2	1.00E-09	-9.00	0.4898
RC#7 ECZ	6	3	1.00E-09	-9.00	0.4434
RC#7 ECZ	7	1	3.30E-10	-9.48	0.6896
RC#7 ECZ	7	2	3.30E-10	-9.48	0.6986
RC#7 ECZ	7	3	3.30E-10	-9.48	0.7425
RC#7 ECZ	8	1	1.00E-10	-10.00	0.7688
RC#7 ECZ	8	2	1.00E-10	-10.00	0.7589
RC#7 ECZ	8	3	1.00E-10	-10.00	0.7348

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-5.00	0.01	-0.10	-0.01
2	-6.00	0.14	0.25	-0.03
3	-7.00	1.03	1.20	1.19
4	-8.00	15.18	15.94	15.25
5	-8.48	37.32	38.20	36.43
6	-9.00	66.39	63.55	57.53
7	-9.48	89.48	90.64	96.34
8	-10.00	99.76	98.47	95.35

Assay Date	8/29/2005	Test Chemical ID	RC#8 CYN	# Concentrations tested	8		
Technician ID	EJB	Replicate #	1	Microsome type	Recombina	Microsome ID	5

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/b*c

2) Calculate total µg ASDN/g soln.

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

= 0.559158 + 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 CM	# Concentrations tested	8					
Technician ID	EJB	Replicate #	1	Microsome type	Recombinant	Microsome ID	5			

								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:	Recombinan	QC 10	QC 100
	0.058	0.071	0.306
	0.058	0.070	0.302
	0.058	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	Variables	Regression results	
0.25	125	1000	0.00025	200	0.0500	0.583	0.544	0.0414	m, b	0.077	0.000
0.125	62.5	1000	0.00013	200	0.0250	0.367	0.328	0.0248	se_m, se_b	0.002	0.000
0.05	25	1000	0.00005	200	0.0100	0.182	0.143	0.0105	r^2, se_y	0.999	0.000
0.025	25	2000	0.00003	200	0.0050	0.111	0.071	0.0051	F, df	2556	3
0.01	5	1000	0.00001	200	0.0020	0.069	0.030	0.0019	ss_{reg}, ss_{resid}	0.000	0.000
0.005	5	2000	0.00001	200	0.0010	0.054	0.015	0.0007			

Regression results are calculated using the function LINEST

Blank	0.040	r^2=	0.999	
		m=	0.077	
		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
Recombinan	0.058	0.019	0.001	200	120	72000	0.003	0.003	3.009
Recombinan	0.058	0.018	0.001	200	120	72000	0.003		
Recombinan	0.058	0.018	0.001	200	120	72000	0.003		
QC 10	0.071	0.031	0.002	200	200	200	0.000	0.000	0.009
QC 10	0.070	0.030	0.002	200	200	200	0.000		
QC 10	0.068	0.029	0.002	200	200	200	0.000		
QC 100	0.306	0.266	0.020	200	200	200	0.000	0.000	0.098
QC 100	0.302	0.262	0.020	200	200	200	0.000		
QC 100	0.295	0.255	0.019	200	200	200	0.000		

Assay Date	8/29/2005	Test Chemical ID	RC#8 CM	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	1

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.009059
Protein Concentration (dilution added to assay, mg/mL):	0.005015

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 CM	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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	9114.543	18229.086	18008.755	36017.51	1	1642378	2.19	35657	0.0430	1	0.003	15	0.5719
			0.5	2	8894.212	17788.424			1					1		15	
	2	2	0.5	1	9781.596	19563.19	19335.863	38671.726	1	1642378	2.35	38311	0.0462	1	0.003	15	0.6145
			0.5	2	9554.268	19108.536			1					1		15	
	3	2	0.5	1	8095.414	16190.828	16339.569	32679.138	1	1642378	1.99	32319	0.0390	1	0.003	15	0.5184
			0.5	2	8244.155	16488.31			1					1		15	
	4	2	0.5	1	7821.448	15642.896	15705.774	31411.548	1	1642378	1.91	31051	0.0375	1	0.003	15	0.4980
			0.5	2	7884.326	15768.652			1					1		15	
Background control	1	2	0.5	1	79.28067	158.56134	194.96877	389.93754	1	1642378	0.02	29	0.0000	1	0.003	15	0.0005
			0.5	2	115.6881	231.3762			1					1		15	
	2	2	0.5	1	87.48212	174.96424	172.87097	345.74194	1	1642378	0.02	-15	0.0000	1	0.003	15	-0.0002
			0.5	2	85.38885	170.7777			1					1		15	
	3	2	0.5	1	90.21593	180.43186	170.09582	340.19164	1	1642378	0.02	-20	0.0000	1	0.003	15	-0.0003
			0.5	2	79.87989	159.75978			1					1		15	
	4	2	0.5	1	88.14333	176.28666	183.11667	366.23334	1	1642378	0.02	6	0.0000	1	0.003	15	0.0001
			0.5	2	94.97334	189.94668			1					1		15	
Positive control	1	2	0.5	1	3821.895	7643.79	7449.877	14899.754	1	1642378	0.91	14539	0.0175	1	0.003	15	0.2332
			0.5	2	3627.982	7255.964			1					1		15	
	2	2	0.5	1	3930.662	7861.324	7855.815	15711.63	1	1642378	0.96	15351	0.0185	1	0.003	15	0.2462
			0.5	2	3925.153	7850.306			1					1		15	
	3	2	0.5	1	3608.656	7217.312	7192.708	14385.416	1	1642378	0.88	14025	0.0169	1	0.003	15	0.2249
			0.5	2	3584.052	7166.104			1					1		15	
	4	2	0.5	1	3512.972	7025.944	7143.394	14286.788	1	1642378	0.87	13926	0.0168	1	0.003	15	0.2234
			0.5	2	3630.422	7260.844			1					1		15	
Negative Control	1	2	0.5	1	7947.912	15895.824	15711.304	31422.608	1	1642378	1.91	31062	0.0375	1	0.003	15	0.4982
			0.5	2	7763.392	15526.784			1					1		15	
	2	2	0.5	1	8319.047	16638.094	16560.491	33120.982	1	1642378	2.02	32760	0.0395	1	0.003	15	0.5254
			0.5	2	8241.444	16482.888			1					1		15	
	3	2	0.5	1	6238.392	12476.784	12712.103	25424.206	1	1642378	1.55	25064	0.0302	1	0.003	15	0.4020
			0.5	2	6473.711	12947.422			1					1		15	
	4	2	0.5	1	6307.79	12615.58	12491.715	24983.43	1	1642378	1.52	24623	0.0297	1	0.003	15	0.3949
			0.5	2	6183.925	12367.85			1					1		15	
RC#8 CM	1-1	2	0.5	1	1462.605	2925.21	2916.993	5833.986	1	1642378	0.36	5473	0.0066	1	0.003	15	0.0878
			0.5	2	1454.388	2908.776			1					1		15	
	1-2	2	0.5	1	1578.341	3156.682	3128.439	6256.878	1	1642378	0.38	5896	0.0071	1	0.003	15	0.0946
			0.5	2	1550.098	3100.196			1					1		15	
	1-3	2	0.5	1	2214.426	4428.852	4362.954	8725.908	1	1642378	0.53	8365	0.0101	1	0.003	15	0.1342
			0.5	2	2148.528	4297.056			1					1		15	
	2-1	2	0.5	1	803.7548	1607.5096	1679.1792	3358.3584	1	1642378	0.20	2998	0.0036	1	0.003	15	0.0481
			0.5	2	875.4244	1750.8488			1					1		15	
	2-2	2	0.5	1	771.2663	1542.5326	1581.0959	3162.1918	1	1642378	0.19	2802	0.0034	1	0.003	15	0.0449
			0.5	2	809.8296	1619.6592			1					1		15	
	2-3	2	0.5	1	645.1909	1290.3818	1358.6122	2717.2244	1	1642378	0.17	2357	0.0028	1	0.003	15	0.0378
			0.5	2	713.4213	1426.8426			1					1		15	
	3-1	2	0.5	1	2051.446	4102.892	4175.655	8351.31	1	1642378	0.51	7991	0.0096	1	0.003	15	0.1282
			0.5	2	2124.209	4248.418			1					1		15	
	3-2	2	0.5	1	2001.185	4002.37	3975.032	7950.064	1	1642378	0.48	7590	0.0092	1	0.003	15	0.1217
			0.5	2	1973.847	3947.694			1					1		15	
	3-3	2	0.5	1	1278.083	2556.166	2664.708	5329.416	1	1642378	0.32	4969	0.0060	1	0.003	15	0.0797
			0.5	2	1386.625	2773.25			1					1		15	
	4-1	2	0.5	1	5931.826	11863.652	11965.509	23931.018	1	1642378	1.46	23570	0.0284	1	0.003	15	0.3780
			0.5	2	6033.683	12067.366			1					1		15	
	4-2	2	0.5	1	5760.242	11520.484	11687.249	23374.496	1	1642378	1.42	23014	0.0278	1	0.003	15	0.3691
			0.5	2	5927.007	11854.014			1					1		15	
	4-3	2	0.5	1	5921.54	11843.08	11865.8	23731.6	1	1642378	1.44	23371	0.0282	1	0.003	15	0.3748
			0.5	2	5944.26	11888.52			1					1		15	
	5-1	2	0.5	1	7234.332	14468.664	14443.52	28887.04	1	1642378	1.76	28527	0.0344	1	0.003	15	0.4575
			0.5	2	7209.188	14418.376			1					1		15	
	5-2	2	0.5	1	7114.935	14229.87	14430.744	28861.488	1	1642378	1.76	28501	0.0344	1	0.003	15	0.4571
			0.5	2	7315.809	14631.618			1					1		15	
	5-3	2	0.5	1	7260.795	14521.59	14250.907	28501.814	1	1642378	1.74	28141	0.0340	1	0.003	15	0.4514
			0.5	2	6990.112	13980.224			1					1		15	
	6-1	2	0.5	1	7475.838	14951.676	15136.502	30273.004	1	1642378	1.84	29912	0.0361	1	0.003	15	0.4798
			0.5	2	7660.664	15321.328			1					1		15	
	6-2	2	0.5	1	7467.55	14935.1	15477.782	30955.564	1	1642378	1.88	30595	0.0369	1	0.003	15	0.4907
			0.5	2	8010.232	16020.464			1					1		15	
	6-3	2	0.5	1	7318.581	14637.162	14798.46	29596.92	1	1642378	1.80	29236	0.0353	1	0.003	15	0.4689
			0.5	2	7479.879	14959.758			1					1		15	
	7-1	2	0.5	1	6730.798	13461.596	13439.298	26878.596	1	1642378	1.64	26518	0.0320	1	0.003	15	0.4253
			0.5	2	6708.5	13417			1					1		15	
	7-2	2	0.5	1	6616.398	13232.796	13183.164	26366.328	1	1642378	1.61	26006	0.0314	1	0.003	15	0.4171
			0.5	2	6566.766	13133.532			1					1		15	
	7-3	2	0.5	1	6373.999	12747.998	12885.725	25771.45	1	1642378	1.57	25411	0.0307	1	0.003	15	0.4076
			0.5	2	6511.726	13023.452			1					1		15	
	8-1	2	0.5	1	7168.218	14336.436	14620.759	29241.518	1	1642378	1.78	28881	0.0348	1	0.003	15	0.4632
			0.5	2	7452.541	14905.082			1					1		15	
	8-2	2	0.5	1	7272.105	14544.21	14709.992	29419.984	1	1642378	1.79	29059	0.0351	1	0.003	15	0.4661
			0.5	2	7437.887	14875.774			1					1		15	
	8-3	2	0.5	1	7380.902	14761.804	14729.241	29458.482	1	1642378	1.79	29098	0.0351	1	0.003	15	0.4667
			0.5	2	7348.339	14696.679			1					1		15	

Assay Date	8/29/2005	Test Chemical ID	RC#8 CM	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.5932	0.0301
Full activity	End	0.5082	0.0144
Full activity	Overall	0.5507	0.0527
Background	Beginning	0.0001	0.000501231
Background	End	-0.0001	0.000295344
Background	Overall	0.0000	0.000362169
Positive	Beginning	0.2397	0.0092
Positive	End	0.2242	0.0011
Positive	Overall	0.2319	0.0105
Negative	Beginning	0.5118	0.0193
Negative	End	0.3985	0.0050
Negative	Overall	0.4551	0.0665

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#8 CM	1	1	1.00E-04	-4.00	0.0878
RC#8 CM	1	2	1.00E-04	-4.00	0.0946
RC#8 CM	1	3	1.00E-04	-4.00	0.1342
RC#8 CM	2	1	3.30E-05	-4.48	0.0481
RC#8 CM	2	2	3.30E-05	-4.48	0.0449
RC#8 CM	2	3	3.30E-05	-4.48	0.0378
RC#8 CM	3	1	1.00E-05	-5.00	0.1282
RC#8 CM	3	2	1.00E-05	-5.00	0.1217
RC#8 CM	3	3	1.00E-05	-5.00	0.0797
RC#8 CM	4	1	1.00E-06	-6.00	0.3780
RC#8 CM	4	2	1.00E-06	-6.00	0.3691
RC#8 CM	4	3	1.00E-06	-6.00	0.3748
RC#8 CM	5	1	1.00E-07	-7.00	0.4575
RC#8 CM	5	2	1.00E-07	-7.00	0.4571
RC#8 CM	5	3	1.00E-07	-7.00	0.4514
RC#8 CM	6	1	1.00E-08	-8.00	0.4798
RC#8 CM	6	2	1.00E-08	-8.00	0.4907
RC#8 CM	6	3	1.00E-08	-8.00	0.4689
RC#8 CM	7	1	1.00E-09	-9.00	0.4253
RC#8 CM	7	2	1.00E-09	-9.00	0.4171
RC#8 CM	7	3	1.00E-09	-9.00	0.4076
RC#8 CM	8	1	1.00E-10	-10.00	0.4632
RC#8 CM	8	2	1.00E-10	-10.00	0.4661
RC#8 CM	8	3	1.00E-10	-10.00	0.4667

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	15.94	17.17	24.36
2	-4.48	8.73	8.16	6.86
3	-5.00	23.27	22.10	14.47
4	-6.00	68.65	67.03	68.07
5	-7.00	83.08	83.01	81.96
6	-8.00	87.12	89.11	85.15
7	-9.00	77.23	75.74	74.01
8	-10.00	84.12	84.64	84.75

Assay Date	9/6/2005	Test Chemical ID	RC#8 CYN	# Concentrations tested	8		
Technician ID	EJB	Replicate #	3	Microsome type	Recombina	Microsome ID	5

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 CYN		# Concentrations tested		8						
Technician ID	EJB	Replicate #	3		Microsome type		Recombinant	Microsome ID	5				
										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:	Recombinan	QC 10	QC 100										
	0.032	0.034	0.115										
	0.031	0.040	0.121										
	0.031	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	0.0450	m, b	0.188	0.000
0.125	62.5	1000	0.00013	200	0.0250	0.158	0.130	0.0248	se_m, se_b	0.004	0.000
0.05	25	1000	0.00005	200	0.0100	0.082	0.054	0.0106	r^2, se_y	0.999	0.000
0.025	25	2000	0.00003	200	0.0050	0.053	0.026	0.0051	F, df	2142	3
0.01	5	1000	0.00001	200	0.0020	0.035	0.007	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
0.005	5	2000	0.00001	200	0.0010	0.031	0.003	0.0009			

Regression results are calculated using the function LINEST

Blank	0.028	r^2=	0.999			
		m=	0.188			
		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
Recombinan	0.032	0.004	0.001	200	120	72000	0.003	0.003	2.846
Recombinan	0.031	0.003	0.001	200	120	72000	0.003		
Recombinan	0.031	0.003	0.001	200	120	72000	0.003		
QC 10	0.034	0.006	0.002	200	200	200	0.000	0.000	0.009
QC 10	0.040	0.012	0.003	200	200	200	0.000		
QC 10	0.033	0.005	0.001	200	200	200	0.000		
QC 100	0.115	0.087	0.017	200	200	200	0.000	0.000	0.087
QC 100	0.121	0.093	0.018	200	200	200	0.000		
QC 100	0.120	0.092	0.018	200	200	200	0.000		

Assay Date	9/6/2005	Test Chemical ID	RC#8 CYN	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID EJB	Replicate # 3

Microsome Dilution Details		
Dilution A	0.12	mL microsome Stock used
	72	mL total volume
	600	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
	600	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	2.845523
Protein Concentration (dilution added to assay, mg/mL):	0.004743

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 CYN	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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 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	10518.38	21036.76	21603.07	43206.14	1	1680873	2.57	42835	0.0509	1	0.002	15	0.7155
			0.5	2	11084.69	22169.38			1					1		15	
	2	2	0.5	1	12210.6	24421.2	23687.16	47374.32	1	1680873	2.82	47003	0.0559	1	0.002	15	0.7851
			0.5	2	11476.56	22953.12			1					1		15	
	3	2	0.5	1	11831.96	23663.92	23676.22	47352.44	1	1680873	2.82	46981	0.0558	1	0.002	15	0.7848
			0.5	2	11844.26	23688.52			1					1		15	
	4	2	0.5	1	11928.59	23857.18	24052.33	48104.66	1	1680873	2.86	47733	0.0567	1	0.002	15	0.7973
			0.5	2	12123.74	24247.48			1					1		15	
Background control	1	2	0.5	1	86.19411	172.38822	165.47478	330.94956	1	1680873	0.02	-40	0.0000	1	0.002	15	-0.0007
			0.5	2	79.28067	158.56134			1					1		15	
	2	2	0.5	1	86.19411	172.38822	172.38822	344.77644	1	1680873	0.02	-27	0.0000	1	0.002	15	-0.0004
			0.5	2	86.19411	172.38822			1					1		15	
	3	2	0.5	1	100.3999	200.7998	207.3596	414.7192	1	1680873	0.02	43	0.0001	1	0.002	15	0.0007
			0.5	2	106.9597	213.9194			1					1		15	
	4	2	0.5	1	91.58124	183.16248	197.40864	394.81728	1	1680873	0.02	24	0.0000	1	0.002	15	0.0004
			0.5	2	105.8274	211.6548			1					1		15	
Positive control	1	2	0.5	1	5076.724	10153.448	9628.552	19257.104	1	1680873	1.15	18896	0.0224	1	0.002	15	0.3155
			0.5	2	4551.828	9103.656			1					1		15	
	2	2	0.5	1	3991.594	7983.188	8207.161	16414.322	1	1680873	0.98	16043	0.0191	1	0.002	15	0.2680
			0.5	2	4215.567	8431.134			1					1		15	
	3	2	0.5	1	5608.149	11216.298	10796.96	21593.92	1	1680873	1.28	21223	0.0252	1	0.002	15	0.3545
			0.5	2	5188.811	10377.622			1					1		15	
	4	2	0.5	1	5544.208	11088.416	10771.292	21542.584	1	1680873	1.28	21171	0.0252	1	0.002	15	0.3536
			0.5	2	5227.084	10454.168			1					1		15	
Negative Control	1	2	0.5	1	6712.402	13424.804	13273.703	26547.406	1	1680873	1.58	26176	0.0311	1	0.002	15	0.4372
			0.5	2	6561.301	13122.602			1					1		15	
	2	2	0.5	1	6080.69	12161.38	12131.849	24263.698	1	1680873	1.44	23892	0.0284	1	0.002	15	0.3991
			0.5	2	6051.159	12102.318			1					1		15	
	3	2	0.5	1	10486.11	20972.22	21256.18	42512.36	1	1680873	2.53	42141	0.0501	1	0.002	15	0.7039
			0.5	2	10770.07	21540.14			1					1		15	
	4	2	0.5	1	10227.26	20454.52	21154.38	42308.76	1	1680873	2.52	41937	0.0498	1	0.002	15	0.7005
			0.5	2	10927.12	21854.24			1					1		15	
RC#8 CYN	1-1	2	0.5	1	1859.024	3718.048	4358.206	8716.412	1	1680873	0.52	8345	0.0099	1	0.002	15	0.1394
			0.5	2	2499.182	4998.364			1					1		15	
	1-2	2	0.5	1	2865.085	5730.17	4743.246	9486.492	1	1680873	0.56	9115	0.0108	1	0.002	15	0.1523
			0.5	2	1878.161	3756.322			1					1		15	
	1-3	2	0.5	1	1365.66	2731.32	2803.67	5607.34	1	1680873	0.33	5236	0.0062	1	0.002	15	0.0875
			0.5	2	1438.01	2876.02			1					1		15	
	2-1	2	0.5	1	3614.823	7229.646	7119.773	14239.546	1	1680873	0.85	13968	0.0165	1	0.002	15	0.2317
			0.5	2	3504.95	7009.9			1					1		15	
	2-2	2	0.5	1	3438.071	6876.142	6872.422	13744.844	1	1680873	0.82	13374	0.0159	1	0.002	15	0.2234
			0.5	2	3434.351	6868.702			1					1		15	
	2-3	2	0.5	1	2739.791	5479.582	5665.42	11330.84	1	1680873	0.67	10960	0.0130	1	0.002	15	0.1831
			0.5	2	2925.629	5851.258			1					1		15	
	3-1	2	0.5	1	6544.86	13089.72	13237.348	26474.696	1	1680873	1.58	26103	0.0310	1	0.002	15	0.4360
			0.5	2	6692.488	13384.976			1					1		15	
	3-2	2	0.5	1	6060.62	12121.24	12476.988	24953.976	1	1680873	1.48	24583	0.0292	1	0.002	15	0.4106
			0.5	2	6416.368	12832.736			1					1		15	
	3-3	2	0.5	1	6182.368	12364.736	12811.299	25622.598	1	1680873	1.52	25251	0.0300	1	0.002	15	0.4218
			0.5	2	6628.931	13257.862			1					1		15	
	4-1	2	0.5	1	8923.253	17846.506	18358.275	36716.55	1	1680873	2.18	36345	0.0432	1	0.002	15	0.6071
			0.5	2	9435.022	18870.044			1					1		15	
	4-2	2	0.5	1	6995.994	13991.988	13904.999	27809.998	1	1680873	1.65	27439	0.0326	1	0.002	15	0.4583
			0.5	2	6909.005	13818.01			1					1		15	
	4-3	2	0.5	1	8396.468	16792.936	17142.248	34284.496	1	1680873	2.04	33913	0.0403	1	0.002	15	0.5665
			0.5	2	8745.78	17491.56			1					1		15	
	5-1	2	0.5	1	11610.66	23221.32	23428.18	46856.36	1	1680873	2.79	46485	0.0552	1	0.002	15	0.7765
			0.5	2	11817.52	23635.04			1					1		15	
	5-2	2	0.5	1	10847.96	21695.92	22620.79	45241.58	1	1680873	2.69	44870	0.0533	1	0.002	15	0.7495
			0.5	2	11772.83	23545.66			1					1		15	
	5-3	2	0.5	1	11394.21	22788.42	22831.83	45663.66	1	1680873	2.72	45292	0.0538	1	0.002	15	0.7568
			0.5	2	11437.62	22875.24			1					1		15	
	6-1	2	0.5	1	11722.55	23445.1	23729.03	47458.06	1	1680873	2.82	47087	0.0560	1	0.002	15	0.7865
			0.5	2	12006.48	24012.96			1					1		15	
	6-2	2	0.5	1	11916.96	23833.92	23306.28	46612.56	1	1680873	2.77	46241	0.0549	1	0.002	15	0.7724
			0.5	2	11389.32	22778.64			1					1		15	
	6-3	2	0.5	1	9884.006	19768.012	21479.076	42958.152	1	1680873	2.56	42587	0.0506	1	0.002	15	0.7114
			0.5	2	11595.07	23190.14			1					1		15	
	7-1	2	0.5	1	11692.78	23385.56	22903.64	45807.28	1	1680873	2.73	45436	0.0540	1	0.002	15	0.7590
			0.5	2	11210.86	22421.72			1					1		15	
	7-2	2	0.5	1	9423.816	18847.632	20975.466	41950.932	1	1680873	2.50	41580	0.0494	1	0.002	15	0.6945
			0.5	2	11551.65	23103.3			1					1		15	
	7-3	2	0.5	1	11286.28	22572.56	22230.47	44460.94	1	1680873	2.65	44090	0.0524	1	0.002	15	0.7365
			0.5	2	10944.19	21888.38			1					1		15	
	8-1	2	0.5	1	12113.8	24227.6	23671.46	47742.92	1	1680873	2.84	47372	0.0563	1	0.002	15	0.7913
			0.5	2	11757.66	23515.32			1					1		15	
	8-2	2	0.5	1	12034.78	24069.56	24003.68	48007.36	1	1680873	2.86	47636	0.0566	1	0.002	15	0.7957
			0.5	2	11968.9	23937.8			1					1		15	
	8-3	2	0.5	1	11815.8	23631.6	23663.06	47326.12	1	1680873	2.82	46955	0.0558	1	0.002	15	0.7843
			0.5	2	11847.26	23694.52			1					1		15	

Assay Date	9/6/2005	Test Chemical ID	RC#8 CYN	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.7503	0.0492
Full activity	End	0.7911	0.0089
Full activity	Overall	0.7707	0.0372
Background	Beginning	-0.0006	0.000163317
Background	End	0.0006	0.000235072
Background	Overall	0.0000	0.000666067
Positive	Beginning	0.2917	0.0336
Positive	End	0.3541	0.0006
Positive	Overall	0.3229	0.0409
Negative	Beginning	0.4182	0.0270
Negative	End	0.7022	0.0024
Negative	Overall	0.5602	0.1647

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#8 CYN	1	1	3.30E-05	-4.48	0.1394
RC#8 CYN	1	2	3.30E-05	-4.48	0.1523
RC#8 CYN	1	3	3.30E-05	-4.48	0.0875
RC#8 CYN	2	1	1.00E-05	-5.00	0.2317
RC#8 CYN	2	2	1.00E-05	-5.00	0.2234
RC#8 CYN	2	3	1.00E-05	-5.00	0.1831
RC#8 CYN	3	1	3.30E-06	-5.48	0.4360
RC#8 CYN	3	2	3.30E-06	-5.48	0.4106
RC#8 CYN	3	3	3.30E-06	-5.48	0.4218
RC#8 CYN	4	1	1.00E-06	-6.00	0.6071
RC#8 CYN	4	2	1.00E-06	-6.00	0.4583
RC#8 CYN	4	3	1.00E-06	-6.00	0.5665
RC#8 CYN	5	1	1.00E-07	-7.00	0.7765
RC#8 CYN	5	2	1.00E-07	-7.00	0.7495
RC#8 CYN	5	3	1.00E-07	-7.00	0.7566
RC#8 CYN	6	1	1.00E-08	-8.00	0.7865
RC#8 CYN	6	2	1.00E-08	-8.00	0.7724
RC#8 CYN	6	3	1.00E-08	-8.00	0.7114
RC#8 CYN	7	1	1.00E-09	-9.00	0.7590
RC#8 CYN	7	2	1.00E-09	-9.00	0.6945
RC#8 CYN	7	3	1.00E-09	-9.00	0.7365
RC#8 CYN	8	1	1.00E-10	-10.00	0.7913
RC#8 CYN	8	2	1.00E-10	-10.00	0.7957
RC#8 CYN	8	3	1.00E-10	-10.00	0.7843

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	18.09	19.76	11.35
2	-5.00	30.06	28.99	23.75
3	-5.48	56.58	53.28	54.73
4	-6.00	78.77	59.47	73.50
5	-7.00	100.75	97.25	98.17
6	-8.00	102.06	100.22	92.30
7	-9.00	98.48	90.12	95.56
8	-10.00	102.67	103.25	101.77

Assay Date	8/15/2005	Test Chemical ID	RC#8 CM	# Concentrations tested	8		
Technician ID	EJB	Replicate #	4	Microsome type	Recombina	Microsome ID	5

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/bc

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#8 CYN	# Concentrations tested	8						
Technician ID	EJB	Replicate #	4	Microsome type	Recombinant	Microsome ID	5				

								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:	Mic 0.008	QC 10	QC 100	Mic 0.08
	0.069	0.086	0.306	0.229
	0.067	0.082	0.319	0.214
	0.066	0.080	0.312	0.230

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

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

Blank	0.058	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
Mic 0.008	0.069	0.011	-0.001	200	120	72000	-0.003	-0.004	-3.643
Mic 0.008	0.067	0.008	-0.001	200	120	72000	-0.004		
Mic 0.008	0.066	0.008	-0.001	200	120	72000	-0.004		
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		
Mic 0.08	0.229	0.170	0.013	200	140	8540	0.004	0.004	3.771
Mic 0.08	0.214	0.155	0.011	200	140	8540	0.003		
Mic 0.08	0.230	0.171	0.013	200	140	8540	0.004		

Assay Date	8/15/2005	Test Chemical ID	RC#8 CYN	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	4

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

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

Protein Concentration (stock microsomes, mg/mL):	3.771282
Protein Concentration (dilution added to assay, mg/mL):	0.006182

Assay Date	8/15/2005	Test Chemical ID	RC#8 CYN	# Concentrations tested	8	Microsome type	Recombinan	Microsome ID	5	Technician ID	EJB	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	9388.386	18776.772	18892.956	37785.912	1	1563166	2.42	37365	0.0476	1	0.003	15	0.5131
			0.5	2	9494.57	18989.14			1					1		15	
	2	2	0.5	1	9432.159	18864.318	19050.883	38101.766	1	1563166	2.44	37701	0.0480	1	0.003	15	0.5177
			0.5	2	9618.724	19237.448			1					1		15	
	3	2	0.5	1	8924.733	17849.466	18047.84	36095.68	1	1563166	2.31	35695	0.0455	1	0.003	15	0.4902
			0.5	2	9123.107	18246.214			1					1		15	
	4	2	0.5	1	9204.225	18408.45	18523.805	37047.61	1	1563166	2.37	36647	0.0467	1	0.003	15	0.5033
			0.5	2	9319.58	18639.16			1					1		15	
Background control	1	2	0.5	1	113.9674	227.9348	222.5078	445.0156	1	1563166	0.03	45	0.0001	1	0.003	15	0.0006
			0.5	2	108.5404	217.0808			1					1		15	
	2	2	0.5	1	105.0491	210.0982	220.8724	441.7448	1	1563166	0.03	41	0.0001	1	0.003	15	0.0006
			0.5	2	115.8233	231.6466			1					1		15	
	3	2	0.5	1	78.11341	156.22682	179.26461	358.52922	1	1563166	0.02	-42	-0.0001	1	0.003	15	-0.0006
			0.5	2	101.1512	202.3024			1					1		15	
	4	2	0.5	1	81.40529	162.81058	178.25451	356.50902	1	1563166	0.02	-44	-0.0001	1	0.003	15	-0.0006
			0.5	2	96.84922	193.69844			1					1		15	
Positive control	1	2	0.5	1	4571.006	9142.012	9213.05	18426.1	1	1563166	1.18	18026	0.0230	1	0.003	15	0.2475
			0.5	2	4642.044	9284.088			1					1		15	
	2	2	0.5	1	4218.49	8436.98	8746.398	17492.796	1	1563166	1.12	17092	0.0218	1	0.003	15	0.2347
			0.5	2	4527.908	9055.816			1					1		15	
	3	2	0.5	1	4495.585	8991.17	9170.987	18341.974	1	1563166	1.17	17942	0.0228	1	0.003	15	0.2464
			0.5	2	4675.402	9350.804			1					1		15	
	4	2	0.5	1	4471.343	8942.686	9099.718	18199.436	1	1563166	1.16	17799	0.0227	1	0.003	15	0.2444
			0.5	2	4628.375	9256.75			1					1		15	
Negative Control	1	2	0.5	1	8127.004	16254.008	16242.761	32485.522	1	1563166	2.08	32085	0.0409	1	0.003	15	0.4406
			0.5	2	8115.757	16231.514			1					1		15	
	2	2	0.5	1	7942.485	15884.97	15796.78	31593.56	1	1563166	2.02	31193	0.0397	1	0.003	15	0.4284
			0.5	2	7854.295	15708.59			1					1		15	
	3	2	0.5	1	7614.148	15228.296	15401.962	30803.924	1	1563166	1.97	30403	0.0387	1	0.003	15	0.4175
			0.5	2	7787.814	15575.628			1					1		15	
	4	2	0.5	1	7627.716	15255.432	15345.32	30690.64	1	1563166	1.96	30290	0.0386	1	0.003	15	0.4160
			0.5	2	7717.604	15435.208			1					1		15	
RC#8 CYN	1-1	2	0.5	1	2781.392	5562.784	5761.299	11522.598	1	1563166	0.74	11122	0.0142	1	0.003	15	0.1527
			0.5	2	2979.907	5959.814			1					1		15	
	1-2	2	0.5	1	2778.678	5557.356	5755.116	11510.232	1	1563166	0.74	11110	0.0141	1	0.003	15	0.1526
			0.5	2	2976.438	5952.876			1					1		15	
	1-3	2	0.5	1	2756.929	5513.858	5709.124	11418.248	1	1563166	0.73	11018	0.0140	1	0.003	15	0.1513
			0.5	2	2952.195	5904.39			1					1		15	
	2-1	2	0.5	1	2597.167	5194.334	5193.646	10387.292	1	1563166	0.66	9987	0.0127	1	0.003	15	0.1371
			0.5	2	2596.479	5192.958			1					1		15	
	2-2	2	0.5	1	2735.261	5470.522	5477.355	10954.71	1	1563166	0.70	10554	0.0134	1	0.003	15	0.1449
			0.5	2	2742.094	5484.188			1					1		15	
	2-3	2	0.5	1	2798.595	5597.19	5413.801	10827.602	1	1563166	0.69	10427	0.0133	1	0.003	15	0.1432
			0.5	2	2615.206	5230.412			1					1		15	
	3-1	2	0.5	1	7299.679	14599.358	14879.493	29758.986	1	1563166	1.90	29359	0.0374	1	0.003	15	0.4032
			0.5	2	7579.814	15159.628			1					1		15	
	3-2	2	0.5	1	7600.661	15201.322	15381.225	30762.45	1	1563166	1.97	30362	0.0387	1	0.003	15	0.4170
			0.5	2	7780.564	15561.126			1					1		15	
	3-3	2	0.5	1	7253.888	14507.776	14804.073	29608.146	1	1563166	1.89	29208	0.0372	1	0.003	15	0.4011
			0.5	2	7550.185	15100.37			1					1		15	
	4-1	2	0.5	1	6763.569	13527.138	13560.965	27121.93	1	1563166	1.74	26721	0.0340	1	0.003	15	0.3670
			0.5	2	6797.396	13594.792			1					1		15	
	4-2	2	0.5	1	7296.73	14593.46	14419.806	28839.612	1	1563166	1.84	28439	0.0362	1	0.003	15	0.3905
			0.5	2	7123.076	14246.152			1					1		15	
	4-3	2	0.5	1	7471.639	14943.278	14601.545	29203.09	1	1563166	1.87	28803	0.0367	1	0.003	15	0.3955
			0.5	2	7129.906	14259.812			1					1		15	
	5-1	2	0.5	1	8531.41	17062.82	16913.574	33827.148	1	1563166	2.16	33427	0.0426	1	0.003	15	0.4590
			0.5	2	8382.164	16764.328			1					1		15	
	5-2	2	0.5	1	8286.329	16572.658	16340.154	32680.308	1	1563166	2.09	32280	0.0411	1	0.003	15	0.4433
			0.5	2	8053.825	16107.65			1					1		15	
	5-3	2	0.5	1	8387.481	16774.962	16482.009	32964.018	1	1563166	2.11	32564	0.0415	1	0.003	15	0.4472
			0.5	2	8094.528	16189.056			1					1		15	
	6-1	2	0.5	1	8395.731	16791.462	16908.969	33817.938	1	1563166	2.16	33417	0.0426	1	0.003	15	0.4589
			0.5	2	8513.238	17026.476			1					1		15	
	6-2	2	0.5	1	8872.794	17745.588	17393.395	34786.79	1	1563166	2.23	34386	0.0438	1	0.003	15	0.4722
			0.5	2	8520.601	17041.202			1					1		15	
	6-3	2	0.5	1	8311.656	16623.312	16694.211	33388.422	1	1563166	2.14	32988	0.0420	1	0.003	15	0.4530
			0.5	2	8382.555	16765.11			1					1		15	
	7-1	2	0.5	1	8652.711	17305.422	17340.963	34681.926	1	1563166	2.22	34281	0.0437	1	0.003	15	0.4708
			0.5	2	8688.252	17376.504			1					1		15	
	7-2	2	0.5	1	8518.752	17037.504	17046.762	34093.524	1	1563166	2.18	33693	0.0429	1	0.003	15	0.4627
			0.5	2	8528.01	17056.02			1					1		15	
	7-3	2	0.5	1	8164.371	16328.742	16105.027	32210.054	1	1563166	2.06	31810	0.0405	1	0.003	15	0.4368
			0.5	2	7940.656	15881.312			1					1		15	
	8-1	2	0.5	1	8252.114	16504.228	16251.177	32502.354	1	1563166	2.08	32102	0.0409	1	0.003	15	0.4408
			0.5	2	7999.063	15998.126			1					1		15	
	8-2	2	0.5	1	7891.053	15782.106	16006.939	32013.878	1	1563166	2.05	31613	0.0403	1	0.003	15	0.4341
			0.5	2	8115.886	16231.772			1					1		15	
	8-3	2	0.5	1	8110.852	16221.704	16063.463	32126.926	1	1563166	2.06	31726	0.0404	1	0.003	15	0.4357
			0.5	2	7952.611	15905.222			1					1		15	

Assay Date	8/15/2005	Test Chemical ID	RC#8 CYN	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	4

Control Type	Portion	Average	SD
Full activity	Beginning	0.5154	0.0033
Full activity	End	0.4967	0.0092
Full activity	Overall	0.5061	0.0122
Background	Beginning	0.0006	3.17611E-05
Background	End	-0.0006	1.96172E-05
Background	Overall	0.0000	0.000681099
Positive	Beginning	0.2411	0.0091
Positive	End	0.2454	0.0014
Positive	Overall	0.2433	0.0058
Negative	Beginning	0.4345	0.0087
Negative	End	0.4167	0.0011
Negative	Overall	0.4256	0.0114

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#8 CYN	1	1	3.30E-05	-4.48	0.1527
RC#8 CYN	1	2	3.30E-05	-4.48	0.1526
RC#8 CYN	1	3	3.30E-05	-4.48	0.1513
RC#8 CYN	2	1	1.00E-05	-5.00	0.1371
RC#8 CYN	2	2	1.00E-05	-5.00	0.1449
RC#8 CYN	2	3	1.00E-05	-5.00	0.1432
RC#8 CYN	3	1	3.30E-06	-5.48	0.4032
RC#8 CYN	3	2	3.30E-06	-5.48	0.4170
RC#8 CYN	3	3	3.30E-06	-5.48	0.4011
RC#8 CYN	4	1	1.00E-06	-6.00	0.3670
RC#8 CYN	4	2	1.00E-06	-6.00	0.3905
RC#8 CYN	4	3	1.00E-06	-6.00	0.3955
RC#8 CYN	5	1	1.00E-07	-7.00	0.4590
RC#8 CYN	5	2	1.00E-07	-7.00	0.4433
RC#8 CYN	5	3	1.00E-07	-7.00	0.4472
RC#8 CYN	6	1	1.00E-08	-8.00	0.4589
RC#8 CYN	6	2	1.00E-08	-8.00	0.4722
RC#8 CYN	6	3	1.00E-08	-8.00	0.4530
RC#8 CYN	7	1	1.00E-09	-9.00	0.4708
RC#8 CYN	7	2	1.00E-09	-9.00	0.4627
RC#8 CYN	7	3	1.00E-09	-9.00	0.4368
RC#8 CYN	8	1	1.00E-10	-10.00	0.4408
RC#8 CYN	8	2	1.00E-10	-10.00	0.4341
RC#8 CYN	8	3	1.00E-10	-10.00	0.4357

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.48	30.18	30.15	29.90
2	-5.00	27.10	28.64	28.29
3	-5.48	79.67	82.39	79.26
4	-6.00	72.51	77.17	78.16
5	-7.00	90.70	87.59	88.36
6	-8.00	90.68	93.31	89.51
7	-9.00	93.02	91.43	86.32
8	-10.00	87.11	85.78	86.09

Assay Date	9/8/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8
Technician ID	EJB	Replicate #	1	Microsome type	Recombina Microsome ID 5

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/b*c

2) Calculate total µg ASDN/g soln.

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

= 0.562705 + 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	EJB	Replicate #	1	Microsome type	Recombinant	Microsome ID	5		

								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:	Mic 0.008	QC 10	QC 100	Mic 0.08
	0.069	0.074	0.307	0.272
	0.069	0.072	0.305	0.282
	0.065	0.067	0.310	0.267

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

Regression results are calculated using the function LINEST

r^2=	0.990	
m=	0.084	
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
Mic 0.008	0.069	0.028	0.000	200	120	72000	0.001	0.001	1.124
Mic 0.008	0.069	0.028	0.000	200	120	72000	0.001		
Mic 0.008	0.065	0.023	0.000	200	120	72000	0.000		
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		
Mic 0.08	0.272	0.231	0.018	200	140	8540	0.005	0.005	5.420
Mic 0.08	0.282	0.241	0.018	200	140	8540	0.006		
Mic 0.08	0.267	0.226	0.017	200	140	8540	0.005		

Assay Date	9/8/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID EJB	Replicate # 1

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.420243
Protein Concentration (dilution added to assay, mg/mL):	0.008886

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	Recombinan	Microsome ID	5	Technician ID	EJB	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol ^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol ^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	16249.85	32499.7	32438.18	64876.36	1	1623262	4.00	64129	0.0788	1	0.004	15	0.5909
			0.5	2	16188.33	32376.66			1					1		15	
	2	2	0.5	1	16173	32346	32265.08	64530.16	1	1623262	3.98	63783	0.0783	1	0.004	15	0.5877
			0.5	2	16092.08	32184.16			1					1		15	
	3	2	0.5	1	16177.56	32355.12	31342.34	62684.68	1	1623262	3.86	61937	0.0761	1	0.004	15	0.5707
			0.5	2	15164.78	30329.56			1					1		15	
	4	2	0.5	1	16379.57	32759.14	33146.35	66292.7	1	1623262	4.08	65545	0.0805	1	0.004	15	0.6040
			0.5	2	16766.78	33533.56			1					1		15	
Background control	1	2	0.5	1	230.9671	461.9342	471.6164	943.2328	1	1623262	0.06	196	0.0002	1	0.004	15	0.0018
			0.5	2	240.6493	481.2986			1					1		15	
	2	2	0.5	1	229.6406	459.2812	448.3459	896.6918	1	1623262	0.06	149	0.0002	1	0.004	15	0.0014
			0.5	2	218.7053	437.4106			1					1		15	
	3	2	0.5	1	134.6783	269.3566	272.7276	545.4552	1	1623262	0.03	-202	-0.0002	1	0.004	15	-0.0019
			0.5	2	138.0493	276.0986			1					1		15	
	4	2	0.5	1	132.7397	265.4794	302.4353	604.8706	1	1623262	0.04	-143	-0.0002	1	0.004	15	-0.0013
			0.5	2	169.6956	339.3912			1					1		15	
Positive control	1	2	0.5	1	8967.941	17935.882	16679.775	33359.55	1	1623262	2.06	32512	0.0401	1	0.004	15	0.3005
			0.5	2	7711.834	15423.668			1					1		15	
	2	2	0.5	1	5660.05	11320.1	12003.431	24006.862	1	1623262	1.48	23259	0.0286	1	0.004	15	0.2143
			0.5	2	6343.381	12686.762			1					1		15	
	3	2	0.5	1	8251.143	16502.286	17110.125	34220.25	1	1623262	2.11	33473	0.0411	1	0.004	15	0.3084
			0.5	2	8858.982	17717.964			1					1		15	
	4	2	0.5	1	8808.177	17616.354	17477.866	34955.732	1	1623262	2.15	34208	0.0420	1	0.004	15	0.3152
			0.5	2	8669.689	17339.378			1					1		15	
Negative Control	1	2	0.5	1	12045.69	24091.38	23894.75	47789.5	1	1623262	2.94	47042	0.0578	1	0.004	15	0.4335
			0.5	2	11849.06	23698.12			1					1		15	
	2	2	0.5	1	10888.55	21777.1	21787.8	43575.6	1	1623262	2.68	42828	0.0526	1	0.004	15	0.3946
			0.5	2	10899.25	21798.5			1					1		15	
	3	2	0.5	1	14664.16	29328.32	29050.57	58101.14	1	1623262	3.58	57354	0.0704	1	0.004	15	0.5285
			0.5	2	14386.41	28772.82			1					1		15	
	4	2	0.5	1	14209.67	28419.34	28459.45	56918.9	1	1623262	3.51	56171	0.0690	1	0.004	15	0.5176
			0.5	2	14249.78	28499.56			1					1		15	
RC#9 DCF	1-1	2	0.5	1	7421.488	14842.976	15001.222	30002.444	1	1623262	1.85	29255	0.0359	1	0.004	15	0.2696
			0.5	2	7579.734	15159.468			1					1		15	
	1-2	2	0.5	1	7796.885	15593.77	14419.313	28838.626	1	1623262	1.78	28091	0.0345	1	0.004	15	0.2589
			0.5	2	6622.428	13244.856			1					1		15	
	1-3	2	0.5	1	7129.906	14259.812	13964.62	27969.24	1	1623262	1.72	27222	0.0334	1	0.004	15	0.2508
			0.5	2	6854.714	13709.428			1					1		15	
	2-1	2	0.5	1	10718.42	21436.84	21220.69	42441.38	1	1623262	2.61	41694	0.0512	1	0.004	15	0.3842
			0.5	2	10502.27	21004.54			1					1		15	
	2-2	2	0.5	1	8641.005	17282.01	17147.331	34294.662	1	1623262	2.11	33547	0.0412	1	0.004	15	0.3091
			0.5	2	8506.326	17012.652			1					1		15	
	2-3	2	0.5	1	9599.682	19199.364	18957.45	37914.9	1	1623262	2.34	37167	0.0456	1	0.004	15	0.3425
			0.5	2	9357.768	18715.536			1					1		15	
	3-1	2	0.5	1	10213.59	20427.18	20458.47	40916.94	1	1623262	2.52	40169	0.0493	1	0.004	15	0.3701
			0.5	2	10244.88	20489.76			1					1		15	
	3-2	2	0.5	1	8899.092	17798.184	17728.707	35457.414	1	1623262	2.18	34710	0.0426	1	0.004	15	0.3198
			0.5	2	8829.615	17659.23			1					1		15	
	3-3	2	0.5	1	8873.271	17746.542	17352.707	34705.414	1	1623262	2.14	33958	0.0417	1	0.004	15	0.3129
			0.5	2	8479.436	16958.872			1					1		15	
	4-1	2	0.5	1	14770.97	29541.94	29456.64	58913.28	1	1623262	3.63	58166	0.0714	1	0.004	15	0.5360
			0.5	2	14685.67	29371.34			1					1		15	
	4-2	2	0.5	1	13833.62	27667.24	27618.4	55236.8	1	1623262	3.40	54489	0.0669	1	0.004	15	0.5021
			0.5	2	13784.78	27569.56			1					1		15	
	4-3	2	0.5	1	14416.12	28832.24	29397.89	58795.78	1	1623262	3.62	58048	0.0713	1	0.004	15	0.5349
			0.5	2	14981.77	29963.54			1					1		15	
	5-1	2	0.5	1	14402.27	28804.54	28486.34	56972.68	1	1623262	3.51	56225	0.0691	1	0.004	15	0.5181
			0.5	2	14084.07	28168.14			1					1		15	
	5-2	2	0.5	1	15637.59	31275.18	30239.57	60479.14	1	1623262	3.73	59732	0.0734	1	0.004	15	0.5504
			0.5	2	14601.96	29203.96			1					1		15	
	5-3	2	0.5	1	14805.07	29610.14	29439.18	58878.36	1	1623262	3.63	58131	0.0714	1	0.004	15	0.5357
			0.5	2	14634.11	29268.22			1					1		15	
	6-1	2	0.5	1	15055.29	30110.58	30392.16	60784.32	1	1623262	3.74	60037	0.0737	1	0.004	15	0.5532
			0.5	2	15336.87	30673.74			1					1		15	
	6-2	2	0.5	1	14161.96	28323.92	28318.49	56636.98	1	1623262	3.49	55889	0.0686	1	0.004	15	0.5150
			0.5	2	14156.53	28313.06			1					1		15	
	6-3	2	0.5	1	13650.87	27301.74	27028.99	54057.96	1	1623262	3.33	53310	0.0655	1	0.004	15	0.4912
			0.5	2	13378.12	26756.24			1					1		15	
	7-1	2	0.5	1	12553.82	25107.64	25030.55	50061.1	1	1623262	3.08	49314	0.0606	1	0.004	15	0.4544
			0.5	2	12476.73	24953.46			1					1		15	
	7-2	2	0.5	1	14917.13	29834.26	30302.89	60605.78	1	1623262	3.73	59858	0.0735	1	0.004	15	0.5516
			0.5	2	15385.76	30771.52			1					1		15	
	7-3	2	0.5	1	14880.28	29760.56	29523.22	59046.44	1	1623262	3.64	58299	0.0716	1	0.004	15	0.5372
			0.5	2	14642.94	29285.88			1					1		15	
	8-1	2	0.5	1	14634.91	29269.82	29681.33	59362.66	1	1623262	3.66	58615	0.0720	1	0.004	15	0.5401
			0.5	2	15046.42	30092.84			1					1		15	
	8-2	2	0.5	1	15523.19	31046.38	30811.26	61622.52	1	1623262	3.80	60875	0.0748	1	0.004	15	0.5609
			0.5	2	15288.07	30576.14			1					1		15	
	8-3	2	0.5	1	14976	29952	30082.35	60164.7	1	1623262	3.71	59417	0.0730	1	0.004	15	0.5475
			0.5	2	15106.35	30212.7			1					1		15	

Assay Date	9/8/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.5893	0.0023
Full activity	End	0.5874	0.0235
Full activity	Overall	0.5883	0.0137
Background	Beginning	0.0016	0.000303251
Background	End	-0.0016	0.000387137
Background	Overall	0.0000	0.001856212
Positive	Beginning	0.2574	0.0609
Positive	End	0.3118	0.0048
Positive	Overall	0.2846	0.0472
Negative	Beginning	0.4141	0.0275
Negative	End	0.5230	0.0077
Negative	Overall	0.4686	0.0650

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#9 DCF	1	1	1.00E-04	-4.00	0.2696
RC#9 DCF	1	2	1.00E-04	-4.00	0.2589
RC#9 DCF	1	3	1.00E-04	-4.00	0.2508
RC#9 DCF	2	1	3.30E-05	-4.48	0.3842
RC#9 DCF	2	2	3.30E-05	-4.48	0.3091
RC#9 DCF	2	3	3.30E-05	-4.48	0.3425
RC#9 DCF	3	1	1.00E-05	-5.00	0.3701
RC#9 DCF	3	2	1.00E-05	-5.00	0.3198
RC#9 DCF	3	3	1.00E-05	-5.00	0.3129
RC#9 DCF	4	1	1.00E-06	-6.00	0.5360
RC#9 DCF	4	2	1.00E-06	-6.00	0.5021
RC#9 DCF	4	3	1.00E-06	-6.00	0.5349
RC#9 DCF	5	1	1.00E-07	-7.00	0.5181
RC#9 DCF	5	2	1.00E-07	-7.00	0.5504
RC#9 DCF	5	3	1.00E-07	-7.00	0.5357
RC#9 DCF	6	1	1.00E-08	-8.00	0.5532
RC#9 DCF	6	2	1.00E-08	-8.00	0.5150
RC#9 DCF	6	3	1.00E-08	-8.00	0.4912
RC#9 DCF	7	1	1.00E-09	-9.00	0.4544
RC#9 DCF	7	2	1.00E-09	-9.00	0.5516
RC#9 DCF	7	3	1.00E-09	-9.00	0.5372
RC#9 DCF	8	1	1.00E-10	-10.00	0.5401
RC#9 DCF	8	2	1.00E-10	-10.00	0.5609
RC#9 DCF	8	3	1.00E-10	-10.00	0.5475

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	45.82	44.00	42.63
2	-4.48	65.30	52.54	58.21
3	-5.00	62.91	54.36	53.19
4	-6.00	91.10	85.34	90.92
5	-7.00	88.06	93.55	91.05
6	-8.00	94.03	87.53	83.50
7	-9.00	77.24	93.75	91.31
8	-10.00	91.80	95.34	93.06

Assay Date	9/12/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8		
Technician ID	EJB	Replicate #	2	Microsome type	Recombina	Microsome ID	5

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/b*c

2) Calculate total µg ASDN/g soln.

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

= 0.562033 + 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	EJB	Replicate #	2	Microsome type	Recombinant

Microsome ID: 5

								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:	Mic 0.008	QC 10	QC 100	Mic 0.08
	0.061	0.073	0.332	0.205
	0.061	0.063	0.315	0.203
	0.059	0.071	0.317	0.201

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
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
Mic 0.008	0.061	0.017	-0.001	200	120	72000	-0.004	-0.004	-3.701
Mic 0.008	0.061	0.017	-0.001	200	120	72000	-0.003		
Mic 0.008	0.059	0.015	-0.001	200	120	72000	-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		
Mic 0.08	0.205	0.161	0.011	200	140	8540	0.003	0.003	3.378
Mic 0.08	0.203	0.159	0.011	200	140	8540	0.003		
Mic 0.08	0.201	0.157	0.011	200	140	8540	0.003		

Assay Date	9/12/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	2

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.377604
Protein Concentration (dilution added to assay, mg/mL):	0.005537

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	9955.42	19910.84	19743.838	39487.676	1	1584999	2.49	38778	0.0487	1	0.003	15	0.5864
			0.5	2	9788.418	19576.836			1					1		15	
	2	2	0.5	1	9421.306	18842.612	18796.322	37592.644	1	1584999	2.37	36883	0.0463	1	0.003	15	0.5577
			0.5	2	9375.016	18750.032			1					1		15	
	3	2	0.5	1	9677.177	19354.354	19656.836	39313.676	1	1584999	2.48	38604	0.0485	1	0.003	15	0.5837
			0.5	2	9979.661	19959.322			1					1		15	
	4	2	0.5	1	9756.096	19512.192	18946.992	37893.984	1	1584999	2.39	37184	0.0467	1	0.003	15	0.5623
			0.5	2	9190.896	18381.792			1					1		15	
Background control	1	2	0.5	1	124.8214	249.6428	287.9349	575.8698	1	1584999	0.04	-134	-0.0002	1	0.003	15	-0.0020
			0.5	2	163.1135	326.227			1					1		15	
	2	2	0.5	1	159.7598	319.5196	309.0028	618.0056	1	1584999	0.04	-92	-0.0001	1	0.003	15	-0.0014
			0.5	2	149.243	298.486			1					1		15	
	3	2	0.5	1	166.0665	332.133	370.7775	741.555	1	1584999	0.05	32	0.0000	1	0.003	15	0.0005
			0.5	2	204.711	409.422			1					1		15	
	4	2	0.5	1	199.3239	398.6478	451.5307	903.0614	1	1584999	0.06	193	0.0002	1	0.003	15	0.0029
			0.5	2	252.2068	504.4136			1					1		15	
Positive control	1	2	0.5	1	4666.649	9333.298	9224.187	18448.374	1	1584999	1.16	17739	0.0223	1	0.003	15	0.2682
			0.5	2	4557.538	9115.076			1					1		15	
	2	2	0.5	1	4127.27	8254.54	8029.318	16058.636	1	1584999	1.01	15349	0.0193	1	0.003	15	0.2321
			0.5	2	3902.048	7804.096			1					1		15	
	3	2	0.5	1	5294.085	10588.17	10163.45	20326.9	1	1584999	1.28	19617	0.0246	1	0.003	15	0.2966
			0.5	2	4869.365	9738.73			1					1		15	
	4	2	0.5	1	5055.85	10111.7	10403.223	20806.446	1	1584999	1.31	20097	0.0252	1	0.003	15	0.3039
			0.5	2	5347.373	10694.746			1					1		15	
Negative Control	1	2	0.5	1	6759.388	13518.776	13755.261	27510.522	1	1584999	1.74	26801	0.0337	1	0.003	15	0.4053
			0.5	2	6995.873	13991.746			1					1		15	
	2	2	0.5	1	5532.875	11065.75	11139.829	22279.658	1	1584999	1.41	21570	0.0271	1	0.003	15	0.3262
			0.5	2	5606.954	11213.908			1					1		15	
	3	2	0.5	1	8268.107	16536.214	16359.622	32719.244	1	1584999	2.06	32010	0.0402	1	0.003	15	0.4840
			0.5	2	8091.515	16183.03			1					1		15	
	4	2	0.5	1	7778.684	15557.368	15226.433	30452.866	1	1584999	1.92	29743	0.0374	1	0.003	15	0.4498
			0.5	2	7447.749	14895.498			1					1		15	
RC#9 DCF	1-1	2	0.5	1	3600.455	7200.91	7403.214	14806.428	1	1584999	0.93	14097	0.0177	1	0.003	15	0.2132
			0.5	2	3802.759	7605.518			1					1		15	
	1-2	2	0.5	1	4562.925	9125.85	9032.099	18064.198	1	1584999	1.14	17355	0.0218	1	0.003	15	0.2624
			0.5	2	4469.174	8938.348			1					1		15	
	1-3	2	0.5	1	3984.269	7968.538	8237.804	16475.608	1	1584999	1.04	15766	0.0198	1	0.003	15	0.2384
			0.5	2	4253.535	8507.07			1					1		15	
	2-1	2	0.5	1	5858.537	11717.074	11265.377	22530.754	1	1584999	1.42	21821	0.0274	1	0.003	15	0.3300
			0.5	2	5406.84	10813.68			1					1		15	
	2-2	2	0.5	1	4425.758	8851.516	8772.549	17545.098	1	1584999	1.11	16835	0.0211	1	0.003	15	0.2546
			0.5	2	4346.791	8693.582			1					1		15	
	2-3	2	0.5	1	4960.322	9920.644	10207.416	20414.832	1	1584999	1.29	19705	0.0247	1	0.003	15	0.2980
			0.5	2	5247.094	10494.188			1					1		15	
	3-1	2	0.5	1	5041.48	10082.96	10389.836	20779.672	1	1584999	1.31	20070	0.0252	1	0.003	15	0.3035
			0.5	2	5348.356	10696.712			1					1		15	
	3-2	2	0.5	1	5022.733	10045.466	10327.7	20655.4	1	1584999	1.30	19946	0.0251	1	0.003	15	0.3016
			0.5	2	5304.967	10609.934			1					1		15	
	3-3	2	0.5	1	4765.104	9530.208	9676.525	19353.05	1	1584999	1.22	18643	0.0234	1	0.003	15	0.2819
			0.5	2	4911.421	9822.842			1					1		15	
	4-1	2	0.5	1	8003.343	16006.686	16137.998	32275.996	1	1584999	2.04	31566	0.0396	1	0.003	15	0.4773
			0.5	2	8134.655	16269.31			1					1		15	
	4-2	2	0.5	1	8249.354	16498.708	16667.169	33334.338	1	1584999	2.10	32625	0.0410	1	0.003	15	0.4933
			0.5	2	8417.815	16835.63			1					1		15	
	4-3	2	0.5	1	8275.08	16550.16	16760.935	33521.87	1	1584999	2.11	32812	0.0412	1	0.003	15	0.4962
			0.5	2	8485.855	16971.71			1					1		15	
	5-1	2	0.5	1	8775.643	17551.286	17764.168	35528.336	1	1584999	2.24	34819	0.0437	1	0.003	15	0.5265
			0.5	2	8988.525	17977.05			1					1		15	
	5-2	2	0.5	1	7575.878	15151.756	15761.711	31523.422	1	1584999	1.99	30814	0.0387	1	0.003	15	0.4659
			0.5	2	8185.833	16371.666			1					1		15	
	5-3	2	0.5	1	8150.414	16300.828	16508.112	33016.224	1	1584999	2.08	32307	0.0406	1	0.003	15	0.4885
			0.5	2	8357.698	16715.396			1					1		15	
	6-1	2	0.5	1	7303.416	14606.832	15346.344	30692.688	1	1584999	1.94	29983	0.0377	1	0.003	15	0.4534
			0.5	2	8042.928	16085.856			1					1		15	
	6-2	2	0.5	1	6847.574	13695.148	14578.19	29156.38	1	1584999	1.84	28447	0.0357	1	0.003	15	0.4302
			0.5	2	7730.616	15461.232			1					1		15	
	6-3	2	0.5	1	7428.428	14856.856	15286.835	30573.67	1	1584999	1.93	29864	0.0375	1	0.003	15	0.4516
			0.5	2	7858.407	15716.814			1					1		15	
	7-1	2	0.5	1	8875.983	17751.966	17387.341	34774.682	1	1584999	2.19	34065	0.0428	1	0.003	15	0.5151
			0.5	2	8511.358	17022.716			1					1		15	
	7-2	2	0.5	1	8908.079	17816.158	17664.954	35329.908	1	1584999	2.23	34620	0.0435	1	0.003	15	0.5235
			0.5	2	8756.875	17513.75			1					1		15	
	7-3	2	0.5	1	8393.24	16786.48	17101.632	34203.264	1	1584999	2.16	33494	0.0421	1	0.003	15	0.5065
			0.5	2	8708.392	17416.784			1					1		15	
	8-1	2	0.5	1	8579.099	17158.198	17319.813	34639.626	1	1584999	2.19	33930	0.0426	1	0.003	15	0.5131
			0.5	2	8740.714	17481.428			1					1		15	
	8-2	2	0.5	1	8855.743	17711.486	17441.379	34882.758	1	1584999	2.20	34173	0.0429	1	0.003	15	0.5167
			0.5	2	8585.636	17171.272			1					1		15	
	8-3	2	0.5	1	7929.942	15859.884	16005.475	32010.95	1	1584999	2.02	31301	0.0393	1	0.003	15	0.4733
			0.5	2	8075.533	16151.066			1					1		15	

Assay Date	9/12/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.5720	0.0203
Full activity	End	0.5730	0.0152
Full activity	Overall	0.5725	0.0146
Background	Beginning	-0.0017	0.000450533
Background	End	0.0017	0.00172689
Background	Overall	0.0000	0.00222103
Positive	Beginning	0.2502	0.0256
Positive	End	0.3003	0.0051
Positive	Overall	0.2752	0.0326
Negative	Beginning	0.3657	0.0559
Negative	End	0.4669	0.0242
Negative	Overall	0.4163	0.0682

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#9 DCF	1	1	1.00E-04	-4.00	0.2132
RC#9 DCF	1	2	1.00E-04	-4.00	0.2624
RC#9 DCF	1	3	1.00E-04	-4.00	0.2384
RC#9 DCF	2	1	3.30E-05	-4.48	0.3300
RC#9 DCF	2	2	3.30E-05	-4.48	0.2546
RC#9 DCF	2	3	3.30E-05	-4.48	0.2980
RC#9 DCF	3	1	1.00E-05	-5.00	0.3035
RC#9 DCF	3	2	1.00E-05	-5.00	0.3016
RC#9 DCF	3	3	1.00E-05	-5.00	0.2819
RC#9 DCF	4	1	1.00E-06	-6.00	0.4773
RC#9 DCF	4	2	1.00E-06	-6.00	0.4933
RC#9 DCF	4	3	1.00E-06	-6.00	0.4962
RC#9 DCF	5	1	1.00E-07	-7.00	0.5265
RC#9 DCF	5	2	1.00E-07	-7.00	0.4659
RC#9 DCF	5	3	1.00E-07	-7.00	0.4885
RC#9 DCF	6	1	1.00E-08	-8.00	0.4534
RC#9 DCF	6	2	1.00E-08	-8.00	0.4302
RC#9 DCF	6	3	1.00E-08	-8.00	0.4516
RC#9 DCF	7	1	1.00E-09	-9.00	0.5151
RC#9 DCF	7	2	1.00E-09	-9.00	0.5235
RC#9 DCF	7	3	1.00E-09	-9.00	0.5065
RC#9 DCF	8	1	1.00E-10	-10.00	0.5131
RC#9 DCF	8	2	1.00E-10	-10.00	0.5167
RC#9 DCF	8	3	1.00E-10	-10.00	0.4733

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	37.23	45.84	41.64
2	-4.48	57.63	44.46	52.04
3	-5.00	53.01	52.68	49.24
4	-6.00	83.37	86.17	86.66
5	-7.00	91.96	81.38	85.33
6	-8.00	79.19	75.13	78.88
7	-9.00	89.97	91.44	88.46
8	-10.00	89.61	90.26	82.67

Assay Date	9/13/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8		
Technician ID	EJB	Replicate #	3	Microsome type	Recombina	Microsome ID	5

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#9 DCF		# Concentrations tested	8					
Technician ID	EJB	Replicate #	3	Microsome type	Recombinant	Microsome ID	5				

								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:	Mic 0.008	QC 10	QC 100	Mic 0.08
	0.055	0.081	0.315	0.190
	0.057	0.075	0.321	0.216
	0.057	0.084	0.324	0.203

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	0.594	0.556	0.0466	m, b	0.087	-0.002
0.125	62.5	1000	0.00013	200	0.0250	0.401	0.363	0.0297	se_m, se_b	0.007	0.002
0.05	25	1000	0.00005	200	0.0100	0.189	0.150	0.0112	r^2, se_y	0.978	0.003
0.025	25	2000	0.00003	200	0.0050	0.122	0.083	0.0054	F, df	177	4
0.01	5	1000	0.00001	200	0.0020	0.073	0.035	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.049	0.011	-0.0010			

Regression results are calculated using the function LINEST

Blank	0.038	r^2=	0.978		
		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
Mic 0.008	0.055	0.017	0.000	200	120	72000	-0.001	-0.001	-1.042
Mic 0.008	0.057	0.019	0.000	200	120	72000	-0.001		
Mic 0.008	0.057	0.018	0.000	200	120	72000	-0.001		
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		
Mic 0.08	0.190	0.152	0.011	200	140	8540	0.003	0.004	3.808
Mic 0.08	0.216	0.178	0.014	200	140	8540	0.004		
Mic 0.08	0.203	0.165	0.012	200	140	8540	0.004		

Assay Date	9/13/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	3

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.808054
Protein Concentration (dilution added to assay, mg/mL):	0.006243

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	13214.87	26429.74	26703.73	53407.46	1	1630418	3.28	52954	0.0646	1	0.003	15	0.6895
			0.5	2	13488.86	26977.72			1					1		15	
	2	2	0.5	1	13312.48	26624.96	27097.11	54194.22	1	1630418	3.32	53741	0.0655	1	0.003	15	0.6997
			0.5	2	13784.63	27569.26			1					1		15	
	3	2	0.5	1	12780.63	25561.26	25461.94	50923.88	1	1630418	3.12	50471	0.0615	1	0.003	15	0.6571
			0.5	2	12681.31	25362.62			1					1		15	
	4	2	0.5	1	12583.76	25167.52	25397.16	50794.32	1	1630418	3.12	50341	0.0614	1	0.003	15	0.6555
			0.5	2	12813.4	25626.8			1					1		15	
Background control	1	2	0.5	1	113.1872	226.3744	213.8798	427.7596	1	1630418	0.03	-25	0.0000	1	0.003	15	-0.0003
			0.5	2	100.6926	201.3852			1					1		15	
	2	2	0.5	1	96.96837	193.93674	226.25957	452.51914	1	1630418	0.03	-1	0.0000	1	0.003	15	0.0000
			0.5	2	129.2912	258.5824			1					1		15	
	3	2	0.5	1	94.97284	189.94568	201.93254	403.86508	1	1630418	0.02	-49	-0.0001	1	0.003	15	-0.0006
			0.5	2	106.9597	213.9194			1					1		15	
	4	2	0.5	1	137.3719	274.7438	263.9702	527.9404	1	1630418	0.03	75	0.0001	1	0.003	15	0.0010
			0.5	2	126.5983	253.1966			1					1		15	
Positive control	1	2	0.5	1	6497.85	12995.7	12768.804	25537.608	1	1630418	1.57	25085	0.0306	1	0.003	15	0.3266
			0.5	2	6270.954	12541.908			1					1		15	
	2	2	0.5	1	6352.36	12704.72	12722.677	25445.354	1	1630418	1.56	24992	0.0305	1	0.003	15	0.3254
			0.5	2	6370.317	12740.634			1					1		15	
	3	2	0.5	1	5737.326	11474.652	11858.373	23716.746	1	1630418	1.45	23264	0.0284	1	0.003	15	0.3029
			0.5	2	6121.047	12242.094			1					1		15	
	4	2	0.5	1	5133.964	10267.928	10273.379	20546.758	1	1630418	1.26	20094	0.0245	1	0.003	15	0.2616
			0.5	2	5139.415	10278.83			1					1		15	
Negative Control	1	2	0.5	1	11808.66	23617.32	23098.31	46196.62	1	1630418	2.83	45744	0.0558	1	0.003	15	0.5956
			0.5	2	11289.65	22579.3			1					1		15	
	2	2	0.5	1	10911.08	21822.16	21895.61	43791.22	1	1630418	2.69	43338	0.0528	1	0.003	15	0.5643
			0.5	2	10984.53	21969.06			1					1		15	
	3	2	0.5	1	9812.103	19624.206	19320.291	38640.582	1	1630418	2.37	38188	0.0466	1	0.003	15	0.4972
			0.5	2	9508.188	19016.376			1					1		15	
	4	2	0.5	1	8102.583	16205.166	16178.031	32356.062	1	1630418	1.98	31903	0.0389	1	0.003	15	0.4154
			0.5	2	8075.448	16150.896			1					1		15	
RC#9 DCF	1-1	2	0.5	1	4226.228	8452.456	8296.228	16592.456	1	1630418	1.02	16139	0.0197	1	0.003	15	0.2101
			0.5	2	4070	8140			1					1		15	
	1-2	2	0.5	1	3924.546	7849.092	7612.225	15224.45	1	1630418	0.93	14771	0.0180	1	0.003	15	0.1923
			0.5	2	3687.679	7375.358			1					1		15	
	1-3	2	0.5	1	3418.153	6836.306	7021.342	14042.684	1	1630418	0.86	13590	0.0166	1	0.003	15	0.1769
			0.5	2	3603.189	7206.378			1					1		15	
	2-1	2	0.5	1	4317.217	8634.434	8696.814	17393.628	1	1630418	1.07	16941	0.0207	1	0.003	15	0.2206
			0.5	2	4379.597	8759.194			1					1		15	
	2-2	2	0.5	1	4127.27	8254.54	8454.924	16909.848	1	1630418	1.04	16457	0.0201	1	0.003	15	0.2143
			0.5	2	4327.654	8655.308			1					1		15	
	2-3	2	0.5	1	3997.021	7994.042	7836.894	15673.788	1	1630418	0.96	15221	0.0186	1	0.003	15	0.1982
			0.5	2	3839.873	7679.746			1					1		15	
	3-1	2	0.5	1	8255.823	16511.646	16624.331	33248.662	1	1630418	2.04	32796	0.0400	1	0.003	15	0.4270
			0.5	2	8368.508	16737.016			1					1		15	
	3-2	2	0.5	1	8514.139	17026.278	16967.189	33934.378	1	1630418	2.08	33481	0.0408	1	0.003	15	0.4359
			0.5	2	8453.05	16906.1			1					1		15	
	3-3	2	0.5	1	7865.968	15733.936	15530.001	31060.002	1	1630418	1.91	30607	0.0373	1	0.003	15	0.3985
			0.5	2	7663.033	15326.066			1					1		15	
	4-1	2	0.5	1	11765.82	23531.24	23350.77	46701.54	1	1630418	2.86	46249	0.0564	1	0.003	15	0.6022
			0.5	2	11585.15	23170.3			1					1		15	
	4-2	2	0.5	1	11615.94	23231.88	22988.3	45976.6	1	1630418	2.82	45524	0.0555	1	0.003	15	0.5927
			0.5	2	11372.36	22744.72			1					1		15	
	4-3	2	0.5	1	11893.44	23786.88	23925.13	47850.26	1	1630418	2.93	47397	0.0578	1	0.003	15	0.6171
			0.5	2	12031.69	24063.38			1					1		15	
	5-1	2	0.5	1	12406.3	24812.6	24827.12	49654.24	1	1630418	3.05	49201	0.0600	1	0.003	15	0.6406
			0.5	2	12420.82	24841.64			1					1		15	
	5-2	2	0.5	1	12562	25124	25225.17	50450.34	1	1630418	3.09	49997	0.0610	1	0.003	15	0.6510
			0.5	2	12663.17	25326.34			1					1		15	
	5-3	2	0.5	1	11158.21	22316.42	22845.72	45691.44	1	1630418	2.80	45238	0.0552	1	0.003	15	0.5890
			0.5	2	11687.51	23375.02			1					1		15	
	6-1	2	0.5	1	12183.19	24366.38	23935.34	47870.68	1	1630418	2.94	47418	0.0578	1	0.003	15	0.6174
			0.5	2	11752.15	23504.3			1					1		15	
	6-2	2	0.5	1	11629.31	23258.62	23561.93	47123.86	1	1630418	2.89	46671	0.0569	1	0.003	15	0.6077
			0.5	2	11932.62	23865.24			1					1		15	
	6-3	2	0.5	1	11670.85	23341.7	23442.79	46885.58	1	1630418	2.88	46433	0.0566	1	0.003	15	0.6046
			0.5	2	11771.94	23543.88			1					1		15	
	7-1	2	0.5	1	11851.82	23703.64	24088.82	48177.64	1	1630418	2.95	47725	0.0582	1	0.003	15	0.6214
			0.5	2	12237	24474			1					1		15	
	7-2	2	0.5	1	11814.75	23629.5	23417.32	46834.64	1	1630418	2.87	46382	0.0565	1	0.003	15	0.6039
			0.5	2	11602.57	23205.14			1					1		15	
	7-3	2	0.5	1	12023.69	24047.38	23964.39	47928.78	1	1630418	2.94	47476	0.0579	1	0.003	15	0.6182
			0.5	2	11940.7	23881.4			1					1		15	
	8-1	2	0.5	1	12329.91	24659.82	24747.06	49494.12	1	1630418	3.04	49041	0.0598	1	0.003	15	0.6385
			0.5	2	12417.15	24834.3			1					1		15	
	8-2	2	0.5	1	12156.1	24312.2	24372.45	48744.9	1	1630418	2.99	48292	0.0589	1	0.003	15	0.6288
			0.5	2	12216.35	24432.7			1					1		15	
	8-3	2	0.5	1	12150.75	24301.5	24567.9	49135.8	1	1630418	3.01	48683	0.0594	1	0.003	15	0.6339
			0.5	2	12417.15	24834.3			1					1		15	

Assay Date	9/13/2005	Test Chemical ID	RC#9 DCF	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	3

Control Type	Portion	Average	SD
Full activity	Beginning	0.6946	0.0072
Full activity	End	0.6563	0.0012
Full activity	Overall	0.6755	0.0225
Background	Beginning	-0.0002	0.000227955
Background	End	0.0002	0.001142332
Background	Overall	0.0000	0.00069986
Positive	Beginning	0.3260	0.0008
Positive	End	0.2823	0.0292
Positive	Overall	0.3041	0.0304
Negative	Beginning	0.5799	0.0221
Negative	End	0.4563	0.0579
Negative	Overall	0.5181	0.0798

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#9 DCF	1	1	1.00E-04	-4.00	0.2101
RC#9 DCF	1	2	1.00E-04	-4.00	0.1923
RC#9 DCF	1	3	1.00E-04	-4.00	0.1769
RC#9 DCF	2	1	3.30E-05	-4.48	0.2206
RC#9 DCF	2	2	3.30E-05	-4.48	0.2143
RC#9 DCF	2	3	3.30E-05	-4.48	0.1982
RC#9 DCF	3	1	1.00E-05	-5.00	0.4270
RC#9 DCF	3	2	1.00E-05	-5.00	0.4359
RC#9 DCF	3	3	1.00E-05	-5.00	0.3985
RC#9 DCF	4	1	1.00E-06	-6.00	0.6022
RC#9 DCF	4	2	1.00E-06	-6.00	0.5927
RC#9 DCF	4	3	1.00E-06	-6.00	0.6171
RC#9 DCF	5	1	1.00E-07	-7.00	0.6406
RC#9 DCF	5	2	1.00E-07	-7.00	0.6510
RC#9 DCF	5	3	1.00E-07	-7.00	0.5890
RC#9 DCF	6	1	1.00E-08	-8.00	0.6174
RC#9 DCF	6	2	1.00E-08	-8.00	0.6077
RC#9 DCF	6	3	1.00E-08	-8.00	0.6046
RC#9 DCF	7	1	1.00E-09	-9.00	0.6214
RC#9 DCF	7	2	1.00E-09	-9.00	0.6039
RC#9 DCF	7	3	1.00E-09	-9.00	0.6182
RC#9 DCF	8	1	1.00E-10	-10.00	0.6385
RC#9 DCF	8	2	1.00E-10	-10.00	0.6288
RC#9 DCF	8	3	1.00E-10	-10.00	0.6339

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	31.11	28.47	26.20
2	-4.48	32.66	31.72	29.34
3	-5.00	63.22	64.54	59.00
4	-6.00	89.15	87.75	91.36
5	-7.00	94.84	96.38	87.20
6	-8.00	91.40	89.96	89.51
7	-9.00	92.00	89.41	91.52
8	-10.00	94.53	93.09	93.84

Assay Date	9/8/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	1	Microsome type	Recombina	Microsome ID	5

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/b*c

2) Calculate total µg ASDN/g soln.

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

= 0.562705 + 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	EJB	Replicate #	1	Microsome type	Recombinant	Microsome ID	5			

								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:	Mic 0.008	QC 10	QC 100	Mic 0.08
	0.069	0.074	0.307	0.272
	0.069	0.072	0.305	0.282
	0.065	0.067	0.310	0.267

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	0.631	0.589	0.0479	m, b	0.084	-0.002
0.125	62.5	1000	0.00013	200	0.0250	0.398	0.357	0.0283	se_m, se_b	0.004	0.001
0.05	25	1000	0.00005	200	0.0100	0.193	0.151	0.0109	r^2, se_y	0.990	0.002
0.025	25	2000	0.00003	200	0.0050	0.123	0.082	0.0051	F, df	390	4
0.01	5	1000	0.00001	200	0.0020	0.075	0.034	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
0.005	5	2000	0.00001	200	0.0010	0.061	0.020	-0.0002			

Regression results are calculated using the function LINEST

Blank	0.041	r^2=	0.990		
		m=	0.084		
		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
Mic 0.008	0.069	0.028	0.000	200	120	72000	0.001	0.001	1.124
Mic 0.008	0.069	0.028	0.000	200	120	72000	0.001		
Mic 0.008	0.065	0.023	0.000	200	120	72000	0.000		
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		
Mic 0.08	0.272	0.231	0.018	200	140	8540	0.005	0.005	5.420
Mic 0.08	0.282	0.241	0.018	200	140	8540	0.006		
Mic 0.08	0.267	0.226	0.017	200	140	8540	0.005		

Assay Date	9/8/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	1

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.420243
Protein Concentration (dilution added to assay, mg/mL):	0.008886

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	Recombinan	Microsome ID	5	Technician ID	EJB	Replicate #	1

Sample ID		Calculate DPM in aqueous portion after extraction							Calculate % turnover			Calculate nmol^3H_2O formed					
Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Background Tubes)	nmol^3H_2O formed	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min
Full activity control	1	2	0.5	1	16249.85	32499.7	32438.18	64876.36	1	1623262	4.00	64129	0.0788	1	0.004	15	0.5909
			0.5	2	16188.33	32376.66			1					1		15	
	2	2	0.5	1	16173	32346	32265.08	64530.16	1	1623262	3.98	63783	0.0783	1	0.004	15	0.5877
			0.5	2	16092.08	32184.16			1					1		15	
	3	2	0.5	1	16177.56	32355.12	31342.34	62684.68	1	1623262	3.86	61937	0.0761	1	0.004	15	0.5707
			0.5	2	15164.78	30329.56			1					1		15	
	4	2	0.5	1	16379.57	32759.14	33146.35	66292.7	1	1623262	4.08	65545	0.0805	1	0.004	15	0.6040
			0.5	2	16766.78	33533.56			1					1		15	
Background control	1	2	0.5	1	230.9671	461.9342	471.6164	943.2328	1	1623262	0.06	196	0.0002	1	0.004	15	0.0018
			0.5	2	240.6493	481.2986			1					1		15	
	2	2	0.5	1	229.6406	459.2812	448.3459	896.6918	1	1623262	0.06	149	0.0002	1	0.004	15	0.0014
			0.5	2	218.7053	437.4106			1					1		15	
	3	2	0.5	1	134.6783	269.3566	272.7276	545.4552	1	1623262	0.03	-202	-0.0002	1	0.004	15	-0.0019
			0.5	2	138.0493	276.0986			1					1		15	
	4	2	0.5	1	132.7397	265.4794	302.4353	604.8706	1	1623262	0.04	-143	-0.0002	1	0.004	15	-0.0013
			0.5	2	169.6956	339.3912			1					1		15	
Positive control	1	2	0.5	1	8967.941	17935.882	16679.775	33359.55	1	1623262	2.06	32612	0.0401	1	0.004	15	0.3005
			0.5	2	7711.834	15423.668			1					1		15	
	2	2	0.5	1	5660.05	11320.1	12003.431	24006.862	1	1623262	1.48	23259	0.0286	1	0.004	15	0.2143
			0.5	2	6343.381	12686.762			1					1		15	
	3	2	0.5	1	8251.143	16502.286	17110.125	34220.25	1	1623262	2.11	33473	0.0411	1	0.004	15	0.3084
			0.5	2	8858.982	17717.964			1					1		15	
	4	2	0.5	1	8808.177	17616.354	17477.536	34955.072	1	1623262	2.15	34208	0.0420	1	0.004	15	0.3152
			0.5	2	8669.359	17338.718			1					1		15	
Negative Control	1	2	0.5	1	12045.69	24091.38	23894.75	47789.5	1	1623262	2.94	47042	0.0578	1	0.004	15	0.4335
			0.5	2	11849.06	23698.12			1					1		15	
	2	2	0.5	1	10888.55	21777.1	21787.8	43575.6	1	1623262	2.68	42828	0.0526	1	0.004	15	0.3946
			0.5	2	10899.25	21798.5			1					1		15	
	3	2	0.5	1	14664.16	29328.32	29050.57	58101.14	1	1623262	3.58	57354	0.0704	1	0.004	15	0.5285
			0.5	2	14386.41	28772.82			1					1		15	
	4	2	0.5	1	14209.67	28419.34	28459.45	56918.9	1	1623262	3.51	56171	0.0690	1	0.004	15	0.5176
			0.5	2	14249.78	28499.56			1					1		15	
RC#10 ATZ	1-1	2	0.5	1	14263.3	28526.6	28505.21	57010.42	1	1623262	3.51	56263	0.0691	1	0.004	15	0.5184
			0.5	2	14241.91	28483.82			1					1		15	
	1-2	2	0.5	1	13977.13	27954.26	28343.56	56687.12	1	1623262	3.49	55940	0.0687	1	0.004	15	0.5155
			0.5	2	14366.43	28732.86			1					1		15	
	1-3	2	0.5	1	14699.83	29399.66	29727.9	59455.8	1	1623262	3.66	58708	0.0721	1	0.004	15	0.5410
			0.5	2	15028.07	30056.14			1					1		15	
	2-1	2	0.5	1	15105.62	30211.24	30128.35	60256.7	1	1623262	3.71	59509	0.0731	1	0.004	15	0.5484
			0.5	2	15022.73	30045.46			1					1		15	
	2-2	2	0.5	1	14455.58	28911.16	30218.58	60437.16	1	1623262	3.72	59690	0.0733	1	0.004	15	0.5500
			0.5	2	15763	31526			1					1		15	
	2-3	2	0.5	1	15960.27	31920.54	30833.25	61666.5	1	1623262	3.80	60919	0.0748	1	0.004	15	0.5613
			0.5	2	14872.98	29745.96			1					1		15	
	3-1	2	0.5	1	15557.53	31115.06	30804.98	61609.96	1	1623262	3.80	60862	0.0747	1	0.004	15	0.5608
			0.5	2	15247.45	30494.9			1					1		15	
	3-2	2	0.5	1	15078.82	30157.64	29758.99	59517.98	1	1623262	3.67	58770	0.0722	1	0.004	15	0.5416
			0.5	2	14680.17	29360.34			1					1		15	
	3-3	2	0.5	1	14950.53	29901.06	29638.43	59276.86	1	1623262	3.65	58529	0.0719	1	0.004	15	0.5393
			0.5	2	14687.9	29375.8			1					1		15	
	4-1	2	0.5	1	13114.89	26229.78	26359.56	56719.12	1	1623262	3.49	55972	0.0687	1	0.004	15	0.5156
			0.5	2	15244.67	30489.34			1					1		15	
	4-2	2	0.5	1	14205.65	28411.3	29424.54	58849.08	1	1623262	3.63	58102	0.0714	1	0.004	15	0.5354
			0.5	2	15218.69	30437.78			1					1		15	
	4-3	2	0.5	1	13894.16	27788.32	29274.58	58549.16	1	1623262	3.61	57802	0.0710	1	0.004	15	0.5326
			0.5	2	15360.42	30760.84			1					1		15	
	5-1	2	0.5	1	14895.66	29791.32	29998.61	59997.22	1	1623262	3.70	59250	0.0728	1	0.004	15	0.5460
			0.5	2	15102.95	30205.9			1					1		15	
	5-2	2	0.5	1	15035.72	30071.44	29526.31	59052.62	1	1623262	3.64	58305	0.0716	1	0.004	15	0.5373
			0.5	2	14490.59	28981.18			1					1		15	
	5-3	2	0.5	1	14916.4	29832.8	29898.25	59796.5	1	1623262	3.68	59049	0.0725	1	0.004	15	0.5441
			0.5	2	14981.85	29963.7			1					1		15	
	6-1	2	0.5	1	14314.11	28628.22	28428.69	56857.38	1	1623262	3.50	56110	0.0689	1	0.004	15	0.5170
			0.5	2	14114.58	28229.16			1					1		15	
	6-2	2	0.5	1	13863.63	27727.26	28145.39	56290.78	1	1623262	3.47	55543	0.0682	1	0.004	15	0.5118
			0.5	2	14281.76	28563.52			1					1		15	
	6-3	2	0.5	1	13491.67	26983.34	27488.25	54976.5	1	1623262	3.39	54229	0.0666	1	0.004	15	0.4997
			0.5	2	13996.58	27993.16			1					1		15	
	7-1	2	0.5	1	15456.01	30912.02	30684.55	61369.1	1	1623262	3.78	60622	0.0745	1	0.004	15	0.5586
			0.5	2	15228.54	30457.08			1					1		15	
	7-2	2	0.5	1	14747.59	29495.18	29740.3	59480.6	1	1623262	3.66	58733	0.0721	1	0.004	15	0.5412
			0.5	2	14992.71	29985.42			1					1		15	
	7-3	2	0.5	1	14941.5	29883	30685.73	61371.46	1	1623262	3.78	60624	0.0745	1	0.004	15	0.5586
			0.5	2	15744.23	31488.46			1					1		15	
	8-1	2	0.5	1	14921.19	29842.38	30936.22	61872.44	1	1623262	3.81	61125	0.0751	1	0.004	15	0.5632
			0.5	2	16015.03	32030.06			1					1		15	
	8-2	2	0.5	1	15497.18	30994.36	31219.86	62439.72	1	1623262	3.85	61692	0.0758	1	0.004	15	0.5685
			0.5	2	15722.68	31445.36			1					1		15	
	8-3	2	0.5	1	15958.49	31916.98	31320.22	62640.44	1	1623262	3.86	61893	0.0760	1	0.004	15	0.5703
			0.5	2	15361.73	30723.46			1					1		15	

Assay Date	9/8/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.5893	0.0023
Full activity	End	0.5874	0.0235
Full activity	Overall	0.5883	0.0137
Background	Beginning	0.0016	0.000303251
Background	End	-0.0016	0.000387137
Background	Overall	0.0000	0.001856212
Positive	Beginning	0.2574	0.0609
Positive	End	0.3118	0.0048
Positive	Overall	0.2846	0.0472
Negative	Beginning	0.4141	0.0275
Negative	End	0.5230	0.0077
Negative	Overall	0.4686	0.0650

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#10 ATZ	1	1	1.00E-04	-4.00	0.5184
RC#10 ATZ	1	2	1.00E-04	-4.00	0.5155
RC#10 ATZ	1	3	1.00E-04	-4.00	0.5410
RC#10 ATZ	2	1	3.30E-05	-4.48	0.5484
RC#10 ATZ	2	2	3.30E-05	-4.48	0.5500
RC#10 ATZ	2	3	3.30E-05	-4.48	0.5613
RC#10 ATZ	3	1	1.00E-05	-5.00	0.5608
RC#10 ATZ	3	2	1.00E-05	-5.00	0.5416
RC#10 ATZ	3	3	1.00E-05	-5.00	0.5393
RC#10 ATZ	4	1	1.00E-06	-6.00	0.5158
RC#10 ATZ	4	2	1.00E-06	-6.00	0.5354
RC#10 ATZ	4	3	1.00E-06	-6.00	0.5326
RC#10 ATZ	5	1	1.00E-07	-7.00	0.5460
RC#10 ATZ	5	2	1.00E-07	-7.00	0.5373
RC#10 ATZ	5	3	1.00E-07	-7.00	0.5441
RC#10 ATZ	6	1	1.00E-08	-8.00	0.5170
RC#10 ATZ	6	2	1.00E-08	-8.00	0.5118
RC#10 ATZ	6	3	1.00E-08	-8.00	0.4997
RC#10 ATZ	7	1	1.00E-09	-9.00	0.5586
RC#10 ATZ	7	2	1.00E-09	-9.00	0.5412
RC#10 ATZ	7	3	1.00E-09	-9.00	0.5586
RC#10 ATZ	8	1	1.00E-10	-10.00	0.5632
RC#10 ATZ	8	2	1.00E-10	-10.00	0.5685
RC#10 ATZ	8	3	1.00E-10	-10.00	0.5703

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	88.12	87.61	91.95
2	-4.48	93.20	93.49	95.41
3	-5.00	95.32	92.05	91.67
4	-6.00	87.66	91.00	90.53
5	-7.00	92.80	91.32	92.48
6	-8.00	87.88	86.99	84.93
7	-9.00	94.95	91.99	94.95
8	-10.00	95.73	96.62	96.94

Assay Date	9/12/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	2	Microsome type	Recombina	Microsome ID	5

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/b*c

2) Calculate total µg ASDN/g soln.

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

= 0.562033 + 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	EJB	Replicate #	2	Microsome type	Recombinant	Microsome ID	5		

								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:	Mic 0.008	QC 10	QC 100	Mic 0.08
	0.061	0.073	0.332	0.205
	0.061	0.063	0.315	0.203
	0.059	0.071	0.317	0.201

Standard concentration (mg/mL)	Volume of stock used	Final volume of Std	mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output
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
Mic 0.008	0.061	0.017	-0.001	200	120	72000	-0.004	-0.004	-3.701
Mic 0.008	0.061	0.017	-0.001	200	120	72000	-0.003		
Mic 0.008	0.059	0.015	-0.001	200	120	72000	-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		
Mic 0.08	0.205	0.161	0.011	200	140	8540	0.003	0.003	3.378
Mic 0.08	0.203	0.159	0.011	200	140	8540	0.003		
Mic 0.08	0.201	0.157	0.011	200	140	8540	0.003		

Assay Date	9/12/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	Recombina	Microsome ID	5	Technician ID	EJB	Replicate #	2

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.377604
Protein Concentration (dilution added to assay, mg/mL):	0.005537

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	9955.42	19910.84	19743.838	39487.676	1	1584999	2.49	38778	0.0487	1	0.003	15	0.5864
			0.5	2	9788.418	19576.836			1					1		15	
	2	2	0.5	1	9421.306	18842.612	18796.322	37592.644	1	1584999	2.37	36883	0.0463	1	0.003	15	0.5577
			0.5	2	9375.016	18750.032			1					1		15	
	3	2	0.5	1	9677.177	19354.354	19656.838	39313.676	1	1584999	2.48	38604	0.0485	1	0.003	15	0.5837
			0.5	2	9979.661	19959.322			1					1		15	
	4	2	0.5	1	9756.096	19512.192	18946.992	37893.984	1	1584999	2.39	37184	0.0467	1	0.003	15	0.5623
			0.5	2	9190.896	18381.792			1					1		15	
Background control	1	2	0.5	1	124.8214	249.6428	287.9349	575.8698	1	1584999	0.04	-134	-0.0002	1	0.003	15	-0.0020
			0.5	2	163.1135	326.227			1					1		15	
	2	2	0.5	1	159.7598	319.5196	309.0028	618.0056	1	1584999	0.04	-92	-0.0001	1	0.003	15	-0.0014
			0.5	2	149.243	298.486			1					1		15	
	3	2	0.5	1	166.0665	332.133	370.7775	741.555	1	1584999	0.05	32	0.0000	1	0.003	15	0.0005
			0.5	2	204.711	409.422			1					1		15	
	4	2	0.5	1	199.3239	398.6478	451.5307	903.0614	1	1584999	0.06	193	0.0002	1	0.003	15	0.0029
			0.5	2	252.2068	504.4136			1					1		15	
Positive control	1	2	0.5	1	4666.649	9333.298	9224.187	18448.374	1	1584999	1.16	17739	0.0223	1	0.003	15	0.2682
			0.5	2	4557.538	9115.076			1					1		15	
	2	2	0.5	1	4127.27	8254.54	8029.318	16058.636	1	1584999	1.01	15349	0.0193	1	0.003	15	0.2321
			0.5	2	3902.048	7804.096			1					1		15	
	3	2	0.5	1	5294.085	10588.17	10163.45	20326.9	1	1584999	1.28	19817	0.0246	1	0.003	15	0.2966
			0.5	2	4869.365	9738.73			1					1		15	
	4	2	0.5	1	5055.85	10111.7	10403.223	20806.446	1	1584999	1.31	20097	0.0252	1	0.003	15	0.3039
			0.5	2	5347.373	10694.746			1					1		15	
Negative Control	1	2	0.5	1	6759.388	13518.776	13755.261	27510.522	1	1584999	1.74	26801	0.0337	1	0.003	15	0.4053
			0.5	2	6995.873	13991.746			1					1		15	
	2	2	0.5	1	5532.875	11065.75	11139.829	22279.658	1	1584999	1.41	21570	0.0271	1	0.003	15	0.3262
			0.5	2	5606.954	11213.908			1					1		15	
	3	2	0.5	1	8268.107	16536.214	16359.622	32719.244	1	1584999	2.06	32010	0.0402	1	0.003	15	0.4840
			0.5	2	8091.515	16183.03			1					1		15	
	4	2	0.5	1	7778.684	15557.368	15226.433	30452.866	1	1584999	1.92	29743	0.0374	1	0.003	15	0.4498
			0.5	2	7447.749	14895.498			1					1		15	
RC#10 ATZ	1-1	2	0.5	1	8224.778	16449.556	16645.066	33290.132	1	1584999	2.10	32581	0.0409	1	0.003	15	0.4927
			0.5	2	8420.288	16840.576			1					1		15	
	1-2	2	0.5	1	7888.401	15776.802	15755.41	31510.82	1	1584999	1.99	30801	0.0387	1	0.003	15	0.4658
			0.5	2	7867.009	15734.018			1					1		15	
	1-3	2	0.5	1	8260.055	16520.11	16468.532	32937.064	1	1584999	2.08	32227	0.0405	1	0.003	15	0.4873
			0.5	2	8208.477	16416.954			1					1		15	
	2-1	2	0.5	1	8864.666	17729.332	17562.328	35124.656	1	1584999	2.22	34415	0.0432	1	0.003	15	0.5204
			0.5	2	8697.662	17395.324			1					1		15	
	2-2	2	0.5	1	9095.829	18191.658	18052.077	36104.154	1	1584999	2.28	35395	0.0445	1	0.003	15	0.5352
			0.5	2	8956.248	17912.496			1					1		15	
	2-3	2	0.5	1	9348.189	18696.378	18009.056	36018.112	1	1584999	2.27	35308	0.0443	1	0.003	15	0.5339
			0.5	2	8660.867	17321.734			1					1		15	
	3-1	2	0.5	1	8751.207	17502.414	17581.107	35162.214	1	1584999	2.22	34453	0.0433	1	0.003	15	0.5210
			0.5	2	8829.9	17659.8			1					1		15	
	3-2	2	0.5	1	8729.499	17458.998	17565.333	35130.666	1	1584999	2.22	34421	0.0432	1	0.003	15	0.5205
			0.5	2	8835.834	17671.668			1					1		15	
	3-3	2	0.5	1	8329.617	16659.234	17005.73	34011.46	1	1584999	2.15	33302	0.0418	1	0.003	15	0.5036
			0.5	2	8676.113	17352.226			1					1		15	
	4-1	2	0.5	1	8474.426	16948.852	15644.397	31288.794	1	1584999	1.97	30579	0.0384	1	0.003	15	0.4624
			0.5	2	7169.971	14339.942			1					1		15	
	4-2	2	0.5	1	8289.904	16579.808	16470.393	32940.786	1	1584999	2.08	32231	0.0405	1	0.003	15	0.4874
			0.5	2	8180.489	16360.978			1					1		15	
	4-3	2	0.5	1	8493.42	16986.84	16798.971	33597.942	1	1584999	2.12	32888	0.0413	1	0.003	15	0.4973
			0.5	2	8305.551	16611.102			1					1		15	
	5-1	2	0.5	1	8933.016	17866.032	17781.584	35563.168	1	1584999	2.24	34854	0.0438	1	0.003	15	0.5270
			0.5	2	8848.568	17697.136			1					1		15	
	5-2	2	0.5	1	9155.576	18311.152	17993.259	35986.518	1	1584999	2.27	35277	0.0443	1	0.003	15	0.5334
			0.5	2	8837.683	17675.366			1					1		15	
	5-3	2	0.5	1	8720.026	17440.052	17600.494	35200.988	1	1584999	2.22	34491	0.0433	1	0.003	15	0.5216
			0.5	2	8880.468	17760.936			1					1		15	
	6-1	2	0.5	1	8634.524	17269.048	17081.843	34163.686	1	1584999	2.16	33454	0.0420	1	0.003	15	0.5059
			0.5	2	8447.319	16894.638			1					1		15	
	6-2	2	0.5	1	8851.101	17702.202	17540.728	35081.456	1	1584999	2.21	34372	0.0432	1	0.003	15	0.5197
			0.5	2	8689.627	17379.254			1					1		15	
	6-3	2	0.5	1	8865.953	17731.906	17458.61	34917.22	1	1584999	2.20	34208	0.0430	1	0.003	15	0.5173
			0.5	2	8592.657	17185.314			1					1		15	
	7-1	2	0.5	1	8708.483	17416.966	17195.914	34391.828	1	1584999	2.17	33682	0.0423	1	0.003	15	0.5093
			0.5	2	8487.431	16974.862			1					1		15	
	7-2	2	0.5	1	8558.59	17117.18	16995.017	33990.034	1	1584999	2.14	33280	0.0418	1	0.003	15	0.5032
			0.5	2	8436.427	16872.854			1					1		15	
	7-3	2	0.5	1	8401.204	16802.408	16816.082	33632.164	1	1584999	2.12	32923	0.0413	1	0.003	15	0.4978
			0.5	2	8414.878	16829.756			1					1		15	
	8-1	2	0.5	1	8137.13	16274.26	16369.819	32739.638	1	1584999	2.07	32030	0.0402	1	0.003	15	0.4843
			0.5	2	8232.689	16465.378			1					1		15	
	8-2	2	0.5	1	8724.645	17449.29	17352.319	34704.638	1	1584999	2.19	33995	0.0427	1	0.003	15	0.5140
			0.5	2	8627.674	17255.348			1					1		15	
	8-3	2	0.5	1	8868.688	17737.376	17782.755	35565.51	1	1584999	2.24	34856	0.0438	1	0.003	15	0.5271
			0.5	2	8914.067	17828.134			1					1		15	

Assay Date	9/12/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	2

Control Type	Portion	Average	SD
Full activity	Beginning	0.5720	0.0203
Full activity	End	0.5730	0.0152
Full activity	Overall	0.5725	0.0146
Background	Beginning	-0.0017	0.000450533
Background	End	0.0017	0.00172689
Background	Overall	0.0000	0.00222103
Positive	Beginning	0.2502	0.0256
Positive	End	0.3003	0.0051
Positive	Overall	0.2752	0.0326
Negative	Beginning	0.3657	0.0559
Negative	End	0.4669	0.0242
Negative	Overall	0.4163	0.0682

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#10 ATZ	1	1	1.00E-04	-4.00	0.4927
RC#10 ATZ	1	2	1.00E-04	-4.00	0.4658
RC#10 ATZ	1	3	1.00E-04	-4.00	0.4873
RC#10 ATZ	2	1	3.30E-05	-4.48	0.5204
RC#10 ATZ	2	2	3.30E-05	-4.48	0.5352
RC#10 ATZ	2	3	3.30E-05	-4.48	0.5339
RC#10 ATZ	3	1	1.00E-05	-5.00	0.5210
RC#10 ATZ	3	2	1.00E-05	-5.00	0.5205
RC#10 ATZ	3	3	1.00E-05	-5.00	0.5036
RC#10 ATZ	4	1	1.00E-06	-6.00	0.4624
RC#10 ATZ	4	2	1.00E-06	-6.00	0.4874
RC#10 ATZ	4	3	1.00E-06	-6.00	0.4973
RC#10 ATZ	5	1	1.00E-07	-7.00	0.5270
RC#10 ATZ	5	2	1.00E-07	-7.00	0.5334
RC#10 ATZ	5	3	1.00E-07	-7.00	0.5216
RC#10 ATZ	6	1	1.00E-08	-8.00	0.5059
RC#10 ATZ	6	2	1.00E-08	-8.00	0.5197
RC#10 ATZ	6	3	1.00E-08	-8.00	0.5173
RC#10 ATZ	7	1	1.00E-09	-9.00	0.5093
RC#10 ATZ	7	2	1.00E-09	-9.00	0.5032
RC#10 ATZ	7	3	1.00E-09	-9.00	0.4978
RC#10 ATZ	8	1	1.00E-10	-10.00	0.4843
RC#10 ATZ	8	2	1.00E-10	-10.00	0.5140
RC#10 ATZ	8	3	1.00E-10	-10.00	0.5271

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	86.05	81.35	85.12
2	-4.48	90.90	93.48	93.25
3	-5.00	90.99	90.91	87.95
4	-6.00	80.76	85.13	86.86
5	-7.00	92.05	93.17	91.10
6	-8.00	88.36	90.78	90.35
7	-9.00	88.96	87.90	86.95
8	-10.00	84.60	89.79	92.06

Assay Date	9/13/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8		
Technician ID	EJB	Replicate #	3	Microsome type	Recombina	Microsome ID	5

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#10 ATZ	# Concentrations tested	8				
Technician ID	EJB	Replicate #	3	Microsome type	Recombinant	Microsome ID	5		

								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:	Mic 0.008	QC 10	QC 100	Mic 0.08
	0.055	0.081	0.315	0.190
	0.057	0.075	0.321	0.216
	0.057	0.084	0.324	0.203

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			
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

Blank	0.038	r^2=	0.978
		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
Mic 0.008	0.055	0.017	0.000	200	120	72000	-0.001	-0.001	-1.042
Mic 0.008	0.057	0.019	0.000	200	120	72000	-0.001		
Mic 0.008	0.057	0.018	0.000	200	120	72000	-0.001		
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		
Mic 0.08	0.190	0.152	0.011	200	140	8540	0.003	0.004	3.808
Mic 0.08	0.216	0.178	0.014	200	140	8540	0.004		
Mic 0.08	0.203	0.165	0.012	200	140	8540	0.004		

Assay Date	9/13/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	Recombin	Microsome ID	5	Technician ID	EJB	Replicate #	3

Microsome Dilution Details		
Dilution A	0.14	mL microsome Stock used
	8.54	mL total volume
	61	dilution factor
Dilution B	7.2	mL microsome Dilution A used
	72	mL total volume
	10	dilution factor
Dilution C (if applicable)		mL microsome Dilution B used
		mL total volume
	NA	dilution factor
	610	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.808054
Protein Concentration (dilution added to assay, mg/mL):	0.006243

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	Recombinan	Microsome ID	5	Technician ID	EJB	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	13214.87	26429.74	26703.73	53407.46	1	1630418	3.28	52954	0.0646	1	0.003	15	0.6895
			0.5	2	13488.86	26977.72			1					1		15	
	2	2	0.5	1	13312.48	26624.96	27097.11	54194.22	1	1630418	3.32	53741	0.0655	1	0.003	15	0.6997
			0.5	2	13784.63	27569.26			1					1		15	
	3	2	0.5	1	12780.83	25561.26	25461.94	50923.88	1	1630418	3.12	50471	0.0615	1	0.003	15	0.6571
			0.5	2	12681.31	25362.62			1					1		15	
	4	2	0.5	1	12583.76	25167.52	25397.16	50794.32	1	1630418	3.12	50341	0.0614	1	0.003	15	0.6555
			0.5	2	12813.4	25626.8			1					1		15	
Background control	1	2	0.5	1	113.1872	226.3744	213.8798	427.7596	1	1630418	0.03	-25	0.0000	1	0.003	15	-0.0003
			0.5	2	100.6926	201.3852			1					1		15	
	2	2	0.5	1	96.96837	193.93674	226.25957	452.51914	1	1630418	0.03	-1	0.0000	1	0.003	15	0.0000
			0.5	2	129.2912	258.5824			1					1		15	
	3	2	0.5	1	94.97284	189.94568	201.93254	403.86508	1	1630418	0.02	-49	-0.0001	1	0.003	15	-0.0006
			0.5	2	106.9597	213.9194			1					1		15	
	4	2	0.5	1	137.3719	274.7438	263.9702	527.9404	1	1630418	0.03	75	0.0001	1	0.003	15	0.0010
			0.5	2	126.5983	253.1966			1					1		15	
Positive control	1	2	0.5	1	6497.85	12995.7	12768.804	25537.608	1	1630418	1.57	25085	0.0306	1	0.003	15	0.3266
			0.5	2	6270.954	12541.908			1					1		15	
	2	2	0.5	1	6352.36	12704.72	12722.677	25445.354	1	1630418	1.56	24992	0.0305	1	0.003	15	0.3254
			0.5	2	6370.317	12740.634			1					1		15	
	3	2	0.5	1	5737.326	11474.652	11858.373	23716.746	1	1630418	1.45	23264	0.0284	1	0.003	15	0.3029
			0.5	2	6121.047	12242.094			1					1		15	
	4	2	0.5	1	5133.964	10267.928	10273.379	20546.758	1	1630418	1.26	20094	0.0245	1	0.003	15	0.2616
			0.5	2	5139.415	10278.83			1					1		15	
Negative Control	1	2	0.5	1	11808.66	23617.32	23098.31	46196.62	1	1630418	2.83	45744	0.0558	1	0.003	15	0.5956
			0.5	2	11289.65	22579.3			1					1		15	
	2	2	0.5	1	10911.08	21822.16	21695.61	43791.22	1	1630418	2.69	43338	0.0528	1	0.003	15	0.5643
			0.5	2	10984.53	21969.06			1					1		15	
	3	2	0.5	1	9812.103	19624.206	19320.291	38640.582	1	1630418	2.37	38188	0.0466	1	0.003	15	0.4972
			0.5	2	9508.188	19016.376			1					1		15	
	4	2	0.5	1	8102.583	16205.166	16178.031	32356.062	1	1630418	1.98	31903	0.0389	1	0.003	15	0.4154
			0.5	2	8075.448	16150.896			1					1		15	
RC#10 ATZ	1-1	2	0.5	1	11728.76	23457.52	23437.88	46875.76	1	1630418	2.88	46423	0.0566	1	0.003	15	0.6044
			0.5	2	11709.12	23418.24			1					1		15	
	1-2	2	0.5	1	11933.3	23866.6	23787.31	47574.62	1	1630418	2.92	47122	0.0575	1	0.003	15	0.6135
			0.5	2	11854.01	23708.02			1					1		15	
	1-3	2	0.5	1	11896.78	23793.56	23627.45	47254.9	1	1630418	2.90	46802	0.0571	1	0.003	15	0.6094
			0.5	2	11730.67	23461.34			1					1		15	
	2-1	2	0.5	1	12158.95	24317.9	24234.26	48468.52	1	1630418	2.97	48015	0.0585	1	0.003	15	0.6252
			0.5	2	12075.31	24150.62			1					1		15	
	2-2	2	0.5	1	11490.93	22981.86	22691.68	45383.36	1	1630418	2.78	44930	0.0548	1	0.003	15	0.5850
			0.5	2	11200.75	22401.5			1					1		15	
	2-3	2	0.5	1	11614.91	23229.82	23653.41	47306.82	1	1630418	2.90	46854	0.0571	1	0.003	15	0.6101
			0.5	2	12038.5	24077			1					1		15	
	3-1	2	0.5	1	12135.01	24270.02	24763.88	49527.76	1	1630418	3.04	49075	0.0598	1	0.003	15	0.6390
			0.5	2	12628.87	25257.74			1					1		15	
	3-2	2	0.5	1	12124.07	24246.14	24679.68	49359.36	1	1630418	3.03	48906	0.0596	1	0.003	15	0.6368
			0.5	2	12555.61	25111.22			1					1		15	
	3-3	2	0.5	1	12544.14	25088.28	25273.42	50546.84	1	1630418	3.10	50094	0.0611	1	0.003	15	0.6522
			0.5	2	12729.28	25458.56			1					1		15	
	4-1	2	0.5	1	12501.94	25003.88	25145.74	50291.48	1	1630418	3.08	49838	0.0608	1	0.003	15	0.6489
			0.5	2	12643.8	25287.6			1					1		15	
	4-2	2	0.5	1	11851.88	23703.76	23883.6	47767.2	1	1630418	2.93	47314	0.0577	1	0.003	15	0.6160
			0.5	2	12031.72	24063.44			1					1		15	
	4-3	2	0.5	1	11698.13	23396.26	23862.47	47724.94	1	1630418	2.93	47272	0.0576	1	0.003	15	0.6155
			0.5	2	12164.34	24328.68			1					1		15	
	5-1	2	0.5	1	12305.96	24611.92	24462.12	48924.24	1	1630418	3.00	48471	0.0591	1	0.003	15	0.6311
			0.5	2	12156.16	24312.32			1					1		15	
	5-2	2	0.5	1	11916.89	23833.78	23828.32	47656.64	1	1630418	2.92	47204	0.0576	1	0.003	15	0.6146
			0.5	2	11911.43	23822.86			1					1		15	
	5-3	2	0.5	1	11973.09	23946.18	24708.47	49416.94	1	1630418	3.03	48964	0.0597	1	0.003	15	0.6375
			0.5	2	12735.38	25470.76			1					1		15	
	6-1	2	0.5	1	11994.64	23989.28	24029.31	48056.62	1	1630418	2.95	47606	0.0580	1	0.003	15	0.6198
			0.5	2	12034.67	24069.34			1					1		15	
	6-2	2	0.5	1	12237.13	24474.26	24611	49222	1	1630418	3.02	48769	0.0595	1	0.003	15	0.6350
			0.5	2	12373.87	24747.74			1					1		15	
	6-3	2	0.5	1	12110.53	24221.06	24557.66	49115.32	1	1630418	3.01	48662	0.0593	1	0.003	15	0.6336
			0.5	2	12447.13	24894.26			1					1		15	
	7-1	2	0.5	1	12441.71	24883.42	25154.22	50308.44	1	1630418	3.09	49855	0.0608	1	0.003	15	0.6491
			0.5	2	12712.51	25425.02			1					1		15	
	7-2	2	0.5	1	12657.33	25314.66	25061.46	50122.92	1	1630418	3.07	49670	0.0606	1	0.003	15	0.6467
			0.5	2	12404.13	24808.26			1					1		15	
	7-3	2	0.5	1	11947.03	23894.06	23424.05	46848.1	1	1630418	2.87	46395	0.0566	1	0.003	15	0.6041
			0.5	2	11477.02	22954.04			1					1		15	
	8-1	2	0.5	1	11500.1	23000.2	23116.88	46233.76	1	1630418	2.84	45781	0.0558	1	0.003	15	0.5961
			0.5	2	11616.78	23233.56			1					1		15	
	8-2	2	0.5	1	11784.6	23569.2	23701.19	47402.38	1	1630418	2.91	46949	0.0572	1	0.003	15	0.6113
			0.5	2	11916.59	23833.18			1					1		15	
	8-3	2	0.5	1	11288.97	22577.94	22995.46	45990.92	1	1630418	2.82	45538	0.0555	1	0.003	15	0.5929
			0.5	2	11706.49	23412.98			1					1		15	

Assay Date	9/13/2005	Test Chemical ID	RC#10 ATZ	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	5	Technician ID	EJB	Replicate #	3

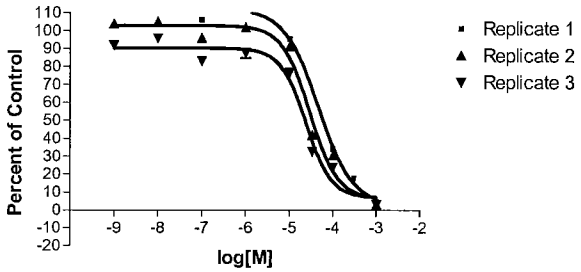
Control Type	Portion	Average	SD
Full activity	Beginning	0.6946	0.0072
Full activity	End	0.6563	0.0012
Full activity	Overall	0.6755	0.0225
Background	Beginning	-0.0002	0.000227955
Background	End	0.0002	0.001142332
Background	Overall	0.0000	0.00069986
Positive	Beginning	0.3260	0.0008
Positive	End	0.2823	0.0292
Positive	Overall	0.3041	0.0304
Negative	Beginning	0.5799	0.0221
Negative	End	0.4563	0.0579
Negative	Overall	0.5181	0.0798

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC#10 ATZ	1	1	1.00E-04	-4.00	0.6044
RC#10 ATZ	1	2	1.00E-04	-4.00	0.6135
RC#10 ATZ	1	3	1.00E-04	-4.00	0.6094
RC#10 ATZ	2	1	3.30E-05	-4.48	0.6252
RC#10 ATZ	2	2	3.30E-05	-4.48	0.5850
RC#10 ATZ	2	3	3.30E-05	-4.48	0.6101
RC#10 ATZ	3	1	1.00E-05	-5.00	0.6390
RC#10 ATZ	3	2	1.00E-05	-5.00	0.6368
RC#10 ATZ	3	3	1.00E-05	-5.00	0.6522
RC#10 ATZ	4	1	1.00E-06	-6.00	0.6489
RC#10 ATZ	4	2	1.00E-06	-6.00	0.6160
RC#10 ATZ	4	3	1.00E-06	-6.00	0.6155
RC#10 ATZ	5	1	1.00E-07	-7.00	0.6311
RC#10 ATZ	5	2	1.00E-07	-7.00	0.6146
RC#10 ATZ	5	3	1.00E-07	-7.00	0.6375
RC#10 ATZ	6	1	1.00E-08	-8.00	0.6198
RC#10 ATZ	6	2	1.00E-08	-8.00	0.6350
RC#10 ATZ	6	3	1.00E-08	-8.00	0.6336
RC#10 ATZ	7	1	1.00E-09	-9.00	0.6491
RC#10 ATZ	7	2	1.00E-09	-9.00	0.6467
RC#10 ATZ	7	3	1.00E-09	-9.00	0.6041
RC#10 ATZ	8	1	1.00E-10	-10.00	0.5961
RC#10 ATZ	8	2	1.00E-10	-10.00	0.6113
RC#10 ATZ	8	3	1.00E-10	-10.00	0.5929

Percent of control values				
	Log[test	Replicate		
Level	substance]	1	2	3
1	-4.00	89.49	90.83	90.22
2	-4.48	92.56	86.61	90.32
3	-5.00	94.60	94.27	96.56
4	-6.00	96.07	91.20	91.12
5	-7.00	93.43	90.99	94.38
6	-8.00	91.77	94.01	93.80
7	-9.00	96.10	95.75	89.43
8	-10.00	88.25	90.50	87.78

Appendix 5: Prism Output for Task 4

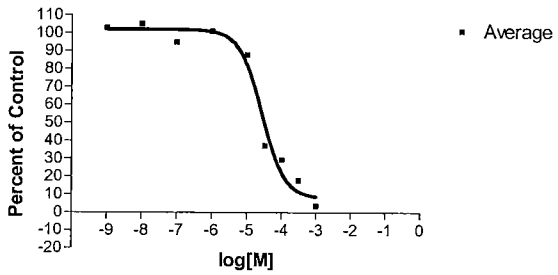
IVT 4-17 Task 4 Aminoglutethimide Replicate 1, 2 and 3



log[Aminoglutethimide]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-3.00	4.99	4.89	4.03	3.09	3.26	3.14	3.07	2.83	2.99
-3.52	17.02	18.89	17.72						
-4.00	34.75	35.10	32.66	31.94	30.22	29.33	24.11	23.31	23.91
-4.48				46.33	41.16	38.72	33.91	31.87	32.47
-5.00	94.59	95.94	95.70	89.49	91.63	92.99	76.38	77.99	76.17
-6.00	111.51	118.84	109.98	104.88	103.64	98.01	92.33	85.08	84.12
-7.00	106.21	107.24	104.88	99.48	96.95	91.33	85.88	83.69	79.28
-8.00	109.34	118.16	115.10	103.80	104.12	107.60	93.66	96.15	97.56

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	2.821	6.385	6.880
TOP	112.1	102.7	90.11
LOGEC50	-4.333	-4.531	-4.617
HILLSLOPE	-1.108	-1.364	-1.491
EC50	4.644e-005	2.945e-005	2.416e-005
Std. Error			
BOTTOM	2.866	4.143	3.520
TOP	1.159	2.001	1.834
LOGEC50	0.04537	0.05620	0.05532
HILLSLOPE	0.09202	0.2097	0.2419
95% Confidence Intervals			
BOTTOM	-3.158 to 8.799	-2.257 to 15.03	-0.4630 to 14.22
TOP	109.7 to 114.5	98.57 to 106.9	86.29 to 93.94
LOGEC50	-4.428 to -4.238	-4.648 to -4.414	-4.732 to -4.501
HILLSLOPE	-1.300 to -0.9164	-1.801 to -0.9264	-1.996 to -0.9863
EC50	3.735e-005 to 5.775e-005	2.248e-005 to 3.858e-005	1.852e-005 to 3.151e-005
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9939	0.9733	0.9719
Absolute Sum of Squares	290.4	912.4	771.3
Sy.x	3.810	6.754	6.210
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

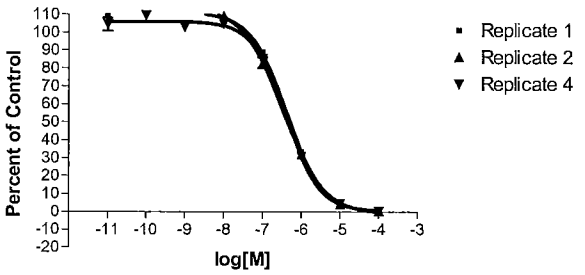
IVT 4-17 Task 4 Aminoglutethimide Replicate 1, 2 and 3



log[Aminoglutethimide]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-3.00	4.99	4.89	4.03	3.09	3.26	3.14	3.07	2.83	2.99
-3.52	17.02	18.89	17.72						
-4.00	34.75	35.10	32.66	31.94	30.22	29.33	24.11	23.31	23.91
-4.48				46.33	41.16	38.72	33.91	31.87	32.47
-5.00	94.59	95.94	95.70	89.49	91.63	92.99	76.38	77.99	76.17
-6.00	111.51	118.84	109.98	104.88	103.64	98.01	92.33	85.08	84.12
-7.00	106.21	107.24	104.88	99.48	96.95	91.33	85.88	83.69	79.28
-8.00	109.34	118.16	115.10	103.80	104.12	107.60	93.66	96.15	97.56

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	7.878
TOP	101.8
LOGEC50	-4.562
HILLSLOPE	-1.328
EC50	2.741e-005
Std. Error	
BOTTOM	3.321
TOP	1.703
LOGEC50	0.05373
HILLSLOPE	0.1787
95% Confidence Intervals	
BOTTOM	1.245 to 14.51
TOP	98.42 to 105.2
LOGEC50	-4.669 to -4.455
HILLSLOPE	-1.685 to -0.9714
EC50	2.141e-005 to 3.510e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9402
Absolute Sum of Squares	6647
Sy.x	9.887
Data	
Number of X values	9
Number of Y replicates	9
Total number of values	72
Number of missing values	9

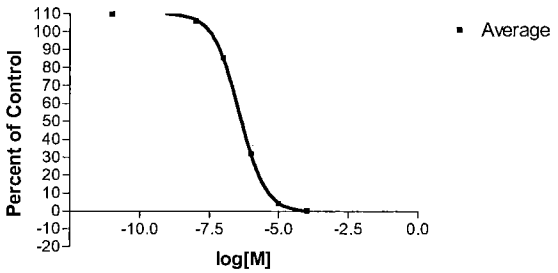
IVT 4-17 Task 4 Ketoconazole Replicate 1, 2 and 4



log[Ketoconazole]	Replicate 1			Replicate 2			Replicate 4		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	0.03	0.48	0.15	0.35	0.31	0.45	0.40	0.15	0.73
-5.00	4.81	4.55	4.43	3.96	4.25	4.29	4.46	4.55	4.34
-6.00	33.81	33.78	32.95	33.41	31.75	31.45	32.84	31.13	28.38
-7.00	90.64	88.43	87.89	87.43	83.89	77.48	85.83	84.44	85.65
-8.00	102.12	105.46	105.08	109.90	108.79	109.02	102.97	105.90	105.18
-9.00	109.06	116.33	116.14	114.63	112.90	112.86	102.50	103.63	102.44
-10.00	113.10	117.32	108.20	114.64	109.66	109.40	102.98	114.38	110.72
-11.00	109.87	107.22	110.26	108.04	106.94	134.08	103.78	110.83	98.80

	Replicate 1	Replicate 2	Replicate 4
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-0.7553	-1.166	0.2061
TOP	111.2	114.2	106.0
LOGEC50	-6.375	-6.476	-6.387
HILLSLOPE	-0.9324	-0.8409	-1.010
EC50	4.218e-007	3.340e-007	4.102e-007
Std. Error			
BOTTOM	1.590	2.954	1.666
TOP	0.9650	1.791	1.049
LOGEC50	0.03463	0.06510	0.03836
HILLSLOPE	0.05623	0.08796	0.07004
95% Confidence Intervals			
BOTTOM	-4.072 to 2.561	-7.328 to 4.995	-3.270 to 3.682
TOP	109.1 to 113.2	110.4 to 117.9	103.8 to 108.2
LOGEC50	-6.447 to -6.303	-6.612 to -6.340	-6.467 to -6.307
HILLSLOPE	-1.050 to -0.8151	-1.024 to -0.6574	-1.156 to -0.8641
EC50	3.572e-007 to 4.982e-007	2.443e-007 to 4.566e-007	3.412e-007 to 4.932e-007
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9964	0.9892	0.9952
Absolute Sum of Squares	186.5	590.9	228.5
Sy.x	3.053	5.436	3.380
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

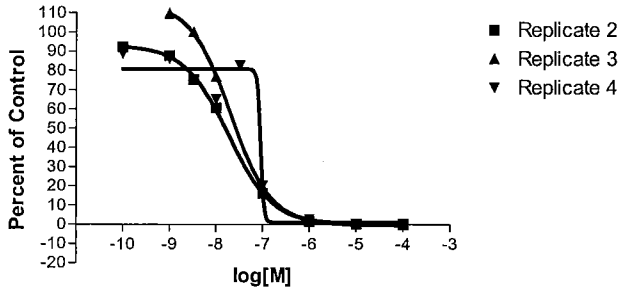
IVT 4-17 Task 4 Ketoconazole Replicate 1, 2 and 4



log[Ketoconazole]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	0.03	0.48	0.15	0.35	0.31	0.45	0.40	0.15	0.73
-5.00	4.81	4.55	4.43	3.96	4.25	4.29	4.46	4.55	4.34
-6.00	33.81	33.78	32.95	33.41	31.75	31.45	32.84	31.13	28.38
-7.00	90.64	88.43	87.89	87.43	83.89	77.48	85.83	84.44	85.65
-8.00	102.12	105.46	105.08	109.90	108.79	109.02	102.97	105.90	105.18
-9.00	109.06	116.33	116.14	114.63	112.90	112.86	102.50	103.63	102.44
-10.00	113.10	117.32	108.20	114.64	109.66	109.40	102.98	114.38	110.72
-11.00	109.87	107.22	110.26	108.04	106.94	134.08	103.78	110.83	98.80

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.6026
TOP	110.4
LOGEC50	-6.412
HILLSLOPE	-0.9191
EC50	3.875e-007
Std. Error	
BOTTOM	1.357
TOP	0.8307
LOGEC50	0.03024
HILLSLOPE	0.04739
95% Confidence Intervals	
BOTTOM	-3.313 to 2.107
TOP	108.7 to 112.1
LOGEC50	-6.472 to -6.351
HILLSLOPE	-1.014 to -0.8245
EC50	3.372e-007 to 4.453e-007
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9910
Absolute Sum of Squares	1390
Sy.x	4.521
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

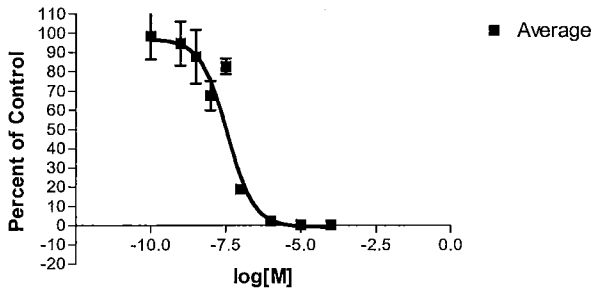
IVT 4-17 Task 4 Prochloraz Replicates 2, 3 and 4



log[Procloraz]	Replicate 2			Replicate 3			Replicate 4		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	0.12	-0.19	-0.27	0.80	0.13	0.56	0.06	0.59	0.34
-5.00	0.36	0.32	0.25	0.41	0.80	0.42	0.75	0.29	0.12
-6.00	1.46	1.78	1.99	2.95	2.00	2.41	2.79	2.66	2.76
-7.00	15.32	16.64	16.84	19.27	20.64	20.20	20.47	21.66	18.22
-7.48							86.46	83.19	78.41
-8.00	61.21	61.54	58.83	80.68	72.58	77.34	64.55	64.87	65.42
-8.48	78.28	74.58	73.35	104.51	99.28	96.52			
-9.00	89.51	86.32	87.47	112.16	110.28	106.73	85.83	87.91	85.42

	Replicate 2	Replicate 3	Replicate 4
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-0.3398	0.4256	1.151
TOP	93.07	114.8	80.74
LOGEC50	-7.724	-7.687	-7.040
HILLSLOPE	-0.9053	-1.012	-12.77
EC50	1.890e-008	2.057e-008	9.130e-008
Std. Error			
BOTTOM	0.6890	0.7883	2.520
TOP	1.023	1.097	2.520
LOGEC50	0.02522	0.02271	65.49
HILLSLOPE	0.03810	0.04046	20702
95% Confidence Intervals			
BOTTOM	-1.777 to 1.097	-1.219 to 2.070	-4.105 to 6.408
TOP	90.94 to 95.21	112.5 to 117.1	75.49 to 86.00
LOGEC50	-7.776 to -7.671	-7.734 to -7.639	-143.6 to 129.6
HILLSLOPE	-0.9848 to -0.8259	-1.096 to -0.9274	-43200 to 43171
EC50	1.674e-008 to 2.133e-008	1.844e-008 to 2.294e-008	
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9981	0.9983	0.9679
Absolute Sum of Squares	68.11	94.73	1143
Sy.x	1.845	2.176	7.560
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

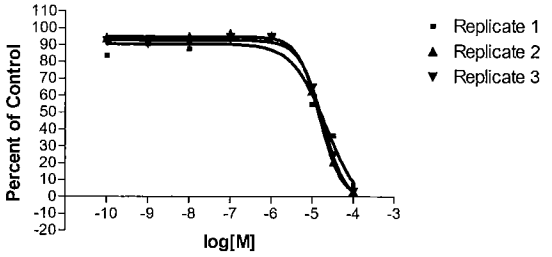
IVT 4-17 Task 4 Prochloraz Replicates 2, 3 and 4



log[Procloraz]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	0.12	-0.19	-0.27	0.80	0.13	0.56	0.06	0.59	0.34
-5.00	0.36	0.32	0.25	0.41	0.80	0.42	0.75	0.29	0.12
-6.00	1.46	1.78	1.99	2.95	2.00	2.41	2.79	2.66	2.76
-7.00	15.32	16.64	16.84	19.27	20.64	20.20	20.47	21.66	18.22
-7.48							86.46	83.19	78.41
-8.00	61.21	61.54	58.83	80.68	72.58	77.34	64.55	64.87	65.42
-8.48	78.28	74.58	73.35	104.51	99.28	96.52			
-9.00	89.51	86.32	87.47	112.16	110.28	106.73	85.83	87.91	85.42

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.9268
TOP	96.86
LOGEC50	-7.440
HILLSLOPE	-0.9714
EC50	3.633e-008
Std. Error	
BOTTOM	2.351
TOP	2.972
LOGEC50	0.07155
HILLSLOPE	0.1311
95% Confidence Intervals	
BOTTOM	-5.624 to 3.770
TOP	90.92 to 102.8
LOGEC50	-7.583 to -7.297
HILLSLOPE	-1.233 to -0.7095
EC50	2.615e-008 to 5.049e-008
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9391
Absolute Sum of Squares	7904
Sy.x	10.78
Data	
Number of X values	9
Number of Y replicates	9
Total number of values	72
Number of missing values	9

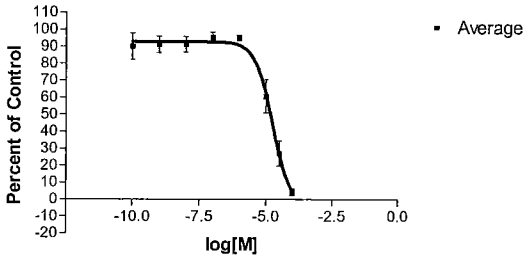
IVT 4-17 Task 4 4-Nonylphenol Replicates 1, 2 and 3



log[4-Nonylphenol]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	8.35	6.91	5.96	2.82	3.43	3.35	2.48	3.38	2.77
-4.48	37.14	38.45	33.02	20.61	20.27	21.28	24.96	27.43	20.80
-5.00	70.56	36.18	57.73	63.86	61.64	62.55	64.57	62.27	66.72
-6.00	93.39	96.47	96.94	96.25	95.81	93.95	95.36	94.43	92.46
-7.00	97.33	96.78	90.66	97.80	97.93	94.50	96.50	96.73	89.19
-8.00	93.24	86.27	83.12	95.29	94.43	94.69	93.12	93.80	86.88
-9.00	97.63	79.94	94.00	90.41	94.06	92.01	90.16	90.48	91.81
-10.00	95.87	83.09	72.09	95.68	93.13	93.46	92.95	94.54	89.18

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-8.451	-1.024	-3.641
TOP	90.71	94.83	92.69
LOGEC50	-4.649	-4.813	-4.740
HILLSLOPE	-1.039	-1.622	-1.520
EC50	2.245e-005	1.538e-005	1.818e-005
Std. Error			
BOTTOM	24.85	1.849	3.416
TOP	2.745	0.4866	0.7615
LOGEC50	0.2626	0.01801	0.03257
HILLSLOPE	0.4239	0.09771	0.1414
95% Confidence Intervals			
BOTTOM	-60.30 to 43.40	-4.881 to 2.833	-10.77 to 3.484
TOP	84.99 to 96.44	93.81 to 95.84	91.10 to 94.28
LOGEC50	-5.196 to -4.101	-4.851 to -4.776	-4.808 to -4.672
HILLSLOPE	-1.923 to -0.1545	-1.826 to -1.418	-1.815 to -1.225
EC50	6.361e-006 to 7.925e-005	1.410e-005 to 1.677e-005	1.555e-005 to 2.126e-005
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9230	0.9977	0.9940
Absolute Sum of Squares	1825	67.65	164.5
Sy.x	9.552	1.839	2.868
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

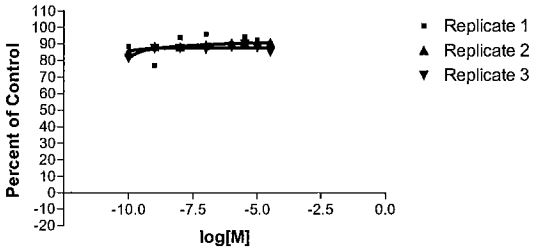
IVT 4-17 Task 4 4-Nonylphenol Replicate 1, 2 and 3



log[4-Nonylphenol]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	8.35	6.91	5.96	2.82	3.43	3.35	2.48	3.38	2.77
-4.48	37.14	38.45	33.02	20.61	20.27	21.28	24.96	27.43	20.80
-5.00	70.56	36.18	57.73	63.86	61.64	62.55	64.57	62.27	66.72
-6.00	93.39	96.47	96.94	96.25	95.81	93.95	95.36	94.43	92.46
-7.00	97.33	96.78	90.66	97.80	97.93	94.50	96.50	96.73	89.19
-8.00	93.24	86.27	83.12	95.29	94.43	94.69	93.12	93.80	86.88
-9.00	97.63	79.94	94.00	90.41	94.06	92.01	90.16	90.48	91.81
-10.00	95.87	83.09	72.09	95.68	93.13	93.46	92.95	94.54	89.18

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-4.206
TOP	92.82
LOGEC50	-4.745
HILLSLOPE	-1.317
EC50	1.798e-005
Std. Error	
BOTTOM	5.245
TOP	0.9639
LOGEC50	0.05125
HILLSLOPE	0.1709
95% Confidence Intervals	
BOTTOM	-14.68 to 6.271
TOP	90.89 to 94.74
LOGEC50	-4.847 to -4.643
HILLSLOPE	-1.658 to -0.9753
EC50	1.421e-005 to 2.276e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9685
Absolute Sum of Squares	2564
Sy.x	6.141
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

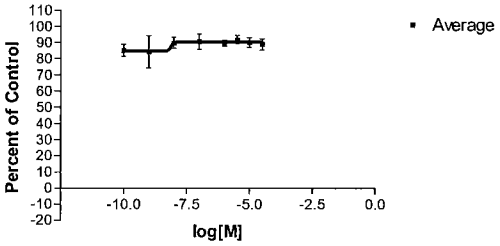
IVT 4-17 Task 4 dibenz[a,h]anthracene Replicates 1, 2 and 3



log[dibenz[a,h]anthracene]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.48	92.26	91.91	87.77	92.82	90.59	89.66	86.85	86.92	82.60
-5.00	92.68	92.25	92.59	90.75	93.31	86.57	85.07	90.59	87.80
-5.48	96.48	94.07	92.96	91.11	90.89	91.17	89.24	92.33	88.56
-6.00	91.40	89.02	90.69	91.80	88.68	91.57	89.65	85.28	90.14
-7.00	98.16	95.76	94.23	85.56	90.84	91.27	85.72	90.31	85.34
-8.00	96.64	91.80	93.44	86.51	90.02	88.20	87.16	87.53	88.03
-9.00	79.83	59.44	92.53	89.59	88.66	87.49	86.64	87.39	87.58
-10.00	85.94	88.03	91.74	83.20	86.48	87.13	83.13	82.64	79.03

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)	Does not converge.		
Best-fit values			
BOTTOM		62.60	74.33
TOP		91.52	87.74
LOGEC50		-13.33	-10.06
HILLSLOPE		0.1866	1.292
EC50		4.697e-014	8.741e-011
Std. Error			
BOTTOM		648.4	789.2
TOP		5.616	0.6190
LOGEC50		80.42	40.50
HILLSLOPE		0.9205	24.21
95% Confidence Intervals			
BOTTOM		-1290 to 1415	-1572 to 1721
TOP		79.80 to 103.2	86.45 to 89.03
LOGEC50		-181.1 to 154.4	-94.53 to 74.42
HILLSLOPE		-1.734 to 2.107	-49.20 to 51.79
EC50			
Goodness of Fit			
Degrees of Freedom		20	20
R^2		0.4527	0.4685
Absolute Sum of Squares		78.97	110.0
Sy.x		1.987	2.345
Data			
Number of X values		8	8
Number of Y replicates		3	3
Total number of values		24	24
Number of missing values		0	0

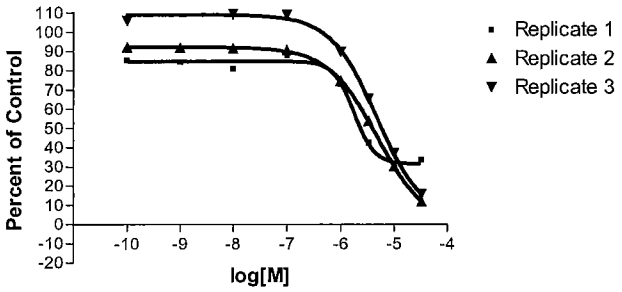
IVT 4-17 Task 4 dibenz[a,h]anthracene Replicates 1, 2 and 3



log[dibenz[a,h]anthracene]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.48	92.26	91.91	87.77	92.82	90.59	89.66	86.85	86.92	82.60
-5.00	92.68	92.25	92.59	90.75	93.31	86.57	85.07	90.59	87.80
-5.48	96.48	94.07	92.96	91.11	90.89	91.17	89.24	92.33	88.56
-6.00	91.40	89.02	90.69	91.80	88.68	91.57	89.65	85.28	90.14
-7.00	98.16	95.76	94.23	85.56	90.84	91.27	85.72	90.31	85.34
-8.00	96.64	91.80	93.44	86.51	90.02	88.20	87.16	87.53	88.03
-9.00	79.83	59.44	92.53	89.59	88.66	87.49	86.64	87.39	87.58
-10.00	85.94	88.03	91.74	83.20	86.48	87.13	83.13	82.64	79.03

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	84.80
TOP	90.34
LOGEC50	-8.121
HILLSLOPE	9.018
EC50	7.563e-009
Std. Error	
BOTTOM	1.539
TOP	0.6882
LOGEC50	180109
HILLSLOPE	1.329e+007
95% Confidence Intervals	
BOTTOM	81.73 to 87.88
TOP	88.96 to 91.71
LOGEC50	-359700 to 359730
HILLSLOPE	-26550000 to 2.655e+007
EC50	
Goodness of Fit	
Degrees of Freedom	68
R^2	0.2182
Absolute Sum of Squares	1449
Sy.x	4.617
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

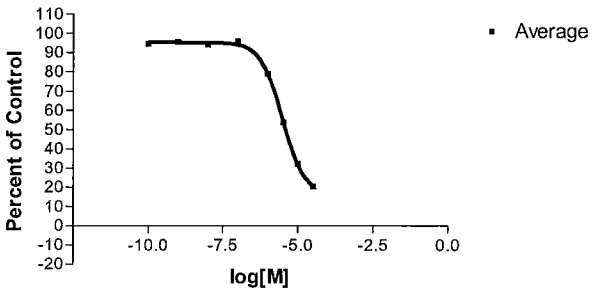
IVT 4-17 Task 4 Fenarimol Replicates 1, 2 and 3



log[Fenarimol]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.48	3.51	49.67	48.24	13.47	11.39	11.16	16.61	15.59	14.44
-5.00	29.29	29.55	29.57	28.79	30.45	31.48	36.89	35.98	38.04
-5.48	42.73	41.83	42.80	51.79	54.66	55.05	61.26	66.97	67.67
-6.00	71.24	73.00	74.71	74.52	74.80	74.20	90.94	86.35	91.86
-7.00	86.47	87.24	90.34	90.11	91.75	89.99	106.78	107.73	112.31
-8.00	82.06	82.80	78.94	92.69	90.32	91.94	110.41	107.26	110.49
-9.00	80.36	85.39	87.61	92.22	93.15	90.51	109.16	112.73	108.51
-10.00	86.83	85.50	84.20	90.04	93.33	93.23	107.75	109.30	100.64

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	31.35	-1.149	2.255
TOP	84.78	92.23	109.1
LOGEC50	-5.751	-5.316	-5.323
HILLSLOPE	-2.269	-0.9373	-0.9985
EC50	1.773e-006	4.828e-006	4.755e-006
Std. Error			
BOTTOM	4.045	2.376	4.997
TOP	2.552	0.4004	0.9195
LOGEC50	0.09697	0.03070	0.05467
HILLSLOPE	0.7764	0.04263	0.08885
95% Confidence Intervals			
BOTTOM	22.91 to 39.79	-6.105 to 3.806	-8.170 to 12.68
TOP	79.46 to 90.10	91.40 to 93.07	107.2 to 111.0
LOGEC50	-5.954 to -5.549	-5.380 to -5.252	-5.437 to -5.209
HILLSLOPE	-3.889 to -0.6498	-1.026 to -0.8484	-1.184 to -0.8132
EC50	1.113e-006 to 2.825e-006	4.166e-006 to 5.596e-006	3.657e-006 to 6.182e-006
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.8942	0.9985	0.9940
Absolute Sum of Squares	1549	31.62	173.5
Sy.x	8.799	1.257	2.946
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

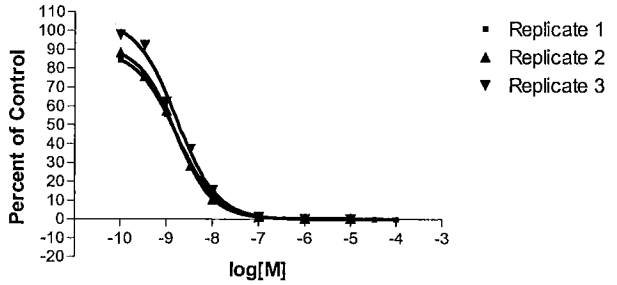
IVT 4-17 Task 4 Fenarimol Replicates 1, 2 and 3



log[Fenarimol]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.48	3.51	49.67	48.24	13.47	11.39	11.16	16.61	15.59	14.44
-5.00	29.29	29.55	29.57	28.79	30.45	31.48	36.89	35.98	38.04
-5.48	42.73	41.83	42.80	51.79	54.66	55.05	61.26	66.97	67.67
-6.00	71.24	73.00	74.71	74.52	74.80	74.20	90.94	86.35	91.86
-7.00	86.47	87.24	90.34	90.11	91.75	89.99	106.78	107.73	112.31
-8.00	82.06	82.80	78.94	92.69	90.32	91.94	110.41	107.26	110.49
-9.00	80.36	85.39	87.61	92.22	93.15	90.51	109.16	112.73	108.51
-10.00	86.83	85.50	84.20	90.04	93.33	93.23	107.75	109.30	100.64

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	16.61
TOP	95.27
LOGEC50	-5.513
HILLSLOPE	-1.216
EC50	3.067e-006
Std. Error	
BOTTOM	5.852
TOP	1.841
LOGEC50	0.08619
HILLSLOPE	0.2523
95% Confidence Intervals	
BOTTOM	4.922 to 28.30
TOP	91.60 to 98.95
LOGEC50	-5.685 to -5.341
HILLSLOPE	-1.720 to -0.7121
EC50	2.064e-006 to 4.559e-006
Goodness of Fit	
Degrees of Freedom	68
R^2	0.8891
Absolute Sum of Squares	7542
Sy.x	10.53
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

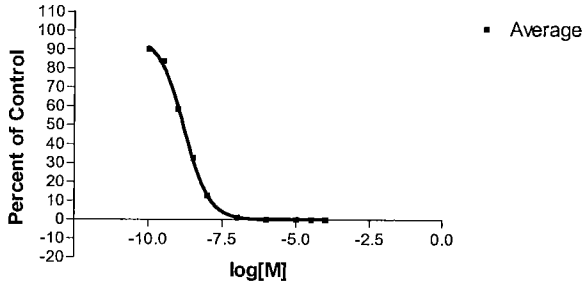
IVT 4-17 Task 4 Econazole Replicates 1,2 and 3



log[Econazole]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	-0.05	0.00	0.02						
-4.48	-0.03	-0.09	-0.06						
-5.00	0.07	0.02	-0.01	0.34	0.82	0.65	0.01	-0.10	-0.01
-6.00	0.06	0.32	0.15	0.45	0.13	0.74	0.14	0.25	-0.03
-7.00	1.31	1.47	1.72	1.27	1.39	1.11	1.03	1.20	1.19
-8.00	12.80	12.75	13.11	6.81	12.18	12.26	15.18	15.94	15.25
-8.48				29.20	28.88	27.61	37.32	38.20	36.43
-9.00	54.45	54.28	58.64	60.96	57.42	55.24	66.39	63.55	57.53

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-0.01227	0.2647	-0.1621
TOP	89.81	92.03	106.0
LOGEC50	-8.783	-8.807	-8.777
HILLSLOPE	-0.9904	-1.080	-0.9838
EC50	1.648e-009	1.559e-009	1.671e-009
Std. Error			
BOTTOM	0.3049	1.136	1.102
TOP	1.075	2.915	3.004
LOGEC50	0.01843	0.04251	0.03918
HILLSLOPE	0.03422	0.09289	0.07148
95% Confidence Intervals			
BOTTOM	-0.6483 to 0.6238	-2.105 to 2.635	-2.462 to 2.137
TOP	87.57 to 92.05	85.95 to 98.12	99.71 to 112.2
LOGEC50	-8.821 to -8.745	-8.896 to -8.719	-8.859 to -8.695
HILLSLOPE	-1.062 to -0.9190	-1.274 to -0.8862	-1.133 to -0.8347
EC50	1.508e-009 to 1.801e-009	1.271e-009 to 1.912e-009	1.384e-009 to 2.017e-009
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9989	0.9923	0.9947
Absolute Sum of Squares	24.76	213.4	187.9
Sy.x	1.113	3.266	3.065
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

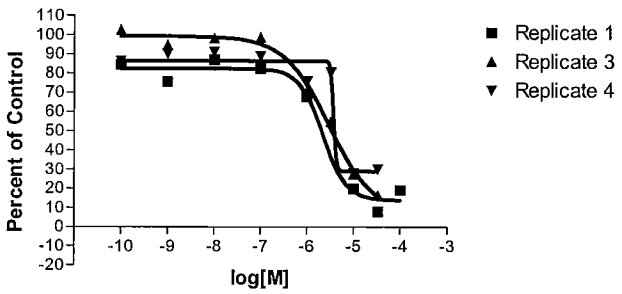
IVT 4-17 Task 4 Econazole Replicate 1, 2 and 3



log[Econazole]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	-0.05	0.00	0.02						
-4.48	-0.03	-0.09	-0.06						
-5.00	0.07	0.02	-0.01	0.34	0.82	0.65	0.01	-0.10	-0.01
-6.00	0.06	0.32	0.15	0.45	0.13	0.74	0.14	0.25	-0.03
-7.00	1.31	1.47	1.72	1.27	1.39	1.11	1.03	1.20	1.19
-8.00	12.80	12.75	13.11	6.81	12.18	12.26	15.18	15.94	15.25
-8.48				29.20	28.88	27.61	37.32	38.20	36.43
-9.00	54.45	54.28	58.64	60.96	57.42	55.24	66.39	63.55	57.53

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.09901
TOP	96.18
LOGEC50	-8.780
HILLSLOPE	-1.037
EC50	1.659e-009
Std. Error	
BOTTOM	0.8043
TOP	2.309
LOGEC50	0.03366
HILLSLOPE	0.06822
95% Confidence Intervals	
BOTTOM	-1.507 to 1.705
TOP	91.57 to 100.8
LOGEC50	-8.847 to -8.713
HILLSLOPE	-1.173 to -0.9003
EC50	1.421e-009 to 1.937e-009
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9855
Absolute Sum of Squares	1308
Sy.x	4.386
Data	
Number of X values	10
Number of Y replicates	9
Total number of values	72
Number of missing values	18

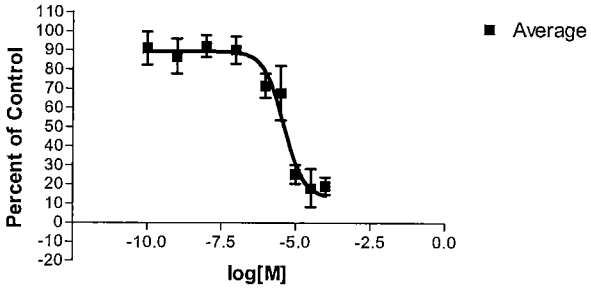
IVT 4-17 Task 4 Chrysin Replicate 1, 3 and 4



log[Chrysin]	Replicate 1			Replicate 3			Replicate 4		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	15.94	17.17	24.36						
-4.48	8.73	8.16	6.86	18.09	19.76	11.35	30.18	30.15	29.90
-5.00	23.27	22.10	14.47	30.06	28.99	23.75	27.10	28.64	28.29
-5.48				56.58	53.28	54.73	79.67	82.39	79.26
-6.00	68.65	67.03	68.07	78.77	59.47	73.50	72.51	77.17	78.16
-7.00	83.08	83.01	81.96	100.75	97.25	98.17	90.70	87.59	88.36
-8.00	87.12	89.11	85.15	102.06	100.22	92.30	90.68	93.31	89.51
-9.00	77.23	75.74	74.01	98.48	90.12	95.56	93.02	91.43	86.32

	Replicate 1	Replicate 3	Replicate 4
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	13.75	5.524	29.04
TOP	82.56	99.41	86.52
LOGEC50	-5.660	-5.510	-5.421
HILLSLOPE	-1.691	-0.8879	-15.72
EC50	2.190e-006	3.091e-006	3.793e-006
Std. Error			
BOTTOM	2.460	8.314	2.969
TOP	1.521	1.822	1.328
LOGEC50	0.09504	0.1147	876.5
HILLSLOPE	0.3911	0.1666	228727
95% Confidence Intervals			
BOTTOM	8.619 to 18.88	-11.82 to 22.87	22.85 to 35.24
TOP	79.38 to 85.73	95.61 to 103.2	83.75 to 89.29
LOGEC50	-5.858 to -5.461	-5.749 to -5.271	-1834 to 1823
HILLSLOPE	-2.507 to -0.8754	-1.235 to -0.5403	-477100 to 477108
EC50	1.387e-006 to 3.456e-006	1.782e-006 to 5.362e-006	
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9776	0.9759	0.9647
Absolute Sum of Squares	539.0	599.8	529.0
Sy.x	5.191	5.476	5.143
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

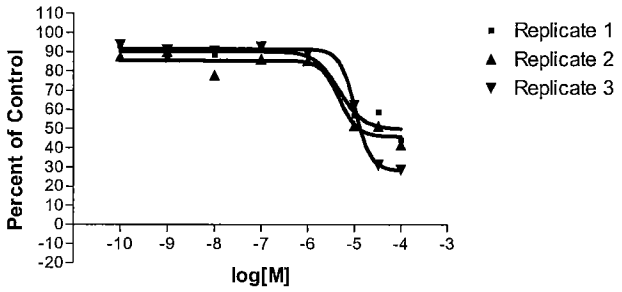
IVT 4-17 Task 4 Chrysin Replicate 1, 3 and 4



log[Chrysin]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	15.94	17.17	24.36						
-4.48	8.73	8.16	6.86	18.09	19.76	11.35	30.18	30.15	29.90
-5.00	23.27	22.10	14.47	30.06	28.99	23.75	27.10	28.64	28.29
-5.48				56.58	53.28	54.73	79.67	82.39	79.26
-6.00	68.65	67.03	68.07	78.77	59.47	73.50	72.51	77.17	78.16
-7.00	83.08	83.01	81.96	100.75	97.25	98.17	90.70	87.59	88.36
-8.00	87.12	89.11	85.15	102.06	100.22	92.30	90.68	93.31	89.51
-9.00	77.23	75.74	74.01	98.48	90.12	95.56	93.02	91.43	86.32

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	12.93
TOP	89.39
LOGEC50	-5.390
HILLSLOPE	-1.303
EC50	4.070e-006
Std. Error	
BOTTOM	4.353
TOP	1.612
LOGEC50	0.07261
HILLSLOPE	0.2255
95% Confidence Intervals	
BOTTOM	4.232 to 21.62
TOP	86.17 to 92.61
LOGEC50	-5.535 to -5.245
HILLSLOPE	-1.754 to -0.8531
EC50	2.915e-006 to 5.684e-006
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9103
Absolute Sum of Squares	6075
Sy.x	9.452
Data	
Number of X values	9
Number of Y replicates	9
Total number of values	72
Number of missing values	9

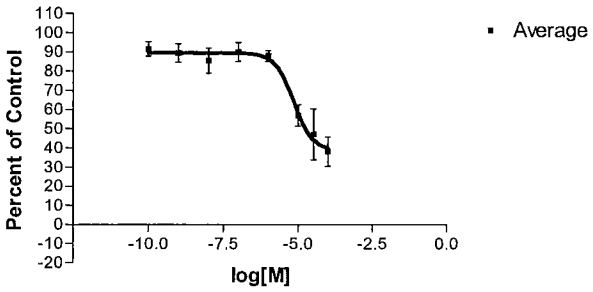
IVT 4-17 Task 4 Dicofol Replicates 1, 2 and 3



log[Dicofol]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	45.82	44.00	42.63	37.23	45.84	41.64	31.11	28.47	26.20
-4.48	65.30	52.54	58.21	57.63	44.46	52.04	32.66	31.72	29.34
-5.00	62.91	54.36	53.19	53.01	52.68	49.24	63.22	64.54	59.00
-6.00	91.10	85.34	90.92	83.37	86.17	86.66	89.15	87.75	91.36
-7.00	88.06	93.55	91.05	91.96	81.38	85.33	94.84	96.38	87.20
-8.00	94.03	87.53	83.50	79.19	75.13	78.88	91.40	89.96	89.51
-9.00	77.24	93.75	91.31	89.97	91.44	88.46	92.00	89.41	91.52
-10.00	91.80	95.34	93.06	89.61	90.26	82.67	94.53	93.09	93.84

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	49.69	45.90	28.23
TOP	90.36	85.58	91.50
LOGEC50	-5.325	-5.320	-4.975
HILLSLOPE	-1.664	-2.236	-2.570
EC50	4.735e-006	4.782e-006	1.059e-005
Std. Error			
BOTTOM	3.155	2.540	1.698
TOP	1.791	1.630	0.6721
LOGEC50	0.1668	0.2135	0.02051
HILLSLOPE	0.6983	1.393	0.6095
95% Confidence Intervals			
BOTTOM	43.11 to 56.27	40.60 to 51.20	24.69 to 31.77
TOP	86.62 to 94.09	82.18 to 88.98	90.09 to 92.90
LOGEC50	-5.673 to -4.977	-5.766 to -4.875	-5.018 to -4.932
HILLSLOPE	-3.121 to -0.2075	-5.142 to 0.6693	-3.841 to -1.298
EC50	2.125e-006 to 1.055e-005	1.715e-006 to 1.333e-005	9.599e-006 to 1.169e-005
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.9100	0.9248	0.9920
Absolute Sum of Squares	754.8	636.7	134.3
Sy.x	6.143	5.642	2.592
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

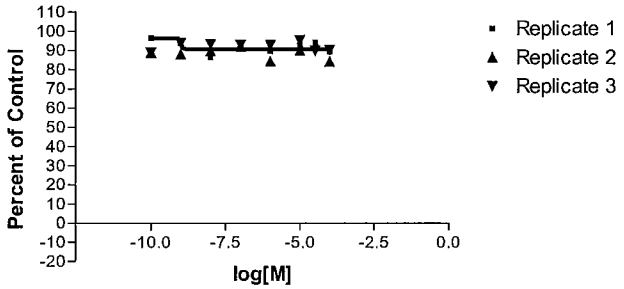
IVT 4-17 Task 4 Dicofol Replicates 1, 2 and 3



log[Dicofol]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	45.82	44.00	42.63	37.23	45.84	41.64	31.11	28.47	26.20
-4.48	65.30	52.54	58.21	57.63	44.46	52.04	32.66	31.72	29.34
-5.00	62.91	54.36	53.19	53.01	52.68	49.24	63.22	64.54	59.00
-6.00	91.10	85.34	90.92	83.37	86.17	86.66	89.15	87.75	91.36
-7.00	88.06	93.55	91.05	91.96	81.38	85.33	94.84	96.38	87.20
-8.00	94.03	87.53	83.50	79.19	75.13	78.88	91.40	89.96	89.51
-9.00	77.24	93.75	91.31	89.97	91.44	88.46	92.00	89.41	91.52
-10.00	91.80	95.34	93.06	89.61	90.26	82.67	94.53	93.09	93.84

	Average
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	38.72
TOP	89.47
LOGEC50	-5.146
HILLSLOPE	-1.380
EC50	7.139e-006
Std. Error	
BOTTOM	2.975
TOP	1.178
LOGEC50	0.07704
HILLSLOPE	0.3637
95% Confidence Intervals	
BOTTOM	32.78 to 44.66
TOP	87.12 to 91.83
LOGEC50	-5.300 to -4.992
HILLSLOPE	-2.106 to -0.6536
EC50	5.009e-006 to 1.017e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9011
Absolute Sum of Squares	3351
Sy.x	7.020
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

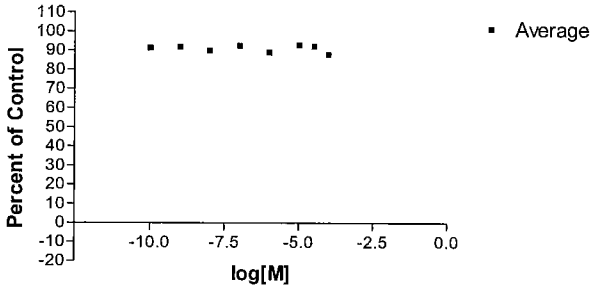
IVT 4-17 Task 4 Atrazine Replicates 1,2 and 3



log[Atrazine]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-4.00	88.12	87.61	91.95	86.05	81.35	85.12	89.49	90.83	90.22
-4.48	93.20	93.49	95.41	90.90	93.48	93.25	92.56	86.61	90.32
-5.00	95.32	92.05	91.67	90.99	90.91	87.95	94.60	94.27	96.56
-6.00	87.66	91.00	90.53	80.76	85.13	86.86	96.07	91.20	91.12
-7.00	92.80	91.32	92.48	92.05	93.17	91.10	93.43	90.99	94.38
-8.00	87.88	86.99	84.93	88.36	90.78	90.35	91.77	94.01	93.80
-9.00	94.95	91.99	94.95	88.96	87.90	86.95	96.10	95.75	89.43
-10.00	95.73	96.62	96.94	84.60	89.79	92.06	88.25	90.50	87.78

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)		Does not converge.	Does not converge.
Best-fit values			
BOTTOM	90.80		
TOP	96.43		
LOGEC50	-8.993		
HILLSLOPE	-14.92		
EC50	1.017e-009		
Std. Error			
BOTTOM	0.6601		
TOP	1.617		
LOGEC50	2.919e+006		
HILLSLOPE	6.014e+009		
95% Confidence Intervals			
BOTTOM	89.42 to 92.18		
TOP	93.06 to 99.80		
LOGEC50	-6090000 to 6.090e+006		
HILLSLOPE	-12550000000 to 1.255e+010		
EC50			
Goodness of Fit			
Degrees of Freedom	20		
R^2	0.3799		
Absolute Sum of Squares	156.9		
Sy.x	2.801		
Data			
Number of X values	8		
Number of Y replicates	3		
Total number of values	24		
Number of missing values	0		

IVT 4-17 Task 4 Atrazine Replicates 1,2 and 3



log[Atrazine]	Average								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-4.00	88.12	87.61	91.95	86.05	81.35	85.12	89.49	90.83	90.22
-4.48	93.20	93.49	95.41	90.90	93.48	93.25	92.56	86.61	90.32
-5.00	95.32	92.05	91.67	90.99	90.91	87.95	94.60	94.27	96.56
-6.00	87.66	91.00	90.53	80.76	85.13	86.86	96.07	91.20	91.12
-7.00	92.80	91.32	92.48	92.05	93.17	91.10	93.43	90.99	94.38
-8.00	87.88	86.99	84.93	88.36	90.78	90.35	91.77	94.01	93.80
-9.00	94.95	91.99	94.95	88.96	87.90	86.95	96.10	95.75	89.43
-10.00	95.73	96.62	96.94	84.60	89.79	92.06	88.25	90.50	87.78

	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

RECOMBINANT AROMATASE VALIDATION STUDY

CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES

INTRALABORATORY STATISTICAL ANALYSIS OF IN VITRO TECHNOLOGIES, INC. DATA

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-17, TASK 4

June 12, 2006

Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
ENDOCRINE DISRUPTOR SCREENING PROGRAM
WASHINGTON, D.C.

Prepared by

BATTELLE
505 King Avenue
Columbus, Ohio 43201

Recombinant Aromatase Validation Study

Conduct Multiple Chemical Studies with Recombinant Microsomes
Intralaboratory Statistical Analysis of
In Vitro Technologies, Inc. Data

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-17, TASK 4

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	6/9/2006	6/9/2006	6/9/2006
Audit draft report	6/9/2006	6/9/2006	6/9/2006

Quality Assurance Unit **Date**

Hillary Flory 12-12-06

This report discusses the methods and results of the intralaboratory statistical analysis of the data collected at In Vitro Technologies, Inc. (IVT) with the recombinant aromatase assay for WA 4-17 Task 4: Conduct Multiple Chemical Studies with Recombinant Microsomes.

Summary and Conclusions

There were ten test substances analyzed in this task: aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, dibenz[a,h]anthracene, fenarimol, econazole, chrysin, dicofol, and atrazine (coded: Chemicals I, II, III, IV, V, VI, VII, VIII, IX, and X, respectively). Recombinant microsomes were prepared by one supplier. In Vitro Technologies, Inc. (IVT) analyzed these ten chemicals and provided summary results from the concentration response curve fits from each chemical for statistical analysis. Three replicates were carried out for each chemical. For each chemical the statistical analyses were based on the data from all the replicates.

Two types of data were obtained: inhibition curve fit data and control activity data. Statistical analyses were carried out separately for these two types of data. The response variable was the "percent of control". Percent of control is defined as the ratio of the background adjusted aromatase activity in the tube under consideration to the average background adjusted aromatase activity among the four full enzyme activity control tubes within the replicate, times 100. Control activity data consists of four types of controls: full enzyme activity control, background activity control, positive control (4-OH ASDN), and negative control (lindane). Statistical analyses were performed separately for these four types of controls.

For the inhibition curve fit data separate analyses were carried out for each of the ten test substances. For each test substance concentration response curves were fitted within each replicate to describe the relation between test substance concentrations and extent of inhibition. The concentration response curves were summarized by four parameters: the baseline response (bottom), the maximum response (top), the concentration that provokes a response halfway between baseline and maximum (IC_{50}), and the slope. Results were compared across replicates within test substance. For the control activity data, statistical analyses were carried out for the controls combined across test substances. Full enzyme activity, background activity, positive, and negative control tube responses were compared between the beginning and the end of each replicate to identify differences within replicates, differences among replicates within test substances, and differences among test substances.

The following results were observed:

1. Test substances V (dibenz[a,h]anthracene) and X (atrazine) are non-inhibitors. For test substance V all responses but one (59.4 percent) were above 79 percent of control. For test substance X all responses were above 80 percent of control.
2. Among the four concentration response curve parameters the top threshold had

the most replicate-to-replicate variation within chemicals.

3. For most of the test substances there was considerable replicate-to-replicate variation within chemicals for at least some of the concentration response curve parameters:
   Chemical I: Aminoglutethimide – Top
   Chemical II: Ketoconazole – Top
   Chemical III: Prochloraz – Top, IC_{50}, Slope
   Chemical VI: Fenarimol – Bottom, Top
   Chemical VII: Econazole – Top
   Chemical VIII: Chrysin – Bottom, Top, Slope
   Chemical IX: Dicofol – Bottom, Top
4. For test substance 9 (dicofol) the bottom threshold was in the range 28 percent to 50 percent.
5. The overall standard errors of the mean of the weighted average estimates of the $\log_{10}IC_{50}$ and the slope were small.
6. For the full enzyme activity controls the differences between the beginning and the end portions, when averaged across replicates, were significant. The end portion was significantly lower than the beginning portion. This implies a reduction in aromatase activity between the beginning and the end of a replicate.
7. For the negative controls the differences between the beginning and the end portions, when averaged across replicates, approached significance ($p=0.054$). The end portion was significantly higher than the beginning portion. This implies an increase in aromatase activity between the beginning and the end of a replicate. It is not understood why there is a difference in the direction of change of activity between the negative controls and the full enzyme activity controls.
8. For the background activity controls and the positive controls the differences between the beginning and the end portions, when averaged across replicates, were not significant.
9. For all control types the average control results did not vary significantly across chemicals. The portion effects within chemicals also did not vary significantly across chemicals
10. There is some ambiguity in the $\log_{10}IC_{50}$ estimate. The "$\log_{10}IC_{50}$" concentration in fact corresponds to the (B+T)/2 percentile, which for some chemicals (e.g. Chemical 6 – fenarimol – (B+T)/2 = 58.1, 45.5, 55.7) deviate somewhat from 50 percent. Such variation in percent inhibition can inflate the replicate-to-replicate variation among the $\log_{10}IC_{50}$ estimates. An alternative (nonstandard) parameterization of the model might be adopted in which the "$\log_{10}IC_{50}$" concentration corresponds to the 50 percentile, irrespective of the top and bottom thresholds.
11. Variation in aromatase activity during the course of a replicate run can result in potential bias or potential loss of precision due to decrease or increase of aromatase activity within replicates. If the inhibition concentration tubes are tested in order of inhibitor concentration, the change in aromatase activity across the replicate can accentuate or can lessen the apparent inhibition, depending on the ordering of the test substance concentrations. If the 24 tubes (8 inhibitor concentrations × 3 repetitions per concentration) were tested in randomized order

then the change in aromatase activity within the replicate would increase the residual variation about the fitted concentration response curves for each replicate but would avoid resulting in biased response curves.

Introduction and Background

In Task 4 of the Recombinant Aromatase Validation Study, each laboratory carried out multiple independent replicates of the recombinant aromatase assay with ten test substances according to a common test protocol. Recombinant microsomes were prepared by one supplier. For each test substance concentration response curves were fitted to the results from each replicate. Graphical displays and analysis of variance summary comparisons of the concentration response curves and the full enzyme activity control, background activity control, positive, and negative controls were prepared for each inhibitor compound.

This report discusses the methods and results of the statistical analyses of the data collected at IVT for the ten test substances: aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, dibenz[a,h]anthracene, fenarimol, econazole, chrysin, dicofol, and atrazine, which were coded by Roman numeric: Chemicals I, II, III, IV, V, VI, VII, VIII, IX, and X, respectively.

Data Used in the Analyses

Aromatase activity levels were determined for the full enzyme activity control, background activity control, positive control, and negative control, and for eight graded concentrations of each of the ten test substances. The specific concentration levels varied for each of the test substances.

Three replicates were carried out for each chemical. Within each replicate of each test substance three repetitions were run at each of the eight concentrations. In addition, two repeat tubes of the full enzyme activity, background activity, positive, and negative controls were run prior to the inhibition concentration runs and two repeat tubes of each the four control types were run following the inhibition concentration runs.

Statistical analyses were carried out on the "percent of control" responses. Percent of control is defined as the ratio of the background adjusted aromatase activity in the tube under consideration to the background adjusted average aromatase activity among the four full enzyme activity control tubes within the replicate, times 100. The average percent of control among the four full enzyme activity control tubes is necessarily 100 percent within each replicate. The average percent of control among the four background activity control tubes is necessarily 0 percent within each replicate.

Nominally for an inhibitor, the percent of control activity values vary between approximately 0 percent near the high inhibition concentrations and approximately 100 percent near the low inhibition concentrations, but this may vary with the inhibitor.

Chemicals I (aminoglutethimide), IV (4-nonylphenol), VI (fenarimol), VIII (chrysin), and IX (dicofol) depart from this behavior with respect to the bottom threshold. Chemicals V (dibenz[a,h]anthracene) and X (atrazine) are apparent non-inhibitors.

The percent of control activity responses at varying inhibitor concentrations within each replicate are displayed in Tables A-1a through A-1j for Chemicals I through X, respectively. The full enzyme activity, background activity, positive, and negative controls background adjusted aromatase activity and the percent of control data within each replicate are displayed in Tables A-2a through A-2j.

Several sets of chemicals were analyzed together on the same day with a single set of controls among them. Therefore, the control values were the same. They were: Chemical I and Chemical II's replicates 1 and 2; Chemical III and Chemical VI's replicates 2 and 3; Chemical IV and Chemical V's replicates 1, 2, and 3; Chemical VII and Chemical VIII's replicates 1 and 3; Chemical IX and Chemical X's replicates 1, 2, and 3, as seen in Tables A-2a to A-2j.

Some potentially outlying responses were identified in the percent of control activity responses inhibitor concentrations data, in that they differed from surrounding data values more than typically:

- Chemical I – Replicate 3 (assay date 7-29-05), level 5, Percent of Control value 92.33
- Chemical III – Replicate 4 (assay date 8-15-05), level 5, Percent of Control values 86.46, 83.19, 78.41; level 6, Percent of Control values 64.55, 64.87, 65.42
- Chemical IV – Replicate 1 (assay date 9-19-05), level 3, Percent of Control values 70.56, 36.18; level 6, Percent of Control values 86.27, 83.12; level 7, Percent of Control value 79.94; level 8, Percent of Control values 83.09, 72.09
- Chemical VI – Replicate 1 (assay date 8-3-05), level 1, Percent of Control values 49.67, 48.24; level 5, Percent of Control value 90.34
- Chemical VII – Replicate 2 (assay date 9-5-05), level 7, Percent of Control value 65.84
- Chemical VIII – Replicate 1 (assay date 8-29-05), level 1, Percent of Control values 15.94, 17.17, 24.36; level 7, Percent of Control values 77.23, 75.74, 74.01; Replicate 3 (assay date 9-6-05), level 4, Percent of Control value 59.47; level 7, Percent of Control value 90.12; Replicate 4 (assay date 8-15-05), level 1, Percent of Control values 30.18, 30.15, 29.90; level 3, Percent of Control values 79.67, 82.39, 79.26
- Chemical IX – Replicate 1 (assay date 9-8-05), level 2, Percent of Control value 65.30; level 3, Percent of Control value 62.91; level 7, Percent of Control value 77.24; Replicate 2 (assay date 9-12-05), level 6, Percent of Control values 79.19, 75.13, 78.88.

One full enzyme activity control value was identified as an outlier: Chemicals III/ VI's replicate 3 (assay date 8-8-05) (Chemicals III and VI shared controls), corrected aromatase activity value 3.28043, percent of control 50.563.

There were no indications for any of these values of departures from protocol test procedures or of clerical errors. It was thus decided to include all these values in the statistical analyses.

Objectives

The primary objectives of the statistical analysis are:

1. Fit concentration curves within each replicate to describe the trend in the percent of control activity across varying inhibitor concentrations for each of the ten test substances.
2. Estimate baseline response, maximum response, the IC_{50} concentration, slope, and associated standard errors within each replicate of each test substance.
3. Combine results across replicates within test substances to determine the average baseline response, average maximum response, average IC_{50} concentration, average slope, and associated standard errors among replicates.
4. Determine whether there are differences between the full enzyme activity, background activity, positive, and negative controls obtained at the beginning and those obtained at the end of each replicate within each test substance.
5. Assess the consistency of test conditions within replicates, across replicates within test substances, and across test substances based on the full enzyme activity, background activity, positive, and negative control values.

Statistical Analysis Methods

Concentration Response Trend Curves

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

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

Y ≡ (background corrected) percent of control in the inhibitor tube
X ≡ logarithm (base 10) of the concentration
DAVG ≡ average (not corrected for background) DPMs across the repeat tubes with the same inhibitor concentration

T ≡ top of plateau (maximum response)
B ≡ bottom of plateau (baseline response)
β ≡ slope of the concentration response curve (β is negative)
$\mu \equiv \log_{10}IC_{50}$ (IC_{50} is the concentration corresponding to the percent of control halfway between baseline and maximum responses)

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

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

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

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

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

For each replicate, the estimated baseline response (**B**) and its associated standard error, the estimated maximum response (**T**) and its associated standard error, the estimated $\log_{10}IC_{50}$ (μ) and its associated standard error, the IC_{50} and its associated geometric standard error, the slope (β) and its associated standard error, and the "Status" of each response curve are reported. The "Status" of each response curve is indicated as "C", complete, if the concentration response curve inhibition ranges from essentially 0 percent to at least 80 percent of control. It is indicated as "I", incomplete, if the concentration response curve inhibition ranges from essentially 100 percent inhibition to less than 50 percent inhibition. It is also indicated as "I", incomplete, if the concentration response curve decreases to between 80 percent and 50 percent of control. If most of the concentration response curve inhibition data do not go below 80 percent of control, the test substance is a non-inhibitor, parameter estimates are not calculated.

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

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

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

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

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

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

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

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

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

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

The control response analyses were carried out with the control data combined across 18 replicates within the ten test substances (several inhibitor chemicals were run simultaneously, with common controls). The control responses were expressed as percent of control. The full enzyme activity, background activity, positive, and negative controls percent of control responses were plotted across replicates, with plotting symbol distinguishing between beginning and end, and with reference line at 0 percent (background activity controls), at 100 percent (full enzyme activity controls), or at 100 percent (negative controls). These plots indicate the extent of consistency across replicates with respect to average value and variability, and provide comparisons of beginning versus end of each replicate. Two separate plots (with the same vertical scale) were generated, one for Chemicals I, II, III, IV, and V, and the other one for Chemicals VI, VII, VIII, IX, and X. Additional plots were prepared displaying the differences of the averages of the first two percent of control values (i.e. those based on the "beginning" tubes) and the average of the last two percent of control values (i.e. those based on the "end" tubes) across replicates. Each plot has a reference line at 0. Again, two separate plots (with the same vertical scale) were generated for Chemicals I to V and for Chemicals VI to X.

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

Round off

Some derived numbers in the results tables may differ from those in the computer printouts or from those obtained using hand calculations by several units in the least significant digit due to round off in intermediate numbers and in intermediate calculations.

Statistical Analysis Results

Concentration Response Trend Curves

Concentration response curves were fitted separately to the repeat tubes data within each replicate and to the averages of the repetitions within each replicate. Percent of control, the response variable for the statistical analyses, is displayed in Tables A-1a through A-1j for Chemicals I through X, respectively. The estimated parameters of the fitted concentration response curves are displayed in Tables 1-1, 2-1, 3-1, 4-1, 6-1, 7-1, 8-1, and 9-1 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively. The

concentration response curves fitted to the averages of the three repetitions within each replicate for Chemicals I, II, III, IV, VI, VII, VIII, and IX are displayed in Figures 1-1, 2-1, 3-1, 4-1, 6-1, 7-1, 8-1, and 9-1.

The parameters of the average concentration response curves, based on random effects analysis of variance model fits with replicate as a random effect are displayed in Tables 1-1, 2-1, 3-1, 4-1, 6-1, 7-1, 8-1, and 9-1 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively. ("Mean" under "Replicate" column). The parameters within each replicate are also displayed. The average concentration response curves, along with the averages of three repetitions within each replicate are plotted together in Figures 1-2, 2-2, 3-2, 4-2, 6-2, 7-2, 8-2, and 9-2 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively.

The parameter estimates for each replicate and the average parameter estimates across replicates and their associated 95 percent confidence intervals are displayed in Tables 1-2, 2-2, 3-2, 4-2, 6-2, 7-2, 8-2, and 9-2 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively. The corresponding graphs for the bottom threshold parameter are presented in Figures 1-3, 2-3, 3-3, 4-3, 6-3, 7-3, 8-3, and 9-3; for the top threshold parameter, 1-4, 2-4, 3-4, 4-4, 6-4, 7-4, 8-4, and 9-4; for $\log_{10}IC_{50}$, Figures 1-5, 2-5, 3-5, 4-5, 6-5, 7-5, 8-5, and 9-5; and for slope, Figures 1-6, 2-6, 3-6, 4-6, 6-6, 7-6, 8-6, and 9-6.

The results of analyses of variance for these estimates are presented in Tables 1-3, 2-3, 3-3, 4-3, 6-3, 7-3, 8-3, and 9-3 for Chemicals I, II, III, IV, VI, VII, VIII, and IX, respectively. For each replicate the squares of the standard errors associated with each parameter are given. These estimates include only within replicate variation. Across replicates, the replicate-to-replicate variation and the square of the standard error of the overall average are displayed. These estimates include both within replicate variation and replicate-to-replicate variation.

Except for one outlying response (replicate 1, $\log_{10}$concentration= -9.00), all other responses were above 79 percent of control for Chemical V (dibenz[a,h]anthracene). All responses were above 80 percent of control for Chemical X (atrazine). Both chemicals were determined to be non-inhibitors. Their parameters were not estimable. Response curve analysis was not performed for these two chemicals. Averages of percent of control of the three repetitions within each replicate are displayed in Figures 5-1 and 10-1 for Chemicals V and X, respectively. Figures 5-2, 5-3, and 5-4 display the percent of control values of the three repetitions for each of the replicates 1, 2, and 3, respectively for Chemical V. Figures 10-2, 10-3, 10-4 display the percent of control values of the three repetitions for each of the replicates 1, 2, and 3, respectively for Chemical X. Percent of control values are shown in Tables A-1e for Chemical V and A-1j for Chemical X.

Chemical I (aminoglutethimide): Response Curve Analysis

Figures 1-1 and 1-2 show that most responses reached or exceeded 100 percent of control at the $\log_{10}$ concentration=-6 for replicates 1 and 2. Replicate 3 had lower

responses and did not reach 100 percent of control. The parameter estimates and their 95 percent confidence intervals are displayed in Table 1-2 and plotted in Figures 1-3 to 1-6. Figure 1-3 shows that the bottom threshold estimates were close to the average and were similar across replicates. Figure 1-4 shows large variation among the top threshold estimates across replicates and a large standard error of the overall mean top threshold estimate. Figure 1-5 shows variation among the $\log_{10}IC_{50}$ estimates for the three replicates and a relatively large standard error of the overall mean. For slope, the estimates were consistent across the replicates, as can be seen in Figure 1-6.

Table 1-3 displays the estimated variance components for the parameter estimates. The replicate-to-replicate variation for bottom parameter was zero, reflecting the consistency across replicates. For the top parameter, the replicate-to-replicate variation attributed 80 percent of overall variation. The replicate-to-replicate variation was about thirty times or more greater than the within replicate variation. The replicate-to-replicate variation was more than six times the within replicate variation for $\log_{10}IC_{50}$. it was comparable to the within replicate variation for slope.

Chemical II (ketoconazole): Response Curve Analysis

Figures 2-1 and 2-2 show that replicates 1, 2, and 4 were in line with one another, except for top threshold where replicate 4 was a bit lower. Responses reached 100 percent of control at $\log_{10}$ concentration=-8.00 for each replicate. Figure 2-3 and Table 2-1 show consistency among the bottom threshold estimates. Figure 2-4 shows some variation among the top threshold estimates, with replicate 4 somewhat lower than replicates 1 and 2. This results in a relatively large standard error for the overall average. The $\log_{10}IC_{50}$ estimates were consistent across replicates, as seen in Figure 2-5. Figure 2-6 shows that slope estimates were also consistent across replicates.

Table 2-3 shows that for the bottom threshold, $\log_{10}IC_{50}$, and slope parameters, the replicate-to-replicate variation was zero. For the top threshold, the replicate-to-replicate variance was large relative to the within replicate variances. For $\log_{10}IC_{50}$ and slope, the within-replicate variances were close to zero.

Chemical III (prochloraz): Response Curve Analysis

Figures 3-1 and 3-2 display considerable variation across replicates. Replicate 4 is out of line with replicates 2 and 3 with respect to slope, $\log_{10}IC_{50}$, and top threshold parameters. Replicate 2 and 3 differ with respect to top threshold. Table 3-1 shows that replicate 4 had a relatively low top threshold and a very steep slope (order of magnitude greater than the other replicates). Figures 3-3, 3-5, and 3-6 show that standard errors for replicate 4 were greater than those for replicates 2 and 3 for bottom threshold, $\log_{10}IC_{50}$, and slope. Therefore they were discounted when calculating weighted averages across replicates. Figure 3-4 shows that the top threshold parameter estimates had relatively low standard errors within replicates and varied considerably across replicates, resulting in a relatively large standard error for the overall average.

Table 3-3 shows that the replicate-to-replicate variation was zero or close to zero for the bottom threshold , $\log_{10}IC_{50}$, and slope. The within replicate variance for replicate 4 was order of magnitude larger than those for replicates 2 and 3, for these parameters. For the top threshold parameter, the replicate-to-replicate variation was order of magnitude grater than the within replicate variation for each replicate.

Chemical IV (4-Nonylphenol): Response Curve Analysis

Figures 4-1 and 4-2 show that the replicates were in line with one another. Table 4-1 and Figure 4-3 show that the bottom threshold estimate was relatively poorly determined in replicate 1 and were near zero in replicates 2 and 3 and for the overall average. Figure 4-4 shows that the top threshold estimate was relatively poorly determined in replicate 1 and were close to one another in replicates 2 and 3 and for the overall average. Figures 4-5 and 4-6 show that the $\log_{10}IC_{50}$ estimate and the slope estimate were relatively poorly determined in replicate 1 and were close to one another in replicates 2 and 3 and the overall average.

Table 4-3 shows that the replicate-to-replicate variation was zero or small for the bottom, $\log_{10}IC_{50}$, and slope parameters. For the top threshold parameter, the replicate-to-replicate variation was comparable to the within replicate variation for replicates 2 and 3. For all the parameters replicate 1's within replicate variation was close to or more than order of magnitude greater than those of the other replicates.

Chemical V (dibenz[a,h]anthracene): Response Curve Analysis

Dibenz[a,h]anthracene is a non-inhibitor chemical. All responses but one (replicate 1) were above 79 percent of control. Response curve analysis was not performed for this chemical since its parameters were not estimable. Averages of percent of control of the three repetitions within each replicate are displayed in Figure 5-1. The percent of control values of the three repetitions for each of the three replicates are displayed in Figures 5-2, 5-3, and 5-4. The individual data values are shown in Tables A-1e.

Chemical VI (fenarimol): Response Curve Analysis

Figures 6-1 and 6-2 show that there is wide variation in the bottom threshold estimates between replicate 1 and the other replicates. Table 6-1 and Figure 6-3 show wide variation among the bottom threshold estimates, with that for replicate 1 at about 30 percent inhibition. This results in a relatively large standard error for the overall mean. Table 6-1 and Figure 6-4 show wide variation in the top threshold estimate. This results in a relatively large standard error for the overall mean. Table 6-1 and Figure 6-5 show that there is relatively wide variation in the $\log_{10}IC_{50}$ estimates between replicate 1 and the other two replicates. This results in a relatively large standard error for the overall mean. Table 6-1 and Figure 6-6 show that replicate 1 has nearly order of magnitude greater variability in the slope estimate than the other replicates.

It should be noted that the "$\log_{10}IC_{50}$" estimate actually estimates the (B+T)/2 percent inhibition concentration of the concentration response relation. For fenarimol this corresponds to 58.1, 45.5, and 55.7 percent inhibition, which influence the variation in the estimates among replicates.

Table 6-3 shows substantially greater replicate-to-replicate variation than within replicate variation for the bottom, top, and $\log_{10}IC_{50}$ estimates. For slope, replicate 1 has order of magnitude greater within replicate variation than the other replicates, or the replicate-to replicate variation which is zero.

Chemical VII (econazole): Response Curve Analysis

Figures 7-1 and 7-2 show that replicate 1 and 2's responses were in line with one other and that replicate 3 had higher responses. Table 7-1 and Figure 7-3 show that the bottom threshold estimates were consistent with one another. Table 7-1 and Figure 7-4 show that for the top threshold parameter replicate 3 differed from replicates 1 and 2, resulting in a relatively large standard error for the overall mean.

Table 7-1 and Figures 7-5 and 7-6 show consistency among the replicates for the $\log_{10}IC_{50}$ and the slope estimates.

Table 7-3 shows that the replicate-to-replicate variation was zero for the bottom threshold, the $\log_{10}IC_{50}$, and the slope parameters. For the top threshold parameter, the replicate-to-replicate variation was more than 7.7 times the within replicate variation.

Chemical VIII (chrysin): Response Curve Analysis

Figures 8-1 and 8-2 show that there is considerable variation among replicates for the top and bottom threshold and the slope parameters. The slope for replicate 4 is very imprecisely determined, as reflected in the very large standard error. Table 8-1 and Figure 8-3 show that the bottom threshold for replicate 3 is relatively imprecisely determined and that for replicate 4 is much higher those for replicates 1 and 3. Both replicates 1 and 3 have relatively high bottom thresholds (13.8 percent and 29.0 percent, respectively). This results in a relatively high standard error for the overall average. Table 8-1 and Figure 8-4 show that the top threshold estimate for replicate 3 is somewhat higher than those for replicates 1 and 4. This results in a relatively high standard error for the overall average. Table 8-1 and Figures 8-5 and 8-6 show very high standard errors for the $\log_{10}IC_{50}$ and slope estimates in replicate 4, compared to those in replicates 1 and 3.

Table 8-3 shows large replicate-to-replicate variation for the bottom and top threshold parameter estimates. For the $\log_{10}IC_{50}$ and the slope the within replicate variances in replicate 4 were so large, that the replicate 4 values were effectively not determined and were excluded from the overall average.

Chemical IX (dicofol): Response Curve Analysis

Figure 9-1 and 9-2 show differences among the replicates for the bottom and the top threshold estimates and for the slope. All the bottom threshold parameter estimates were high: 49.7, 45.9, and 28.2 percent, for replicates 1, 2, and 3 respectively. Table 9-1 and Figure 9-3 show variability between the bottom threshold parameter for replicate 3 and those for replicates 1 and 2. This results in a relatively high standard error for the overall average. Table 9-1 and Figure 9-4 show some variability among the top threshold parameter estimates. This results in some increase in the variability for the overall average. Table 9-1 and Figures 9-5 and 9-6 show consistency among replicates for the $\log_{10}IC_{50}$ and the slope parameter estimates. Replicate 2 has the largest within replicate variation, particularly for slope.

Table 9-3 shows that for the bottom threshold parameter, the replicate-to-replicate variation was an order of magnitude larger than the within replicate variation. For the top threshold parameter, the replicate-to-replicate variation was also large and was more than twice the within replicate variation.

For $\log_{10}IC_{50}$ and slope, the within-replicate variances for replicates 1, 2, and 3 were close to zero, with replicate 2 having the largest variance among the three replicates. For slope, the replicate-to-replicate variance was zero.

Chemical X (atrazine): Response Curve Analysis

Atrazine is a non-inhibitor chemical. All responses were above 80 percent of control for three replicates. Response curve analysis was not performed for this chemical since its parameters were not estimable. Averages of percent of control of the three repetitions within each replicate are displayed in Figure 10-1. The percent of control values of the three repetitions for each of the three replicates are displayed in Figures 10-2, 10-3, and 10-4 and the individual data 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

Inhibitors were tested in pairs (as shown in Table 11), with common controls. For some test substances there was partial commonality of controls. For example for test substances I/II there were four replicates carried out among the two substances, three replicates per substance. Across the ten substances there were 18 replicates.

The control data were combined across the 18 replicates among the ten test substances. The control responses were expressed as percent of control. The full enzyme activity, background activity, positive, and negative control responses for each replicate are displayed in Tables A-2a through A-2j for Chemicals I through X, respectively. The percent of control data are plotted by replicate in Figures 11a, 11b, 12a, 12b, 13a, 13b, 14a, and 14b with plotting symbol distinguishing between beginning and end of the replicate. Figures 15a, 15b, 16a, 16b, 17a, 17b, 18a, and 18b show the differences

between the averages at the beginning and at the end within each replicate (end minus beginning). In the horizontal axis of these figures, "I-1" stands for replicate 1 of Chemical I, "V-2" stands for replicate 2 of Chemical V, and so on. The chemicals in these figures were grouped by the first five chemicals and the last five chemicals. Figures 11a, 12a, 13a, 14a, 15a, 16a, 17a, and 18a display the results for Chemicals I, II, III, IV, and V; Figures 11b, 12b, 13b, 14b, 15b, 16b, 17b, and 18b display the results for Chemicals VI, VII, VIII, IX, and X. For the same figure number, "a" plots and "b" plots have the same vertical scale.

Several sets of chemicals/replicates were analyzed together on the same day with one set of controls between them. Therefore, the control values were the same. They were: Chemical I and Chemical II's replicates 1 and 2; Chemical III and Chemical VI's replicates 2 and 3; Chemical IV and Chemical V's replicates 1, 2, and 3; Chemical VII and Chemical VIII's replicates 1 and 3; Chemical IX and Chemical X's replicates 1, 2, and 3.

Mixed effects analysis of variance models were fitted to the full enzyme activity, background activity, positive, and negative controls percent of control data. The fixed effect factors in the analysis of variance were test substance, portion (beginning or end), and portion by test substance interaction. The random effects were replicate nested within test substance and portion by replicate within test substance interaction. The residual error variation corresponded to variation among repetitions within test substance, replicate, and portion. For the background and full enzyme activity control responses the component of variation due to replicate is constrained to be 0 by the definitions of these control responses. The analysis results for the four types of controls are displayed in Table 11. The left panel of the table displays the results of the tests for the differences between the responses collected at the beginning and at the end of a replicate. The right panel displays the estimated variance components across replicate within test substances and across portion by replicate interaction within test substances.

The chemical main effect and the chemical by portion interaction effect were not significant for any of the control types.

Figures 11a and 11b show that for the full enzyme activity controls the majority of the control responses were lower at the end of a replicate than at the beginning. This is reflected in Figures 15a and 15b, with that the majority of the curves falling below zero and in the significant portion effects for the full enzyme activity control in Table 11 ($p=0.003$ and estimate of end minus beginning=-6.354 percent).

Figures 11a and 11b and Table 11 show that the average levels and the variability about the average levels were consistent for across all the replicates for the full enzyme activity for test substances I, II, IV, V, VII, VIII, IX, and X (i.e. all but test substances III/VI). Test conditions appear to have been consistent among those test substances and the replicates within test substances for the full enzyme activity. Chemicals III/VI replicate 3 had one control response at the end at about 50 percent of control and two control responses at the beginning at more than 120 percent of control. These outlying

responses resulted in high replicate by portion interaction variation for Chemicals III/ VI. The remaining replicate and replicate*portion variances were zero. The large majority of the full enzyme activity control values were between 90 and 110 percent of control.

Figures 12a, 12b, 16a, and 16b and Table 11 show that the background activity controls except for several isolated replicates (replicate 1 of Chemicals I/II, VI, IX/X, and replicate 2 of Chemicals IX/X), there was little variability for both the beginning and the end. The difference between the beginning and the end was close to zero and was not significant. There was very little variation for the replicate main effect and the replicate by portion interaction.

Figures 13a and 13b show that the majority of positive controls were between 40 percent and 55 percent of control. The end and the beginning portions showed no consistent difference. The portion effect (i.e. end minus beginning) was not significant. Table 11 shows that the replicate-to-replicate variation was high for Chemicals III /VI. This was due to the relatively high responses in replicate 3.

Figures 14a and 14b show that the negative control values were for the most part between 70 percent and 110 percent of control. The ends of portions tended to be greater than the beginnings. The average difference (end minus beginning) was positive and approaching significance ($p=0.054$). Table 11 shows that the replicate-to-replicate variance for the negative controls was high for Chemicals I/II and III/VI. The replicate by portion interaction variance was high for Chemicals III/VI, VII/VIII, and IX,/X.

With the exception of several test substances and replicates, the test system was consistent across chemicals and replicates within chemicals. For the full enzyme activity controls there was a significant average decrease in control activity between the beginning and the end portions across all the test replicates. For the negative controls there was an average increase in control activity between the beginning and the end portions for the mean across all the test replicates. The average increase approached significance ($p=0.054$).

Variation in aromatase activity during the course of a replicate run can result in potential bias or potential loss of precision due to decrease or increase of aromatase activity within replicates. If the inhibition concentration tubes are tested in order of inhibitor concentration, the change in aromatase activity across the replicate can accentuate or can lessen the apparent inhibition, depending on the ordering of the test substance concentrations. If the 24 tubes (8 inhibitor concentrations × 3 repetitions per concentration) were tested in randomized order then the change in aromatase activity within the replicate would increase the residual variation about the fitted concentration response curves for each replicate but would avoid resulting in biased response curves.

Table 1-1. Reference Chemical I: Aminoglutethimide. Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates. Percent of Control Activity. Recombinant Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	2.778 (2.862)	112.100 (1.156)	-4.333 (0.045)	4.648E-05 (1.110)	-1.109 (0.092)	C
2	6.424 (4.152)	102.700 (2.007)	-4.532 (0.056)	2.936E-05 (1.139)	-1.365 (0.211)	C
3	6.938 (3.521)	90.110 (1.838)	-4.618 (0.055)	2.407E-05 (1.136)	-1.496 (0.244)	C
Mean [c]	4.876 (1.958)	101.693 (6.394)	-4.492 (0.086)	3.223E-05 (1.218)	-1.248 (0.125)	C
Average Values [b]						
1	2.778 (5.070)	112.100 (2.048)	-4.333 (0.080)	4.648E-05 (1.203)	-1.109 (0.163)	C
2	6.446 (8.670)	102.700 (4.195)	-4.533 (0.118)	2.934E-05 (1.311)	-1.366 (0.441)	C
3	6.938 (7.436)	90.110 (3.882)	-4.618 (0.117)	2.407E-05 (1.309)	-1.496 (0.514)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 1-2. Reference Chemical I: Aminoglutethimide. Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals. Percent of Control Activity. Recombinant Aromatase Assay.

Parameter	Estimate (95 percent CI)			
	Replicate 1[a]	Replicate 2[a]	Replicate 3[a]	Mean[b]
Bottom	2.778 (-3.192,8.748)	6.424 (-2.237,15.085)	6.938 (-0.407,14.283)	4.876 (0.952,8.800)
Top	112.100 (109.689,114.511)	102.700 (98.513,106.887)	90.110 (86.276,93.944)	101.693 (74.380,129.007)
$Log_{10}IC_{50}$	-4.333 (-4.427,-4.239)	-4.532 (-4.650,-4.414)	-4.618 (-4.733,-4.503)	-4.492 (-4.850,-4.133)
Slope	-1.109 (-1.300,-0.918)	-1.365 (-1.804,-0.926)	-1.496 (-2.004,-0.988)	-1.248 (-1.641,-0.855)

a. Parameter estimates and their associated 95 percent confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95 percent confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 1-3. Reference Chemical I: Aminoglutethimide.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 2	Replicate 3	Random Replicate (p-value) [d]	Variance of Mean
Bottom	8.19104 /df=20	17.23910 /df=20	12.39744 /df=20	0.00000 /df=2 (p=NA)	3.83503 /df=55.251
Top	1.33634 /df=20	4.02805 /df=20	3.37824 /df=20	119.72972 /df=2 (p=0.164)	40.87772 /df=2.015
$Log_{10}IC_{50}$	0.00205 /df=20	0.00318 /df=20	0.00306 /df=20	0.01923 /df=2 (p=0.187)	0.00733 /df=2.057
Slope	0.00842 /df=20	0.04435 /df=20	0.05929 /df=20	0.01972 /df=2 (p=0.332)	0.01567 /df=3.073

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \Sigma(Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 2-1. Reference Chemical II: Ketoconazole. Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates. Percent of Control Activity. Recombinant Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-0.753 (1.590)	111.200 (0.965)	-6.375 (0.035)	4.218E-07 (1.083)	-0.933 (0.056)	C
2	-1.168 (2.954)	114.200 (1.792)	-6.476 (0.065)	3.340E-07 (1.162)	-0.841 (0.088)	C
4	0.206 (1.666)	106.000 (1.049)	-6.387 (0.038)	4.102E-07 (1.092)	-1.010 (0.070)	C
Mean [c]	-0.411 (1.072)	110.321 (2.366)	-6.393 (0.024)	4.043E-07 (1.057)	-0.939 (0.039)	C
Average Values [b]						
1	-0.753 (2.517)	111.200 (1.528)	-6.375 (0.055)	4.218E-07 (1.135)	-0.933 (0.089)	C
2	-1.168 (1.837)	114.200 (1.114)	-6.476 (0.040)	3.340E-07 (1.098)	-0.841 (0.055)	C
4	0.206 (2.073)	106.000 (1.304)	-6.387 (0.048)	4.102E-07 (1.116)	-1.010 (0.087)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 2-2. Reference Chemical II: Ketoconazole.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay.

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 4 [a]	Mean [b]
Bottom	-0.753 (-4.070,2.563)	-1.168 (-7.330,4.994)	0.206 (-3.270,3.681)	-0.411 (-2.572,1.750)
Top	111.200 (109.186,113.214)	114.200 (110.462,117.938)	106.000 (103.812,108.188)	110.321 (99.982,120.660)
$Log_{10}IC_{50}$	-6.375 (-6.447,-6.303)	-6.476 (-6.612,-6.340)	-6.387 (-6.467,-6.307)	-6.393 (-6.441,-6.345)
Slope	-0.933 (-1.050,-0.815)	-0.841 (-1.024,-0.657)	-1.010 (-1.156,-0.864)	-0.939 (-1.017,-0.860)

a. Parameter estimates and their associated 95 percent confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95 percent confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 2-3. Reference Chemical II: Ketoconazole.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 2	Replicate 4	Random Replicate (p-value) [d]	Variance of Mean
Bottom	2.52810 /df=20	8.72612 /df=20	2.77556 /df=20	0.00000 /df=2 (p=NA)	1.14884 /df=43.625
Top	0.93200 /df=20	3.21126 /df=20	1.10040 /df=20	15.11208 /df=2 (p=0.187)	5.59959 /df=1.969
$Log_{10}IC_{50}$	0.00120 /df=20	0.00424 /df=20	0.00147 /df=20	0.00000 /df=2 (p=NA)	0.00057 /df=44.274
Slope	0.00317 /df=20	0.00774 /df=20	0.00491 /df=20	0.00000 /df=2 (p=NA)	0.00154 /df=53.192

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \Sigma(Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

**Table 3-1. Reference Chemical III: Prochloraz.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay.**

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
2	-0.345 (0.691)	93.080 (1.027)	-7.724 (0.025)	1.889E-08 (1.060)	-0.905 (0.038)	C
3	0.423 (0.788)	114.800 (1.096)	-7.687 (0.023)	2.056E-08 (1.054)	-1.011 (0.040)	C
4	1.151 (2.520)	80.740 (2.520)	-7.040 (59.720)	9.122E-08 (5.248E59)	-12.640 (18462.000)	C
Mean [c]	0.037 (0.509)	96.295 (9.942)	-7.704 (0.018)	1.978E-08 (1.043)	-0.957(0.053)	C
Average Values [b]						
2	-0.345 (0.732)	93.080 (1.086)	-7.724 (0.027)	1.889E-08 (1.064)	-0.905 (0.040)	C
3	0.423 (0.397)	114.800 (0.552)	-7.687 (0.011)	2.056E-08 (1.027)	-1.011 (0.020)	C
4	1.151 (5.449)	80.740 (5.449)	-7.040 (131.500)	9.123E-08 (3.16E131)	-12.660 (40783.000)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

**Table 3-2. Reference Chemical III: Prochloraz.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay.**

Parameter	Estimate (95 percent CI)			
	Replicate 2 [a]	Replicate 3 [a]	Replicate 4 [a]	Mean [b]
Bottom	-0.345 (-1.787,1.097)	0.423 (-1.220,2.066)	1.151 (-4.106,6.408)	0.037 (-1.007,1.081)
Top	93.080 (90.938,95.222)	114.800 (112.514,117.086)	80.740 (75.483,85.997)	96.295 (53.412,139.178)
$Log_{10}IC_{50}$	-7.724 (-7.777,-7.671)	-7.687 (-7.734,-7.640)	-7.040 (-131.614,117.534)	-7.704 (-7.742,-7.665)
Slope	-0.905 (-0.984,-0.825)	-1.011 (-1.095,-0.927)	-12.640 (-38523.7,38498.42)	-0.957 (-1.068,-0.846)

a. Parameter estimates and their associated 95 percent confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95 percent confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

**Table 3-3. Reference Chemical III: Prochloraz.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay.**

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 2	Replicate 3	Replicate 4	Random Replicate (p-value) [d]	Variance of Mean
Bottom	0.47790 /df=20	0.62031 /df=20	6.35040 /df=20	0.00000 /df=2 (p=NA)	0.25893 /df=27.103
Top	1.05473 /df=20	1.20122 /df=20	6.35040 /df=20	293.69899 /df=2 (p=0.161)	98.84915 /df=1.995
$Log_{10}IC_{50}$	0.00064 /df=20	0.00052 /df=20	3566.47840 /df=20	0.00011 /df=2 (p=0.456)	0.00034 /df=20.000
Slope	0.00146 /df=20	0.00163 /df=20	3.4×10^{8} /df=20	0.00410 /df=2 (p=0.304)	0.00282 /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. Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates. Percent of Control Activity. Recombinant Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-8.134 (24.870)	90.700 (2.745)	-4.652 (0.263)	2.226E-05 (1.832)	-1.045 (0.423)	C
2	-0.968 (1.835)	94.820 (0.486)	-4.814 (0.018)	1.534E-05 (1.042)	-1.628 (0.098)	C
3	-3.551 (3.386)	92.690 (0.761)	-4.742 (0.032)	1.813E-05 (1.077)	-1.526 (0.142)	C
Mean [c]	-1.582 (1.610)	93.493 (1.014)	-4.781 (0.035)	1.656E-05 (1.084)	-1.576 (0.079)	C
Average Values [b]						
1	-8.186 (33.370)	90.700 (3.676)	-4.652 (0.353)	2.229E-05 (2.252)	-1.045 (0.566)	C
2	-0.968 (3.269)	94.820 (0.866)	-4.814 (0.032)	1.534E-05 (1.076)	-1.628 (0.174)	C
3	-3.552 (3.939)	92.690 (0.885)	-4.742 (0.038)	1.813E-05 (1.090)	-1.526 (0.165)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

**Table 4-2. Reference Chemical IV: 4-Nonylphenol.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay.**

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	-8.134 (-60.012,43.744)	-0.968 (-4.796,2.860)	-3.551 (-10.614,3.512)	-1.582 (-4.930,1.766)
Top	90.700 (84.974,96.426)	94.820 (93.806,95.834)	92.690 (91.104,94.276)	93.493 (90.795,96.190)
$Log_{10}IC_{50}$	-4.652 (-5.201,-4.103)	-4.814 (-4.851,-4.777)	-4.742 (-4.809,-4.675)	-4.781 (-4.854,-4.708)
Slope	-1.045 (-1.927,-0.163)	-1.628 (-1.832,-1.424)	-1.526 (-1.821,-1.231)	-1.576 (-1.738,-1.414)

a. Parameter estimates and their associated 95 percent confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95 percent confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 4-3. Reference Chemical IV: 4-Nonylphenol. Variances Associated with Estimated Parameters of Concentration Response Curves. Percent of Control Activity. Recombinant Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 2	Replicate 3	Random Replicate (p-value) [d]	Variance of Mean
Bottom	618.51690 /df=20	3.36723 /df=20	11.46500 /df=20	0.00000 /df=2 (p=NA)	2.59189 /df=20.963
Top	7.53503 /df=20	0.23620 /df=20	0.57836 /df=20	1.91257 /df=2 (p=0.264)	1.02808 /df=4.493
$Log_{10}IC_{50}$	0.06917 /df=20	0.00032 /df=20	0.00104 /df=20	0.00186 /df=2 (p=0.295)	0.00122 /df=19.776
Slope	0.17859 /df=20	0.00956 /df=20	0.02002 /df=20	0.00000 /df=2 (p=NA)	0.00624 /df=26.762

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \Sigma(Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 5-1. Reference Chemical V: Dibenz[a, h]anthracene. Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates. Percent of Control Activity. Recombinant Aromatase Assay.

Only a few data points were below 80 percent (79.8, 79.0, 59.4) 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.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	31.360 (4.039)	84.780 (2.551)	-5.752 (0.097)	1.770E-06 (1.249)	-2.275 (0.778)	C
2	-1.169 (2.383)	92.230 (0.400)	-5.317 (0.031)	4.823E-06 (1.073)	-0.937 (0.043)	C
3	2.230 (5.010)	109.100 (0.920)	-5.323 (0.055)	4.749E-06 (1.134)	-0.998 (0.089)	C
Mean [c]	10.747 (10.347)	95.493 (7.177)	-5.452 (0.138)	3.531E-06 (1.373)	-0.952 (0.038)	C
Average Values [b]						
1	31.360 (2.471)	84.780 (1.562)	-5.752 (0.059)	1.770E-06 (1.146)	-2.275 (0.476)	C
2	-1.169 (1.559)	92.230 (0.262)	-5.317 (0.020)	4.823E-06 (1.047)	-0.937 (0.028)	C
3	2.230 (5.989)	109.100 (1.099)	-5.323 (0.066)	4.749E-06 (1.163)	-0.998 (0.106)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

**Table 6-2. Reference Chemical VI: Fenarimol.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay.**

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	31.360 (22.935,39.785)	-1.169 (-6.140,3.802)	2.230 (-8.221,12.681)	10.747 (-33.515,55.008)
Top	84.780 (79.459,90.101)	92.230 (91.395,93.065)	109.100 (107.182,111.018)	95.493 (64.502,126.483)
$Log_{10}IC_{50}$	-5.752 (-5.954,-5.550)	-5.317 (-5.381,-5.253)	-5.323 (-5.437,-5.209)	-5.452 (-6.100,-4.805)
Slope	-2.275 (-3.897,-0.653)	-0.937 (-1.026,-0.848)	-0.998 (-1.184,-0.813)	-0.952 (-1.032,-0.872)

a. Parameter estimates and their associated 95 percent confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95 percent confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 6-3. Reference Chemical VI: Fenarimol.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 2	Replicate 3	Random Replicate (p-value) [d]	Variance of Mean
Bottom	16.31352 /df=20	5.67869 /df=20	25.10010 /df=20	305.70927 /df=2 (p=0.170)	107.06997 /df=2.012
Top	6.50760 /df=20	0.16032 /df=20	0.84548 /df=20	152.06448 /df=2 (p=0.163)	51.50575 /df=1.993
$Log_{10}IC_{50}$	0.00934 /df=20	0.00095 /df=20	0.00300 /df=20	0.05271 /df=2 (p=0.189)	0.01897 /df=1.833
Slope	0.60466 /df=20	0.00182 /df=20	0.00790 /df=20	0.00000 /df=2 (p=NA)	0.00148 /df=20.645

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \Sigma(Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 7-1. Reference Chemical VII: Econazole.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	-0.013 (0.305)	89.810 (1.075)	-8.783 (0.018)	1.648E-09 (1.043)	-0.990 (0.034)	C
2	0.267 (1.137)	92.020 (2.918)	-8.808 (0.043)	1.557E-09 (1.103)	-1.081 (0.093)	C
3	-0.157 (1.097)	106.000 (2.991)	-8.778 (0.039)	1.668E-09 (1.094)	-0.984 (0.071)	C
Mean [c]	-0.005 (0.284)	95.719 (5.015)	-8.786 (0.016)	1.639E-09 (1.036)	-0.998 (0.029)	C
Average Values [b]						
1	-0.013 (0.016)	89.810 (0.056)	-8.783 (0.001)	1.648E-09 (1.002)	-0.990 (0.002)	C
2	0.267 (0.664)	92.020 (1.704)	-8.808 (0.025)	1.557E-09 (1.059)	-1.081 (0.054)	C
3	-0.157 (1.849)	106.000 (5.045)	-8.778 (0.066)	1.668E-09 (1.164)	-0.984 (0.120)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

**Table 7-2. Reference Chemical VII: Econazole.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay.**

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 2 [a]	Replicate 3 [a]	Mean [b]
Bottom	-0.013 (-0.649,0.623)	0.267 (-2.105,2.639)	-0.157 (-2.446,2.131)	-0.005 (-0.579,0.568)
Top	89.810 (87.568,92.052)	92.020 (85.933,98.107)	106.000 (99.761,112.239)	95.719 (73.799,117.639)
$Log_{10}IC_{50}$	-8.783 (-8.821,-8.745)	-8.808 (-8.897,-8.719)	-8.778 (-8.859,-8.697)	-8.786 (-8.817,-8.754)
Slope	-0.990 (-1.062,-0.919)	-1.081 (-1.275,-0.887)	-0.984 (-1.133,-0.836)	-0.998 (-1.057,-0.939)

a. Parameter estimates and their associated 95 percent confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95 percent confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 7-3. Reference Chemical VII: Econazole.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 2	Replicate 3	Random Replicate (p-value) [d]	Variance of Mean
Bottom	0.09290 /df=20	1.29277 /df=20	1.20341 /df=20	0.00000 /df=2 (p=NA)	0.08085 /df=42.859
Top	1.15563 /df=20	8.51472 /df=20	8.94608 /df=20	69.41526 /df=2 (p=0.181)	25.14865 /df=1.968
$Log_{10}IC_{50}$	0.00034 /df=20	0.00181 /df=20	0.00152 /df=20	0.00000 /df=2 (p=NA)	0.00024 /df=47.233
Slope	0.00117 /df=20	0.00868 /df=20	0.00507 /df=20	0.00000 /df=2 (p=NA)	0.00086 /df=43.471

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \Sigma(Si^4/df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 8-1. Reference Chemical VIII: Chrysin.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	13.750 (2.457)	82.560 (1.521)	-5.660 (0.095)	2.190E-06 (1.246)	-1.690 (0.396)	C
3	5.536 (8.304)	99.410 (1.819)	-5.511 (0.115)	3.085E-06 (1.302)	-0.889 (0.167)	C
4	29.040 (2.970)	86.520 (1.328)	-5.423 (1063.000)	3.776E-06 (NA)	-15.840 (282689.0)	C
Mean [c]	17.399 (6.684)	89.447 (5.057)	-5.599 (0.073)	2.518E-06 (1.184)	-1.209 (0.393)	C
Average Values [b]						
1	13.750 (4.936)	82.560 (3.055)	-5.660 (0.191)	2.190E-06 (1.554)	-1.690 (0.794)	C
3	5.534 (11.600)	99.410 (2.542)	-5.511 (0.160)	3.085E-06 (1.445)	-0.889 (0.233)	C
4	29.050 (6.229)	86.520 (2.786)	-5.387 (2.504)	4.105E-06 (319.154)	-9.607 (192.700)	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. The weighted average for the slope parameter could not be estimated since the slope's one-way analysis of variance model did not converge.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

**Table 8-2. Reference Chemical VIII: Chrysin.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay.**

Parameter	Estimate (95 percent CI)			
	Replicate 1 [a]	Replicate 3 [a]	Replicate 4 [a]	Mean [b]
Bottom	13.750 (8.625,18.875)	5.536 (-11.786,22.858)	29.040 (22.845,35.235)	17.399 (-10.257,45.054)
Top	82.560 (79.387,85.733)	99.410 (95.616,103.204)	86.520 (83.750,89.290)	89.447 (67.441,111.453)
$Log_{10}IC_{50}$	-5.660 (-5.859,-5.461)	-5.511 (-5.750,-5.272)	-5.423 (-2222.80,2211.956)	-5.599 (-5.752,-5.446)
Slope	-1.690 (-2.516,-0.864)	-0.889 (-1.236,-0.541)	-15.840 (-589695,589663.1)	-1.209 (-2.028,-0.390)

a. Parameter estimates and their associated 95 percent confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95 percent confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect. The mean and its 95 percent confidence interval for the slope parameter could not be estimated since the slope's analysis of variance model did not converge.

Table 8-3. Reference Chemical VIII: Chrysin.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
				Overall	
	Replicate 1	Replicate 3	Replicate 4	Random Replicate (p-value) [d]	Variance of Mean
Bottom	6.03685 /df=20	68.95642 /df=20	8.82090 /df=20	111.35619 /df=2 (p=0.207)	44.67203 /df=2.086
Top	2.31344 /df=20	3.30876 /df=20	1.76358 /df=20	74.25723 /df=2 (p=0.168)	25.57128 /df=1.977
$Log_{10}IC_{50}$	0.00910 /df=20	0.01311 /df=20	1129969.00 /df=20	0.00000 /df=2 (p=NA)	0.00537 /df=20.000
Slope	0.15682 /df=20	0.02779 /df=20	7.99131E10 /df=20	0.22882 /df=2 (p=0.307)	0.15408 /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). The variance of mean for slope parameter could not be estimated since the slope's analysis of variance model did not converge.
d. p-value is based on the Wald Z-test result.

Table 9-1. Reference Chemical IX: Dicofol.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay.

Replicate	Bottom (SE)	Top (SE)	$Log_{10}IC_{50}$ (SE)	IC'_{50} (GSE) [d]	Slope (SE)	Status
Individual Values [a]						
1	49.660 (3.169)	90.360 (1.791)	-5.324 (0.166)	4.747E-06 (1.467)	-1.659 (0.691)	C
2	45.900 (2.541)	85.590 (1.630)	-5.322 (0.215)	4.764E-06 (1.642)	-2.225 (1.426)	C
3	28.240 (1.694)	91.500 (0.672)	-4.975 (0.020)	1.059E-05 (1.048)	-2.580 (0.613)	C
Mean [c]	41.074 (6.670)	89.324 (1.814)	-5.151 (0.135)	7.066E-06 (1.365)	-2.179 (0.436)	C
Average Values [b]						
1	49.670 (4.841)	90.360 (2.739)	-5.324 (0.255)	4.745E-06 (1.797)	-1.660 (1.059)	C
2	45.900 (4.448)	85.590 (2.853)	-5.321 (0.374)	4.781E-06 (2.366)	-2.234 (2.442)	C
3	28.240 (2.013)	91.500 (0.799)	-4.975 (0.024)	1.059E-05 (1.057)	-2.580 (0.728)	C

a. Concentration response curve fitted to the data collected within each replicate, with three repetitions at each concentration level.
b. Concentration response curve fitted to the averages of the three repetitions at each concentration level within each replicate.
c. Weighted averages of the parameter estimates across the three replicates.
d. 10 to the power of $log_{10}IC_{50}$ and 10 to the power of its associated standard error.

Table 9-2. Reference Chemical IX: Dicofol.
Parameter Estimates of the Concentration Response Curves and Associated 95 percent Confidence Intervals.
Percent of Control Activity. Recombinant Aromatase Assay.

Parameter	Estimate (95 percent CI)			
	Replicate 1[a]	Replicate 2[a]	Replicate 3[a]	Mean[b]
Bottom	49.660 (43.050,56.270)	45.900 (40.600,51.200)	28.240 (24.706,31.774)	41.074 (12.938,69.209)
Top	90.360 (86.624,94.096)	85.590 (82.190,88.990)	91.500 (90.098,92.902)	89.324 (81.794,96.854)
$Log_{10}IC_{50}$	-5.324 (-5.671,-4.977)	-5.322 (-5.771,-4.873)	-4.975 (-5.018,-4.932)	-5.151 (-5.582,-4.720)
Slope	-1.659 (-3.100,-0.218)	-2.225 (-5.200,0.750)	-2.580 (-3.858,-1.302)	-2.179 (-3.063,-1.295)

a. Parameter estimates and their associated 95 percent confidence intervals for each replicate, based on the concentration response curves fitted to the individual repetition values within replicates.
b. Mean and its associated 95 percent confidence interval, based on a one-way analysis of variance model with replicate treated as a random effect.

Table 9-3. Reference Chemical IX: Dicofol.
Variances Associated with Estimated Parameters of Concentration Response Curves.
Percent of Control Activity. Recombinant Aromatase Assay.

Parameter	Variance/Degree of Freedom [a,b,c]				
	Replicate 1	Replicate 2	Replicate 3	Overall	
				Random Replicate (p-value) [d]	Variance of Mean
Bottom	10.04256 /df=20	6.45668 /df=20	2.86964 /df=20	127.07115 /df=2 (p=0.168)	44.48773 /df=2.042
Top	3.20768 /df=20	2.65690 /df=20	0.45172 /df=20	7.91619 /df=2 (p=0.210)	3.28968 /df=2.078
$Log_{10}IC_{50}$	0.02766 /df=20	0.04640 /df=20	0.00042 /df=20	0.03650 /df=2 (p=0.232)	0.01827 /df=2.987
Slope	0.47707 /df=20	2.03348 /df=20	0.37516 /df=20	0.00000 /df=2 (p=NA)	0.19035 /df=36.982

a. The variance estimates for each replicate were based on the concentration response curves fitted to the individual repetition results within each concentration level.
b. Variance estimates for the random replicate were estimated based on a one-way random effects analysis of variance. The variances for each replicate were fixed at their reported values.
c. Degrees of freedom for the variance of mean were estimated by $2*((1/K)* \Sigma(S_r^2 + S_i^2))^2/(var(S_r^2) +(2/K^2)* \Sigma(Si^4 /df_i))$, where S_r^2 is random replicate variance, S_i^2 and df_i are estimated variance and degree of freedom for a given replicate, $var(S_r^2)$ is the variance associated with the estimation of S_r^2 and K is the number of replicates (Hartung and Makambi, 2001).
d. p-value is based on the Wald Z-test result.

Table 10-1. Reference Chemical X: Atrazine.
Estimated Parameters of the Concentration Response Curve Fits by Replicate and Averaged Across Replicates.
Percent of Control Activity. Recombinant Aromatase Assay.

All data points were above 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)
				I / II (4 replicates)	III / VI (4 replicates)	IV / V (3 replicates)	VII / VIII (4 replicates)	IX / X (3 replicates)	
Full Enzyme Activity Control	-6.354 (1.923)	0.0031 /df=23.19	Replicate	0.00	0.00	0.00	0.00	0.00	41.26
			Replicate * Portion	0.00	67.98	0.00	0.00	0.00	
Background Activity Control	-0.049 (0.067)	0.4745 /df=13.99	Replicate	0.00	0.00	0.00	0.00	0.00	0.02
			Replicate * Portion	0.04	0.03	0.00	0.00	0.07	
Positive Control	0.619 (1.519)	0.6888 /df=16.94	Replicate	0.00	69.34	7.05	2.69	0.00	10.30
			Replicate * Portion	35.20	18.82	0.24	7.49	18.25	
Negative Control	7.479 (3.603)	0.0542 /df=16.17	Replicate	180.34	87.47	0.00	0.00	0.00	29.45
			Replicate * Portion	47.25	169.86	19.75	178.41	107.84	

Note: The chemical main effect and the chemical by portion interaction effect were not significant for all four types of controls.
Note: There were two common replicates of controls and one different replicate of controls between chemicals I and II, between chemicals III and VI, and between chemicals VII and VIII. Therefore, the combined I / II, III / VI, and VII / VIII each had four replicates of controls. All three replicates of controls were the same for chemicals IV and V. Also all three replicates of controls were the same for chemicals IX and X.

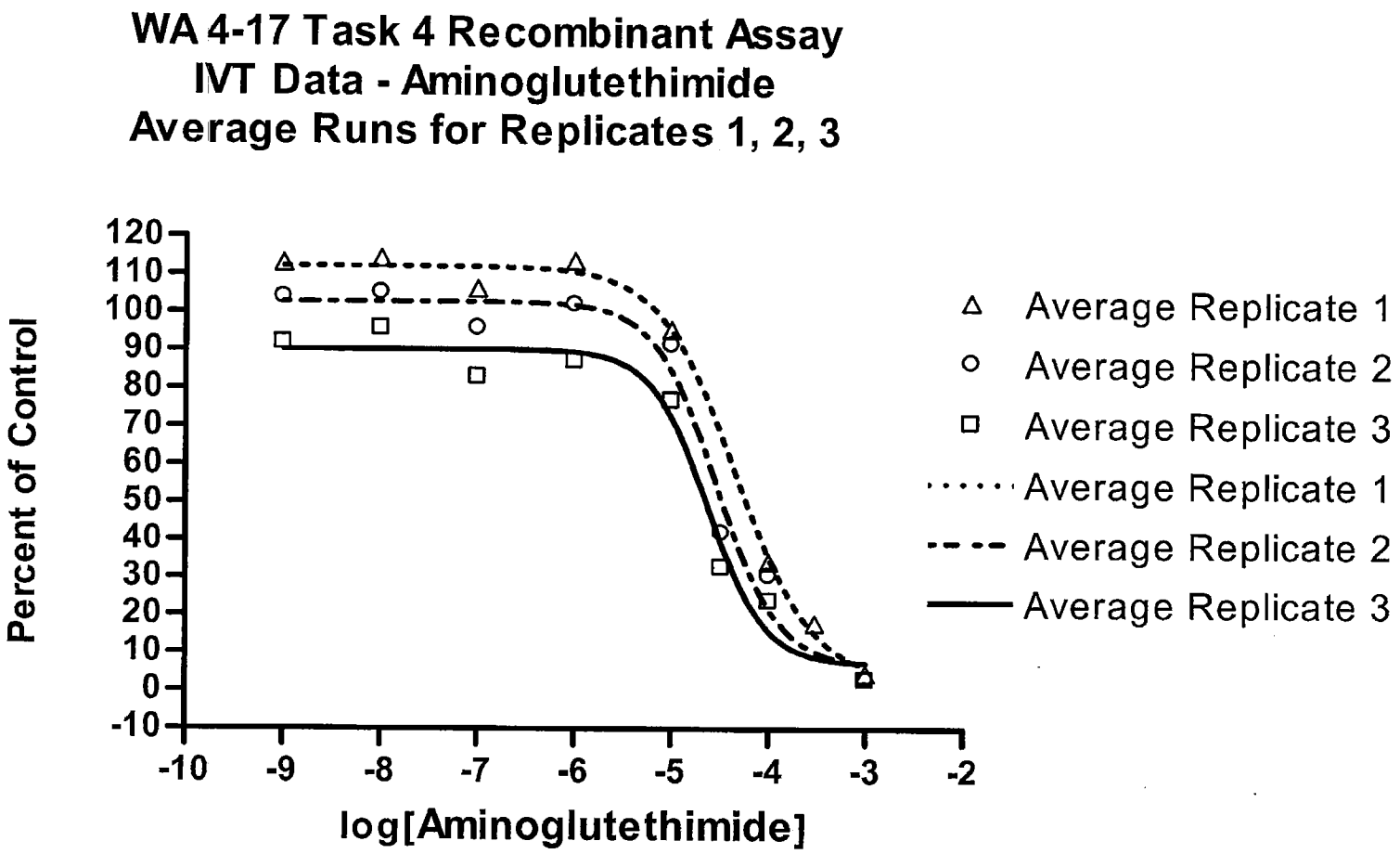


Figure 1-1. **Reference Chemical I: Aminoglutethimide.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

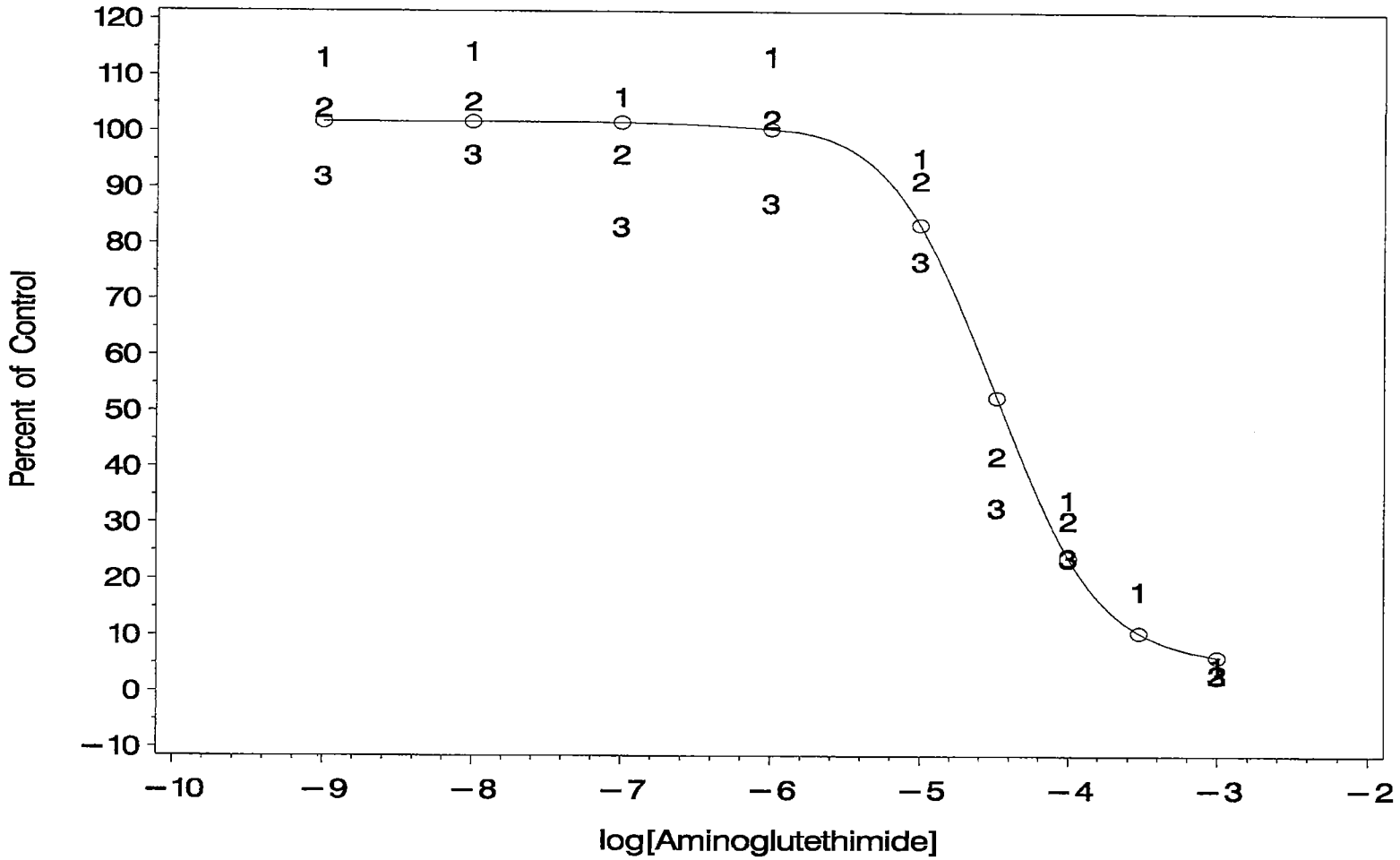


Figure 1-2. **Reference Chemical I: Aminoglutethimide. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.**

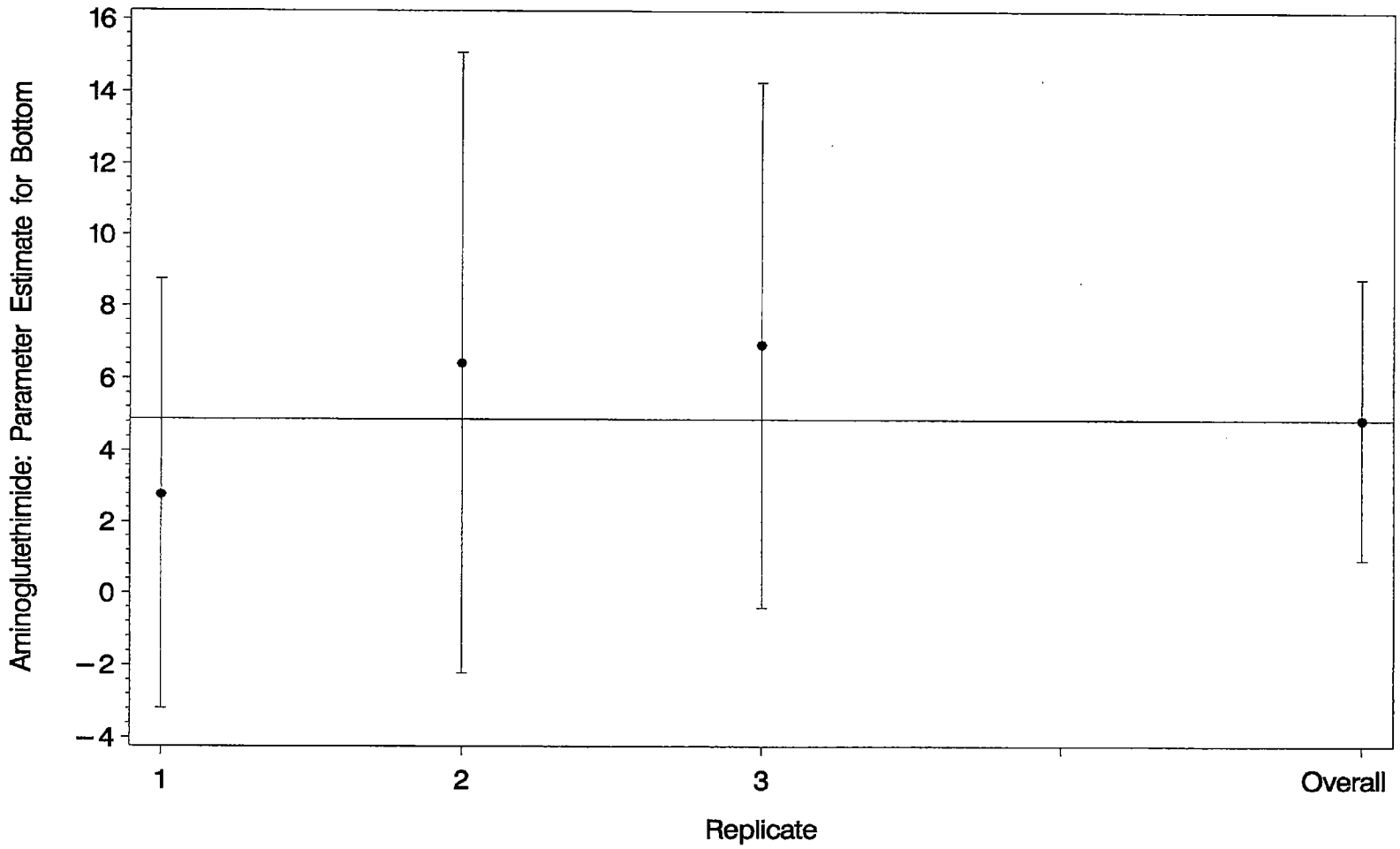


Figure 1-3. Reference Chemical I: Aminoglutethimide. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

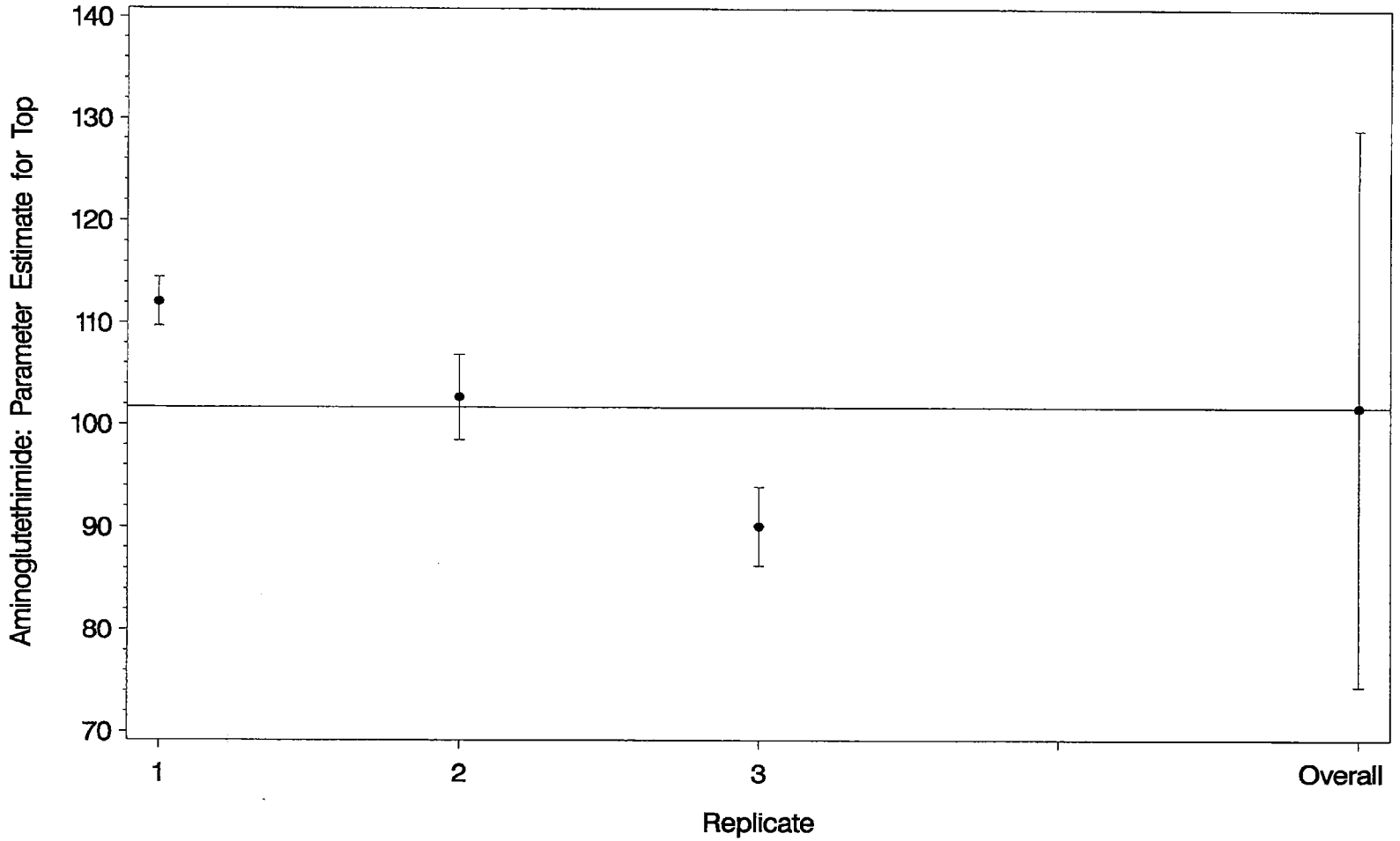


Figure 1-4. Reference Chemical I: Aminoglutethimide. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

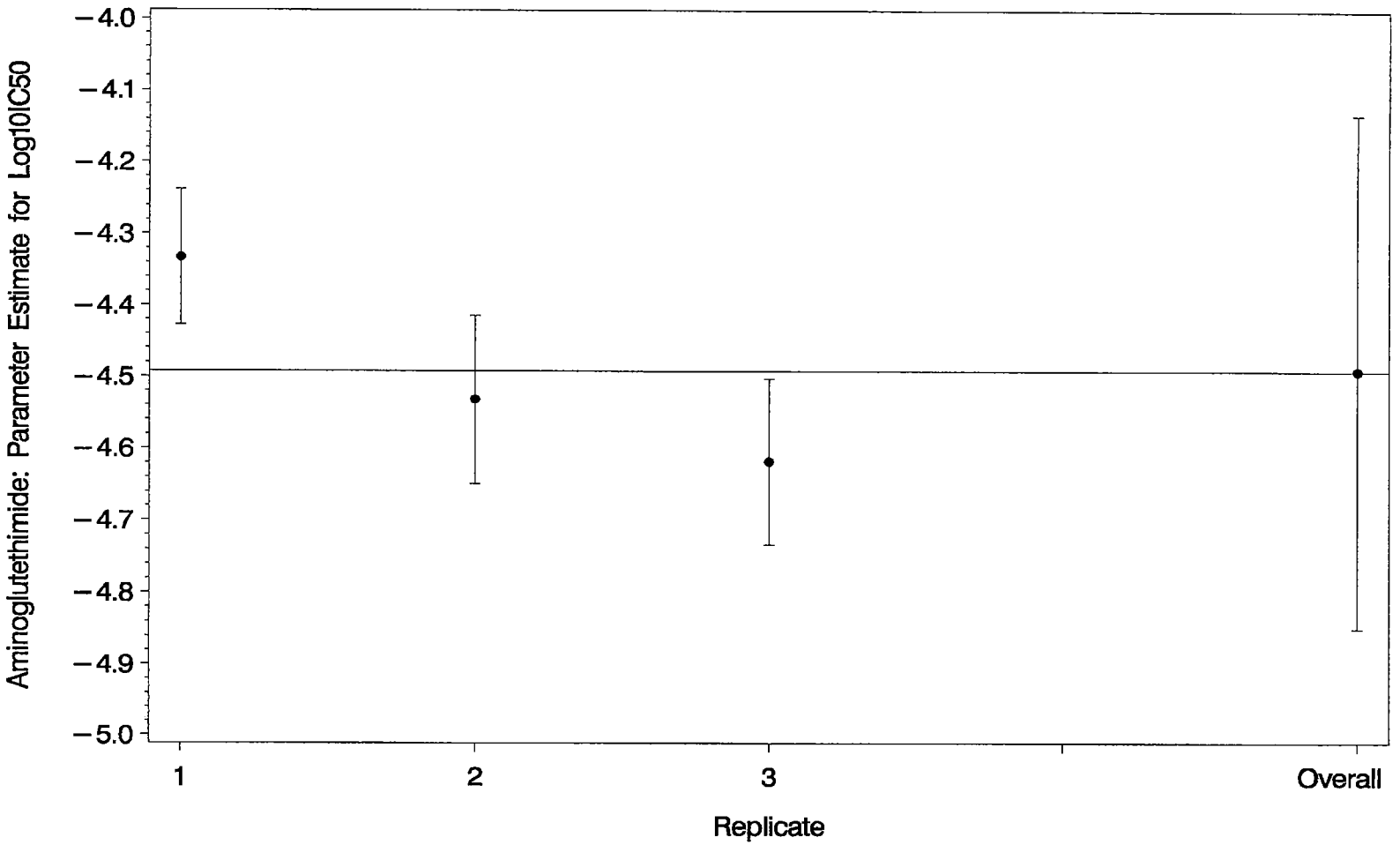


Figure 1-5. Reference Chemical I: Aminoglutethimide. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

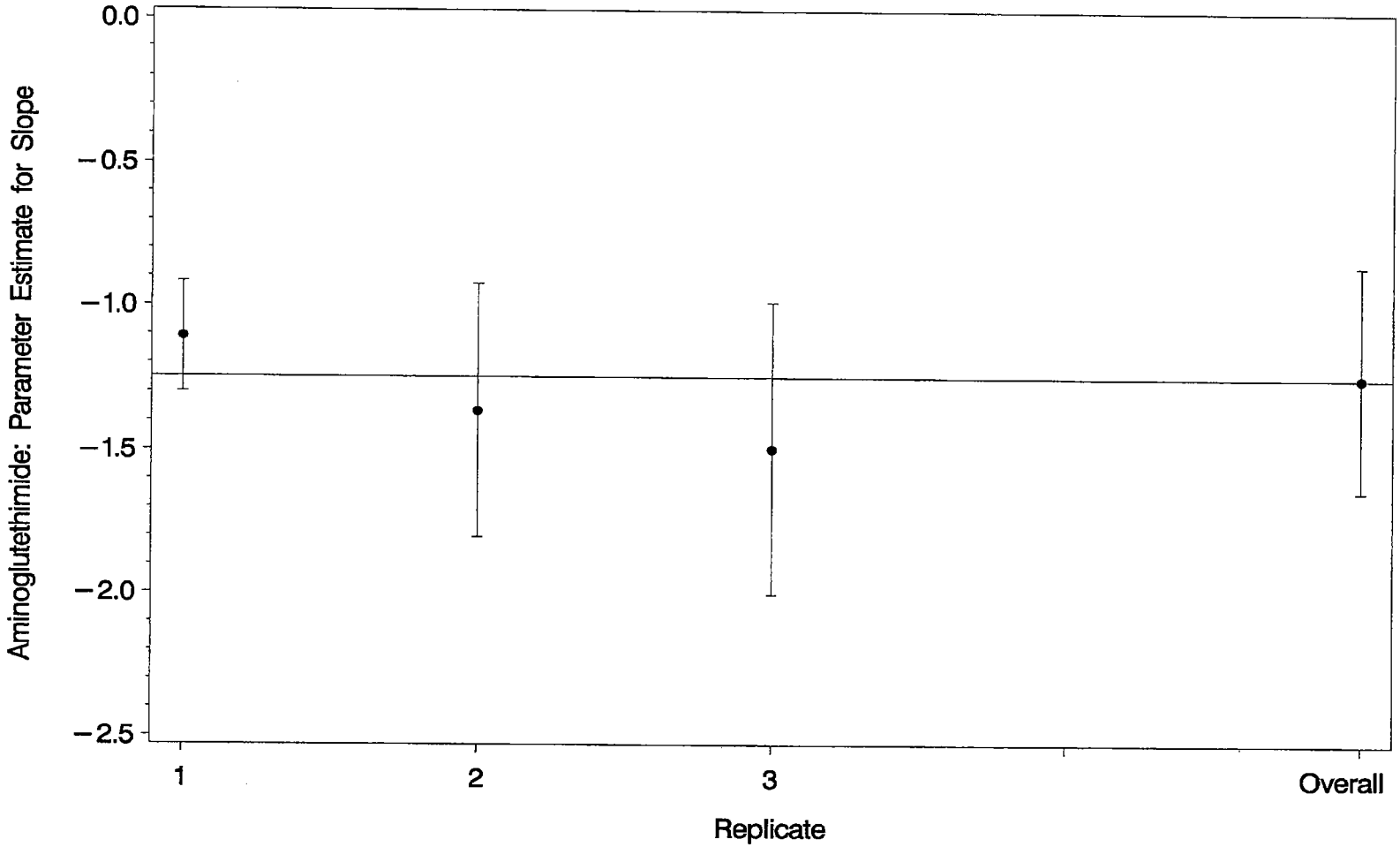


Figure 1-6. Reference Chemical I: Aminoglutethimide. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

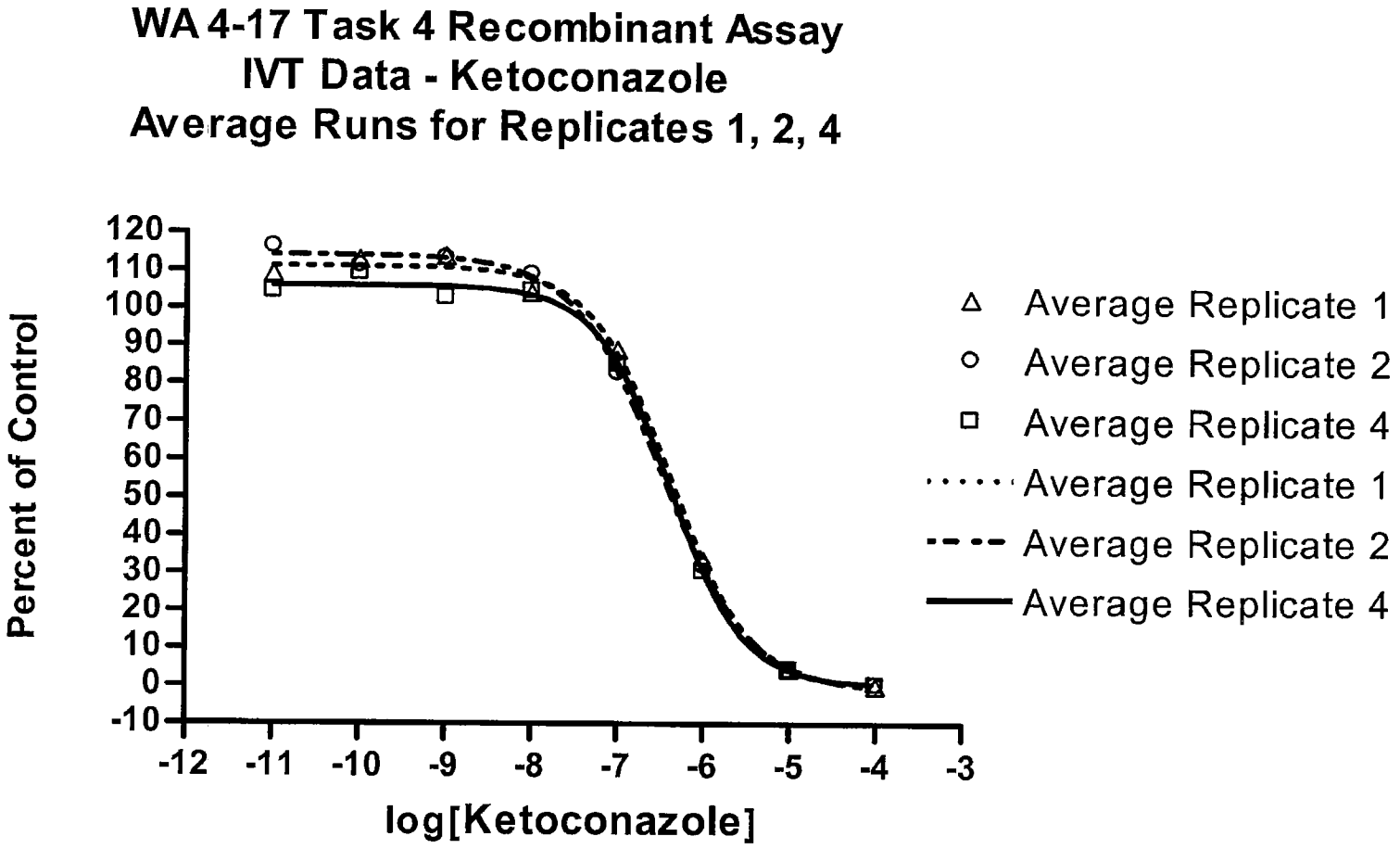


Figure 2-1. **Reference Chemical II: Ketoconazole.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

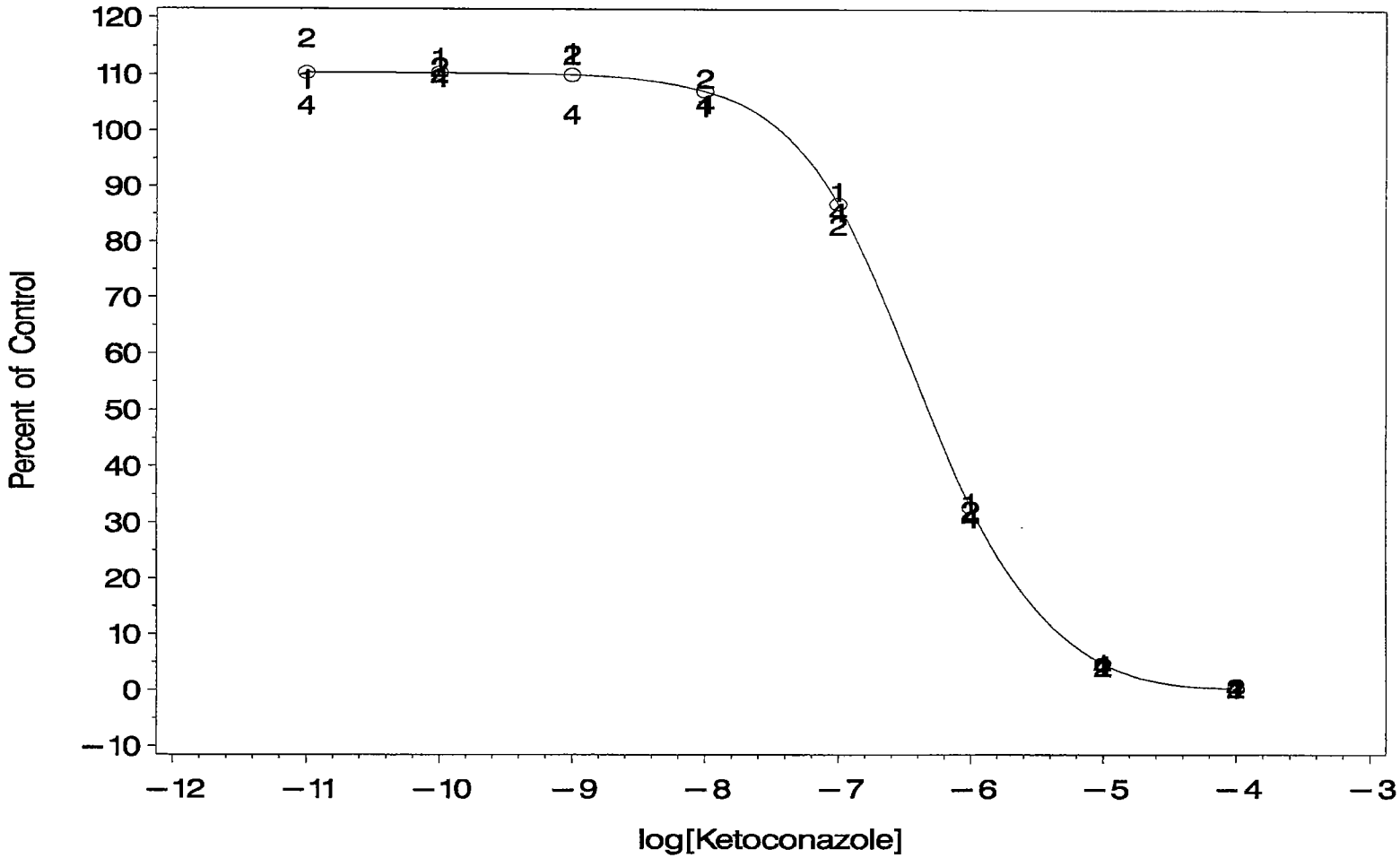


Figure 2-2. Reference Chemical II: Ketoconazole. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

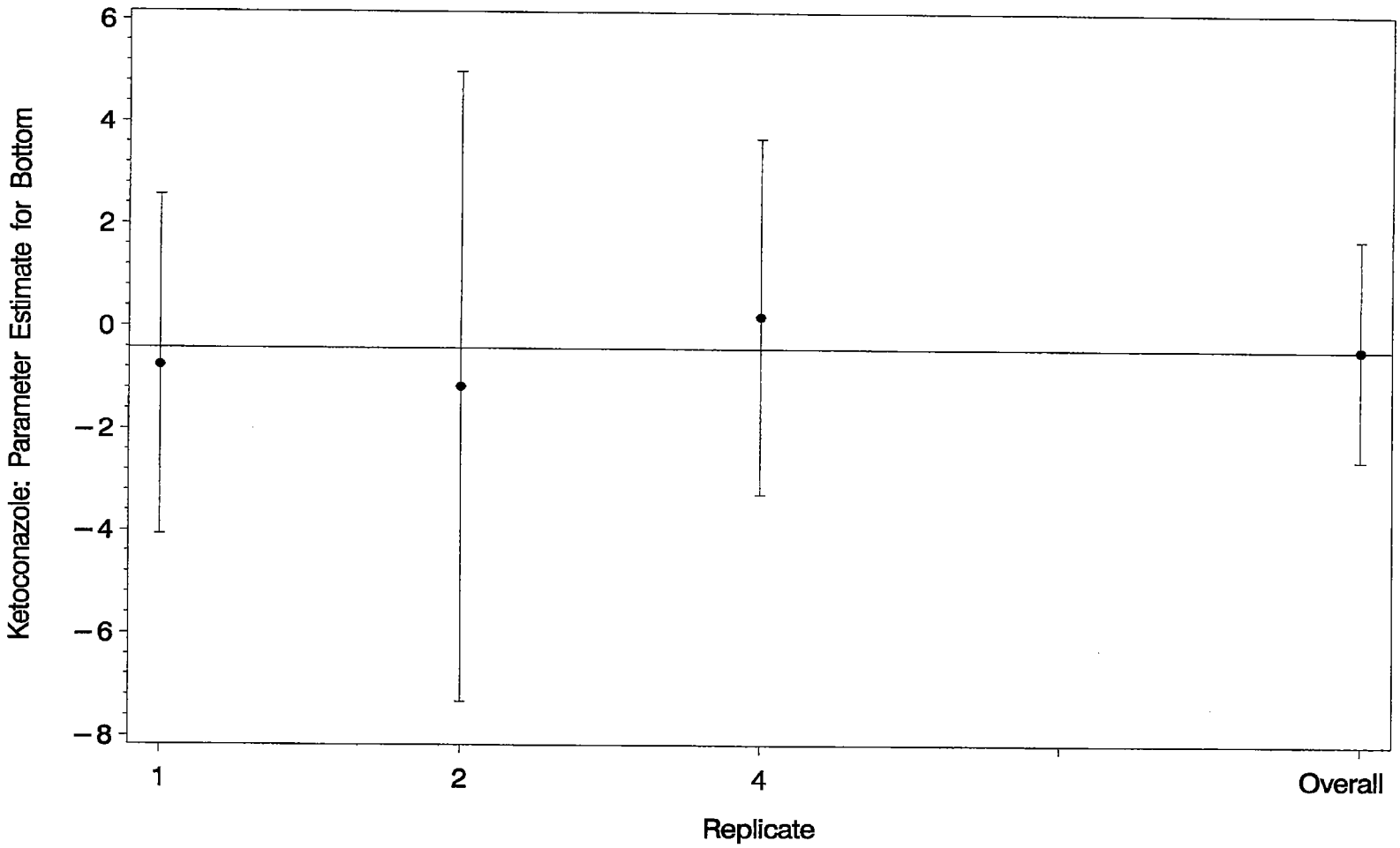


Figure 2-3. **Reference Chemical II: Ketoconazole. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

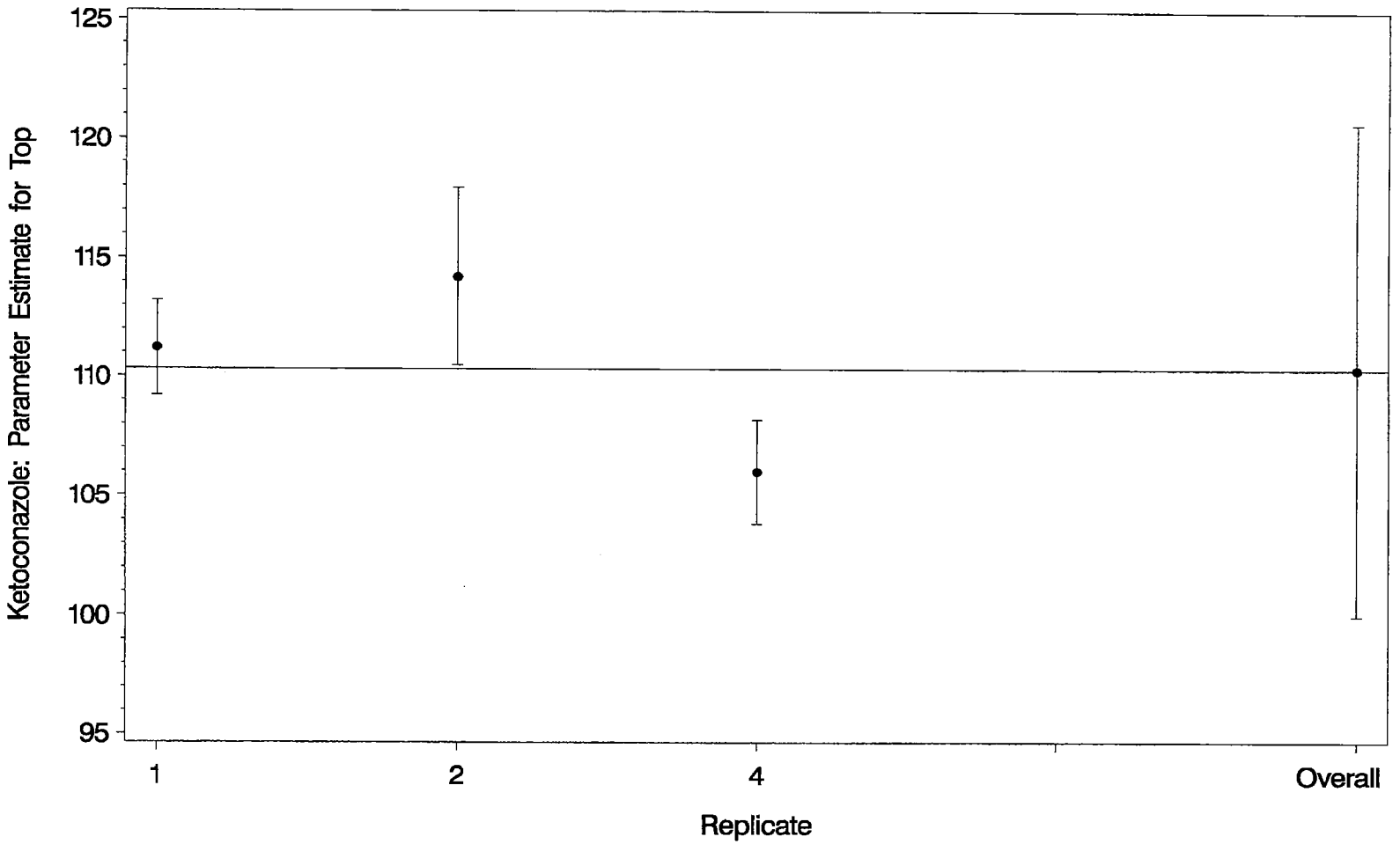


Figure 2-4. Reference Chemical II: Ketoconazole. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

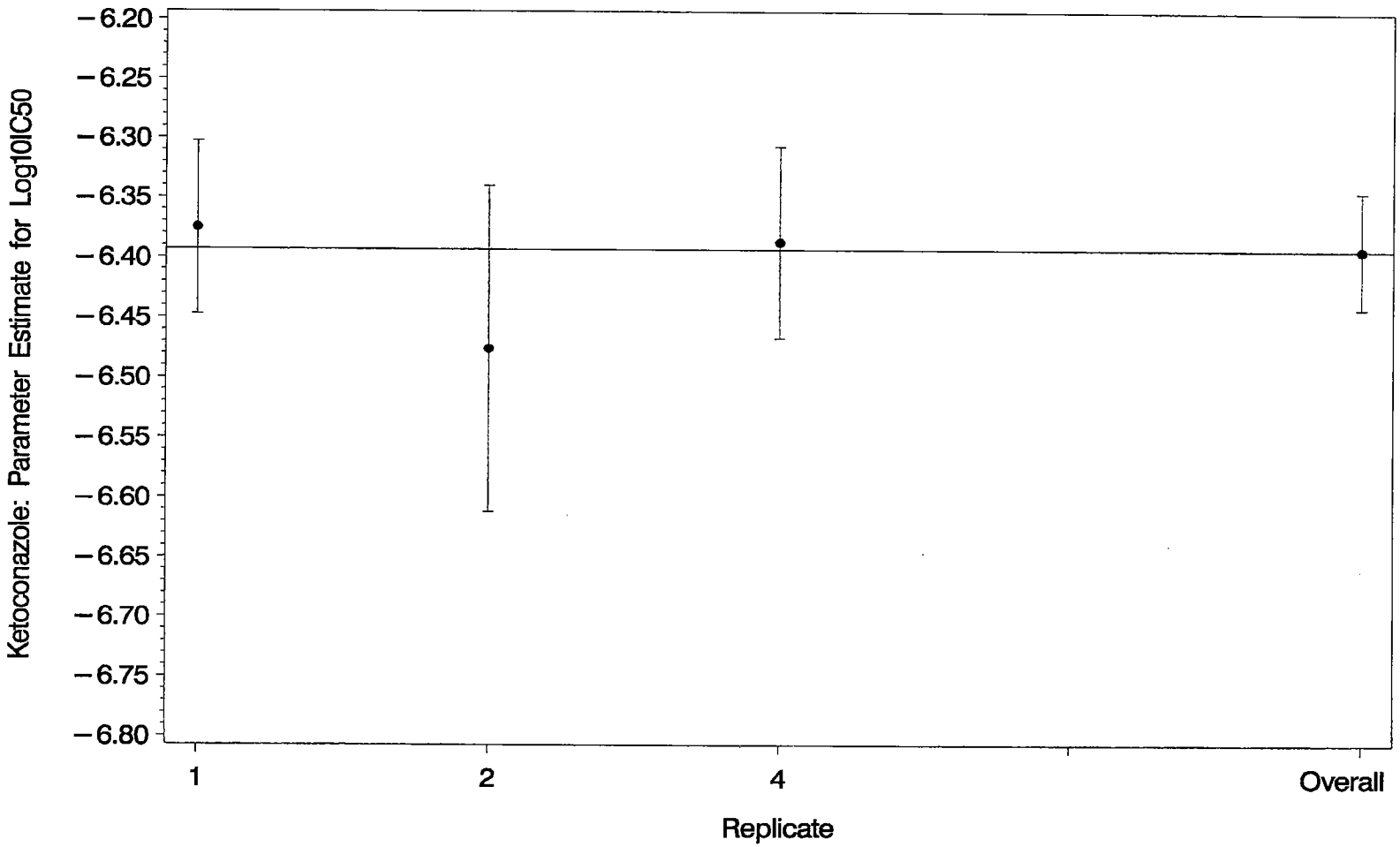


Figure 2-5. Reference Chemical II: Ketoconazole. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

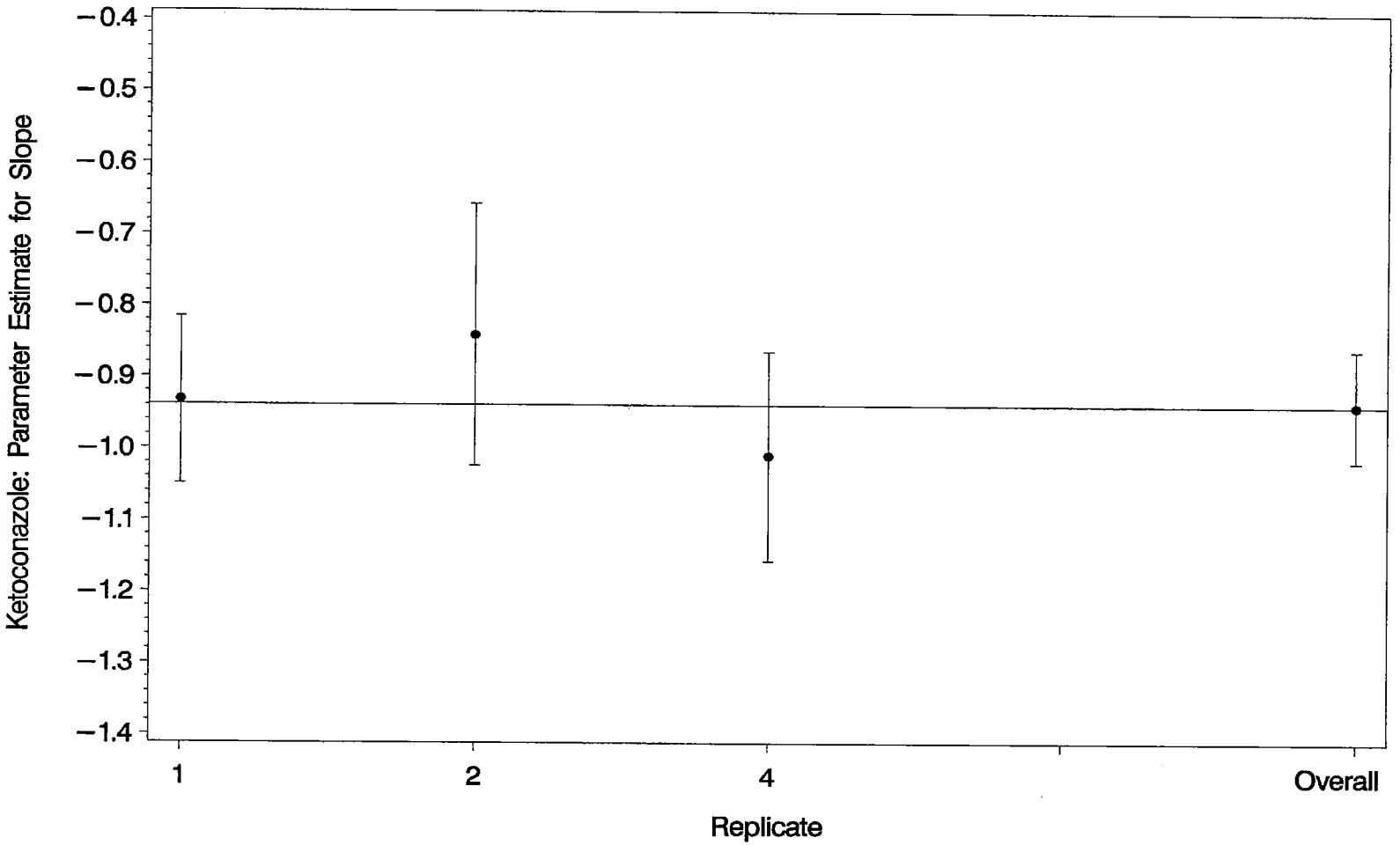


Figure 2-6. Reference Chemical II: Ketoconazole. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

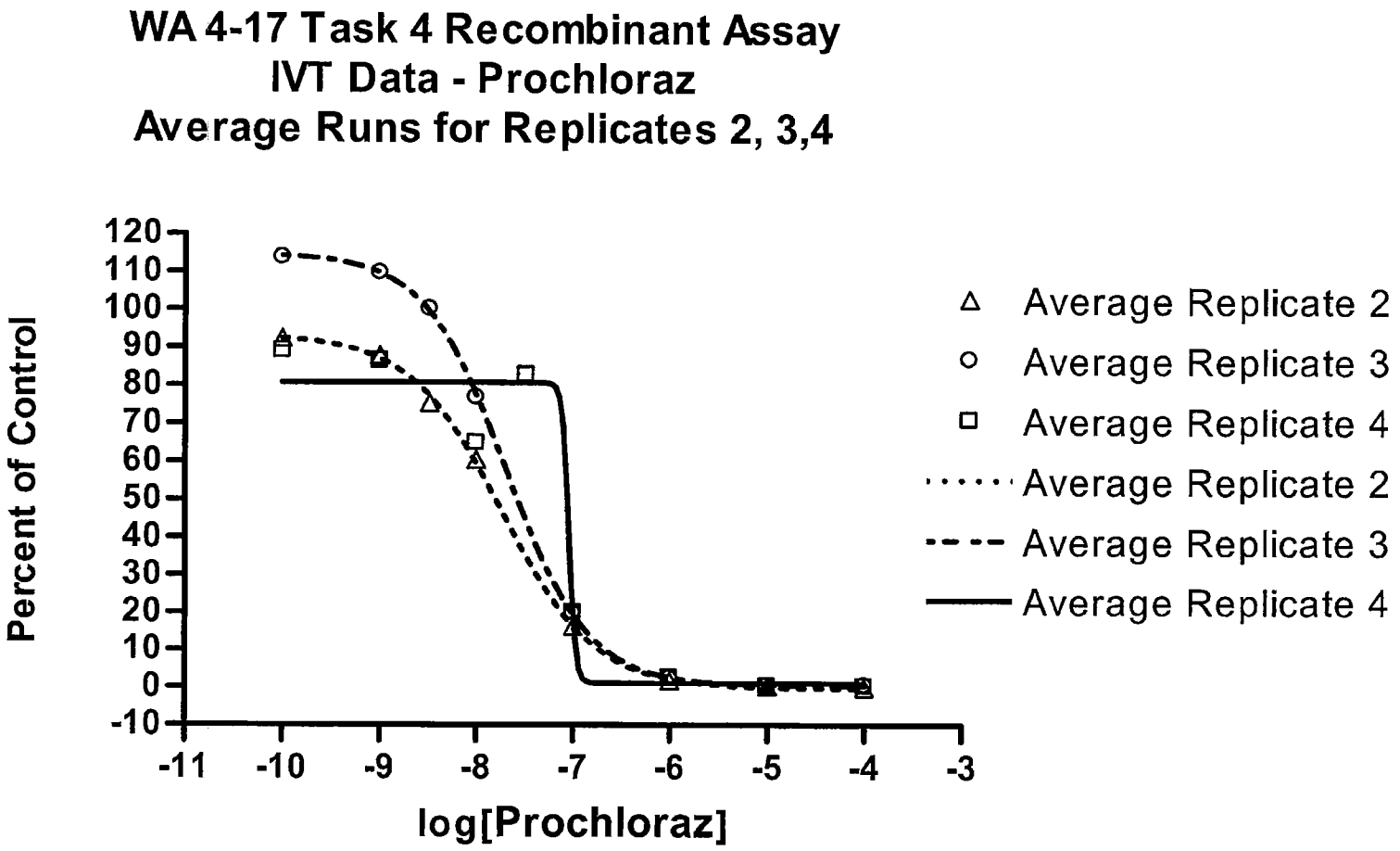


Figure 3-1. **Reference Chemical III: Prochloraz.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

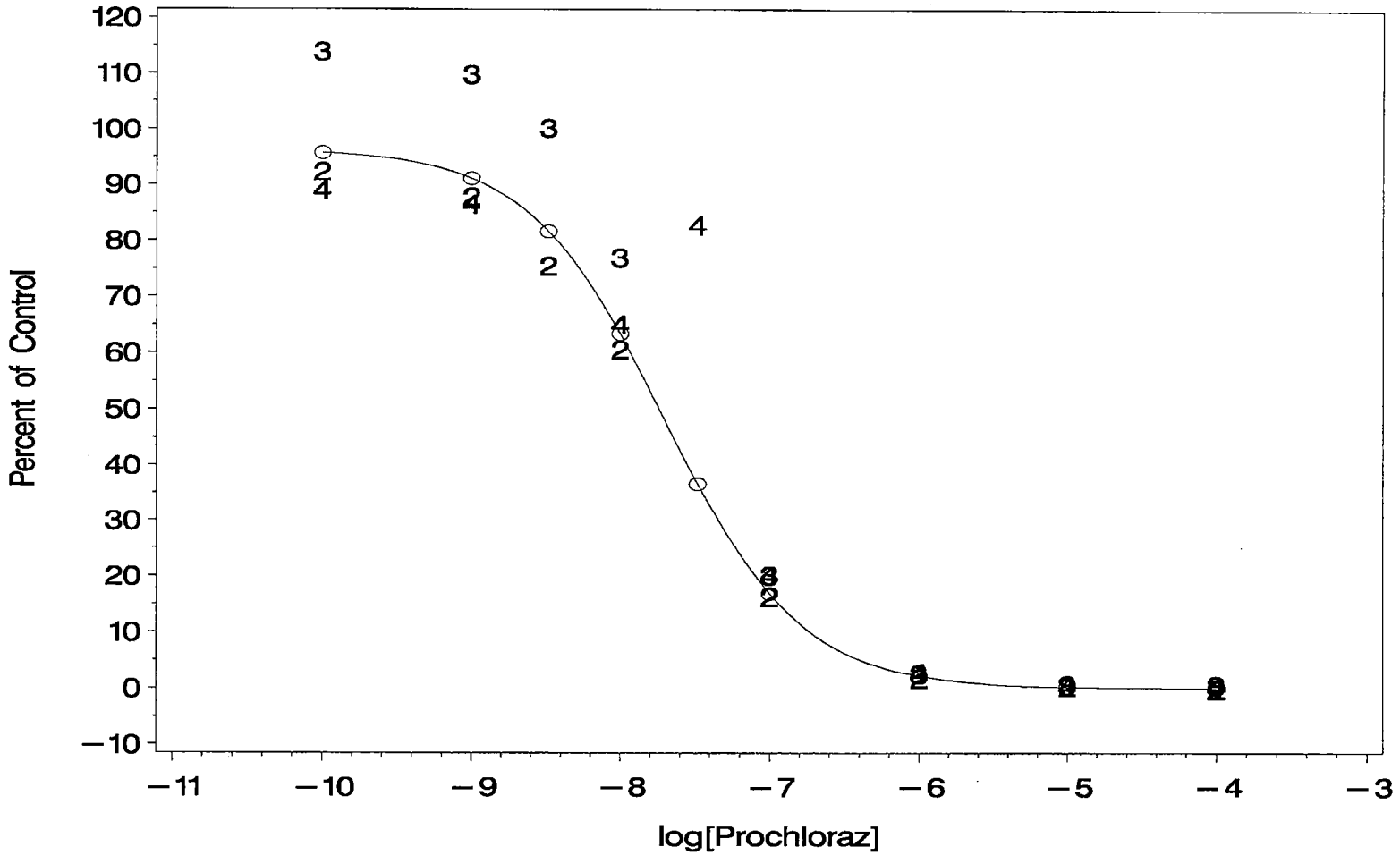


Figure 3-2. Reference Chemical III: Prochloraz. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

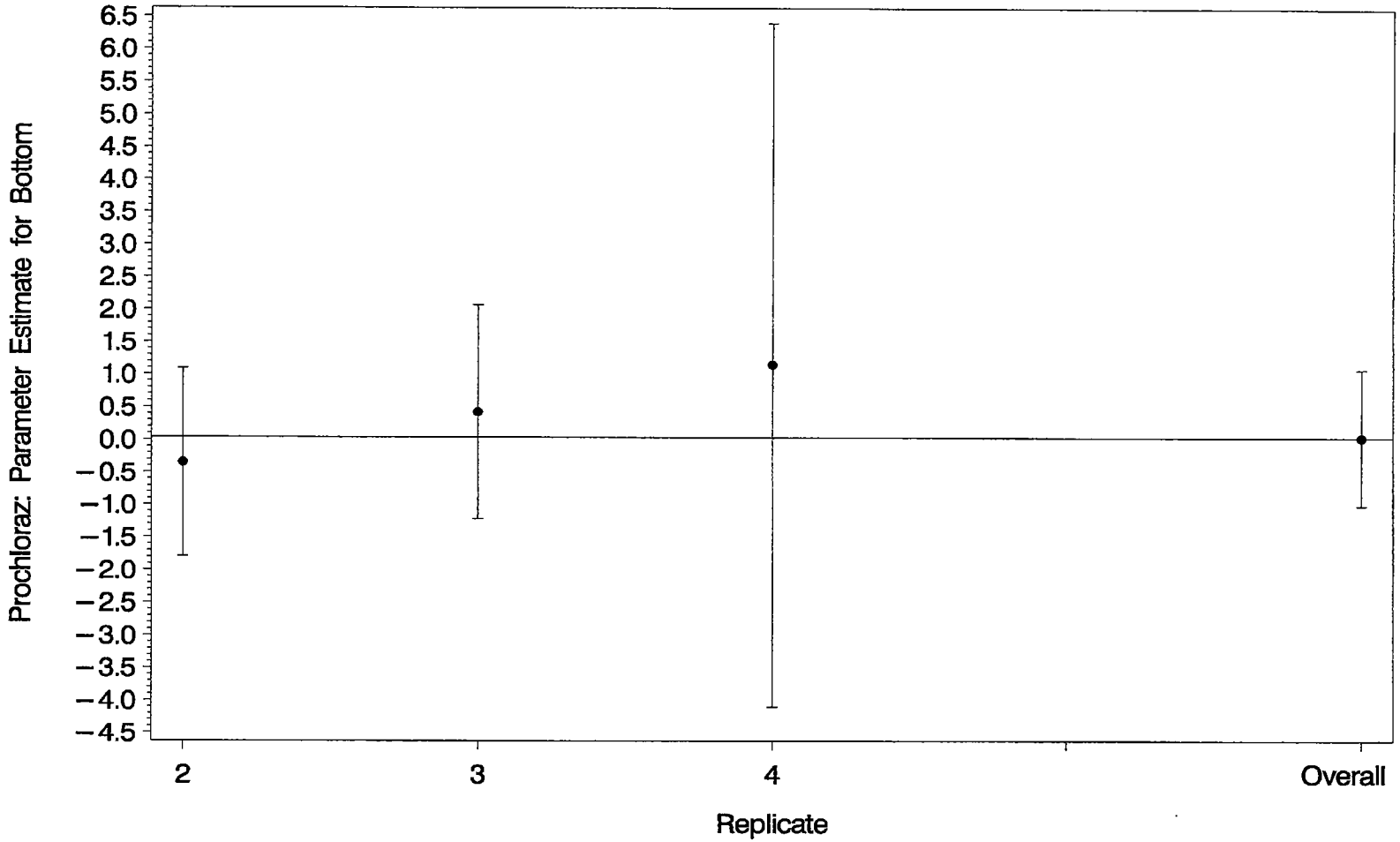


Figure 3-3. Reference Chemical III: Prochloraz. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

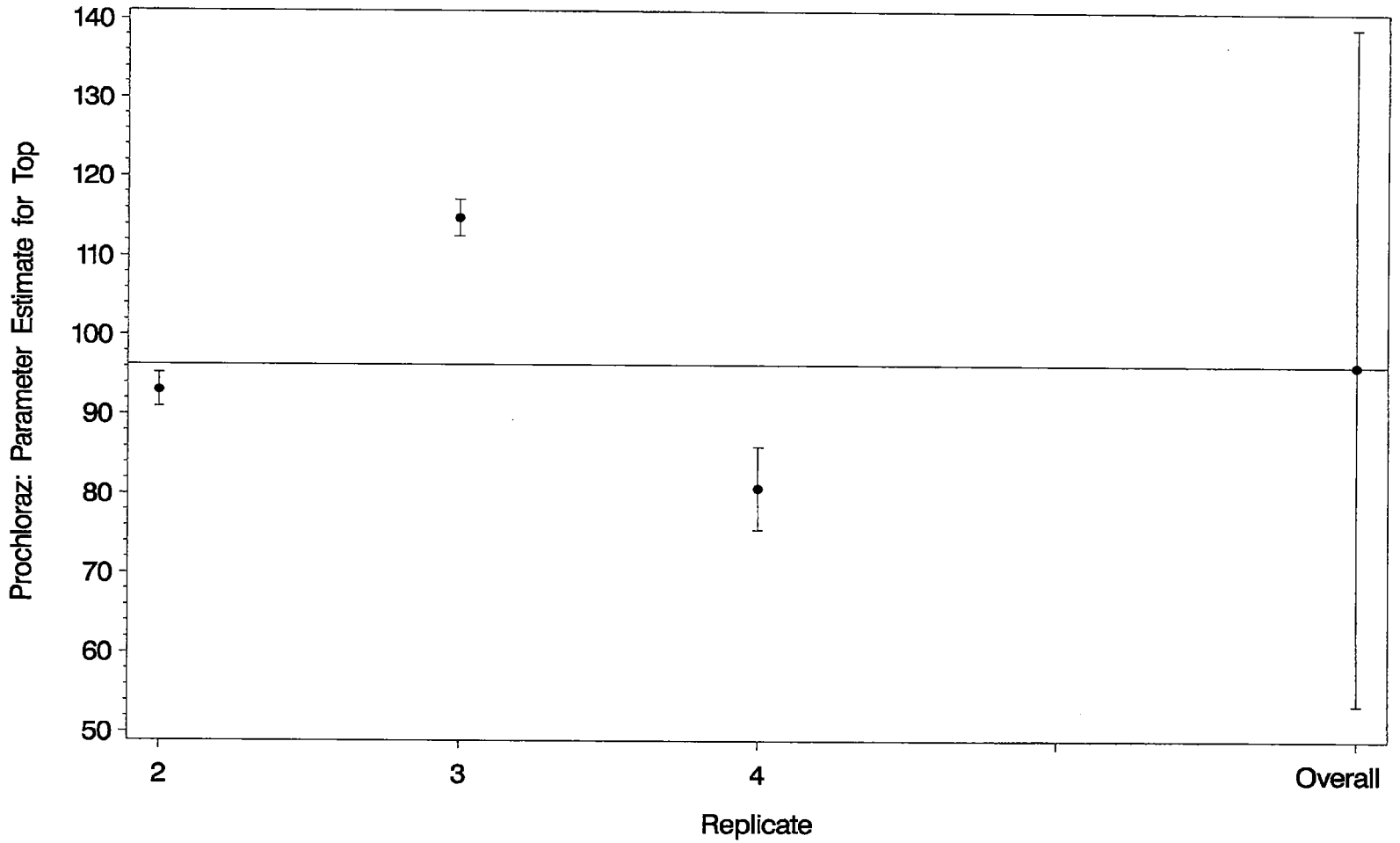


Figure 3-4. Reference Chemical III: Prochloraz. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

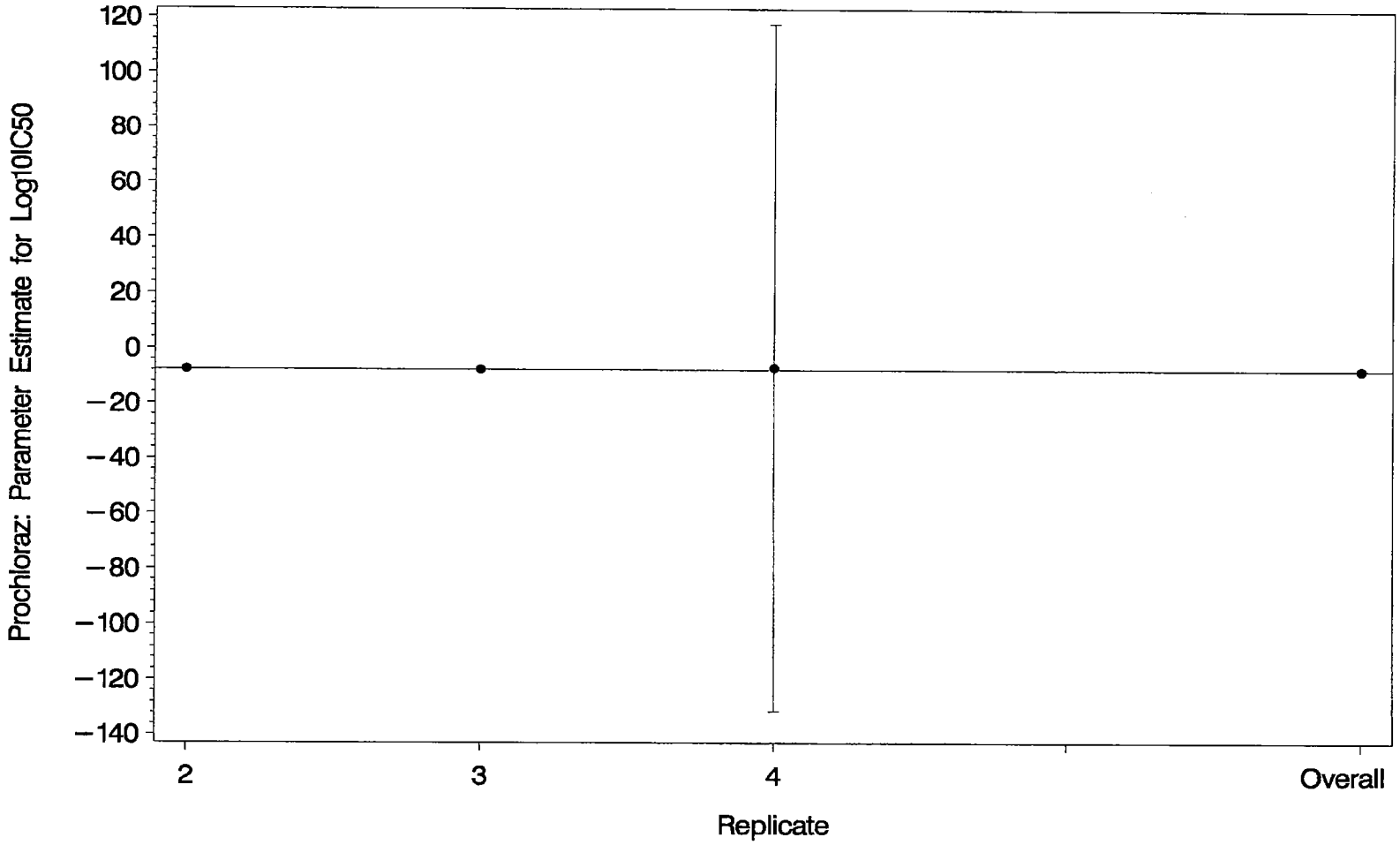


Figure 3-5. Reference Chemical III: Prochloraz. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

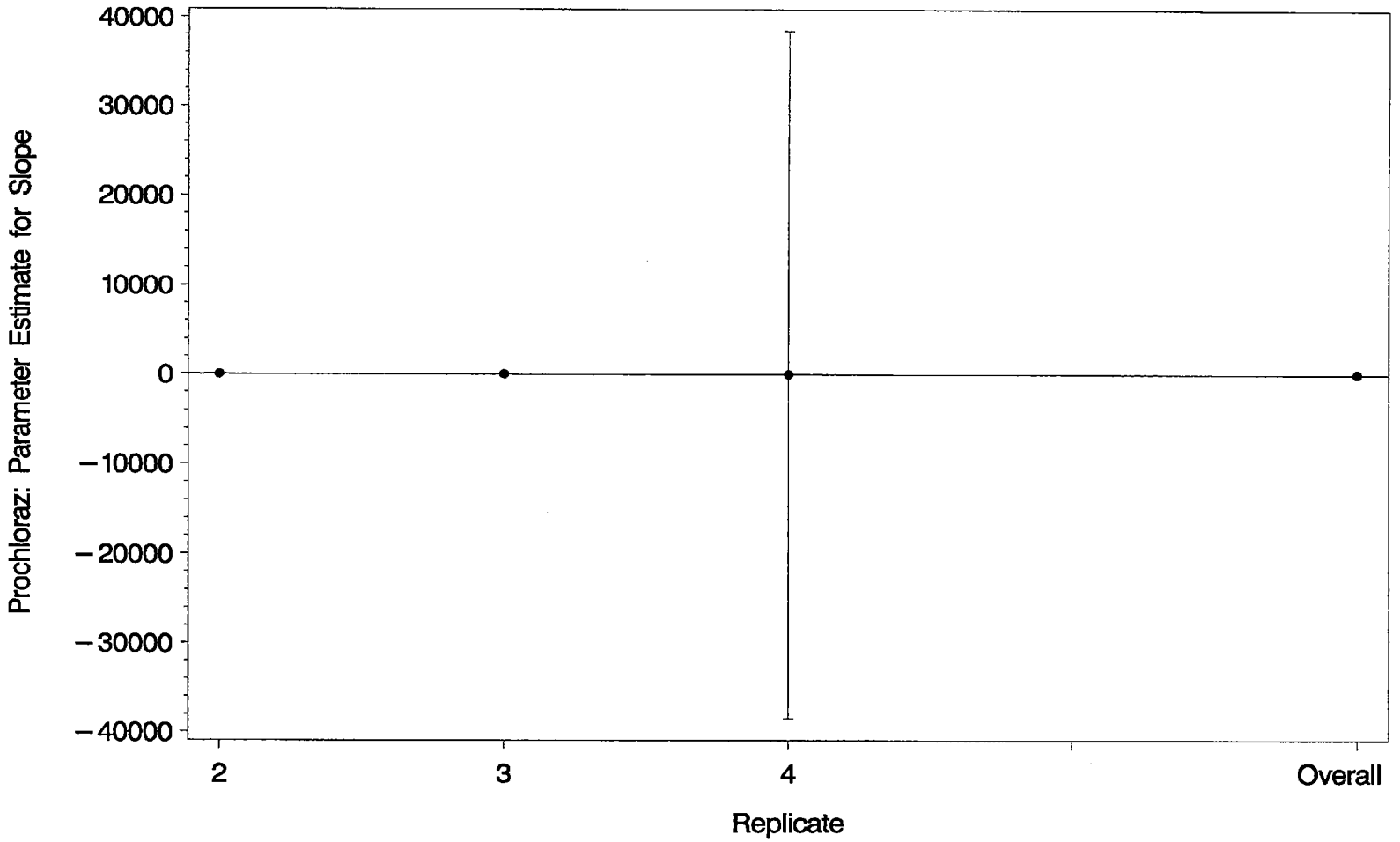


Figure 3-6. Reference Chemical III: Prochloraz. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

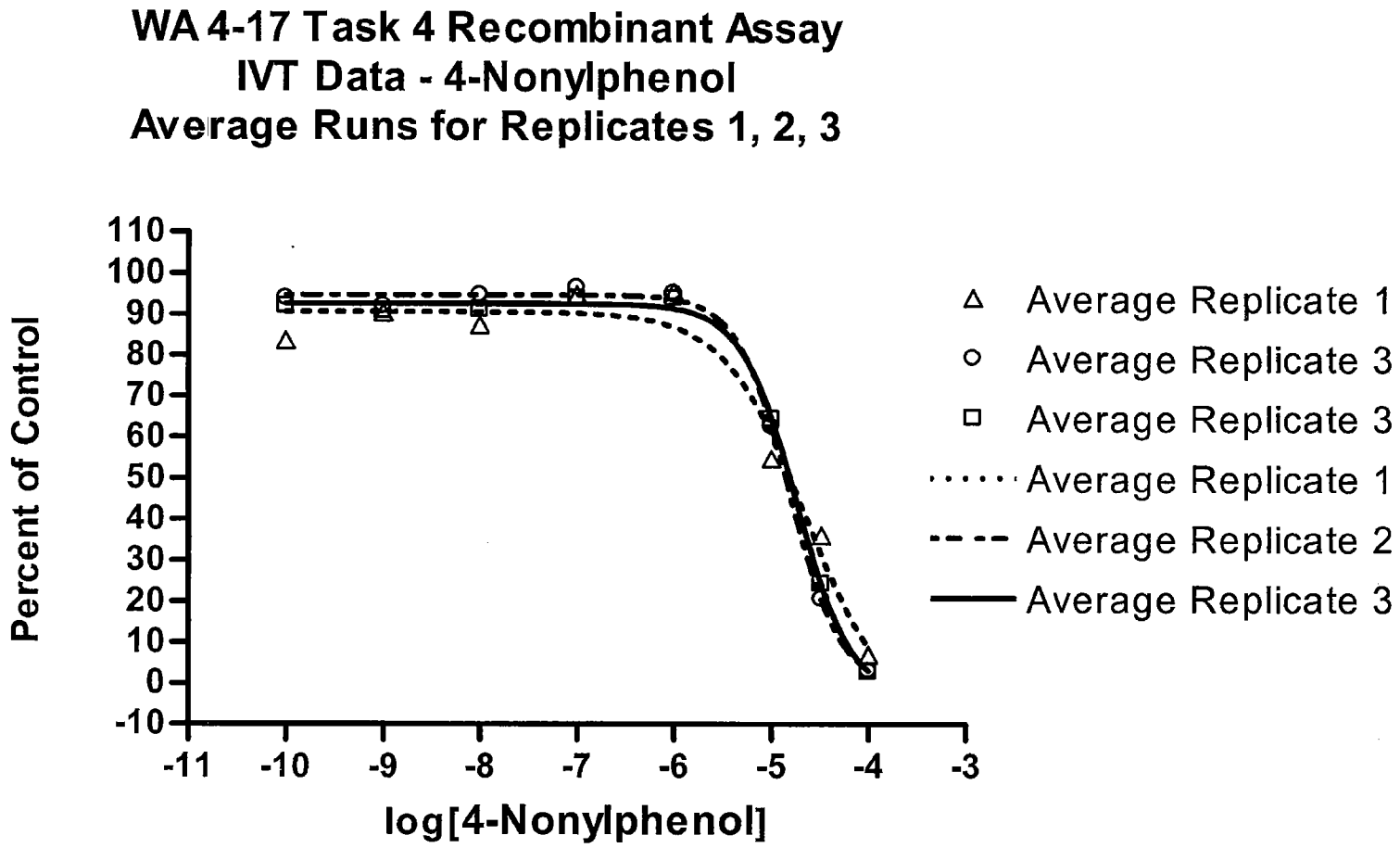


Figure 4-1. **Reference Chemical IV: 4-Nonylphenol.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

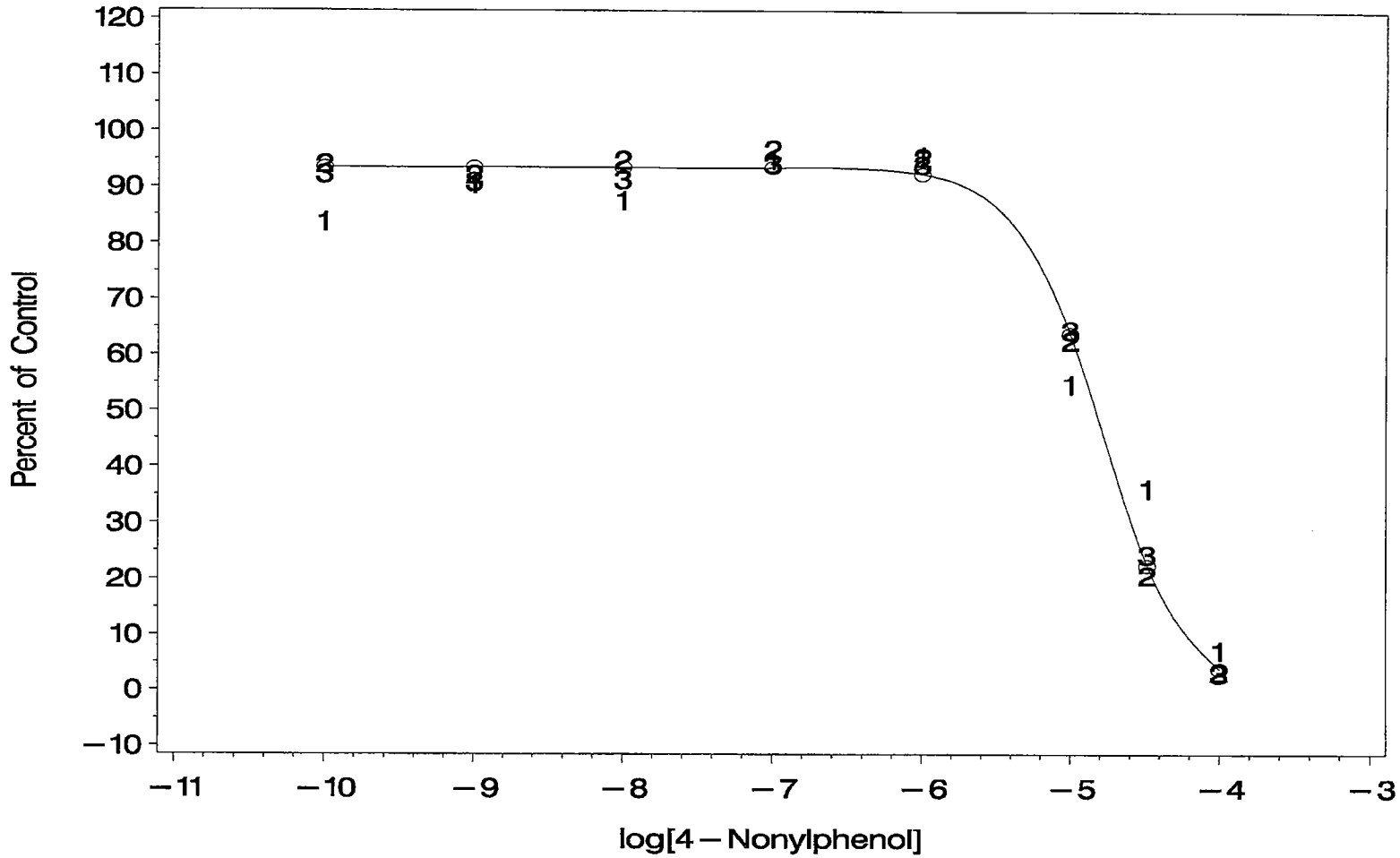


Figure 4-2. Reference Chemical IV: 4-Nonylphenol. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

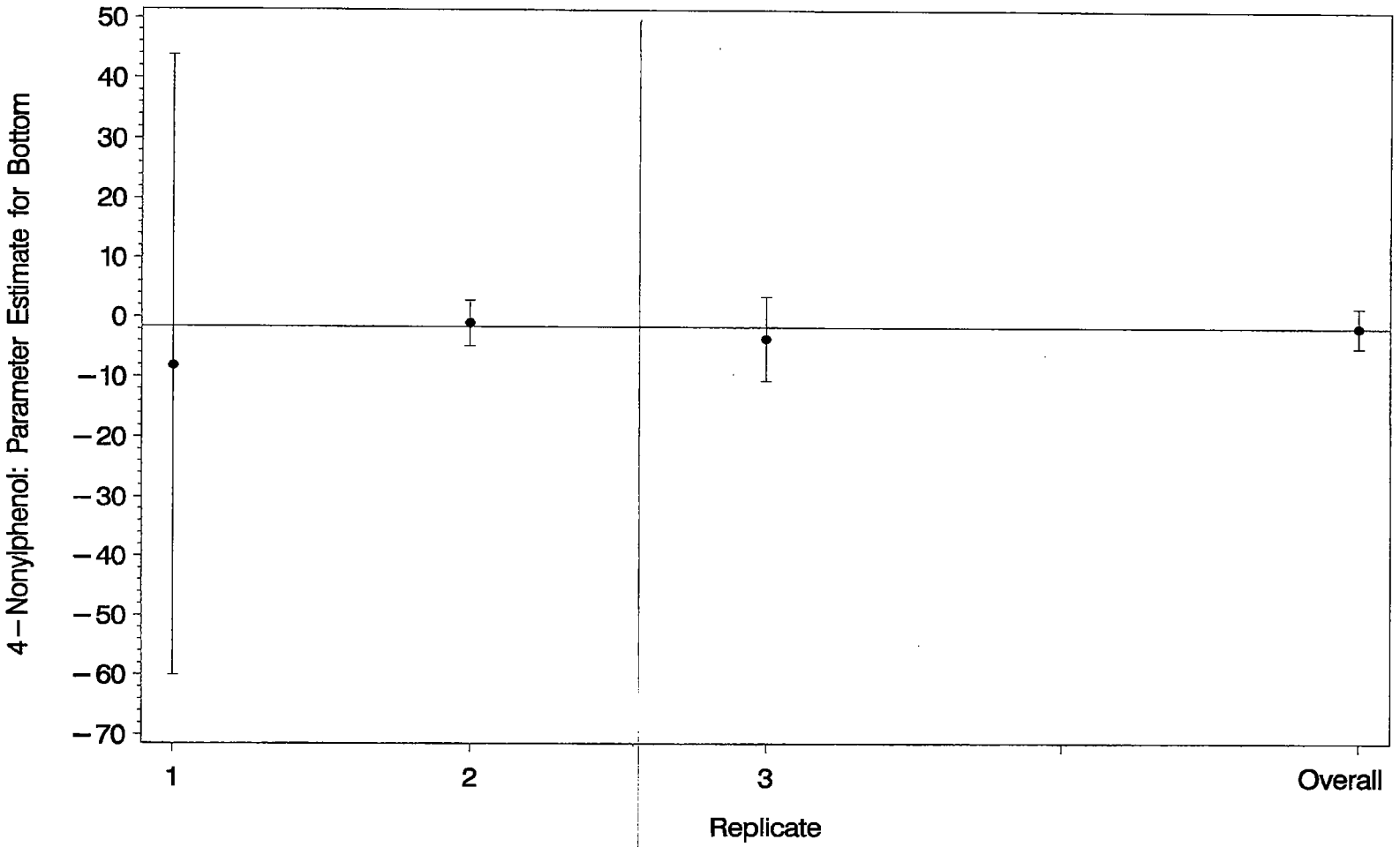


Figure 4-3. Reference Chemical IV: 4-Nonylphenol. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

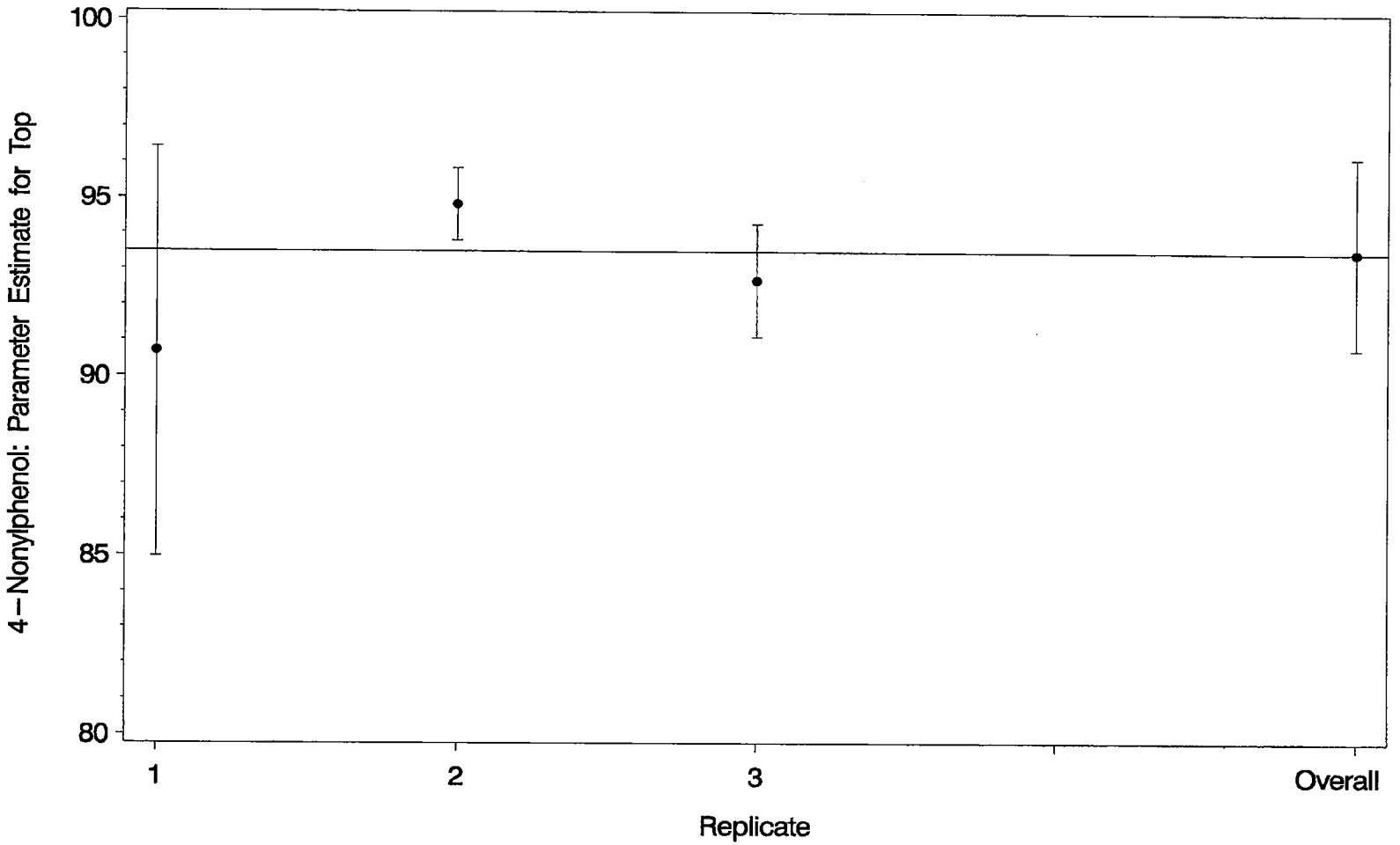


Figure 4-4. **Reference Chemical IV: 4-Nonylphenol. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

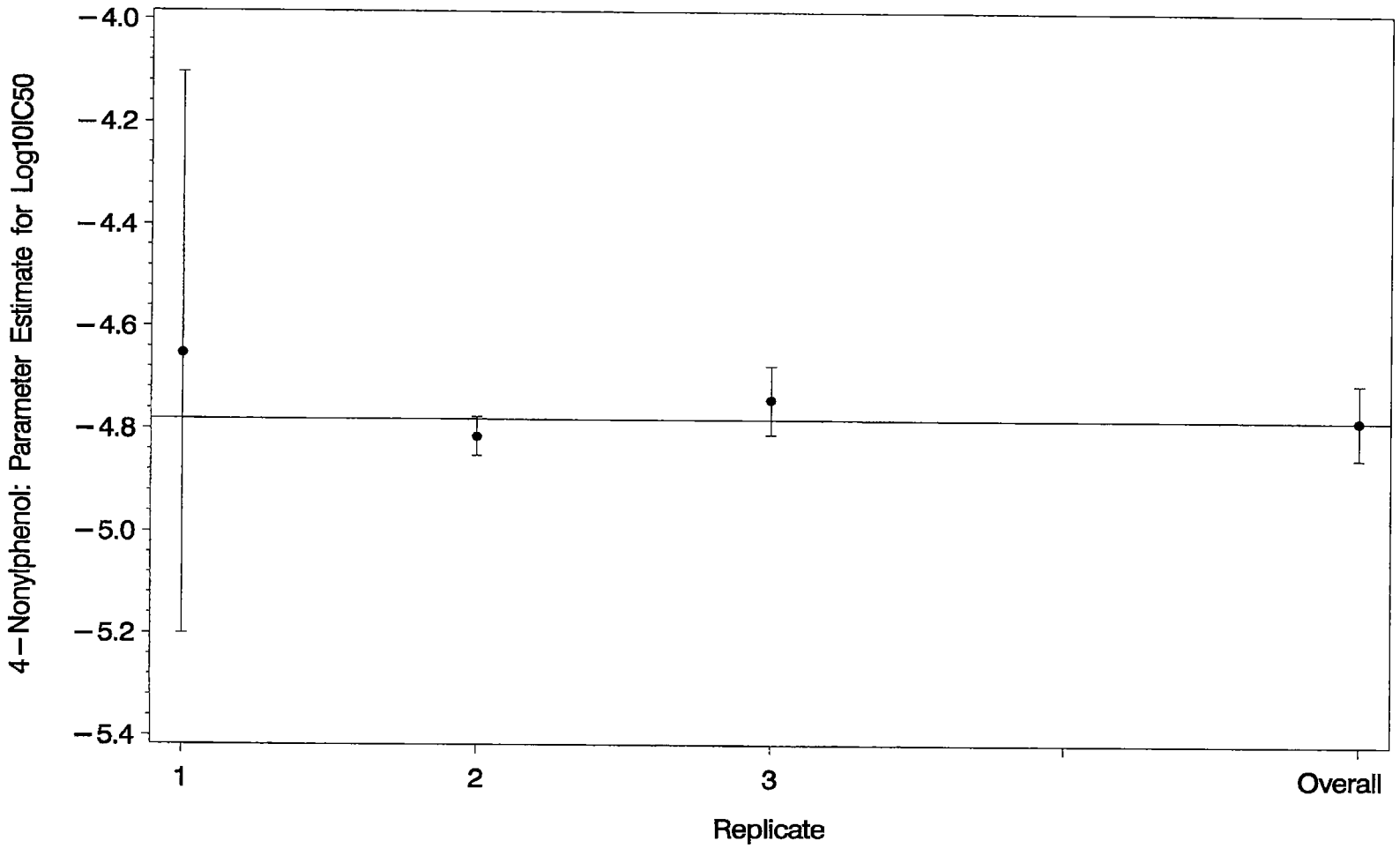


Figure 4-5. **Reference Chemical IV: 4-Nonylphenol. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

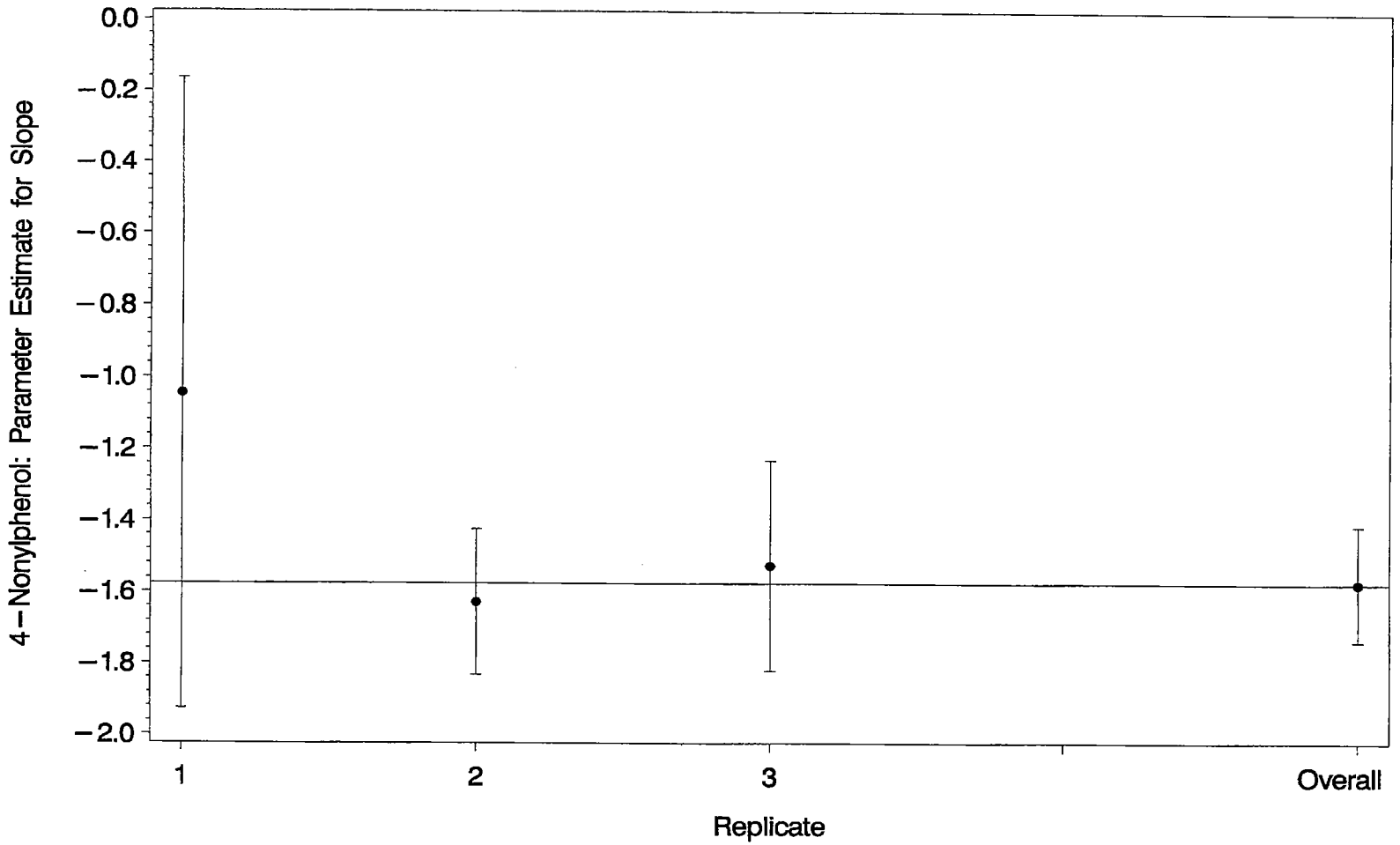


Figure 4-6. **Reference Chemical IV: 4-Nonylphenol. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

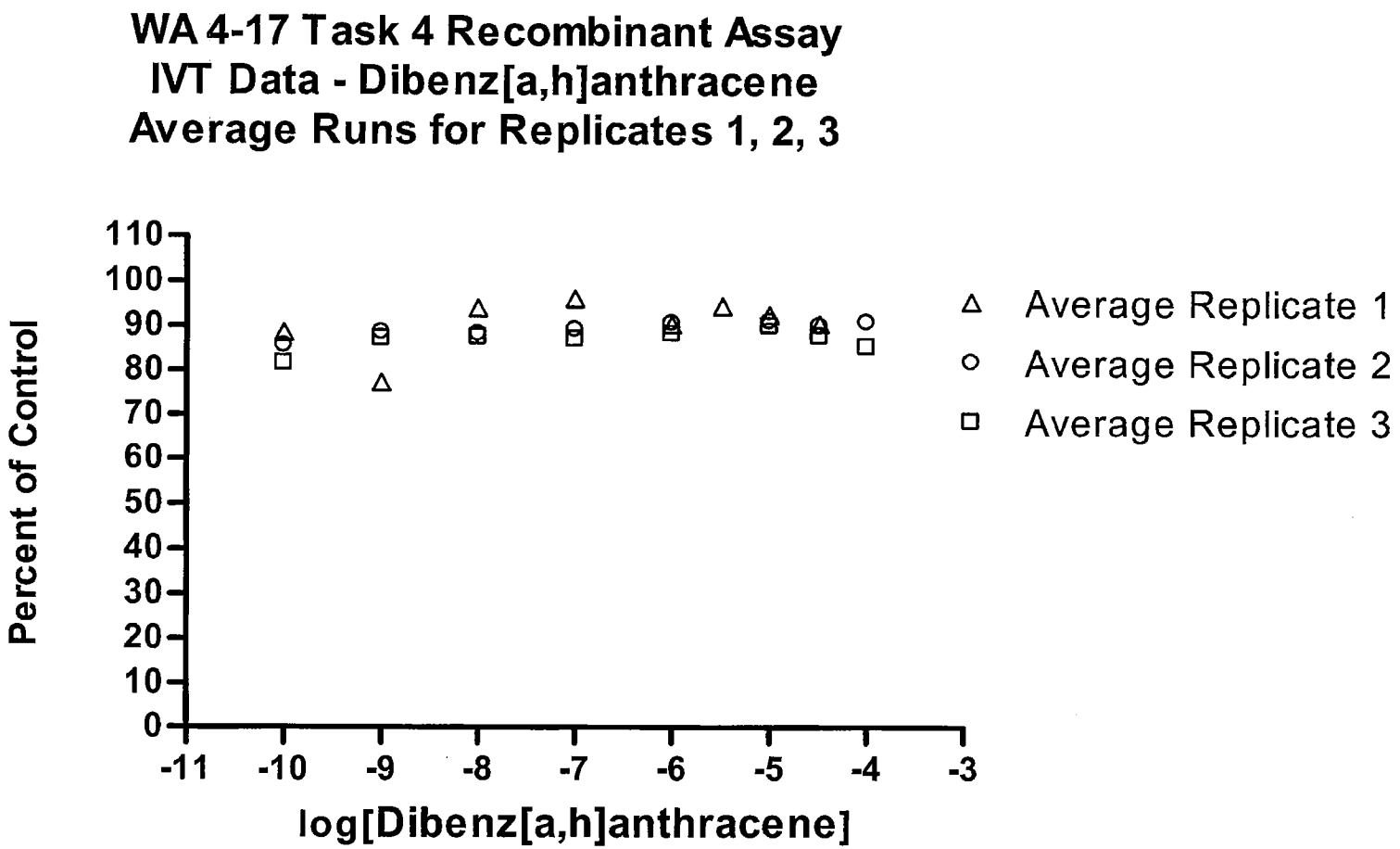


Figure 5-1. **Reference Chemical V: Dibenz[a, h]anthracene.**
Scatter Plot for Averages of Repetitions within Concentration for Each Replicate.
Recombinant Aromatase Assay.

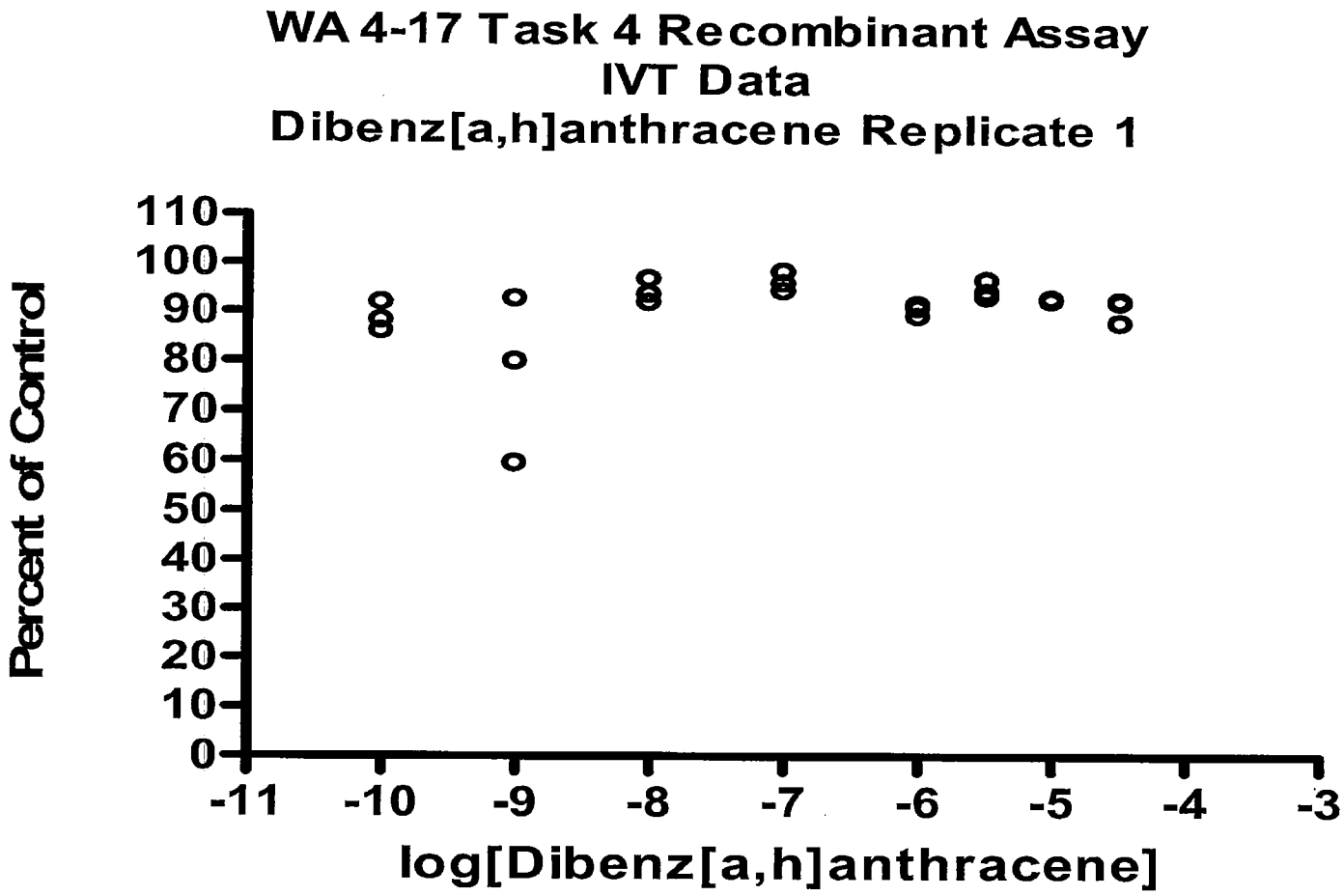


Figure 5-2. **Reference Chemical V: Dibenz[a, h]anthracene. Scatter Plot for Replicate 1 Data. Recombinant Aromatase Assay.**

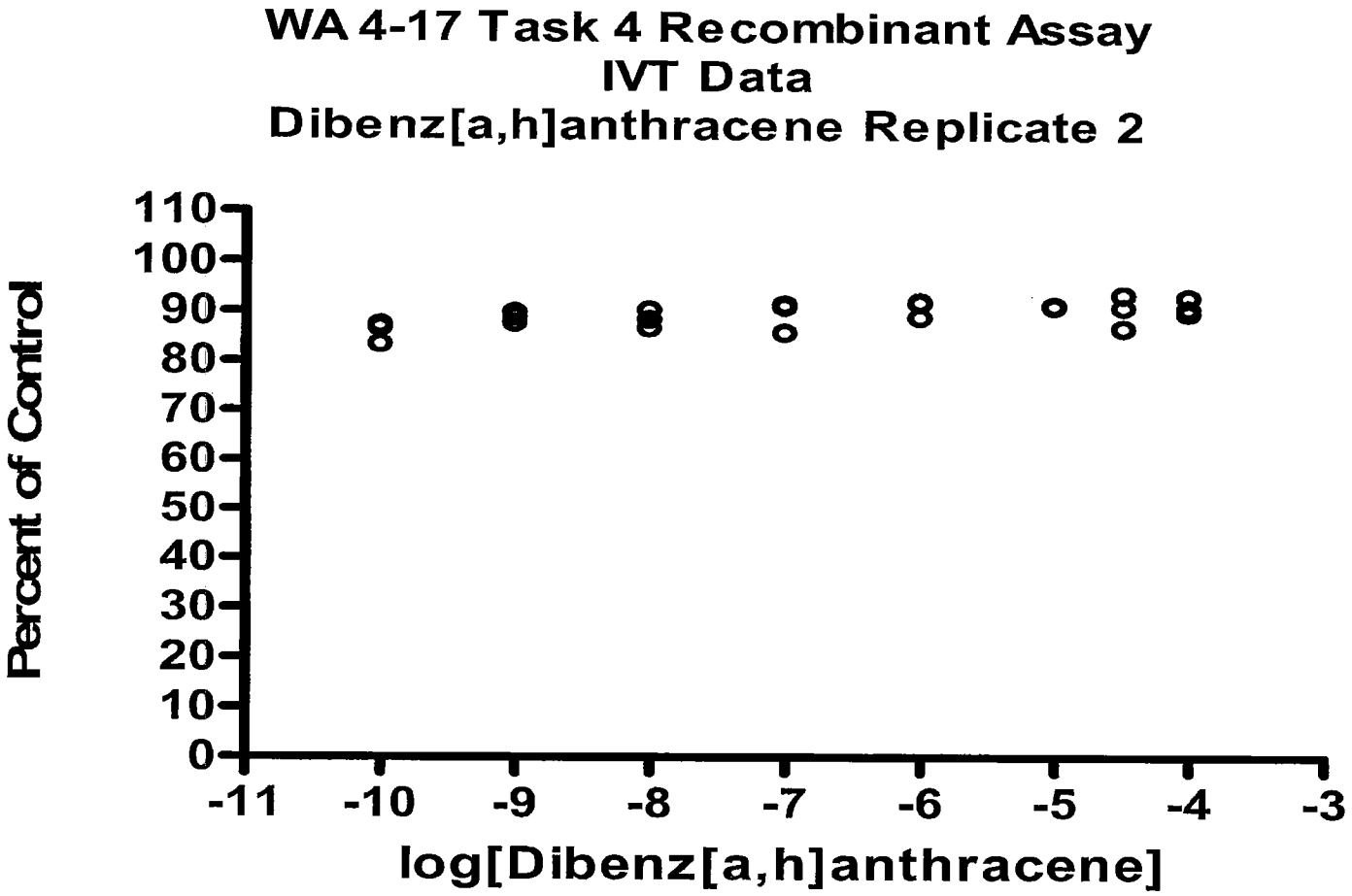


Figure 5-3. **Reference Chemical V: Dibenz[a, h]anthracene. Scatter Plot for Replicate 2 Data. Recombinant Aromatase Assay.**

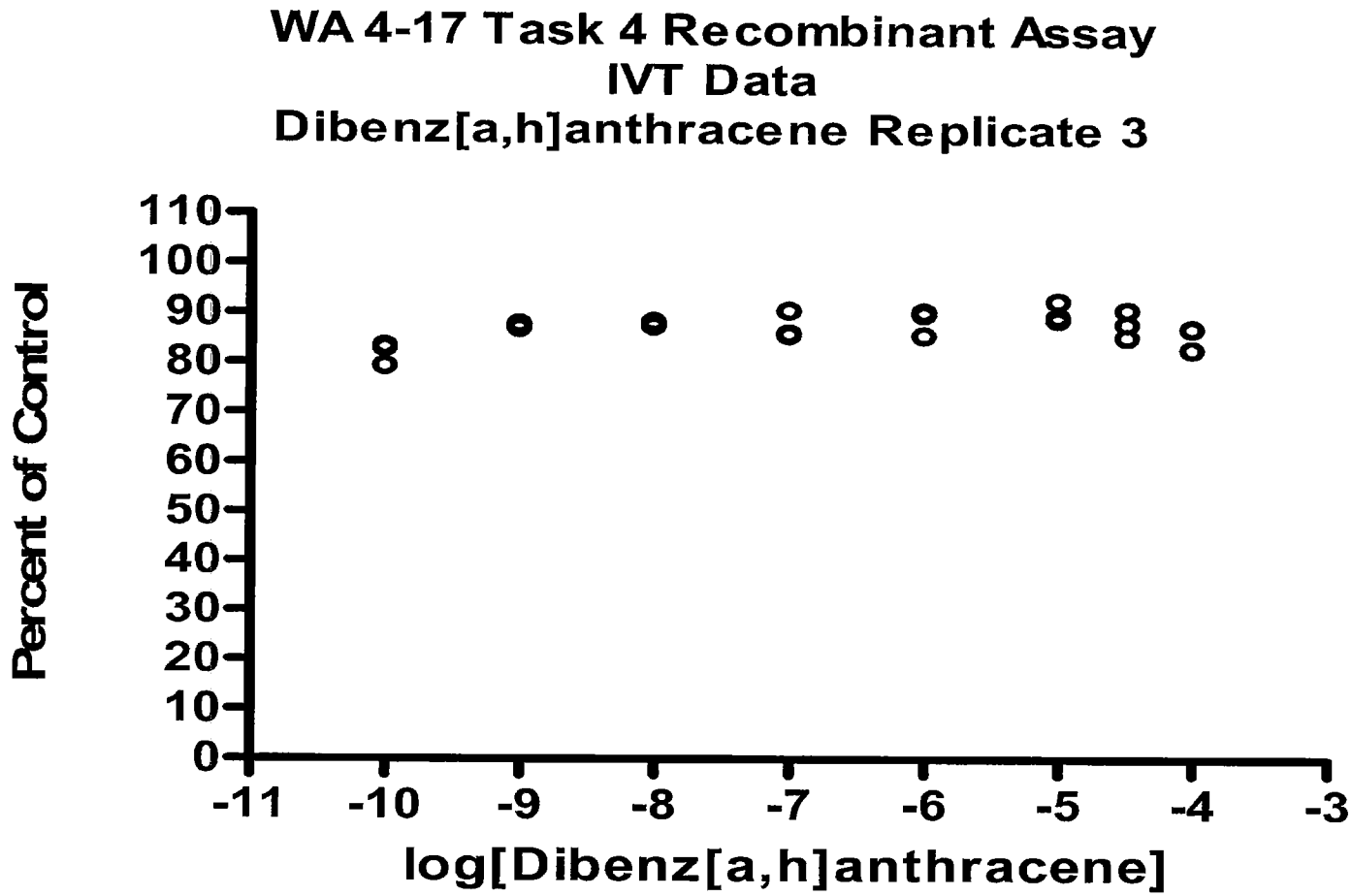


Figure 5-4. Reference Chemical V: Dibenz[a, h]anthracene.
Scatter Plot for Replicate 3 Data. Recombinant Aromatase Assay.

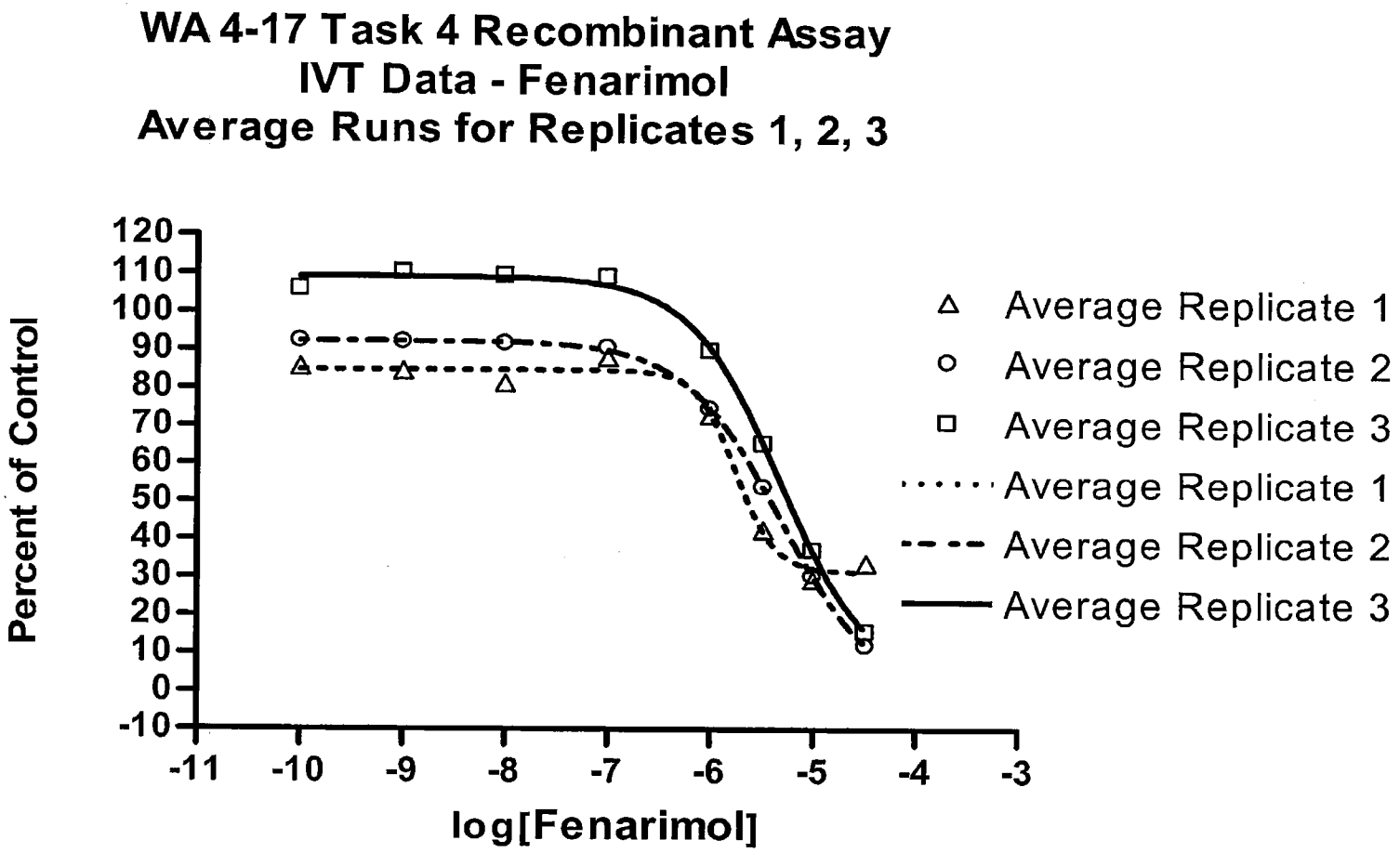


Figure 6-1. **Reference Chemical VI: Fenarimol.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

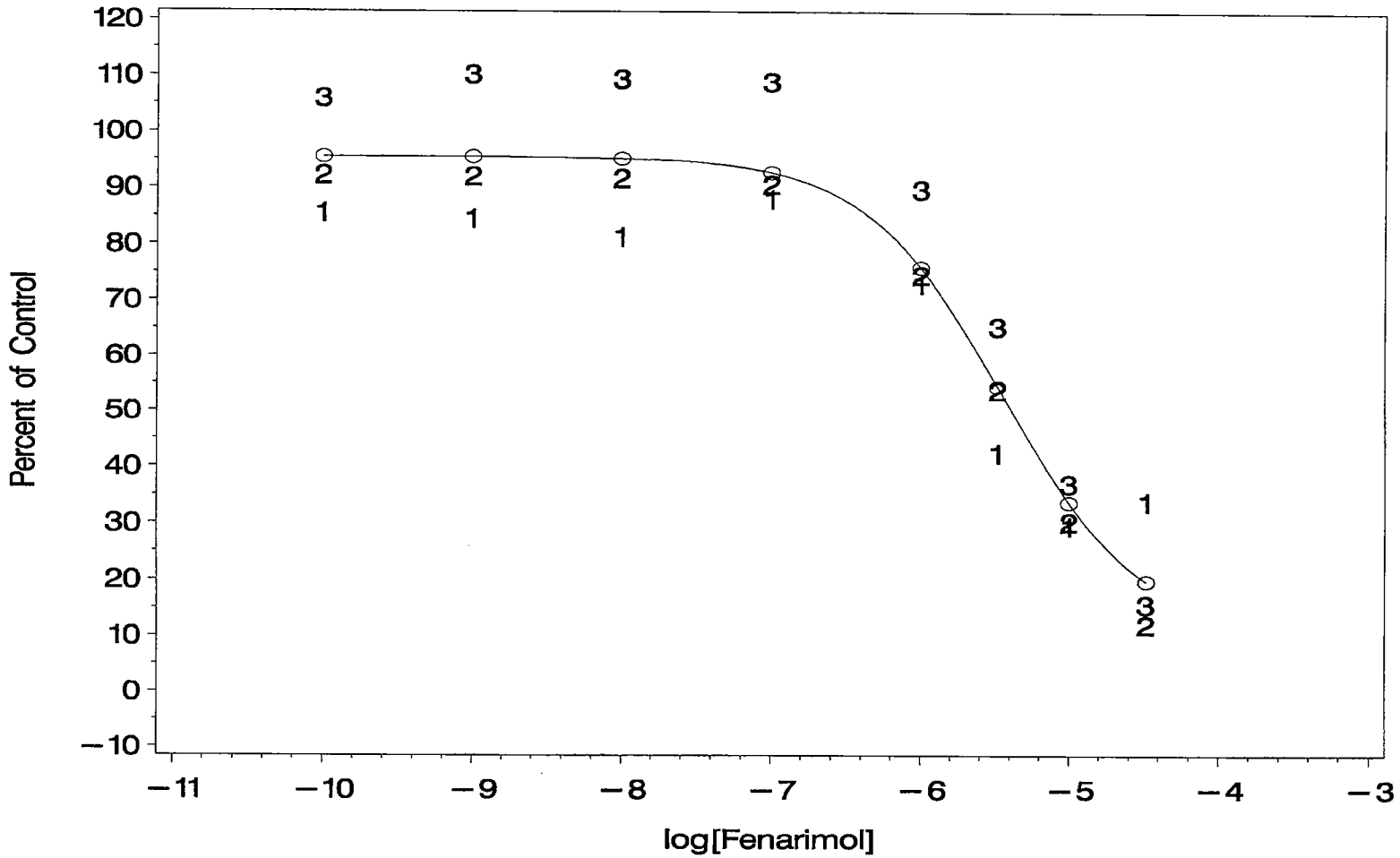


Figure 6-2. Reference Chemical VI: Fenarimol. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

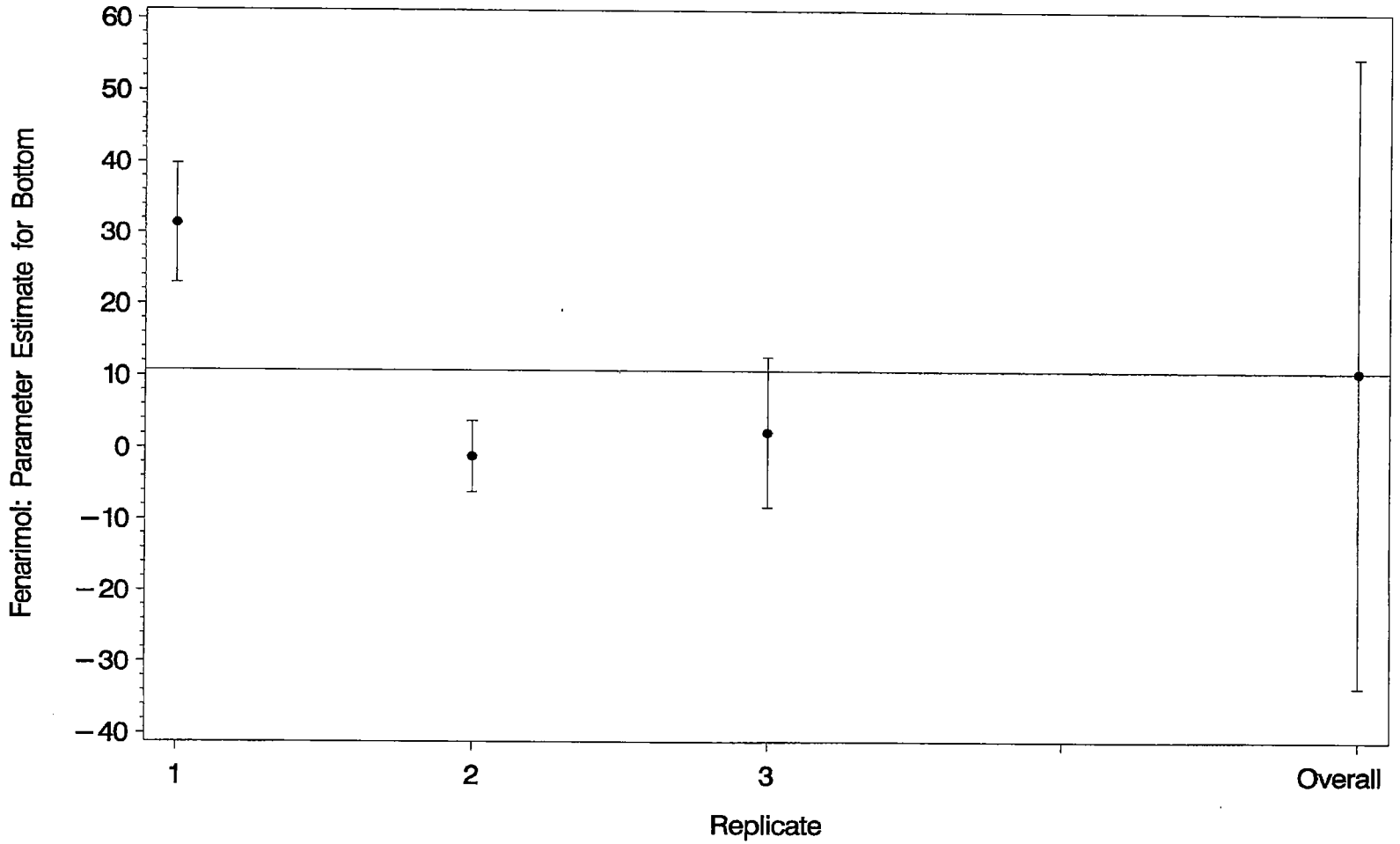


Figure 6-3. Reference Chemical VI: Fenarimol. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

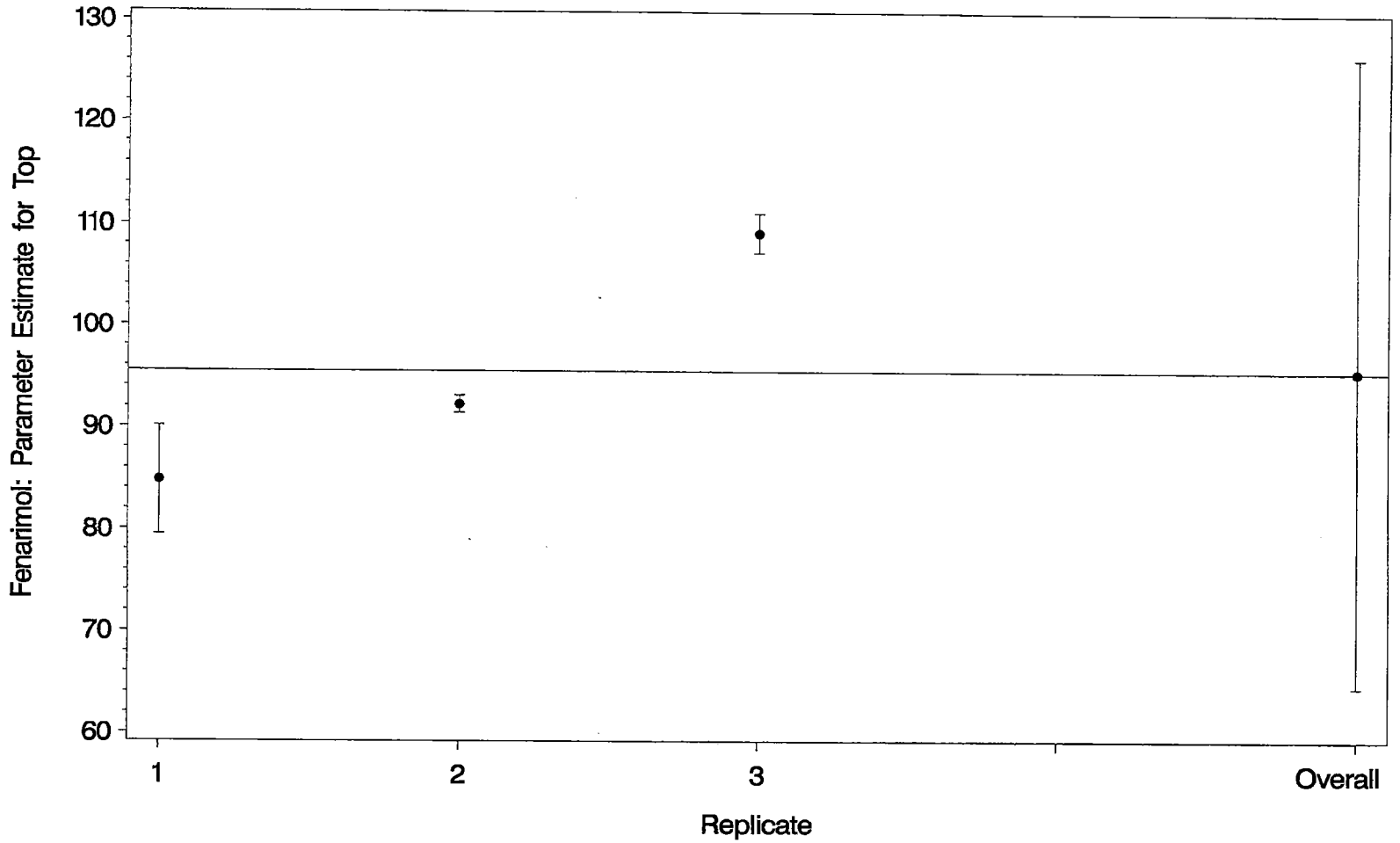


Figure 6-4. **Reference Chemical VI: Fenarimol. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

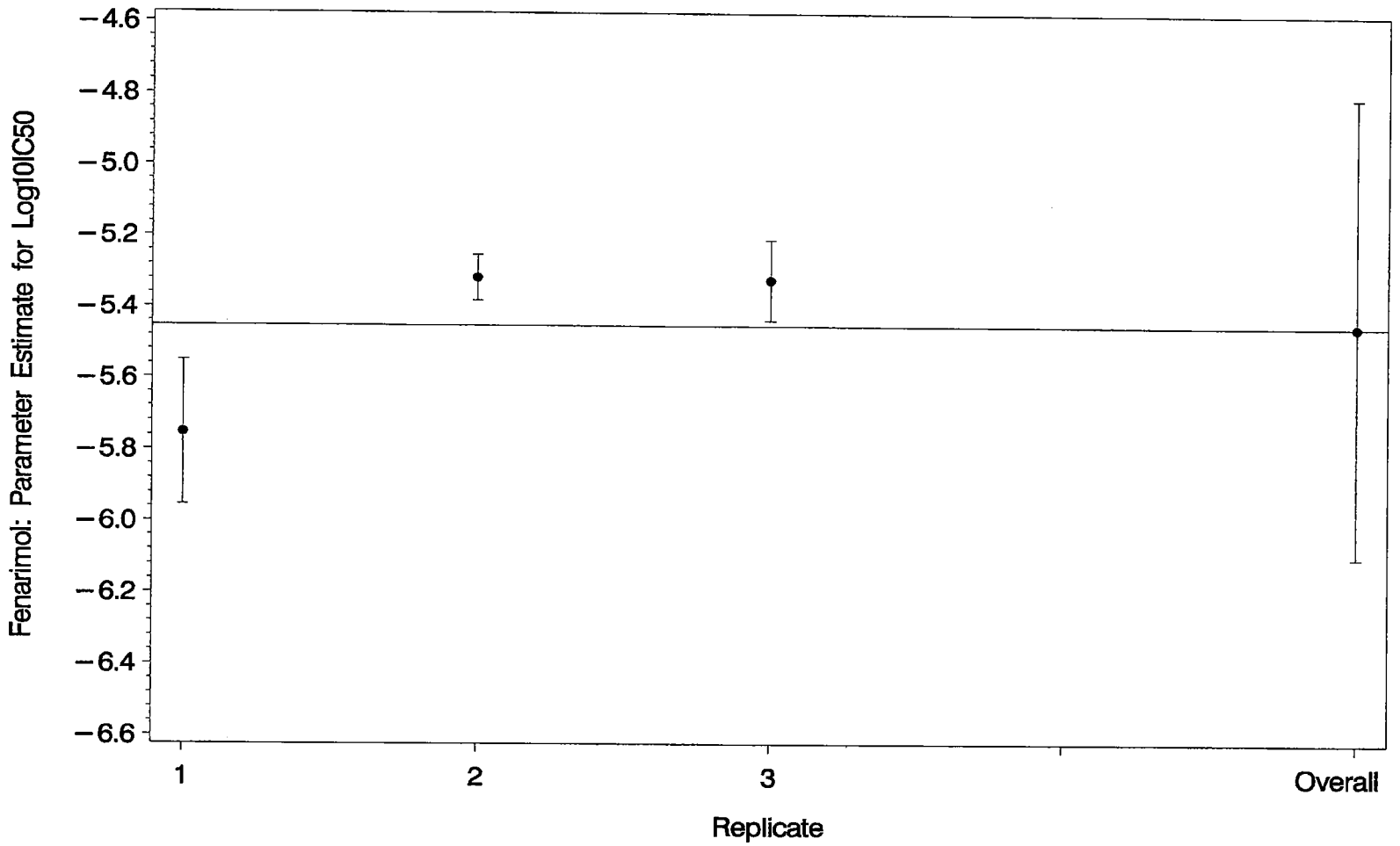


Figure 6-5. Reference Chemical VI: Fenarimol. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

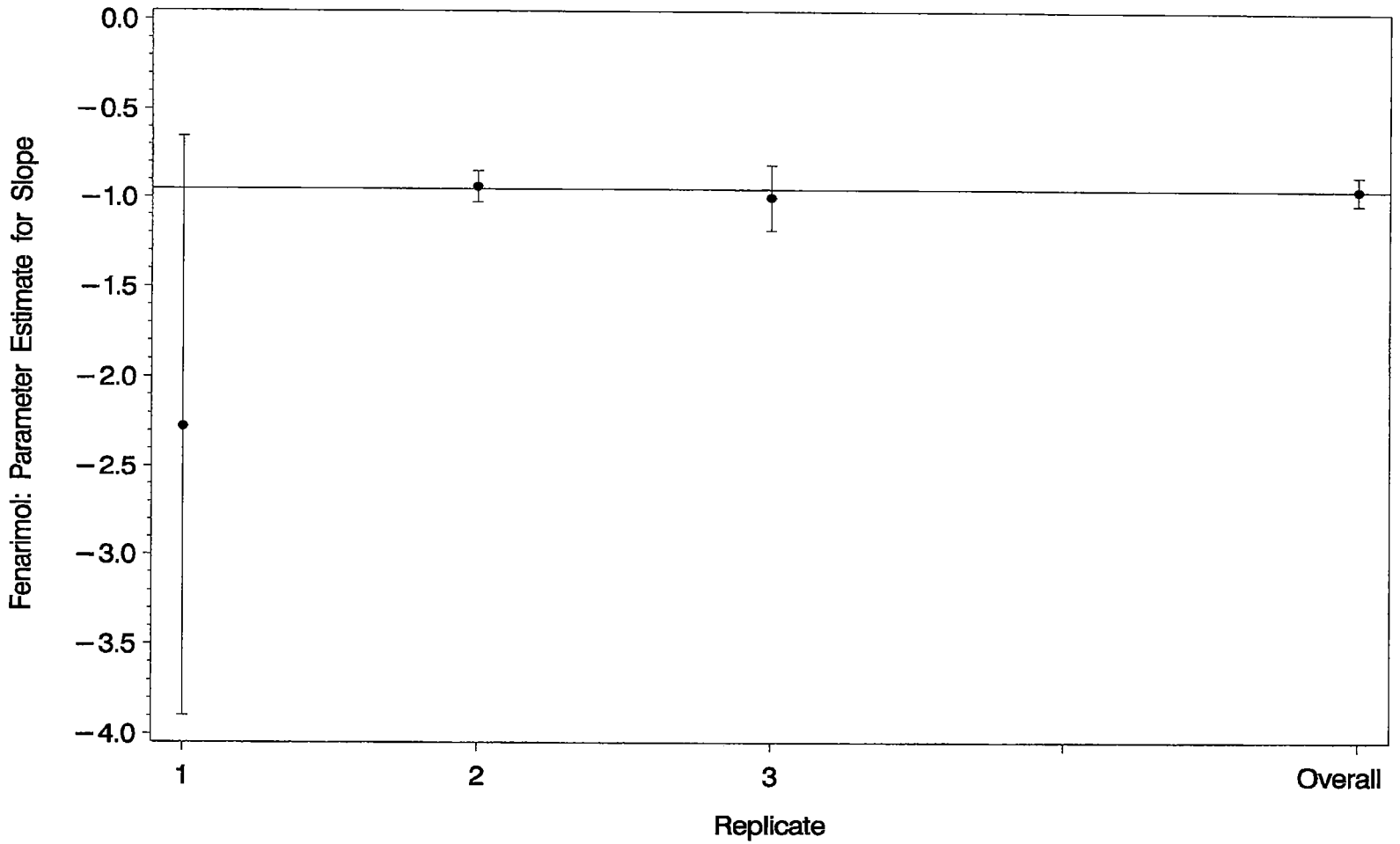


Figure 6-6. Reference Chemical VI: Fenarimol. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

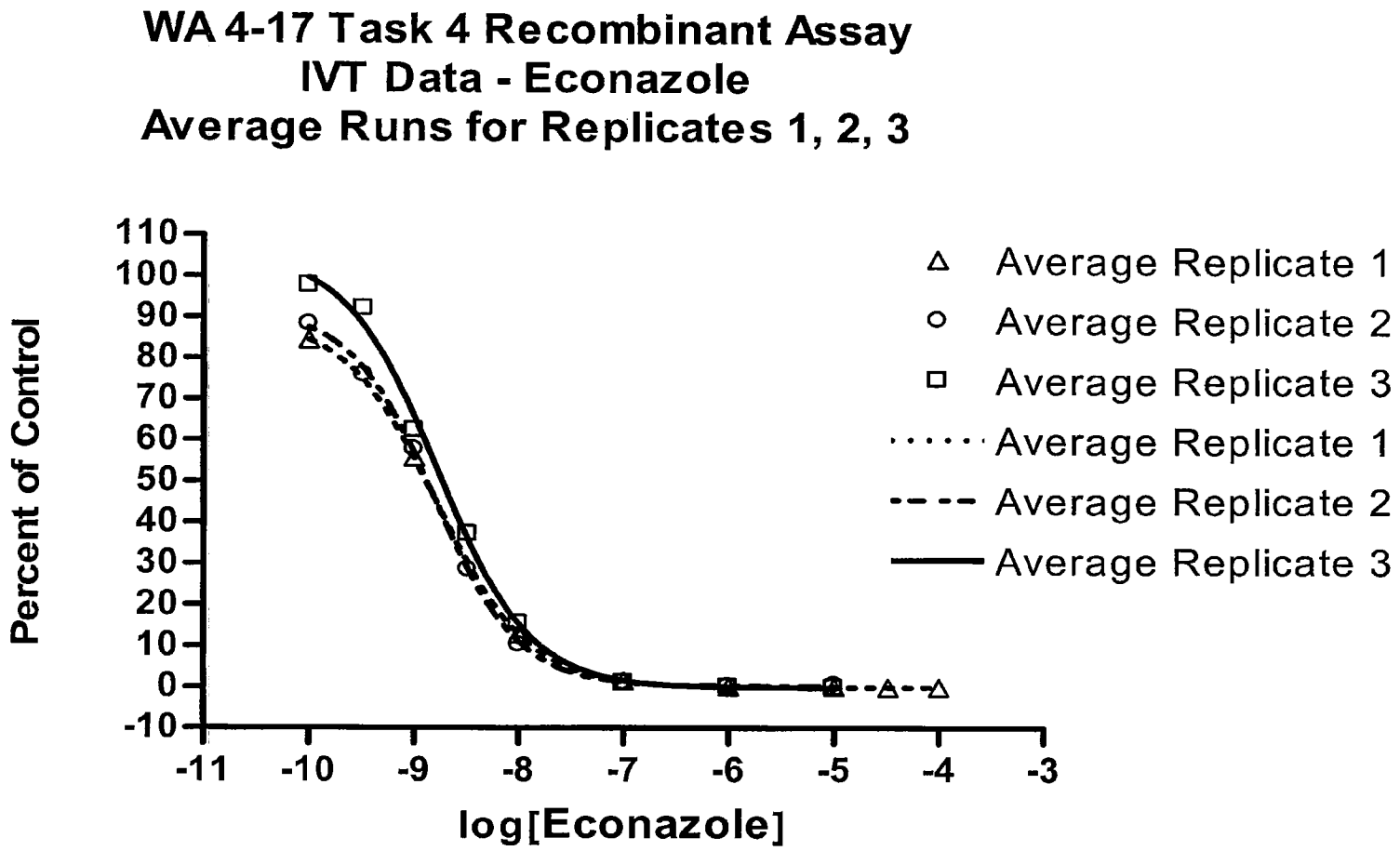


Figure 7-1. **Reference Chemical VII: Econazole.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

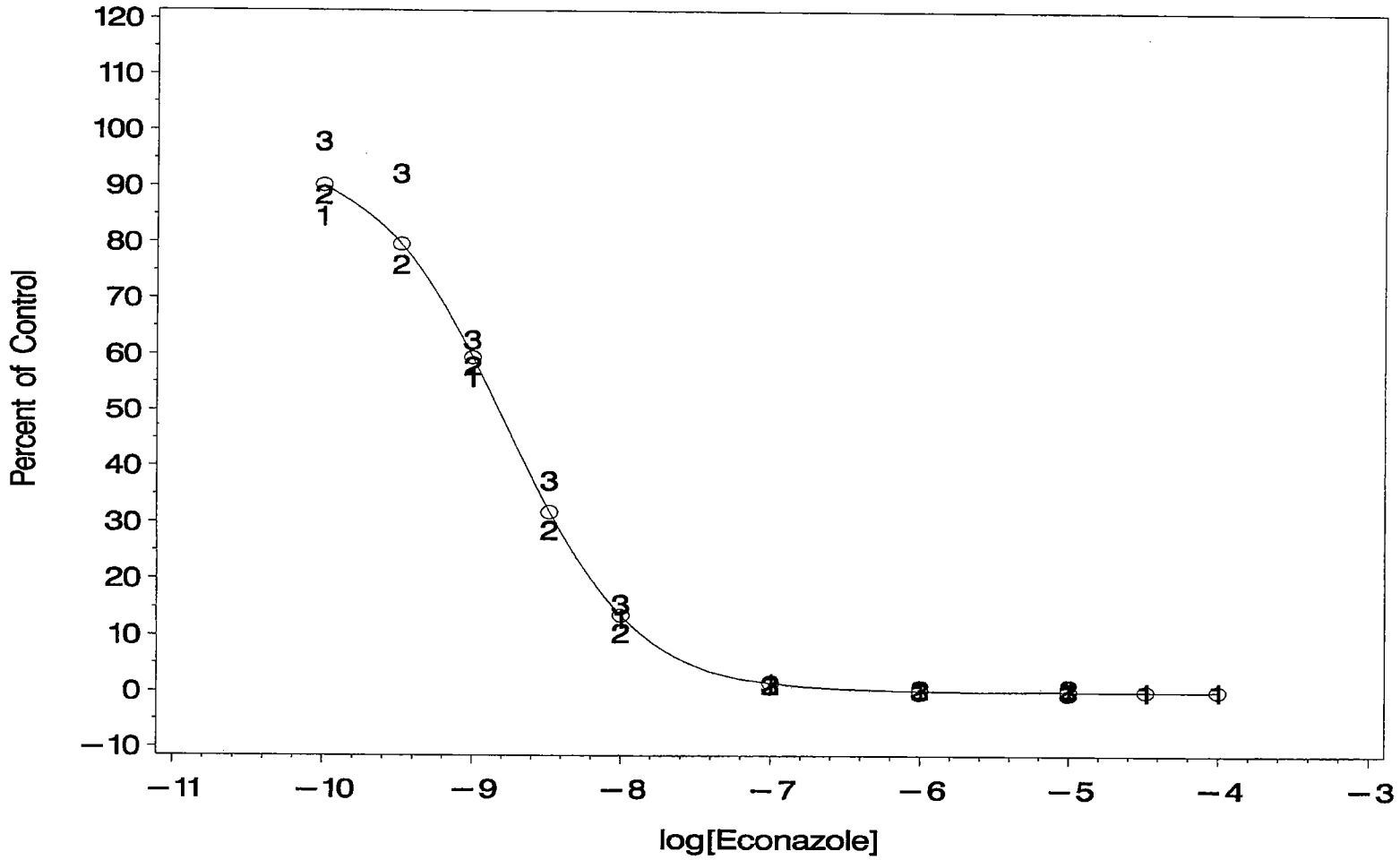


Figure 7-2. Reference Chemical VII: Econazole. Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

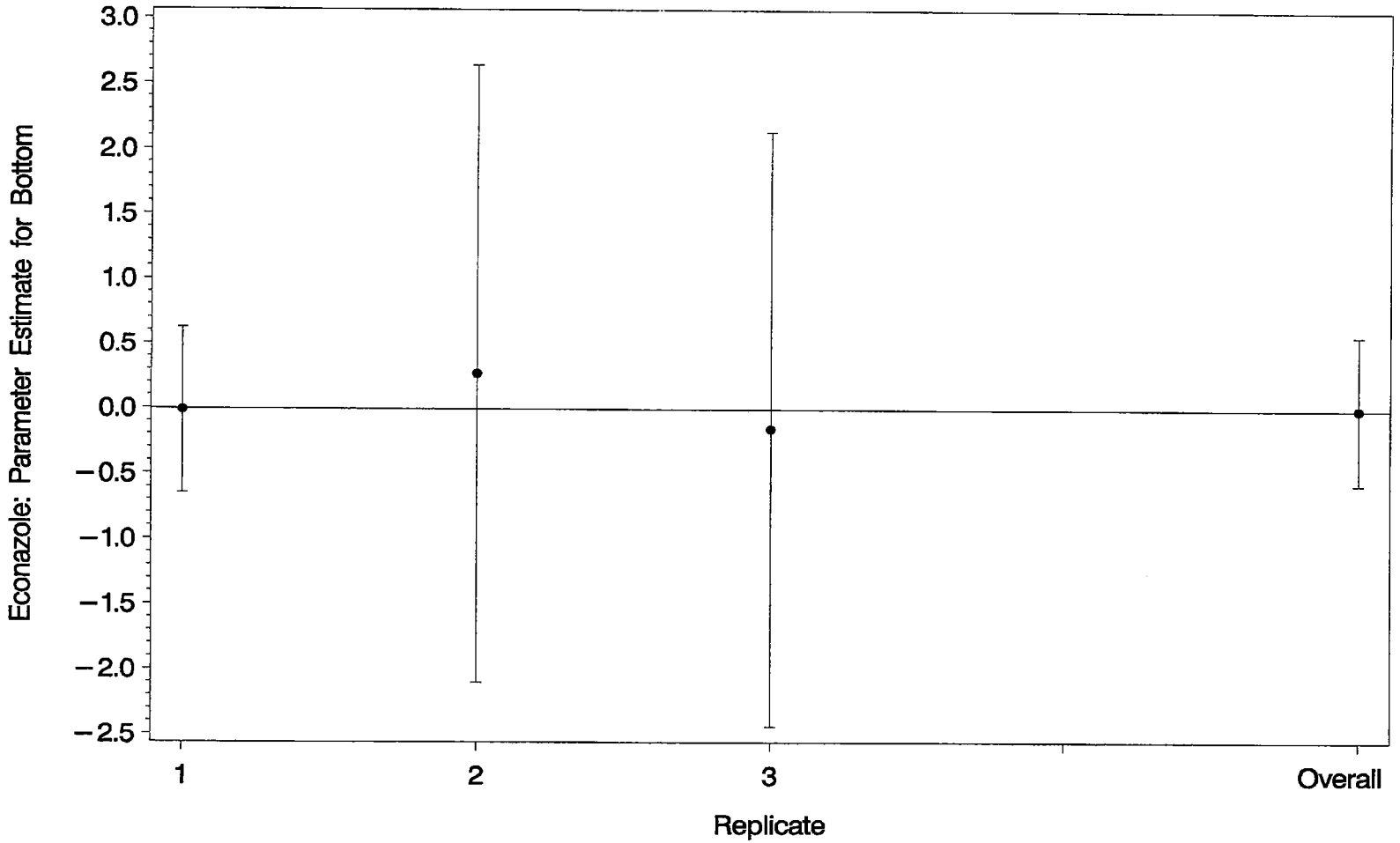


Figure 7-3. Reference Chemical VII: Econazole. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

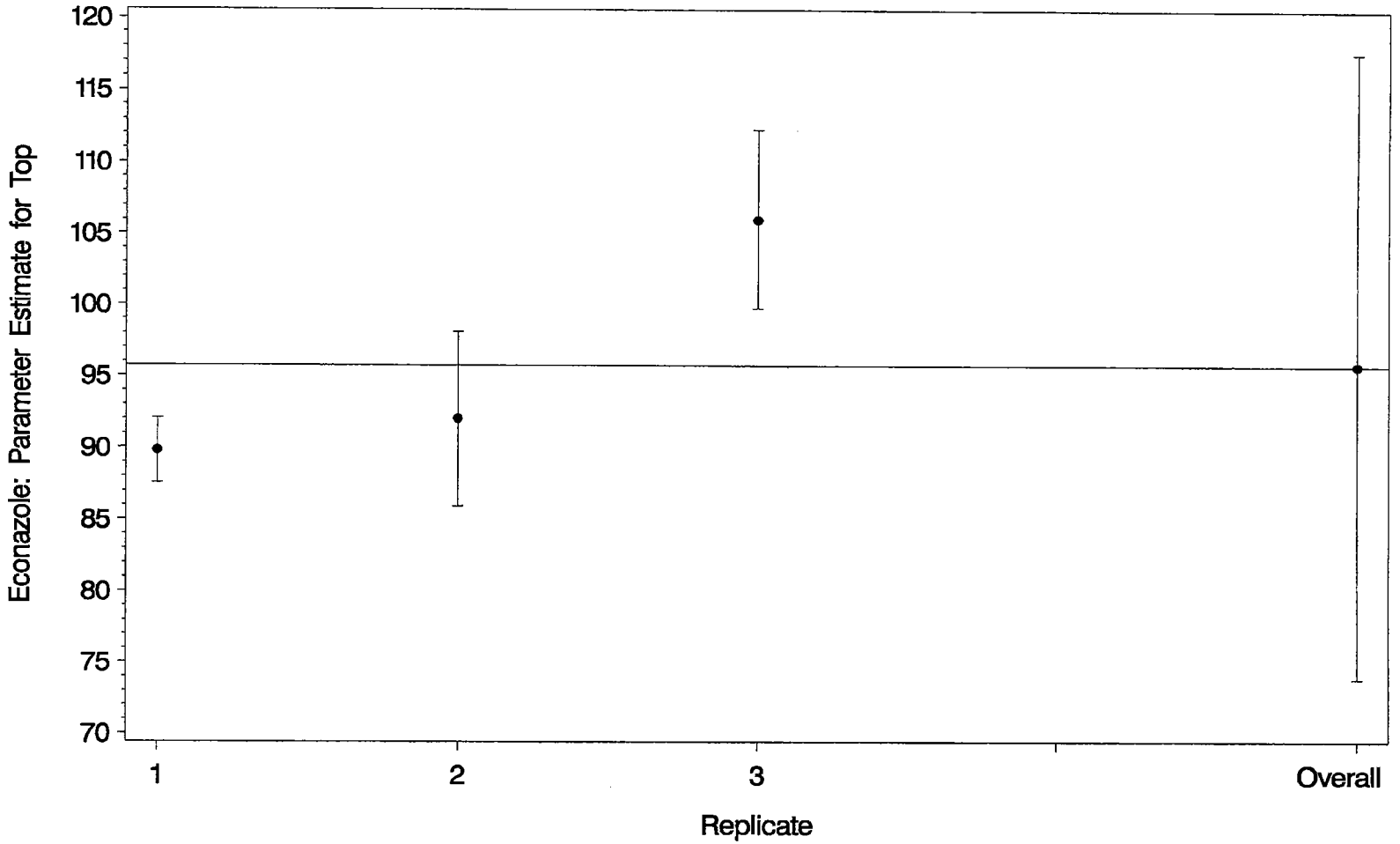


Figure 7-4. Reference Chemical VII: Econazole. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

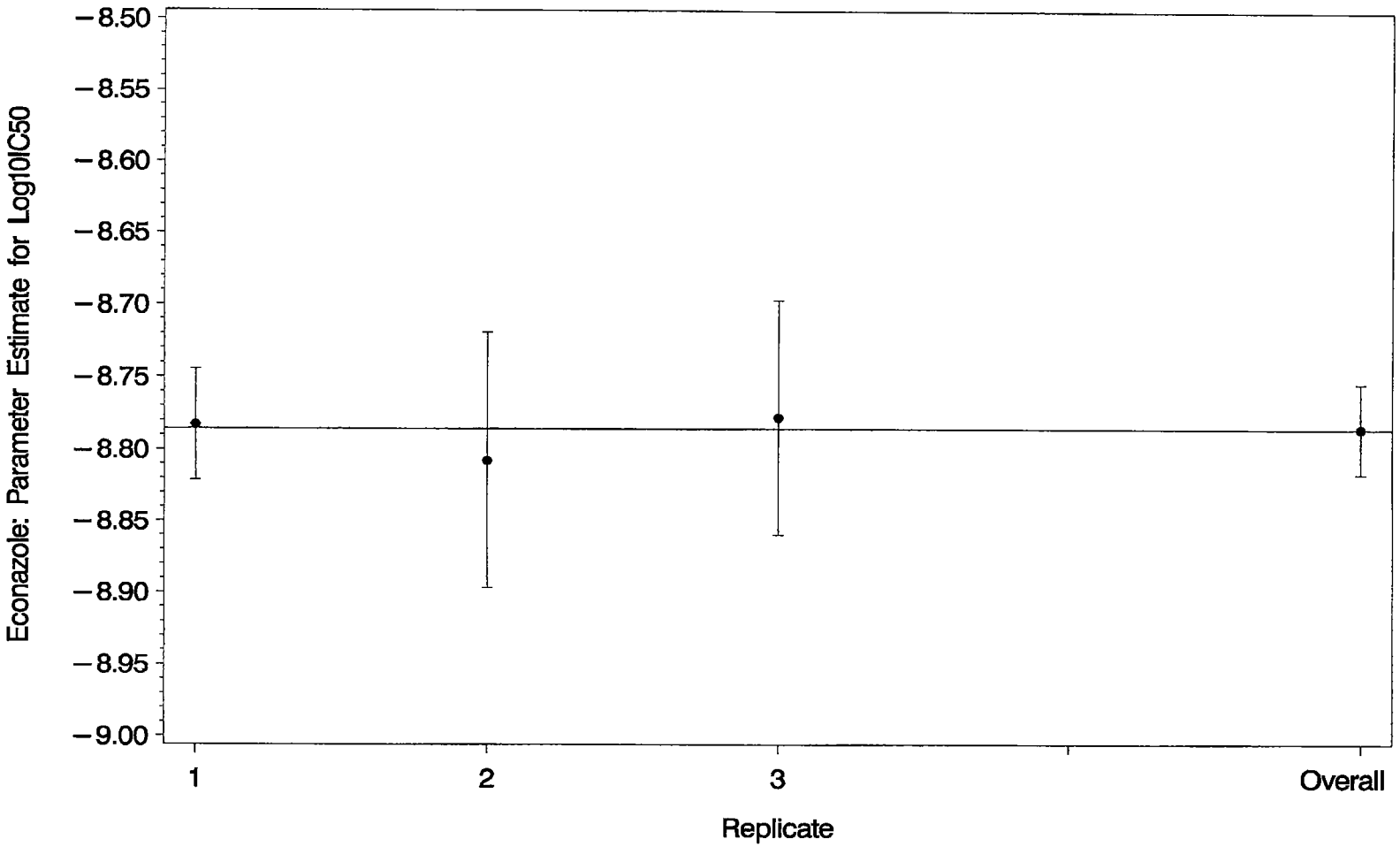


Figure 7-5. Reference Chemical VII: Econazole. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

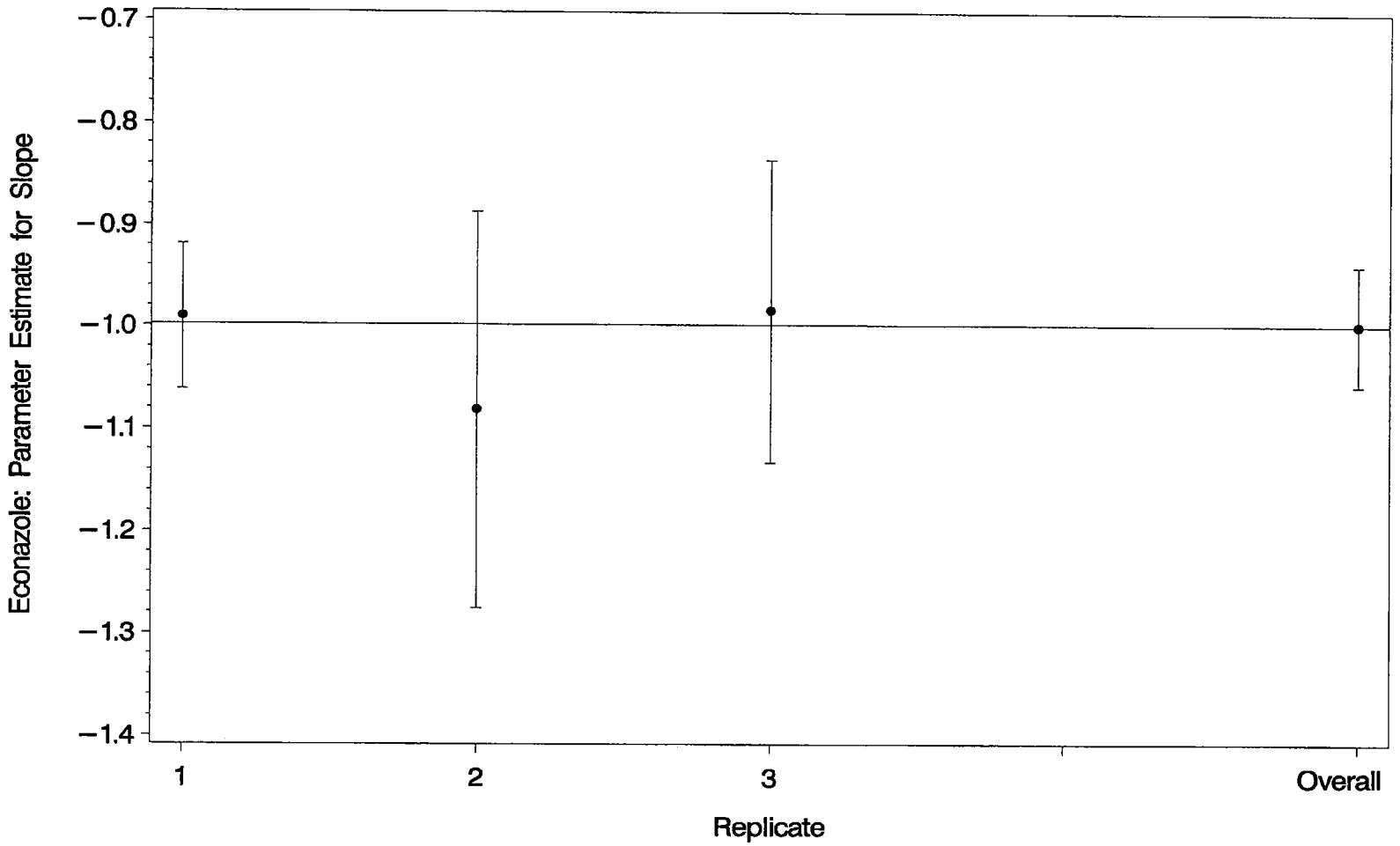


Figure 7-6. Reference Chemical VII: Econazole. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

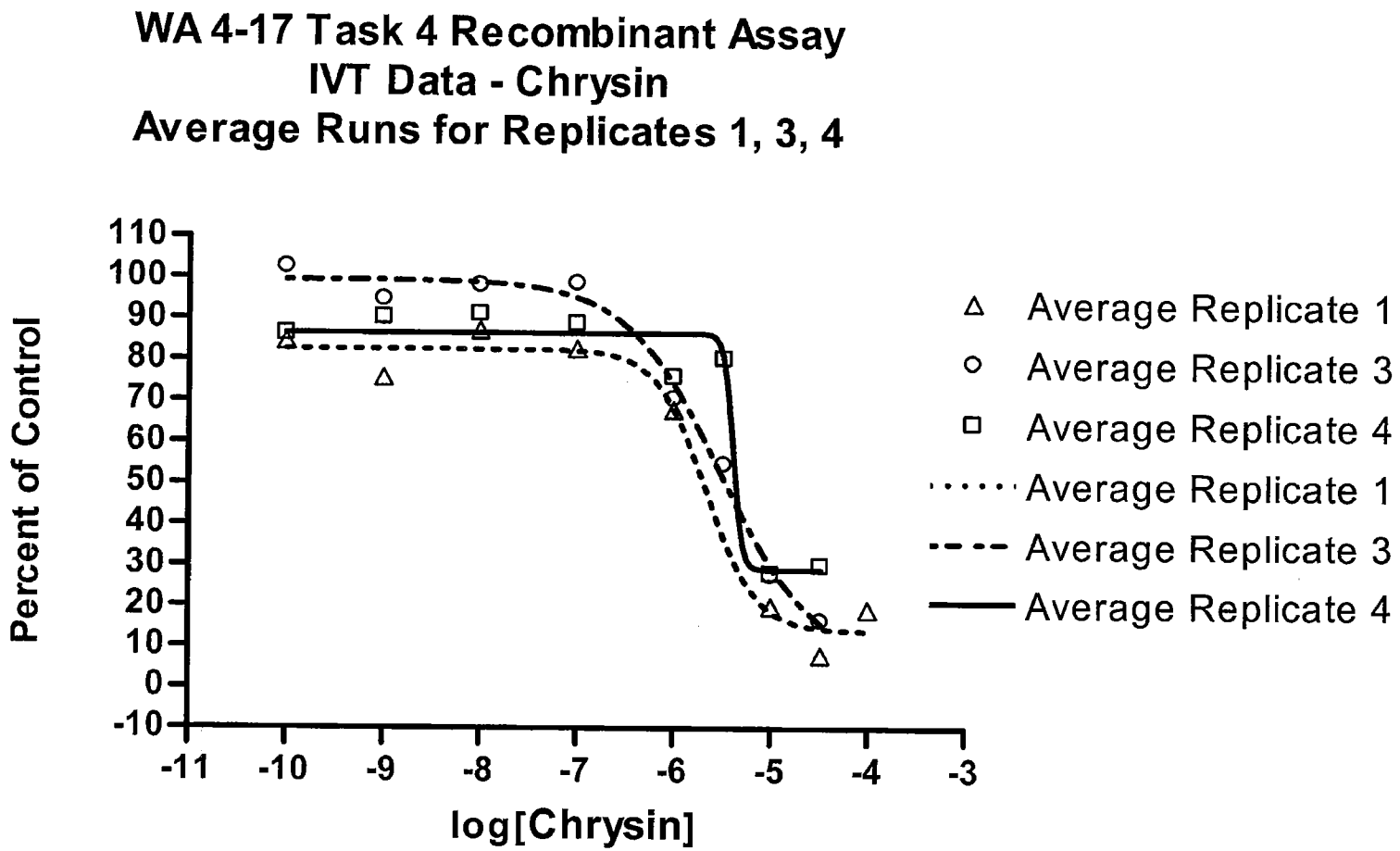


Figure 8-1. **Reference Chemical VIII: Chrysin.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

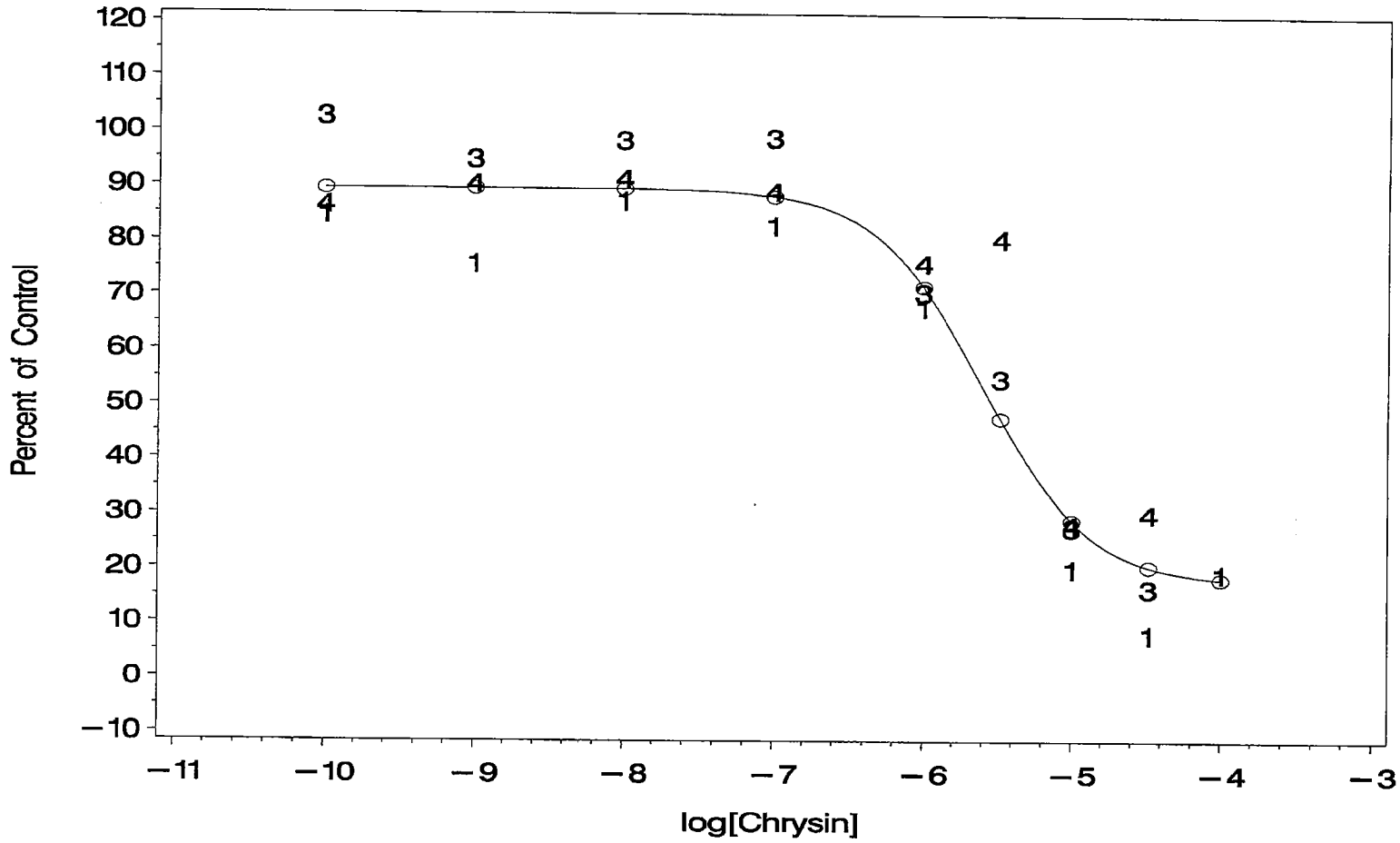


Figure 8-2. Reference Chemical VIII: Chrysin.
Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay.

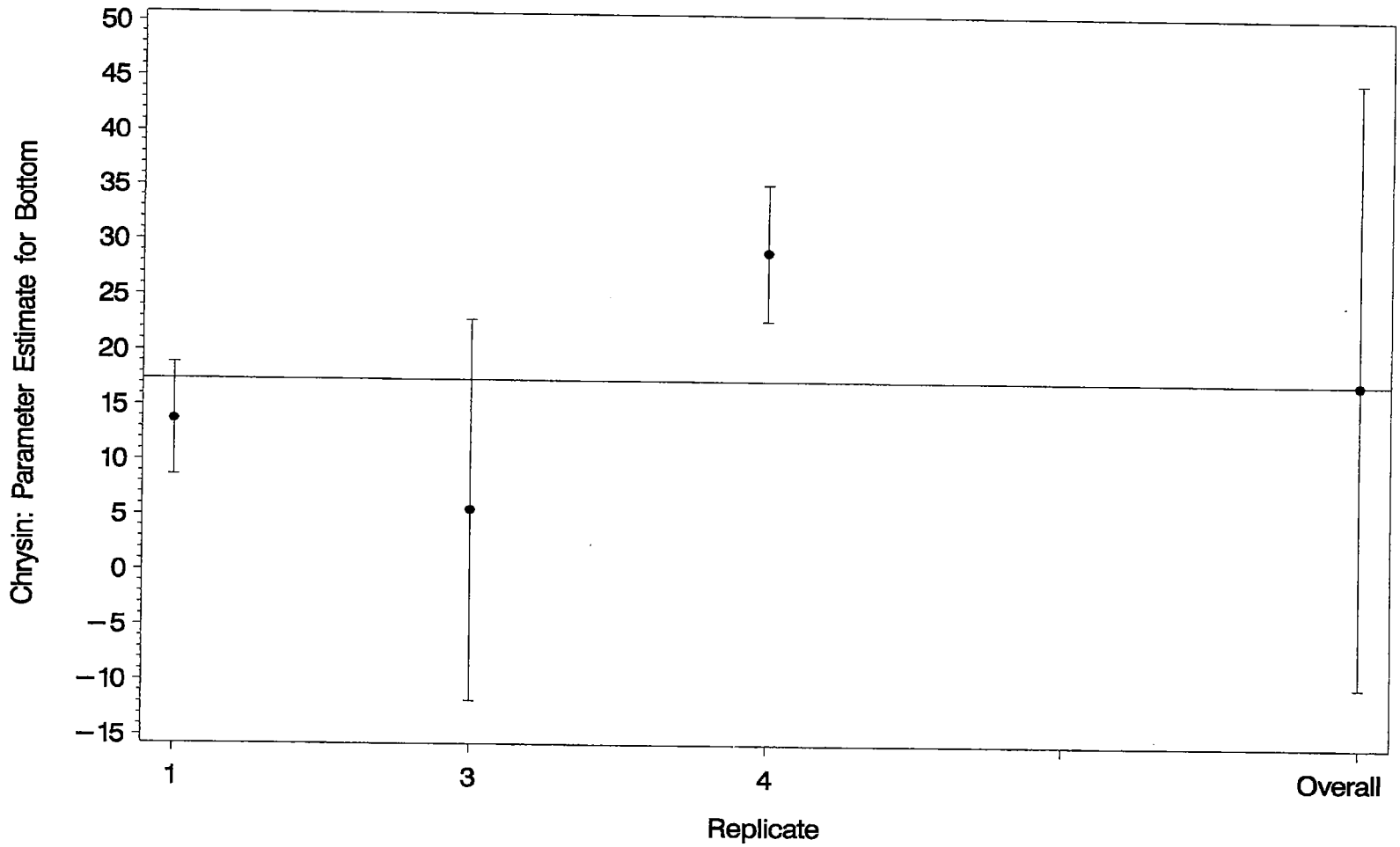


Figure 8-3. Reference Chemical VIII: Chrysin. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

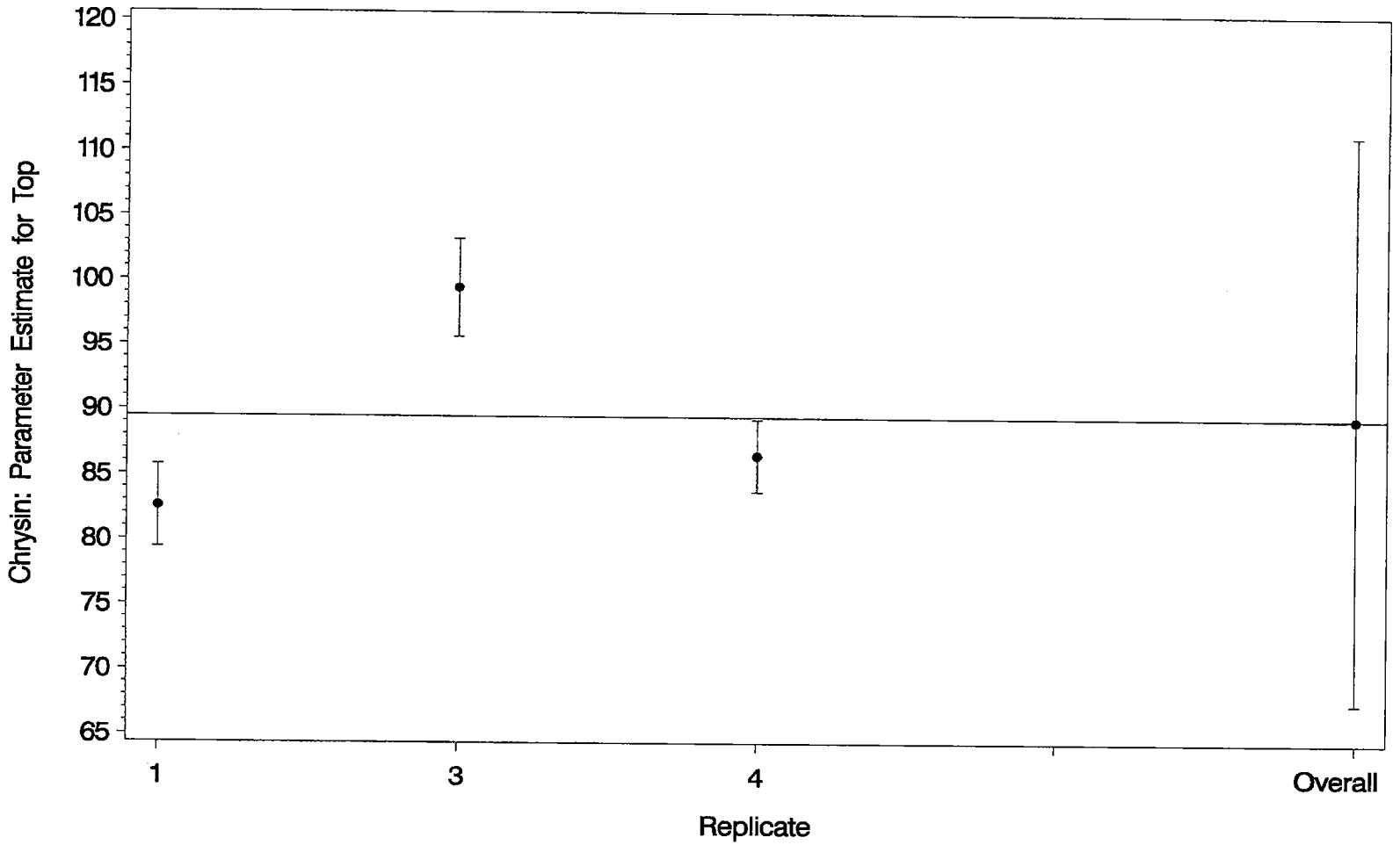


Figure 8-4. **Reference Chemical VIII: Chrysin. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

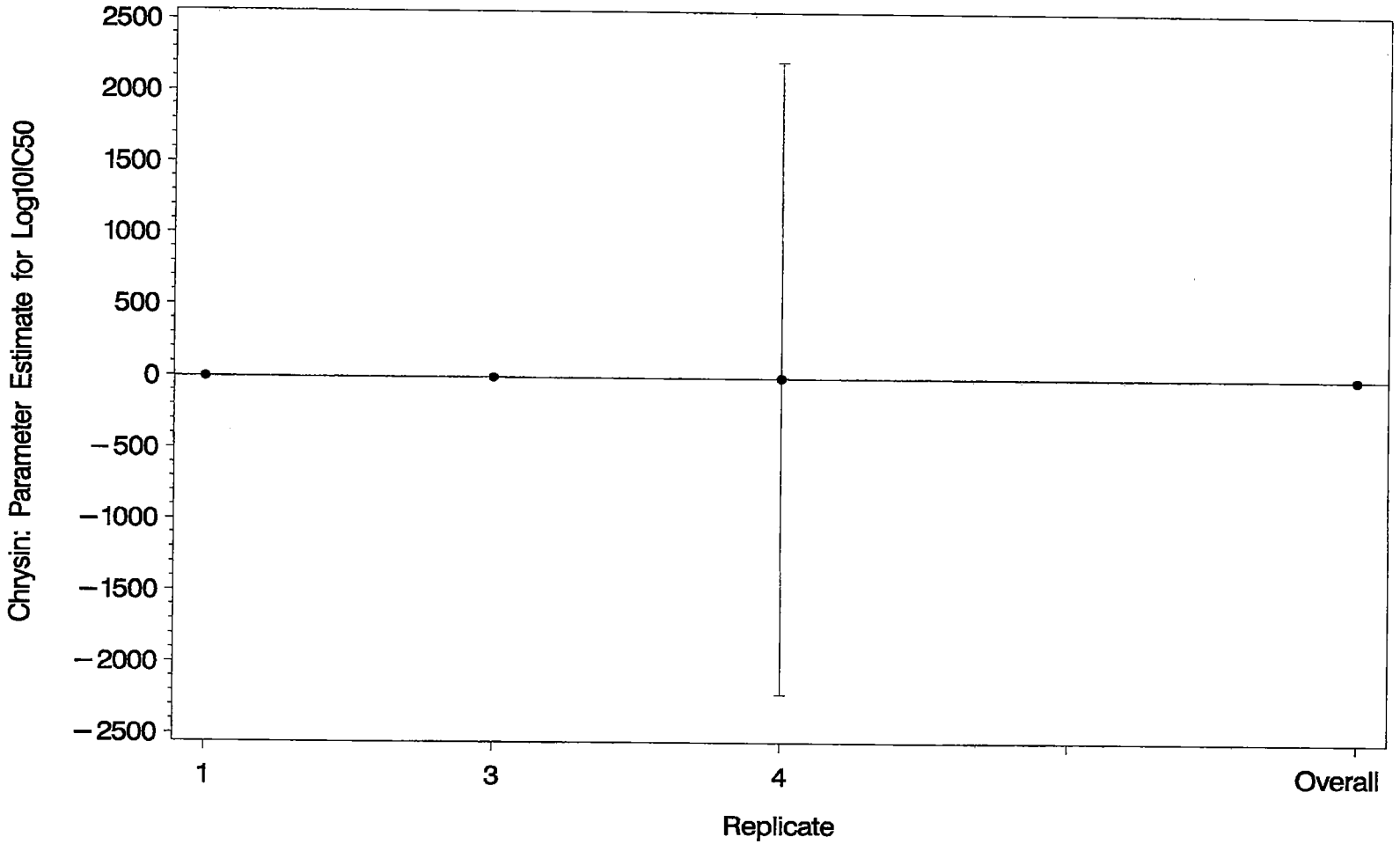


Figure 8-5. Reference Chemical VIII: Chrysin. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

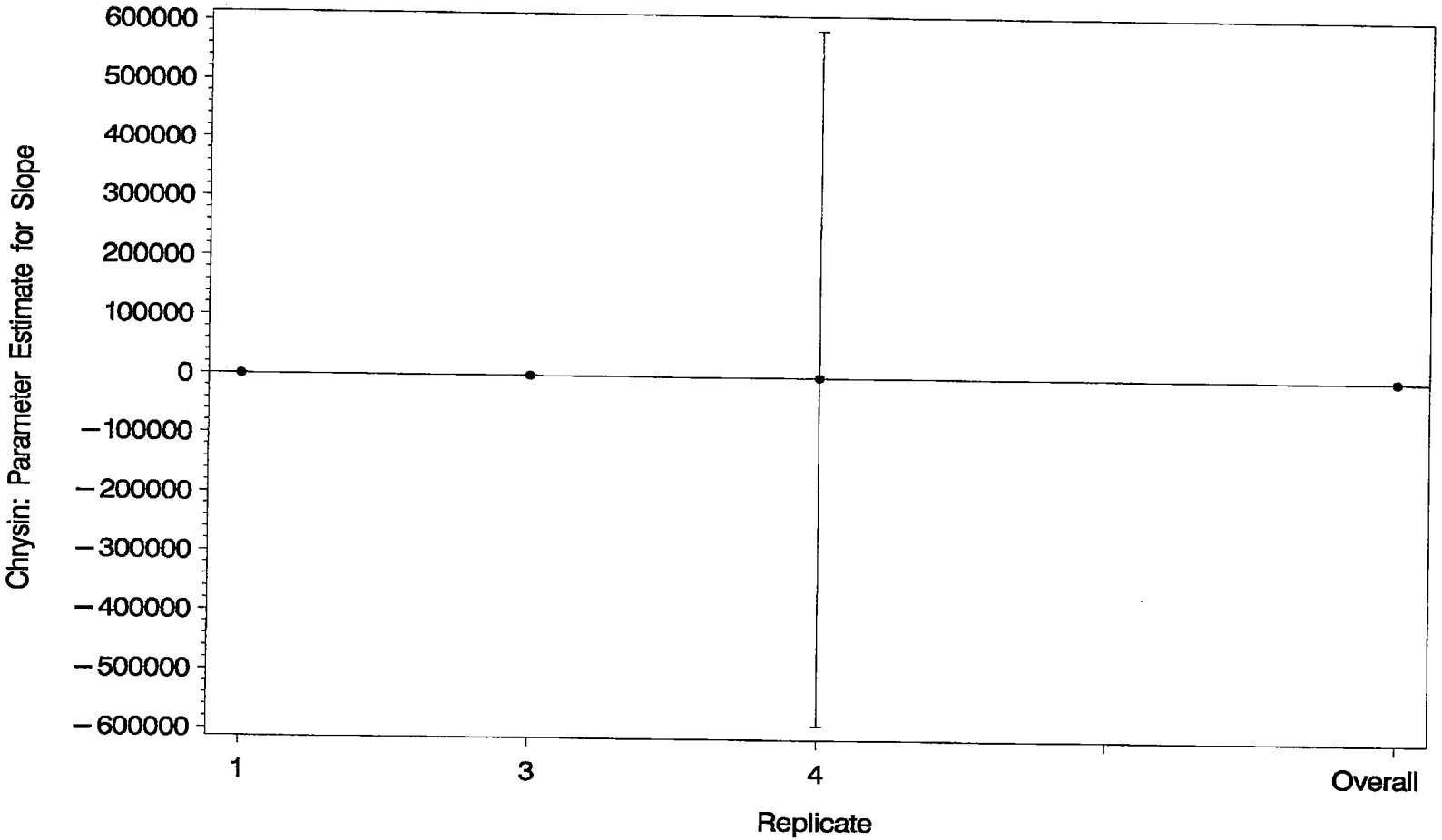


Figure 8-6. Reference Chemical VIII: Chrysin. Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay.

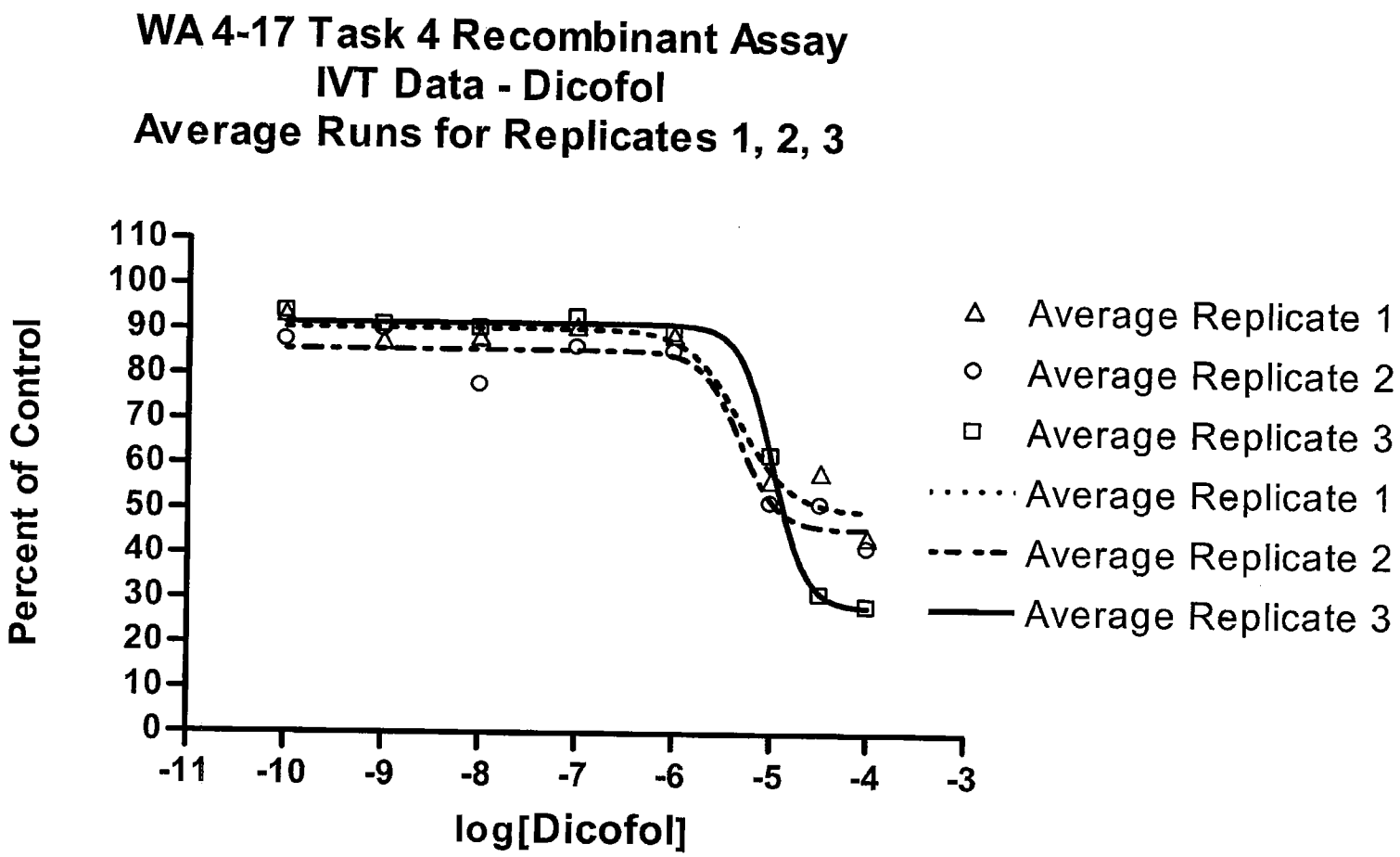


Figure 9-1. **Reference Chemical IX: Dicofol.**
Concentration Response Curves and Averages of Repetitions within Each Concentration.
Recombinant Aromatase Assay.

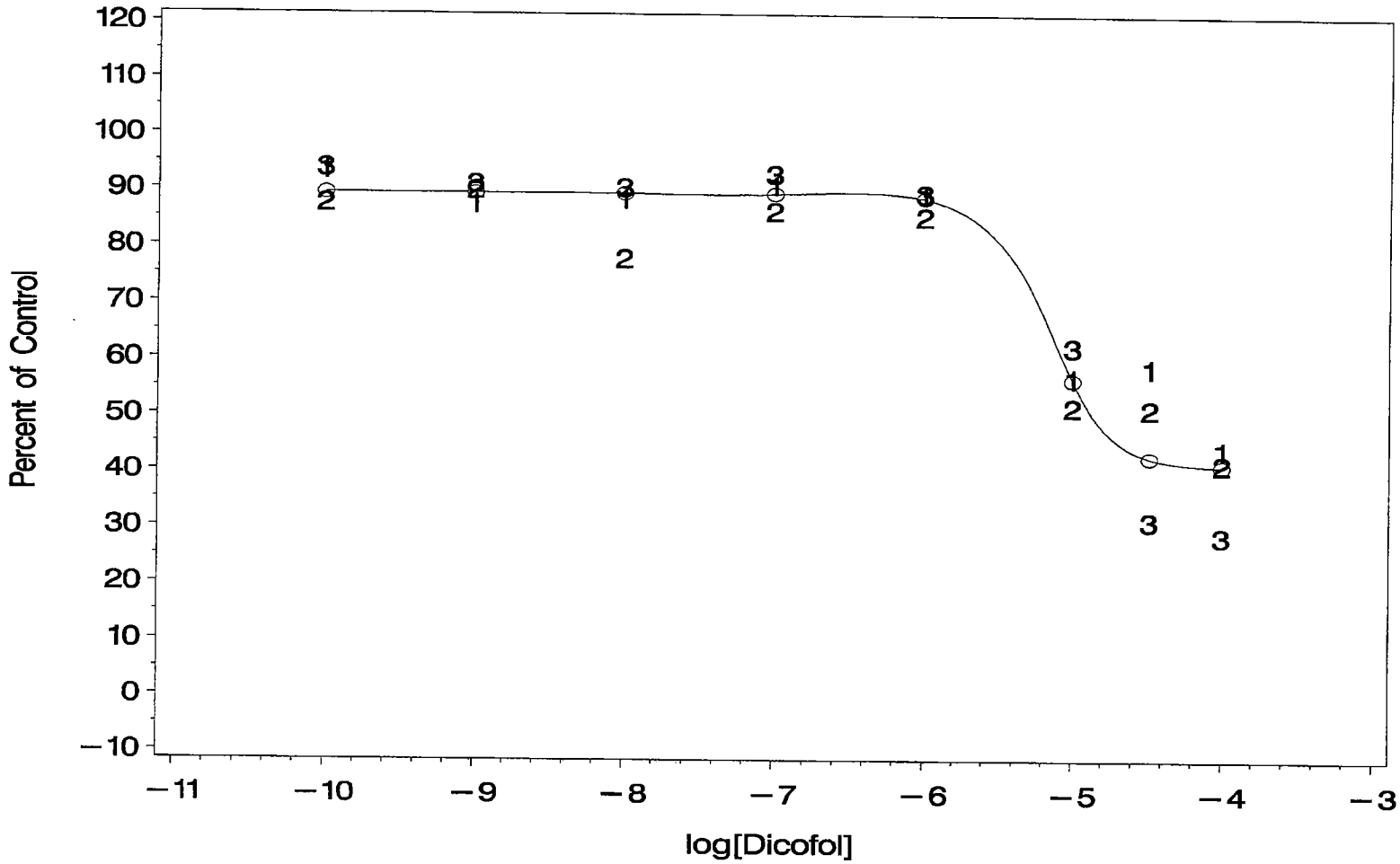


Figure 9-2. **Reference Chemical IX: Dicofol.**
Overall Average Concentration Response Curve Across Replicates and Average Responses Across Repetitions Within Chemical Concentrations. Recombinant Aromatase Assay. Parameters of Average Curve Based on One-Way Analysis of Variance Across Replicate Parameter Values.

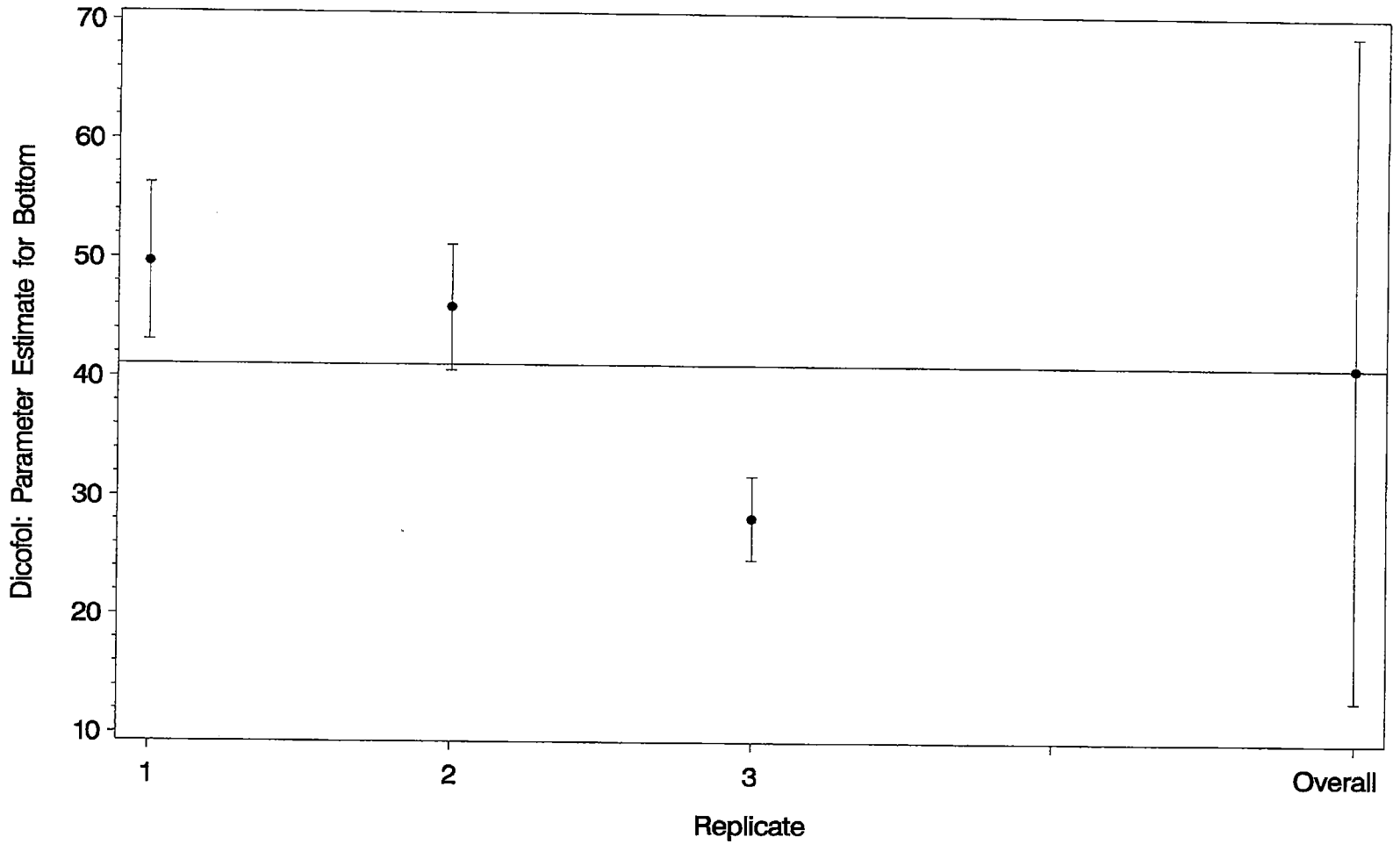


Figure 9-3. Reference Chemical IX: Dicofol. Bottom Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

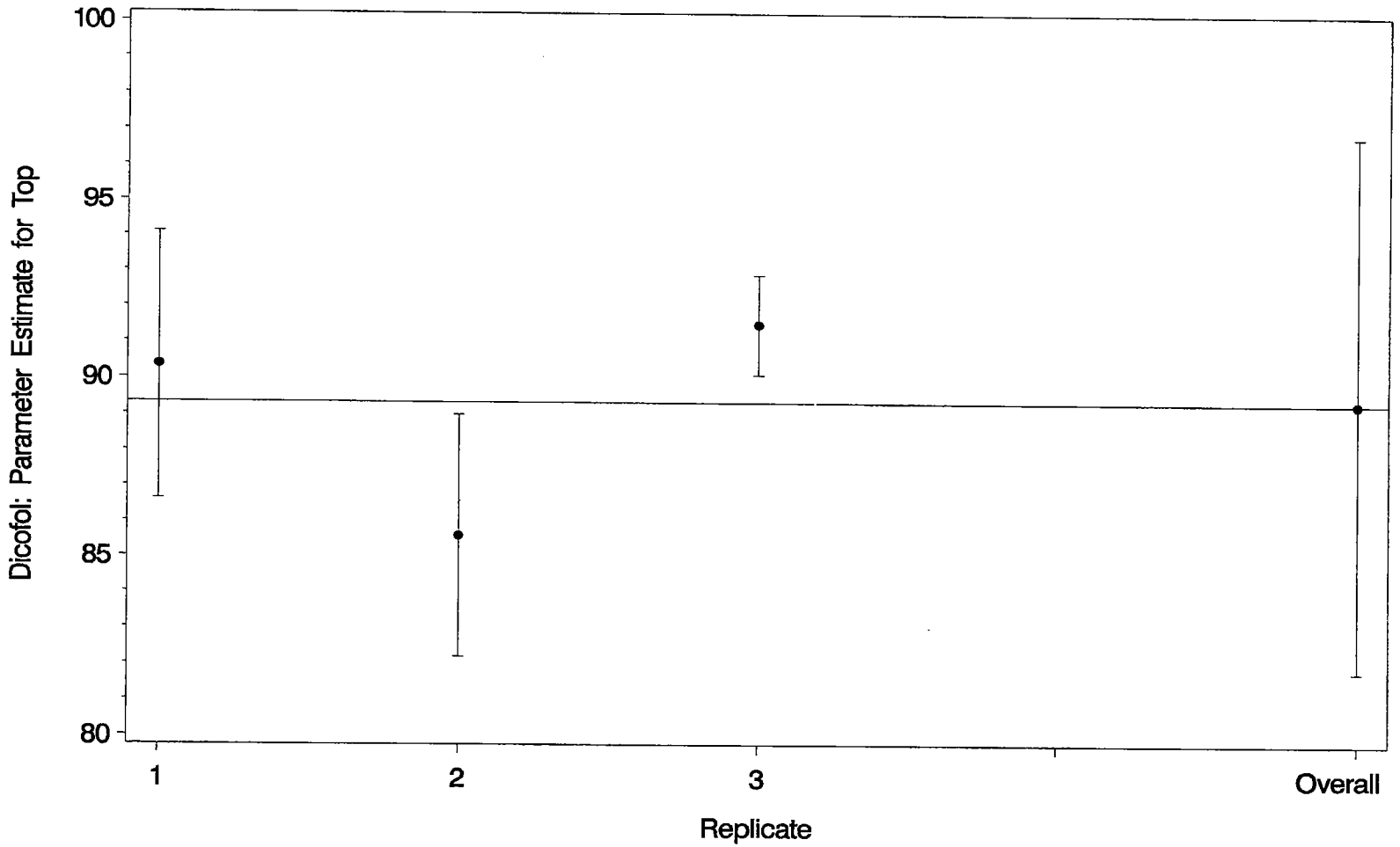


Figure 9-4. **Reference Chemical IX: Dicofol. Top Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.**

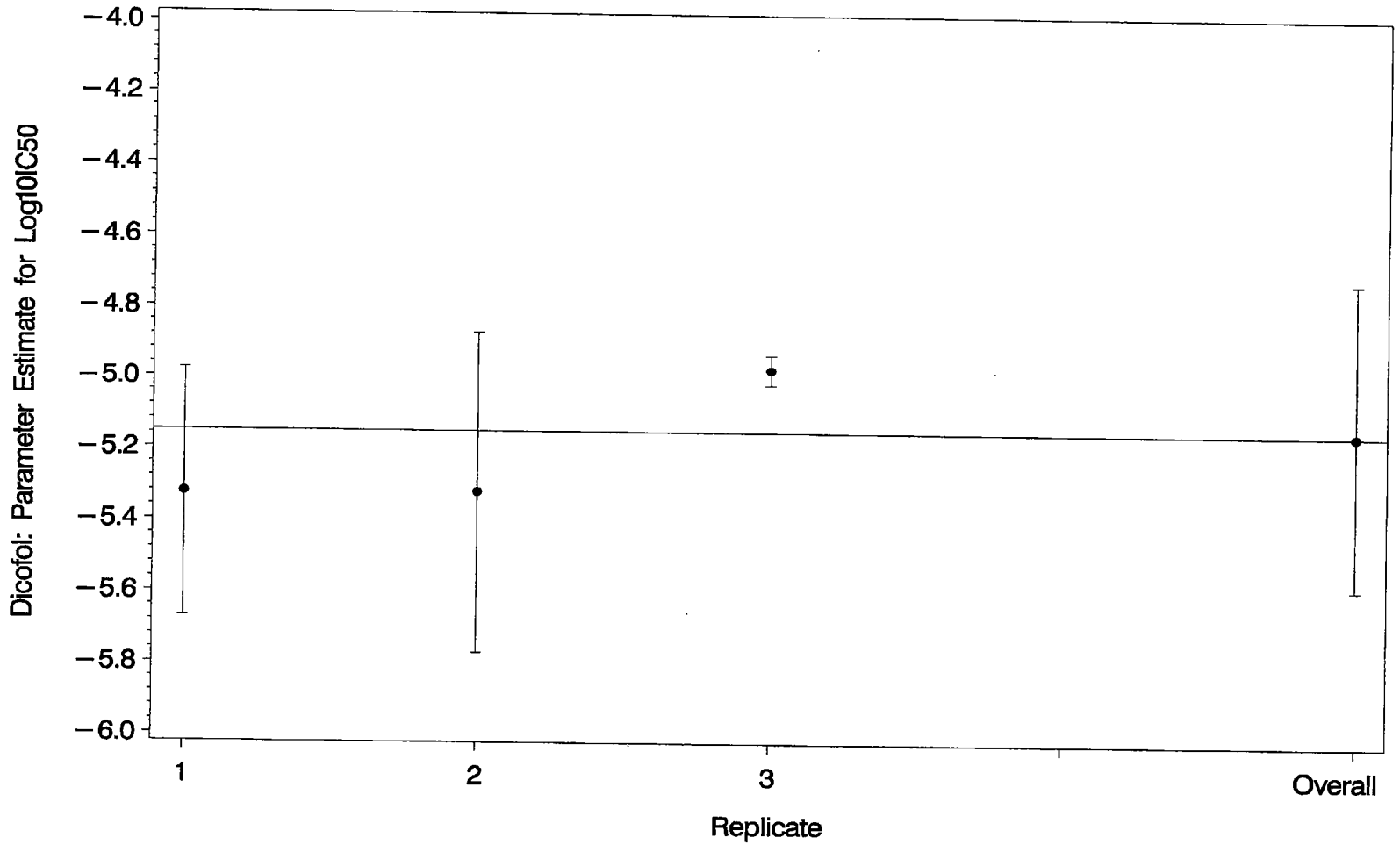


Figure 9-5. Reference Chemical IX: Dicofol. $Log_{10}IC_{50}$ Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

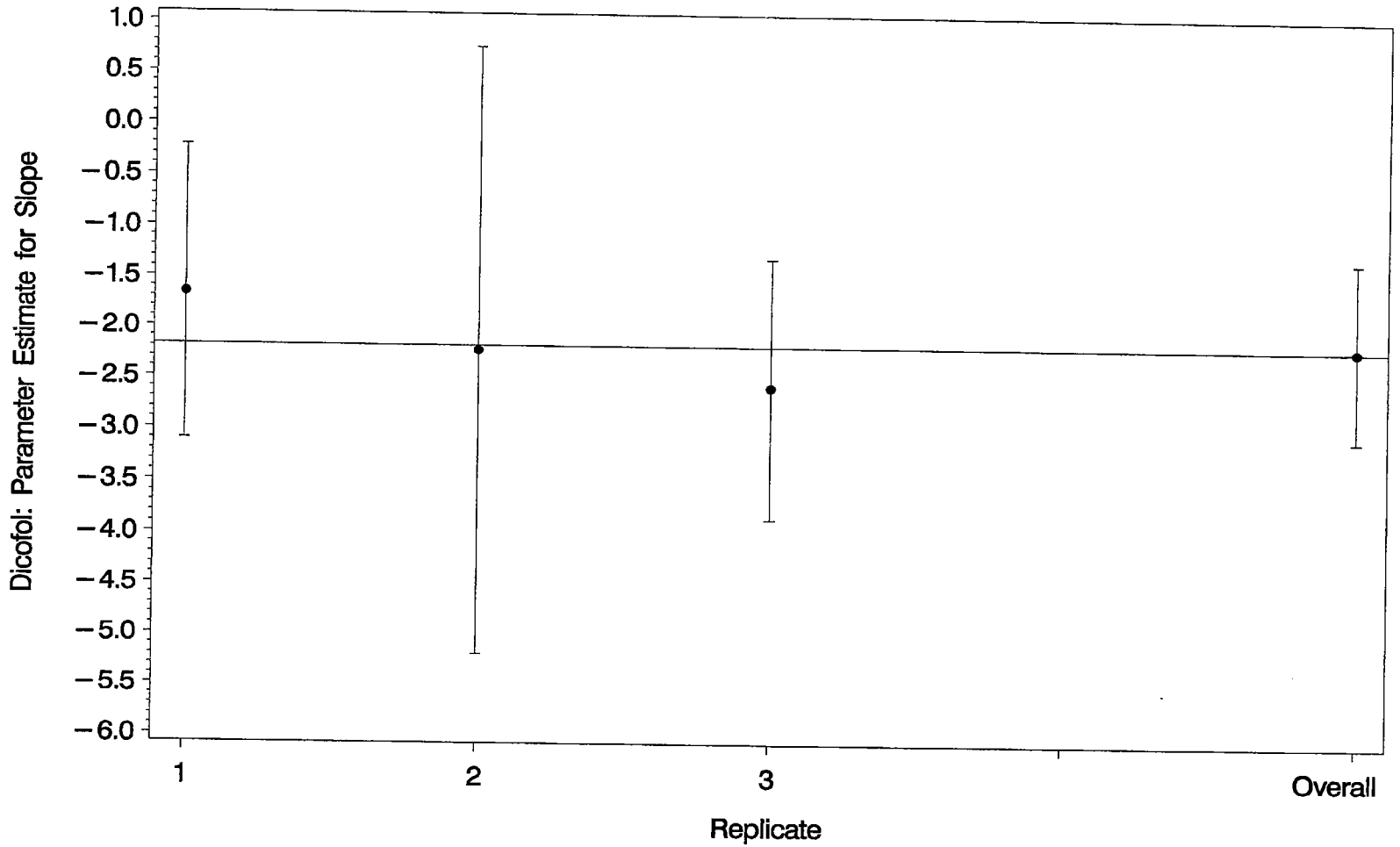


Figure 9-6. Reference Chemical IX: Dicofol.
Slope Parameter Estimates and Their Associated 95 percent Confidence Intervals for Each Replicate and Across Replicates. Recombinant Aromatase Assay. The Solid Reference Line Corresponds to the Average Across Replicates.

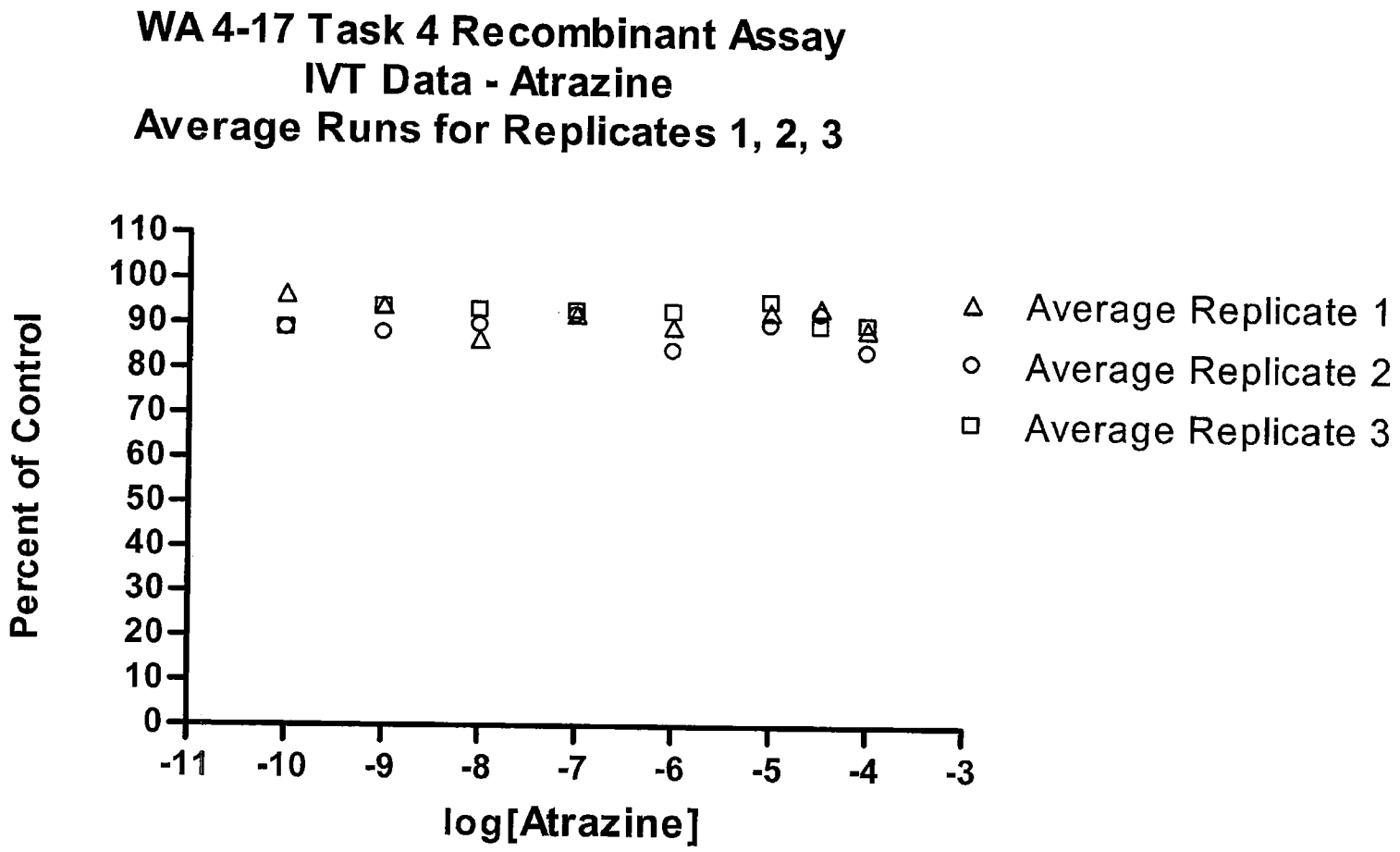


Figure 10-1. **Reference Chemical X: Atrazine.**
Scatter Plot for Averages of Repetitions within Concentration for Each Replicate.
Recombinant Aromatase Assay.

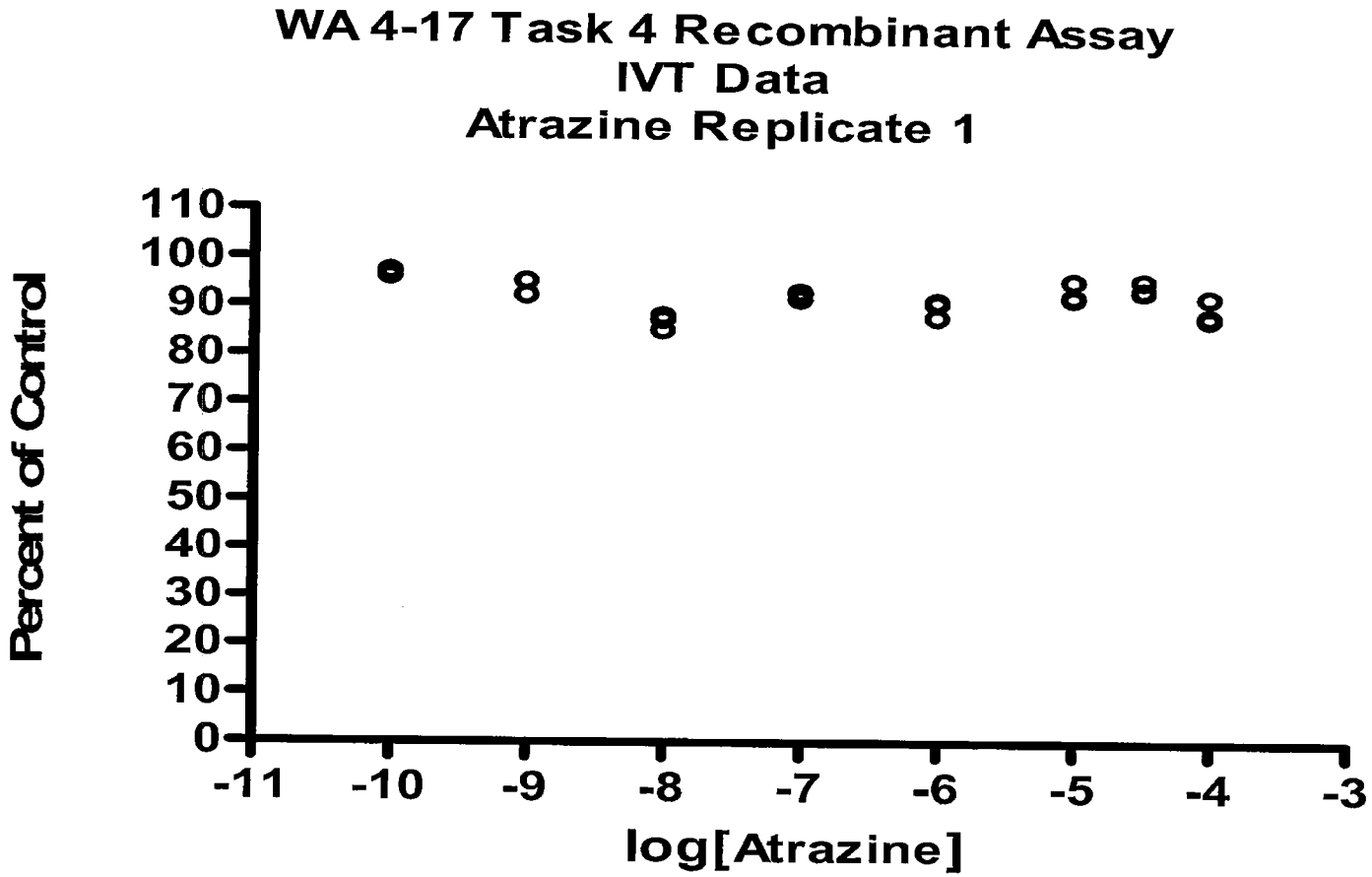


Figure 10-2. Reference Chemical X: Atrazine.
Scatter Plot for Replicate 1 Data. Recombinant Aromatase Assay.

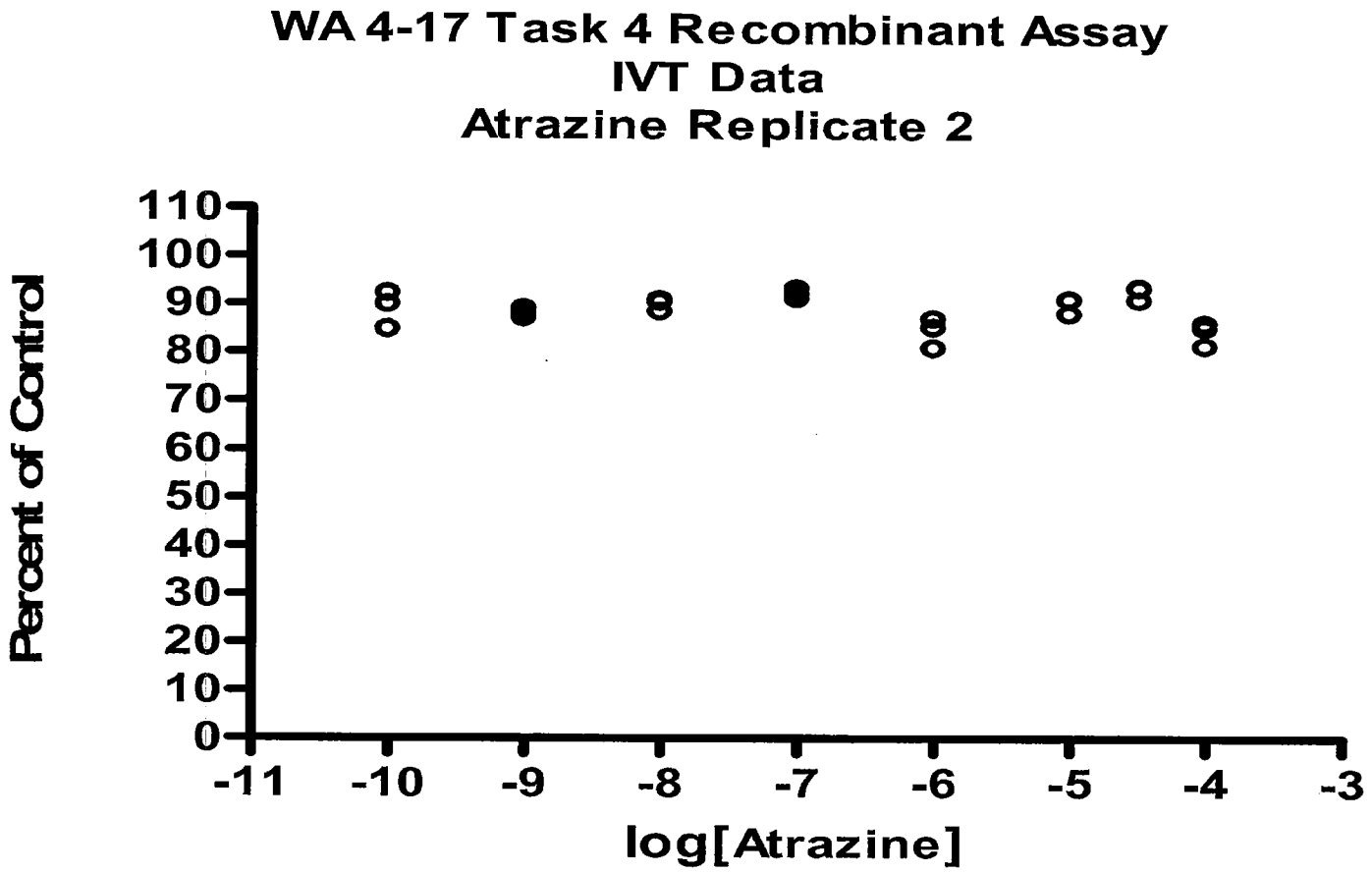


Figure 10-3. Reference Chemical X: Atrazine.
Scatter Plot for Replicate 2 Data. Recombinant Aromatase Assay.

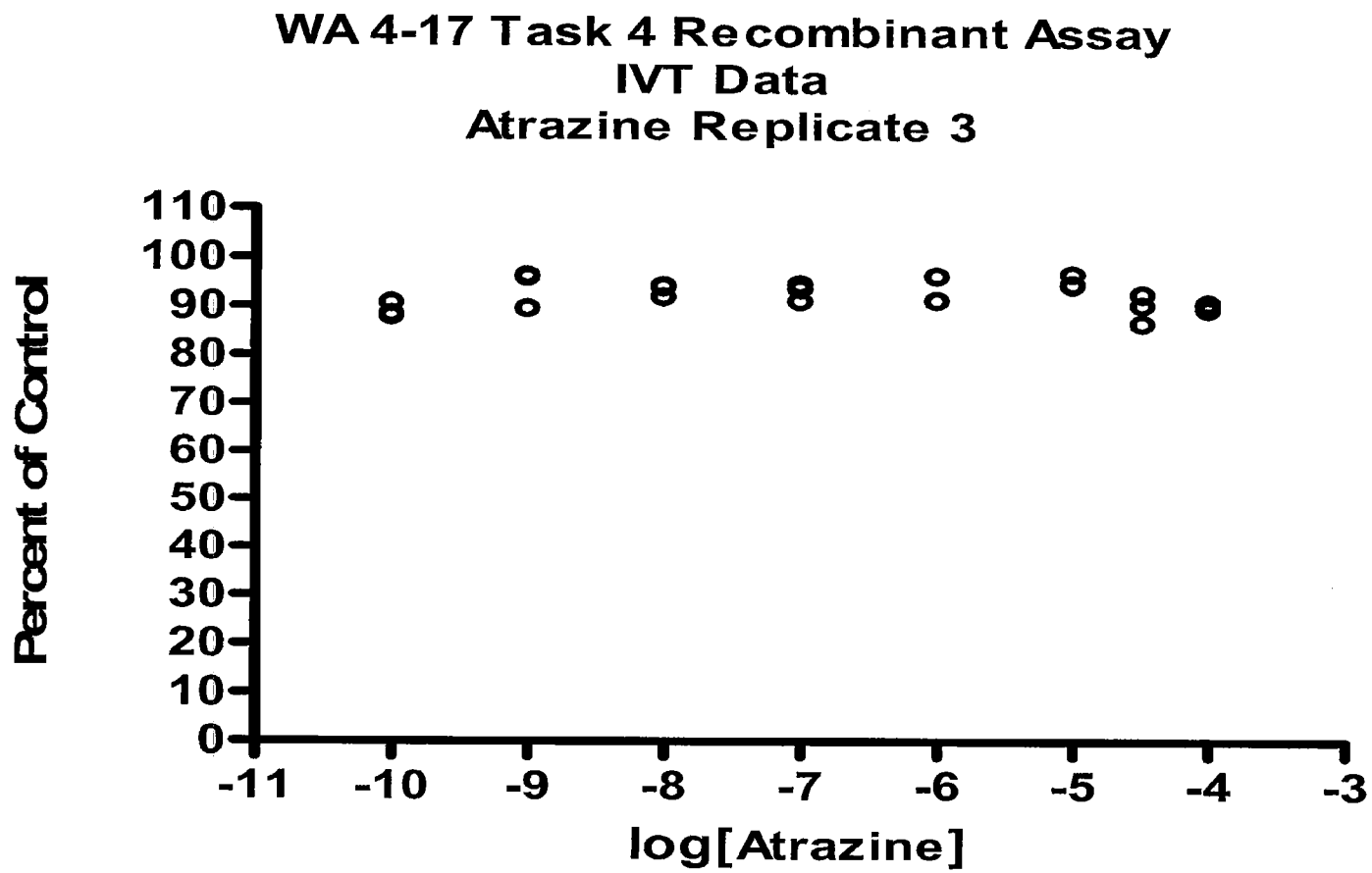


Figure 10-4. Reference Chemical X: Atrazine.
Scatter Plot for Replicate 3 Data. Recombinant Aromatase Assay.

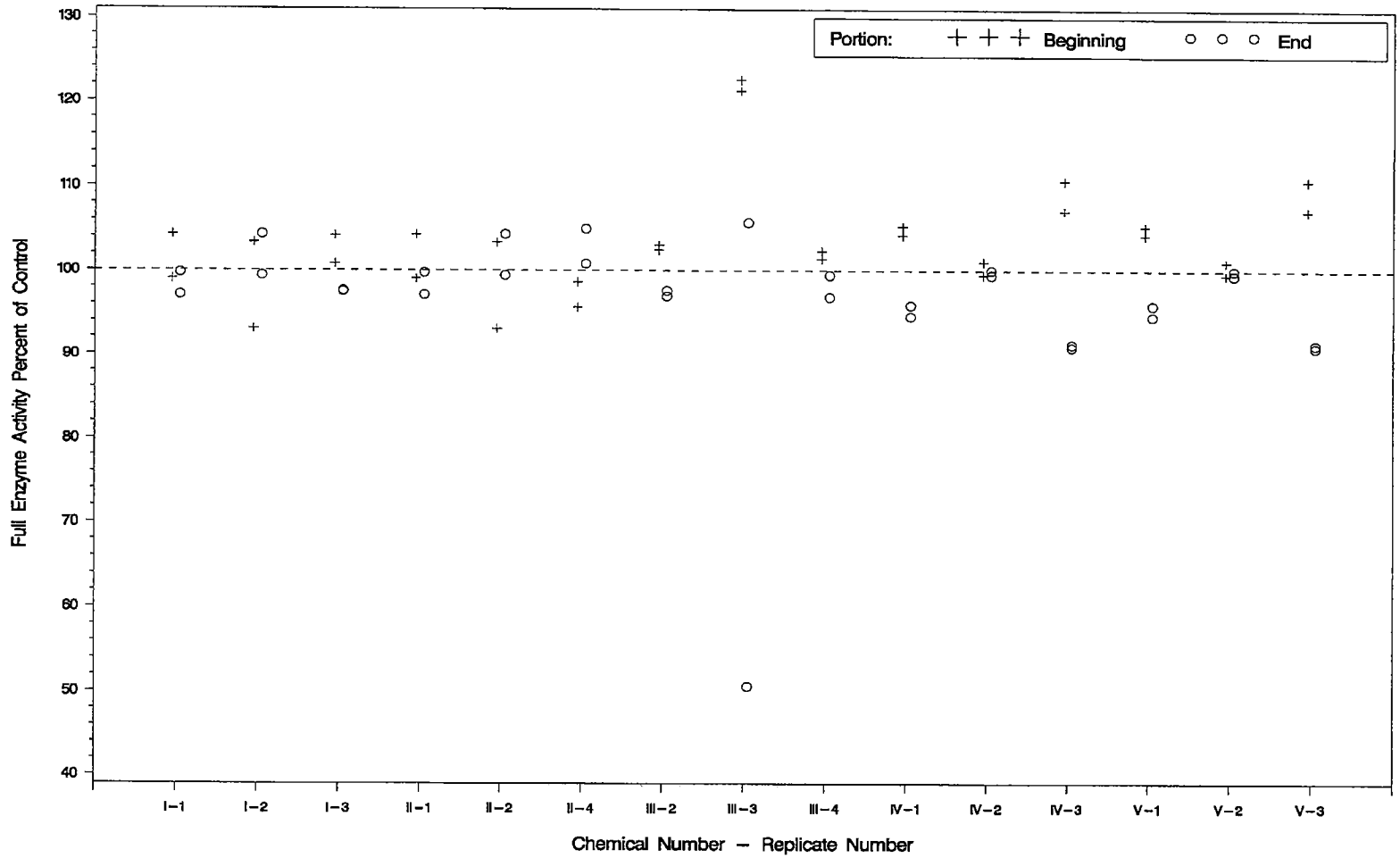


Figure 11a. **Full Enzyme Activity Control Data. Chemicals I to V. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 100 percent.**

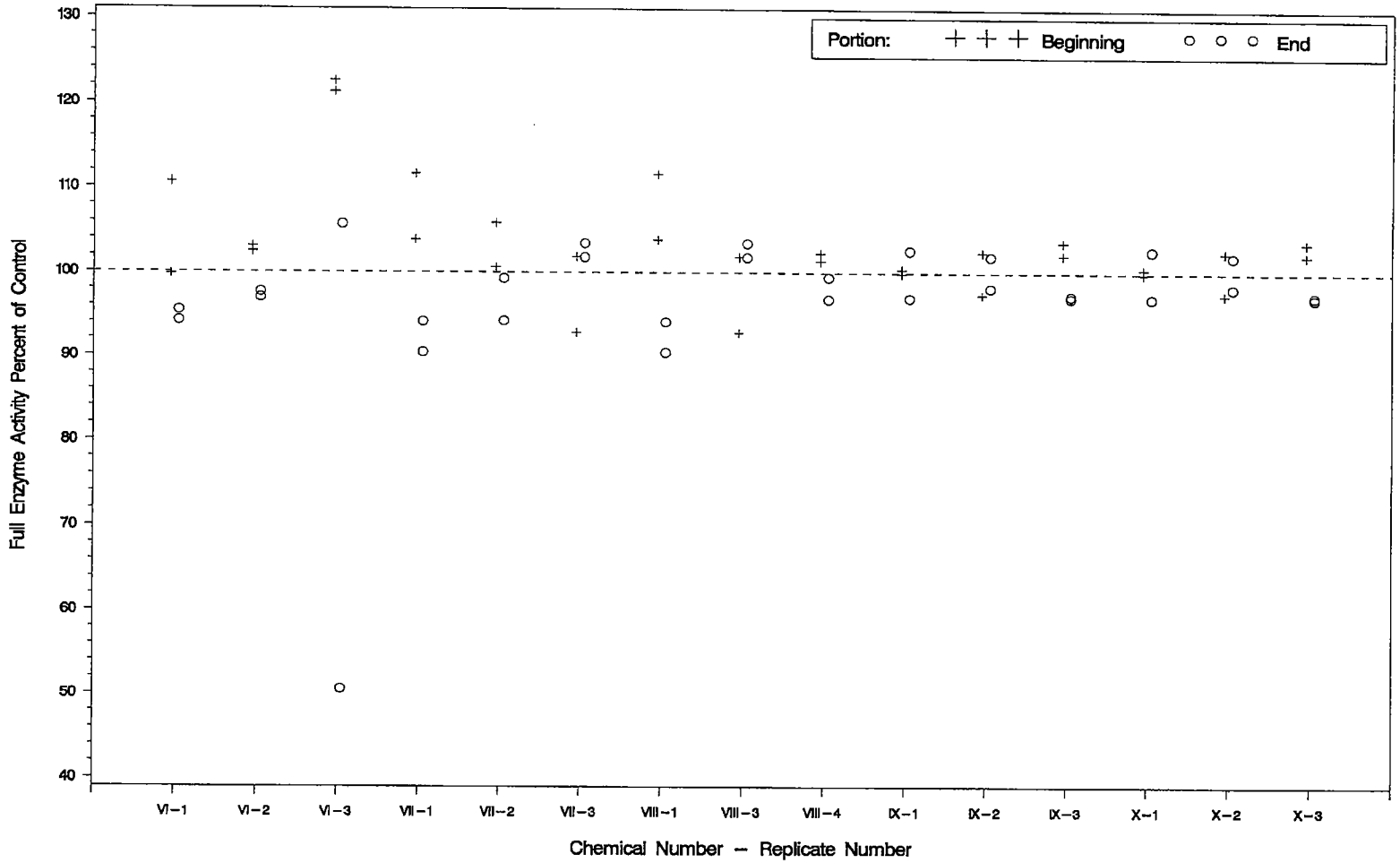


Figure 11b. **Full Enzyme Activity Control Data. Chemicals VI to X. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 100 percent.**

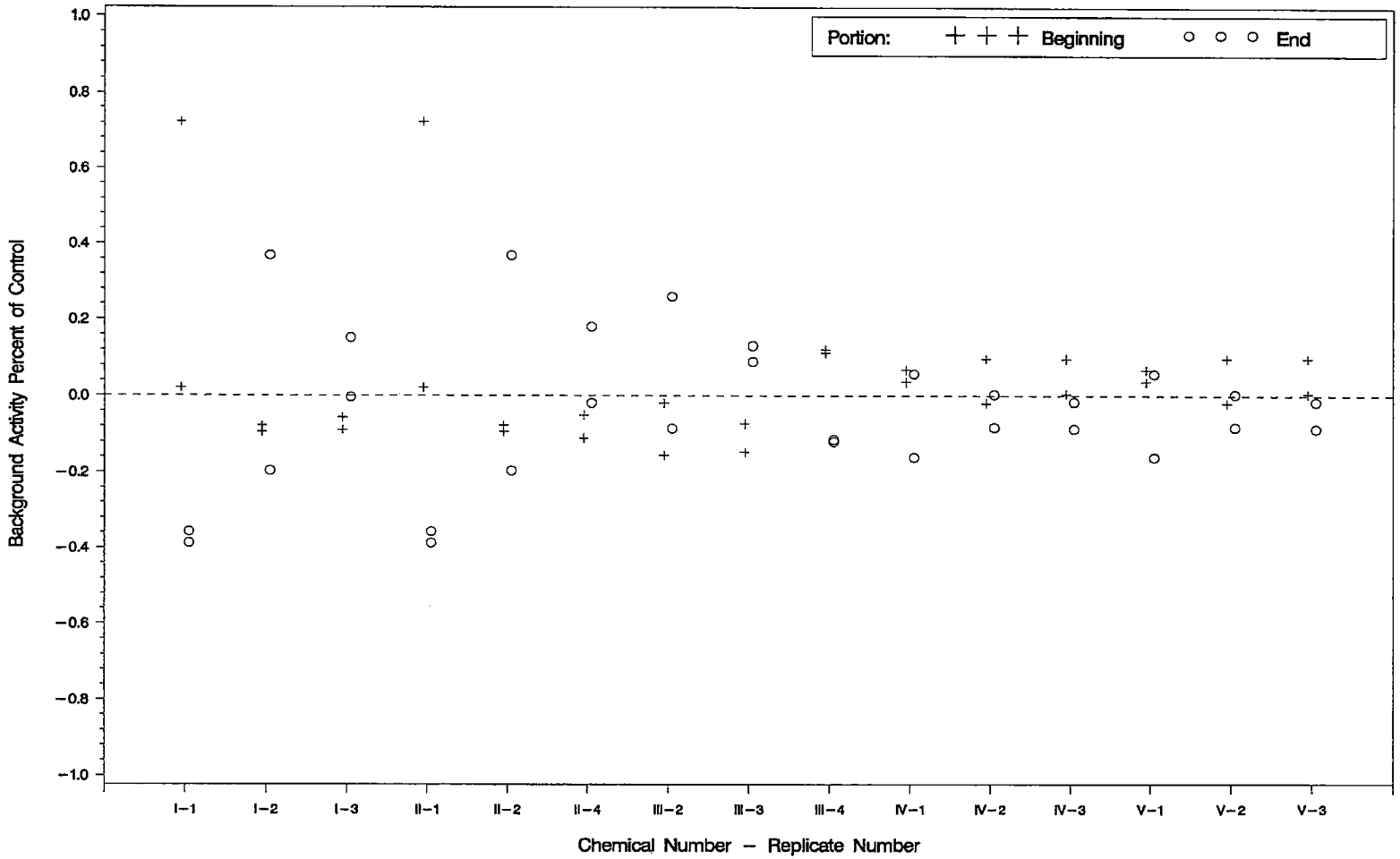


Figure 12a. **Background Activity Control Data. Chemicals I to V. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 0 percent.**

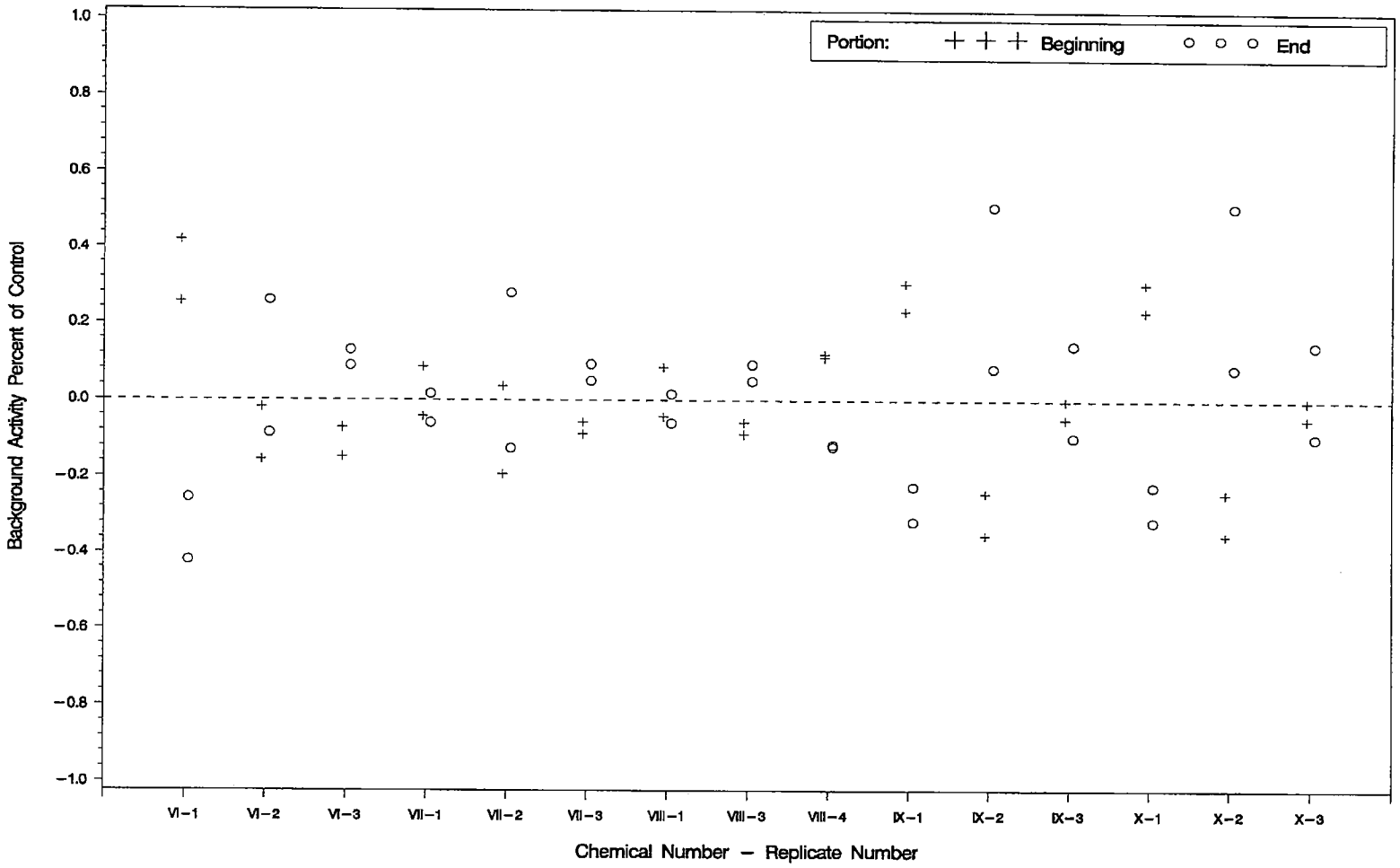


Figure 12b. **Background Activity Control Data. Chemicals VI to X. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 0 percent.**

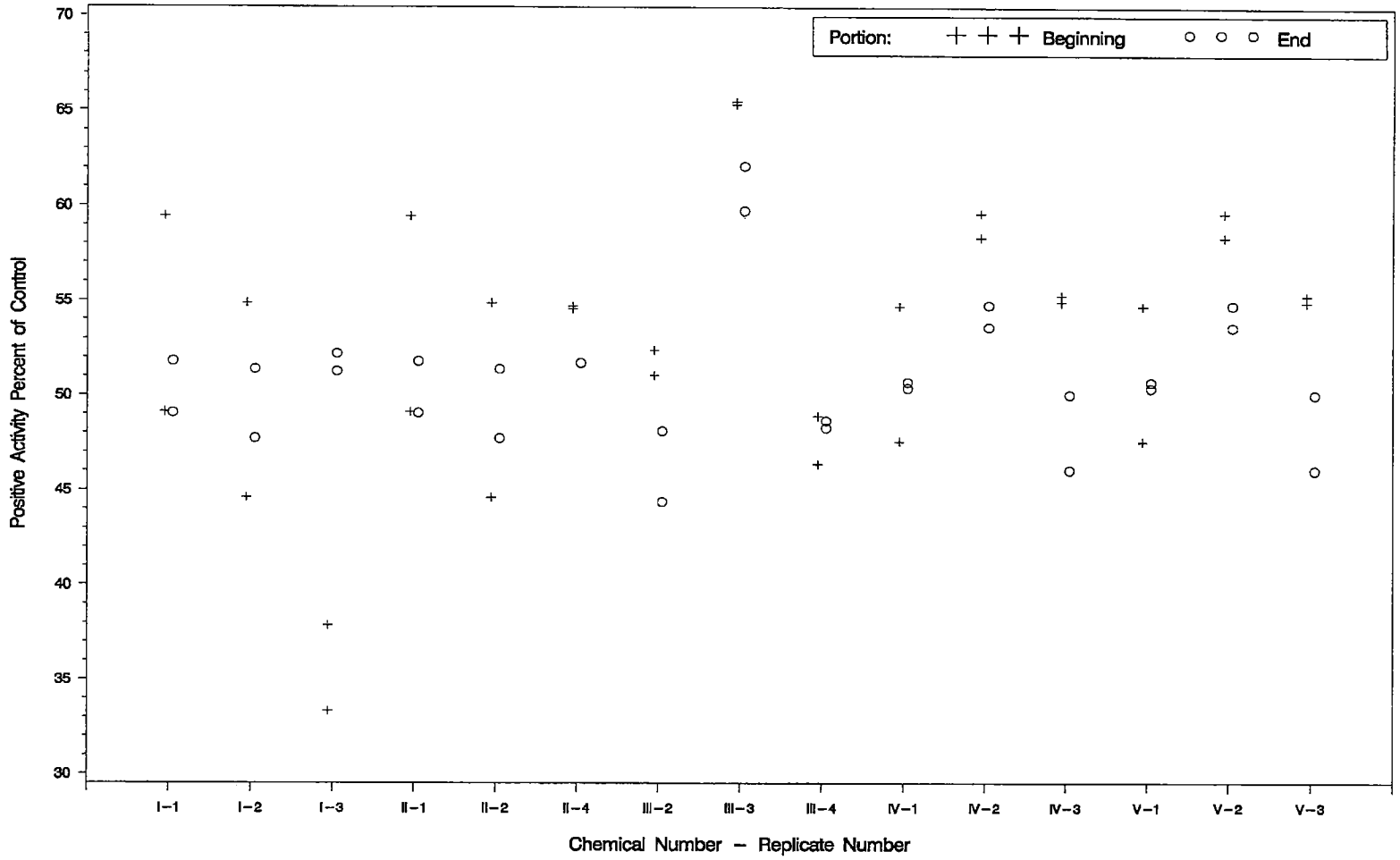


Figure 13a. **Positive Control Data. Chemicals I to V. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay.**

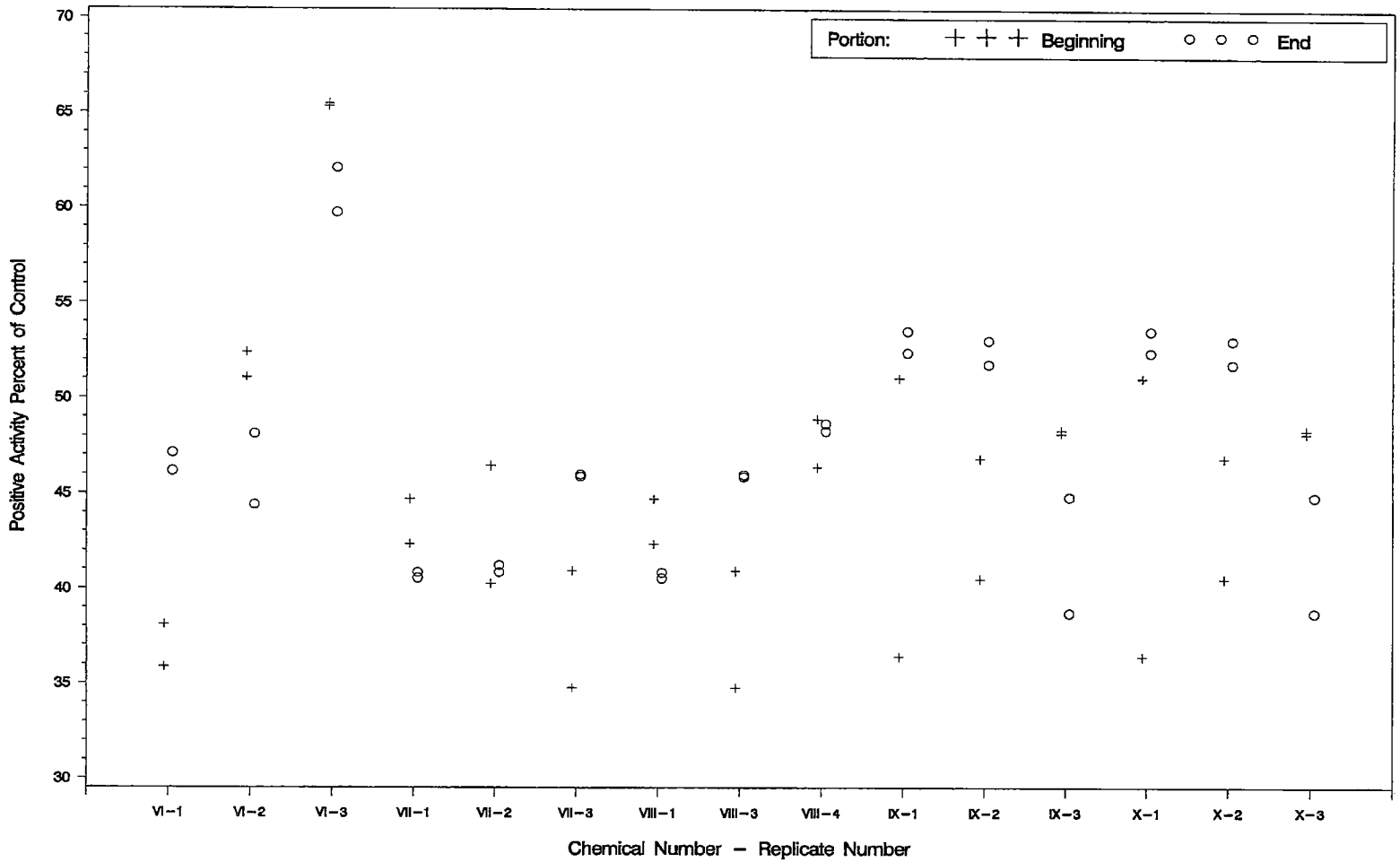


Figure 13b. **Positive Control Data. Chemicals VI to X. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay.**

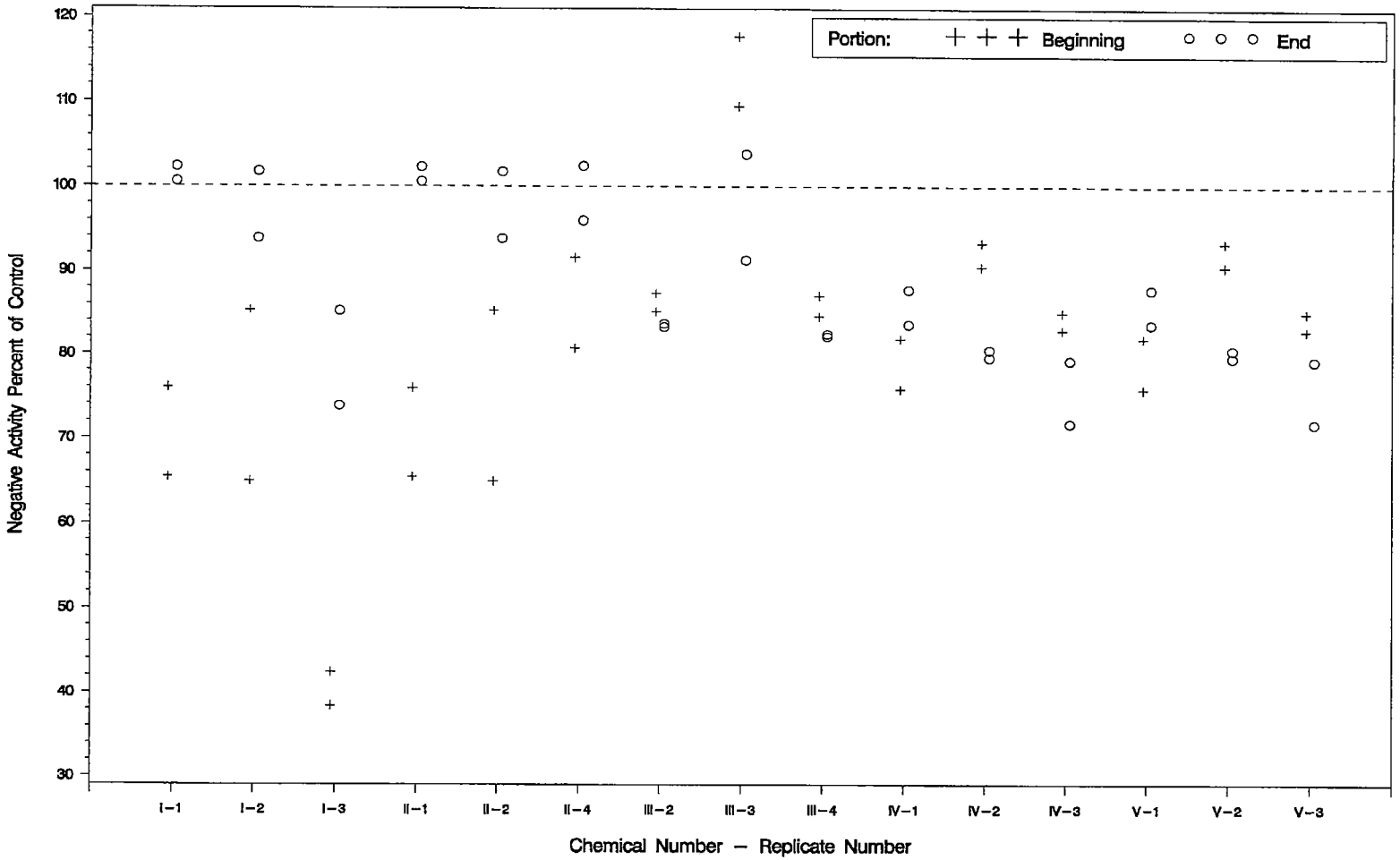


Figure 14a. **Negative Control Data. Chemicals I to V. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 100 percent.**

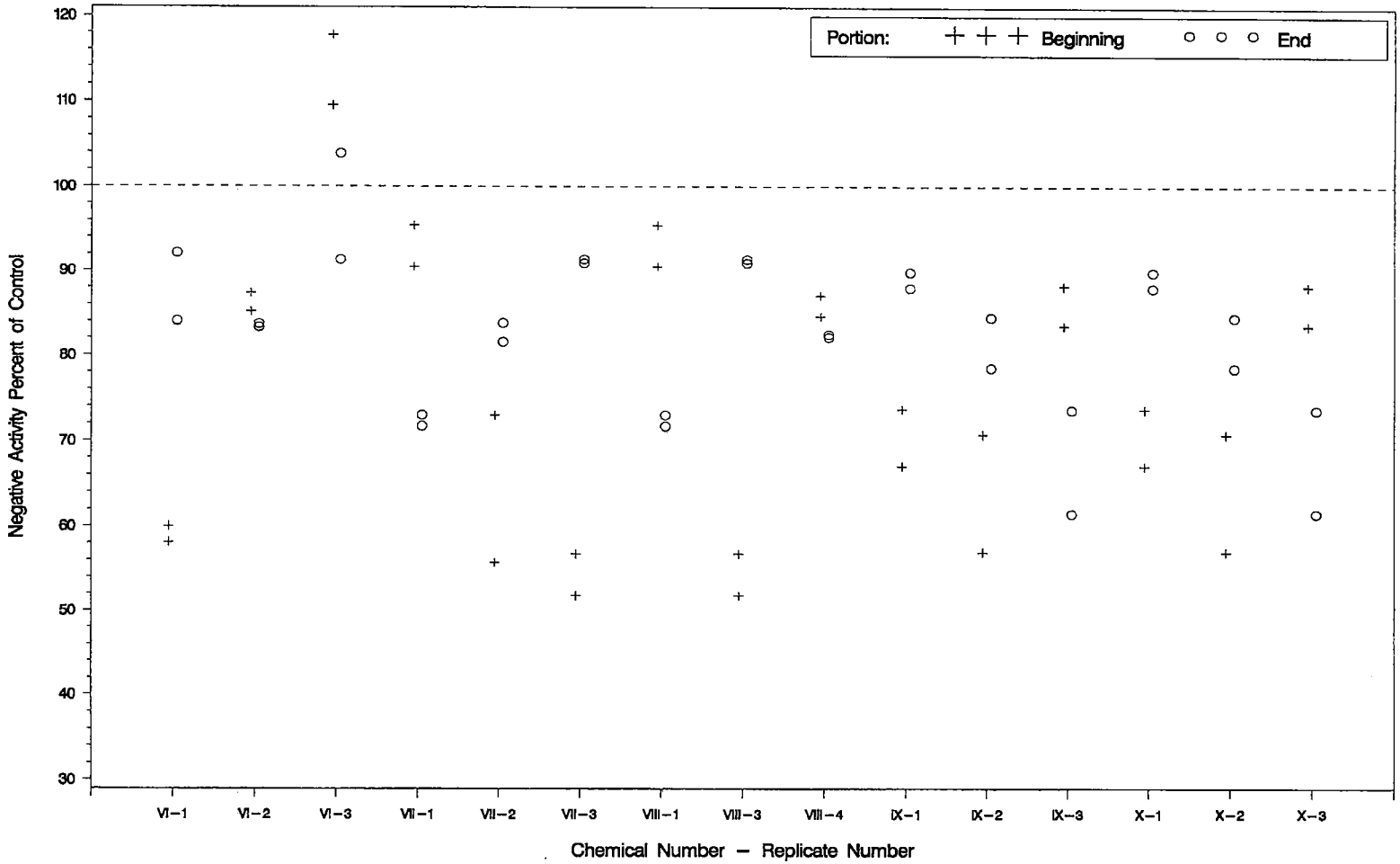


Figure 14b. **Negative Control Data. Chemicals VI to X. Percent of Control by Replicate and Portion of Replicate (Beginning or End). Recombinant Aromatase Assay. Reference Line at 100 percent.**

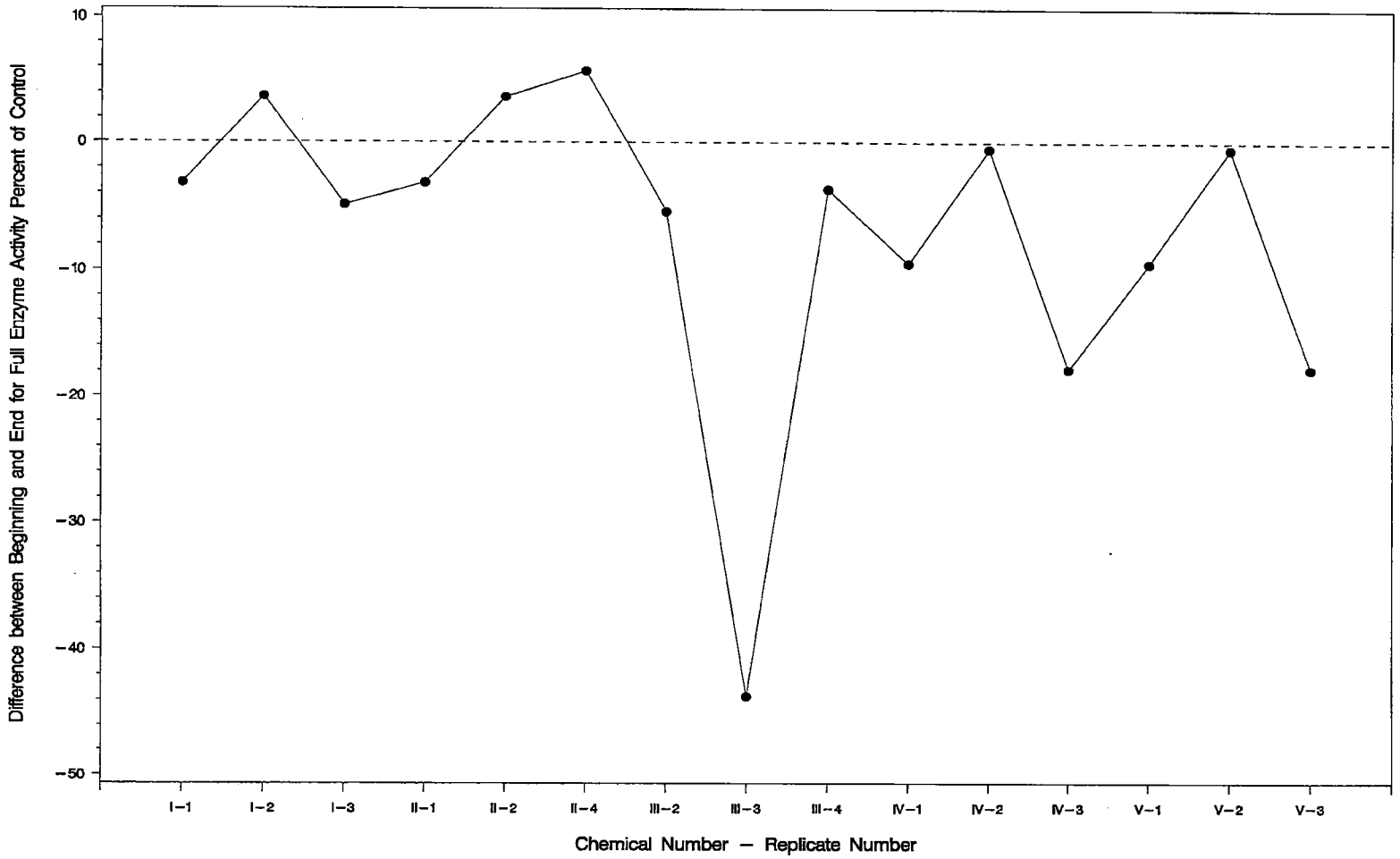


Figure 15a. **Full Enzyme Activity Control Data. Chemicals I to V. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Recombinant Aromatase Assay. Reference Line at 0 percent.**

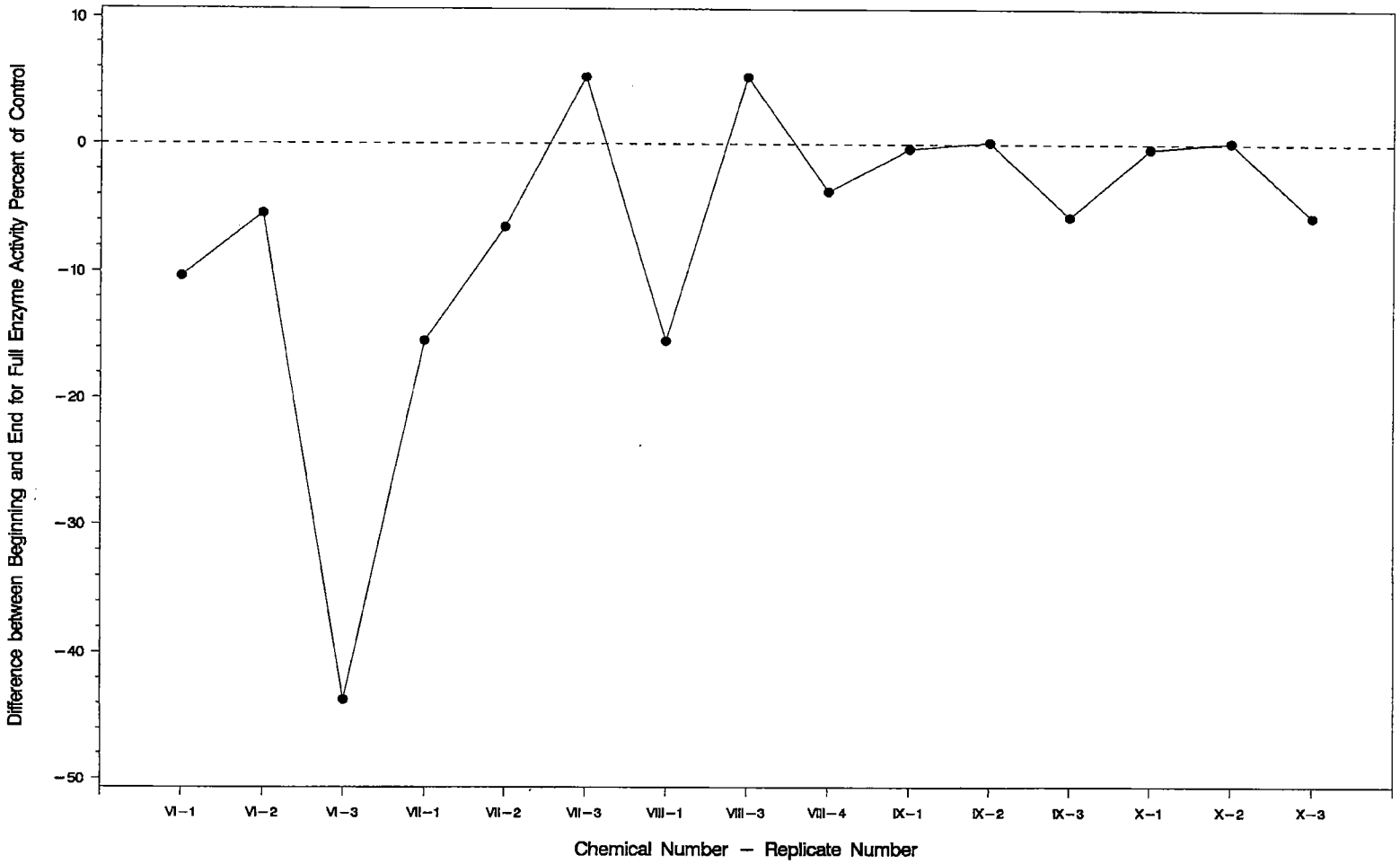


Figure 15b. **Full Enzyme Activity Control Data. Chemicals VI to X. Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning). Recombinant Aromatase Assay. Reference Line at 0 percent.**

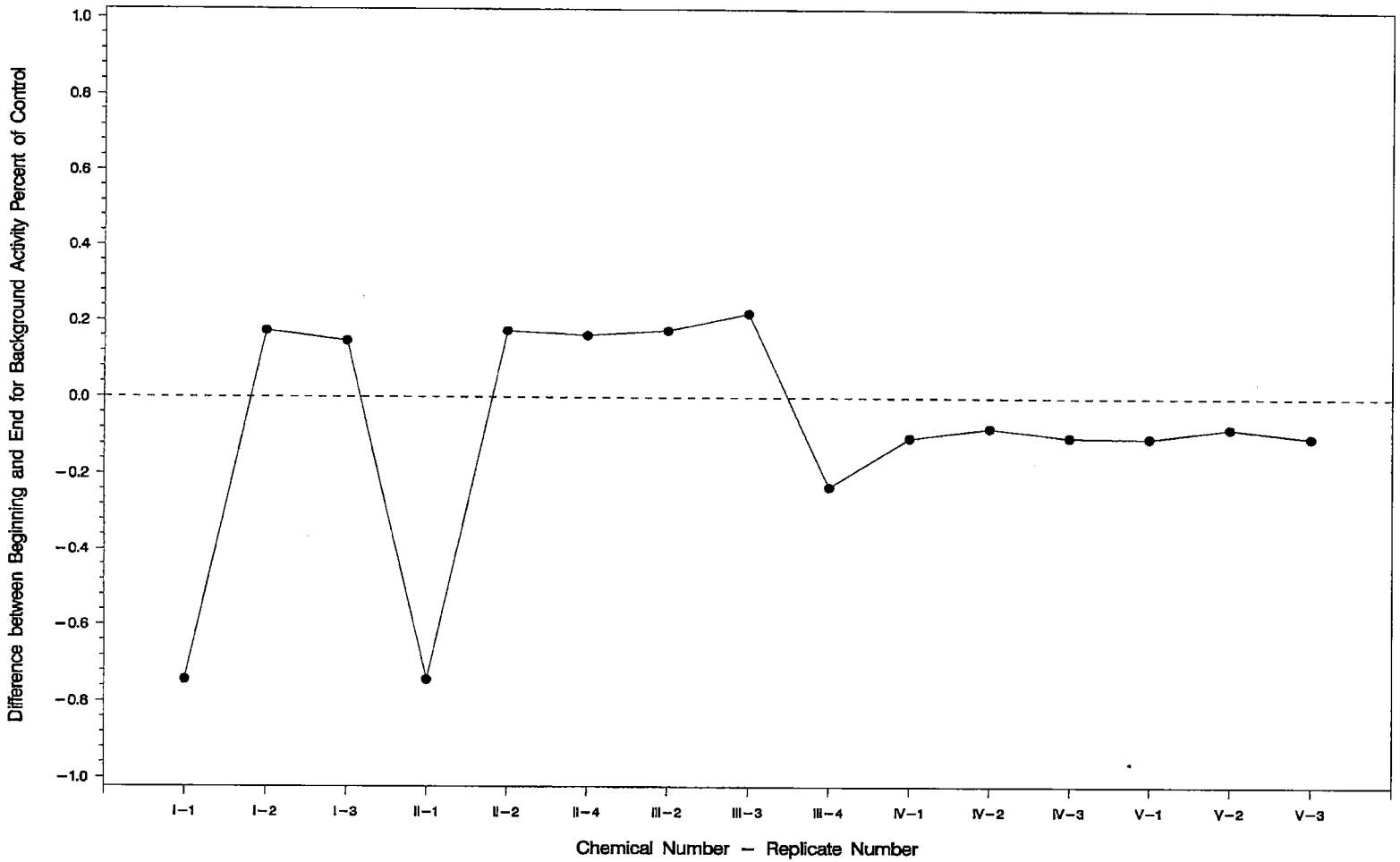


Figure 16a. **Background Activity Control Data. Chemicals I to V.**
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Recombinant Aromatase Assay. Reference Line at 0 percent.

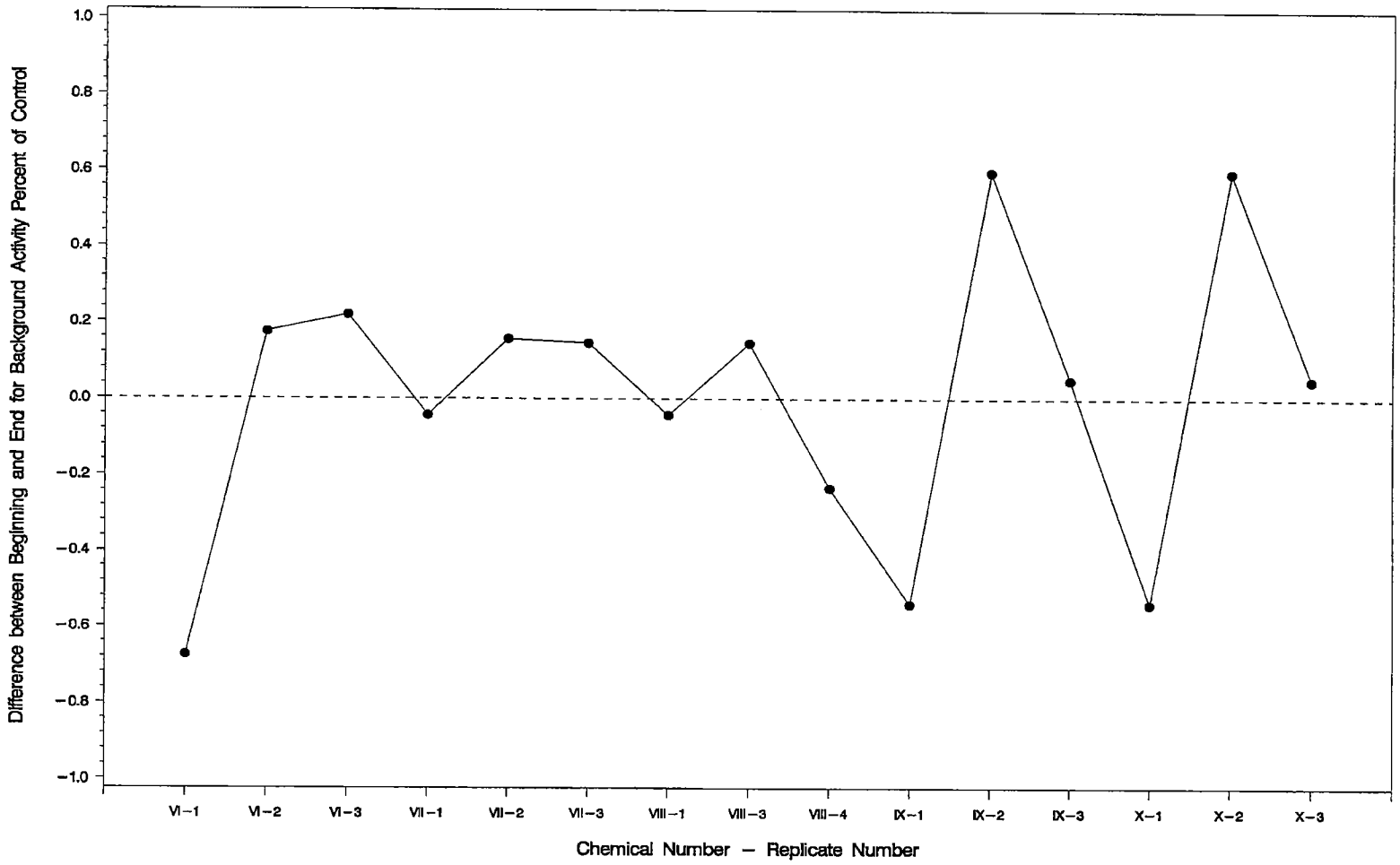


Figure 16b. **Background Activity Control Data. Chemicals VI to X.
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Recombinant Aromatase Assay. Reference Line at 0 percent.**

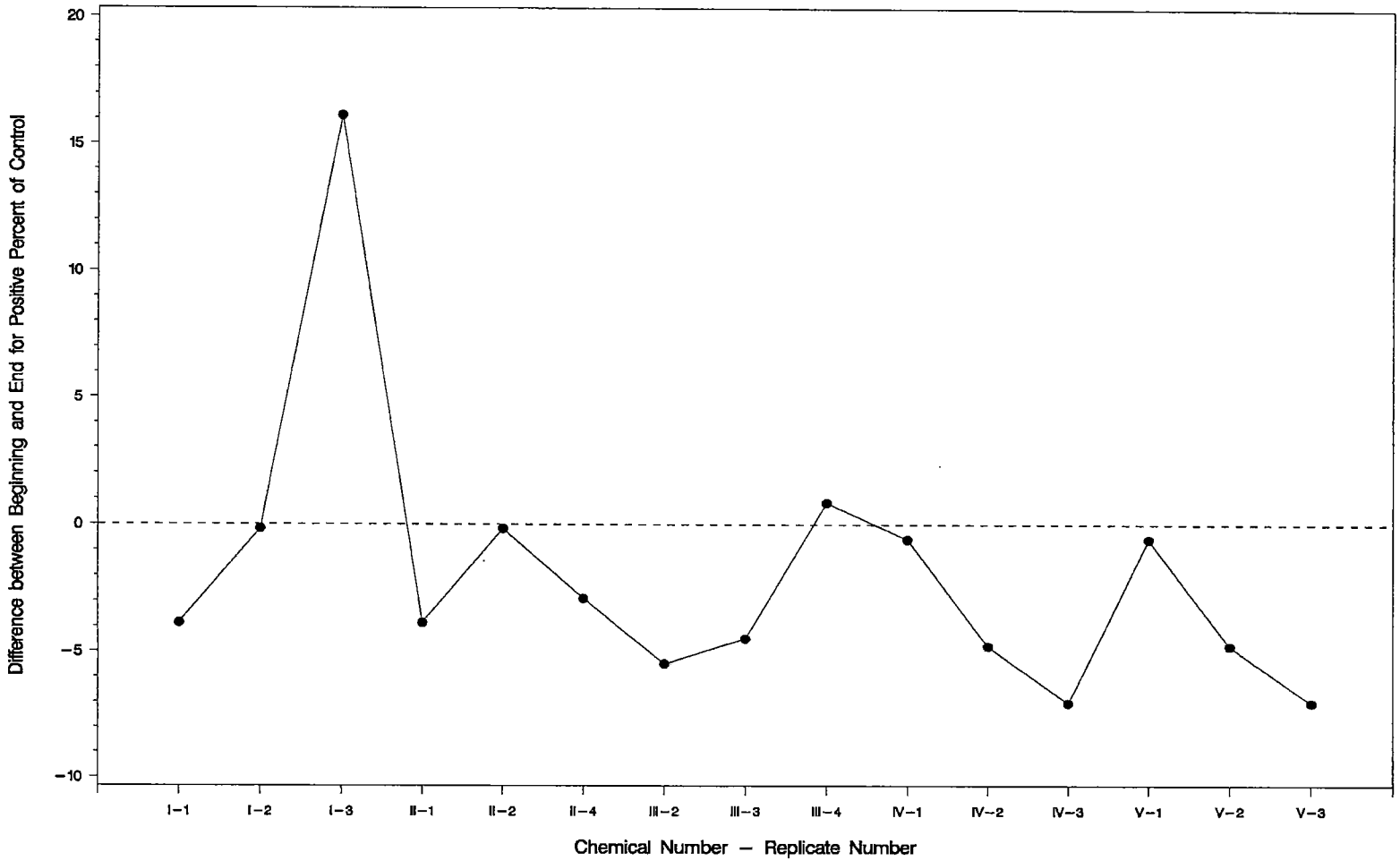


Figure 17a. **Positive Control Data. Chemicals I to V.**
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Recombinant Aromatase Assay. Reference Line at 0 percent.

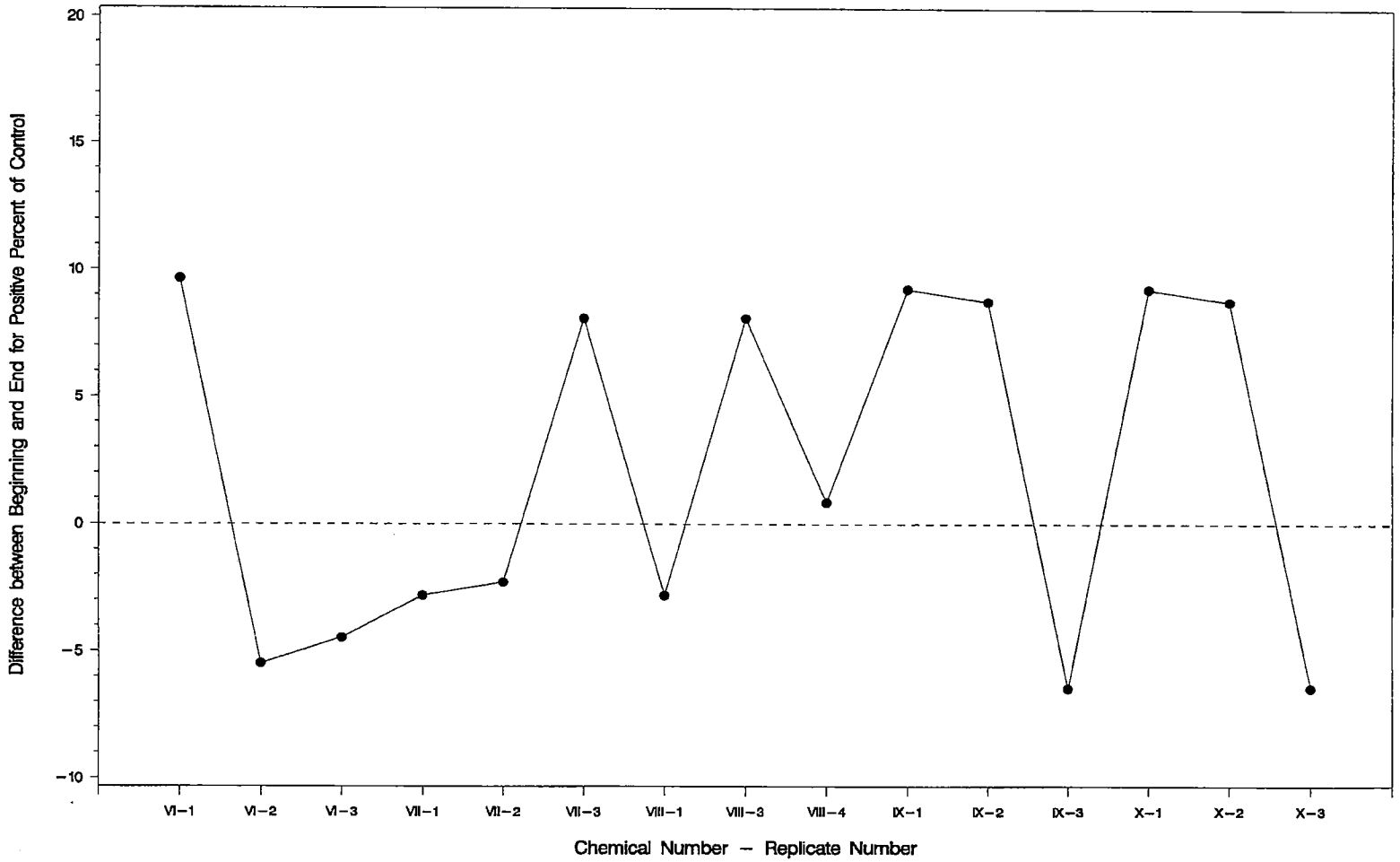


Figure 17b. **Positive Control Data. Chemicals VI to X.**
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Recombinant Aromatase Assay. Reference Line at 0 percent.

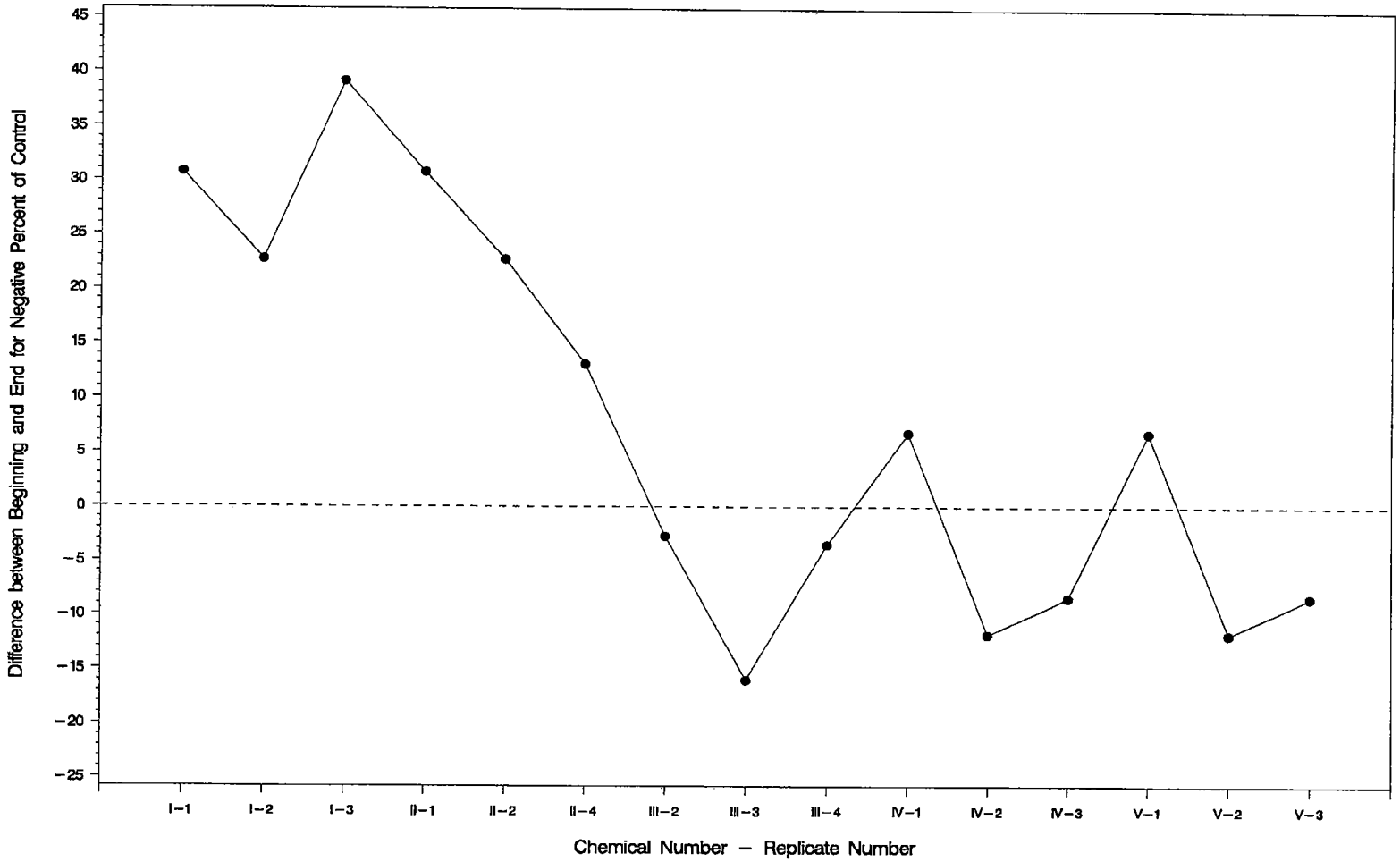


Figure 18a. **Negative Control Data. Chemicals I to V.**
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Recombinant Aromatase Assay. Reference Line at 0 percent.

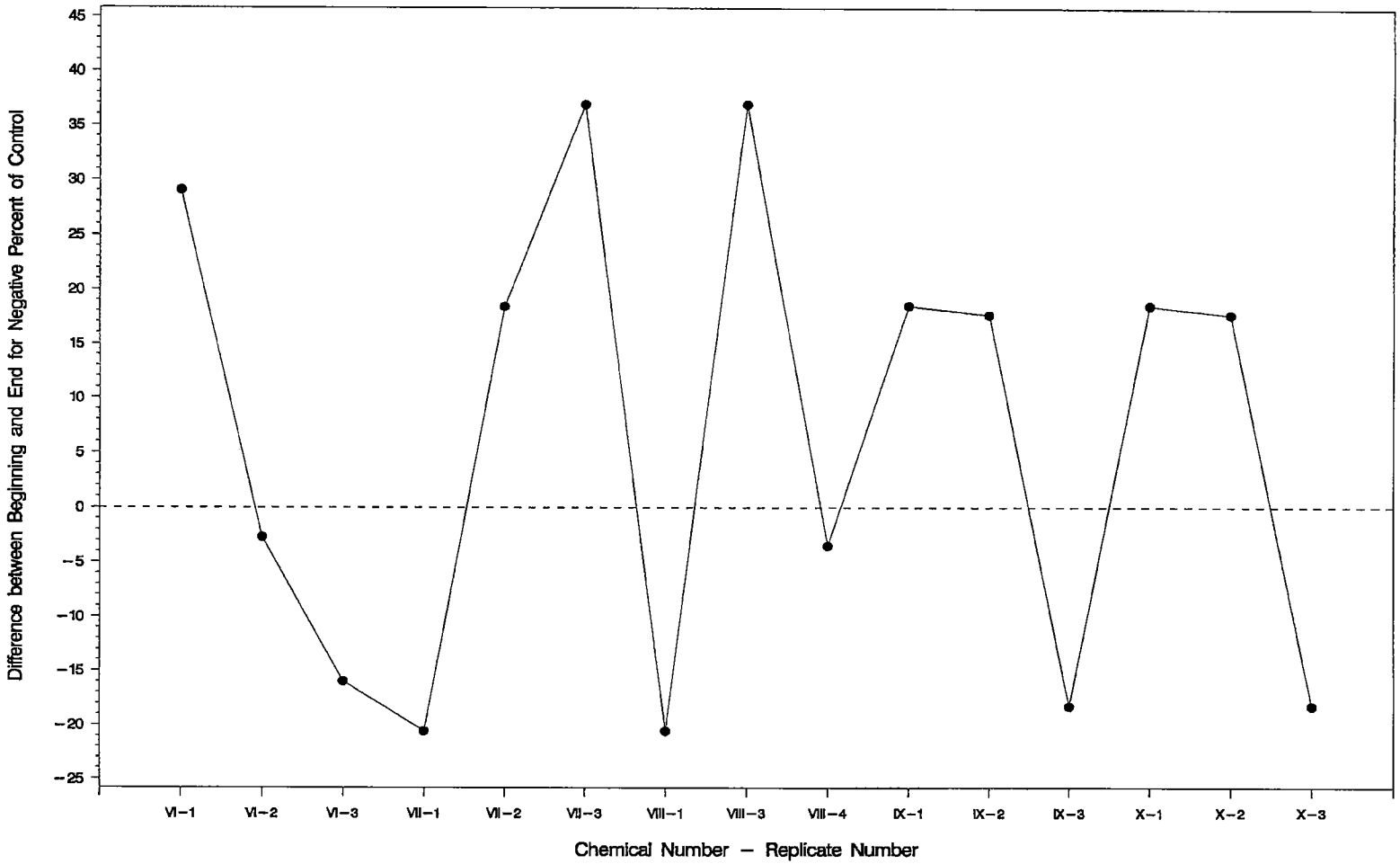


Figure 18b. **Negative Control Data. Chemicals VI to X.**
Difference Between the Averages of the Two End Percent of Control Responses and the Average of the Two Beginning Responses by Replicate (End Minus Beginning).
Recombinant Aromatase Assay. Reference Line at 0 percent.

Table A-1a. Reference Chemical I: Aminoglutethimide Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Aminoglutethimide]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-3.0000	4.987	4.887	4.031
	-3.5229	17.022	18.886	17.717
	-4.0000	34.752	35.104	32.661
	-5.0000	94.588	95.940	95.701
	-6.0000	111.508	118.845	109.977
	-7.0000	106.212	107.243	104.882
	-8.0000	109.339	118.158	115.096
	-9.0000	114.013	114.844	110.092
2	-3.0000	3.093	3.260	3.143
	-4.0000	31.936	30.219	29.328
	-4.4815	46.325	41.163	38.725
	-5.0000	89.486	91.631	92.993
	-6.0000	104.883	103.637	98.011
	-7.0000	99.483	96.951	91.328
	-8.0000	103.805	104.119	107.601
	-9.0000	103.739	102.467	105.701
3	-3.0000	3.072	2.833	2.992
	-4.0000	24.113	23.306	23.909
	-4.4815	33.910	31.875	32.472
	-5.0000	76.376	77.986	76.174
	-6.0000	92.327	85.084	84.120
	-7.0000	85.877	83.692	79.278
	-8.0000	93.665	96.145	97.564
	-9.0000	91.941	93.893	89.769

Table A-1b. Reference Chemical II: Ketoconazole
Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Ketoconazole]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	0.034	0.483	0.145
	-5.0000	4.811	4.553	4.433
	-6.0000	33.809	33.775	32.949
	-7.0000	90.642	88.426	87.894
	-8.0000	102.116	105.461	105.077
	-9.0000	109.061	116.333	116.141
	-10.0000	113.104	117.322	108.196
	-11.0000	109.866	107.222	110.258
2	-4.0000	0.347	0.314	0.446
	-5.0000	3.961	4.248	4.288
	-6.0000	33.412	31.751	31.452
	-7.0000	87.432	83.887	77.483
	-8.0000	109.904	108.795	109.021
	-9.0000	114.634	112.905	112.859
	-10.0000	114.640	109.661	109.395
	-11.0000	108.045	106.939	134.084
4	-4.0000	0.400	0.147	0.734
	-5.0000	4.458	4.545	4.343
	-6.0000	32.837	31.132	28.383
	-7.0000	85.828	84.442	85.647
	-8.0000	102.969	105.904	105.178
	-9.0000	102.500	103.625	102.440
	-10.0000	102.983	114.380	110.718
	-11.0000	103.779	110.830	98.799

Table A-1c. Reference Chemical III: Prochloraz
Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Prochloraz]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
2	-4.0000	0.117	-0.193	-0.273
	-5.0000	0.356	0.317	0.245
	-6.0000	1.459	1.783	1.992
	-7.0000	15.319	16.636	16.840
	-8.0000	61.210	61.536	58.830
	-8.4815	78.282	74.582	73.346
	-9.0000	89.511	86.320	87.466
	-10.0000	95.652	93.074	88.180
3	-4.0000	0.799	0.135	0.557
	-5.0000	0.414	0.799	0.422
	-6.0000	2.953	1.999	2.409
	-7.0000	19.265	20.637	20.205
	-8.0000	80.681	72.579	77.340
	-8.4815	104.515	99.278	96.522
	-9.0000	112.157	110.276	106.725
	-10.0000	111.942	114.053	115.572
4	-4.0000	0.058	0.594	0.343
	-5.0000	0.752	0.286	0.122
	-6.0000	2.786	2.663	2.758
	-7.0000	20.466	21.655	18.224
	-7.4815	86.460	83.188	78.411
	-8.0000	64.550	64.874	65.421
	-9.0000	85.829	87.915	85.425
	-10.0000	91.240	91.202	84.414

Table A-1d. Reference Chemical IV: 4-Nonylphenol Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [4-Nonylphenol]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	8.350	6.912	5.961
	-4.4815	37.140	38.448	33.019
	-5.0000	70.557	36.183	57.726
	-6.0000	93.390	96.465	96.937
	-7.0000	97.326	96.782	90.665
	-8.0000	93.236	86.273	83.125
	-9.0000	97.628	79.944	93.999
	-10.0000	95.873	83.093	72.089
2	-4.0000	2.817	3.427	3.350
	-4.4815	20.615	20.266	21.283
	-5.0000	63.861	61.642	62.545
	-6.0000	96.250	95.814	93.952
	-7.0000	97.804	97.931	94.500
	-8.0000	95.291	94.428	94.692
	-9.0000	90.415	94.057	92.006
	-10.0000	95.676	93.129	93.464
3	-4.0000	2.481	3.376	2.771
	-4.4815	24.963	27.426	20.802
	-5.0000	64.566	62.269	66.720
	-6.0000	95.356	94.431	92.459
	-7.0000	96.502	96.726	89.190
	-8.0000	93.123	93.802	86.885
	-9.0000	90.156	90.482	91.807
	-10.0000	92.947	94.540	89.177

Table A-1e. Reference Chemical V: Dibenz[a,h]anthracene Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Dibenze[a,h]anthracene]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.4815	92.256	91.908	87.773
	-5.0000	92.682	92.250	92.587
	-5.4815	96.483	94.068	92.959
	-6.0000	91.396	89.016	90.692
	-7.0000	98.163	95.756	94.230
	-8.0000	96.643	91.803	93.442
	-9.0000	79.834	59.439	92.530
	-10.0000	85.942	88.026	91.742
2	-4.0000	92.819	90.591	89.663
	-4.4815	90.752	93.315	86.575
	-5.0000	91.112	90.893	91.169
	-6.0000	91.800	88.681	91.573
	-7.0000	85.562	90.839	91.271
	-8.0000	86.509	90.018	88.202
	-9.0000	89.593	88.661	87.493
	-10.0000	83.202	86.480	87.127
3	-4.0000	86.854	86.918	82.601
	-4.4815	85.070	90.593	87.805
	-5.0000	89.235	92.334	88.562
	-6.0000	89.647	85.282	90.135
	-7.0000	85.723	90.314	85.342
	-8.0000	87.164	87.532	88.026
	-9.0000	86.636	87.393	87.578
	-10.0000	83.130	82.641	79.028

Table A-1f. Reference Chemical VI: Fenarimol
Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Fenarimol]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.4776	3.511	49.672	48.237
	-5.0000	29.287	29.553	29.567
	-5.4815	42.732	41.832	42.798
	-6.0000	71.244	72.996	74.710
	-7.0000	86.468	87.242	90.336
	-8.0000	82.056	82.802	78.936
	-9.0000	80.361	85.393	87.607
	-10.0000	86.828	85.499	84.202
2	-4.4815	13.473	11.395	11.156
	-5.0000	28.786	30.452	31.481
	-5.4815	51.795	54.660	55.047
	-6.0000	74.518	74.798	74.202
	-7.0000	90.114	91.754	89.991
	-8.0000	92.690	90.316	91.941
	-9.0000	92.215	93.148	90.510
	-10.0000	90.035	93.328	93.227
3	-4.4815	16.609	15.590	14.437
	-5.0000	36.886	35.977	38.044
	-5.4815	61.261	66.974	67.672
	-6.0000	90.944	86.353	91.863
	-7.0000	106.781	107.733	112.308
	-8.0000	110.415	107.264	110.488
	-9.0000	109.164	112.726	108.507
	-10.0000	107.751	109.304	100.641

Table A-1g. Reference Chemical VII: Econazole
Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Econazole]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	-0.055	-0.003	0.022
	-4.4815	-0.032	-0.095	-0.058
	-5.0000	0.069	0.017	-0.015
	-6.0000	0.061	0.319	0.153
	-7.0000	1.311	1.473	1.721
	-8.0000	12.799	12.745	13.107
	-9.0000	54.450	54.277	58.636
	-10.0000	86.361	81.711	85.549
2	-5.0000	0.337	0.818	0.648
	-6.0000	0.449	0.126	0.745
	-7.0000	1.273	1.387	1.108
	-8.0000	6.811	12.182	12.257
	-8.4815	29.200	28.879	27.610
	-9.0000	60.958	57.423	55.240
	-9.4815	65.835	81.277	80.732
	-10.0000	90.589	87.768	86.793
3	-5.0000	0.007	-0.102	-0.005
	-6.0000	0.142	0.251	-0.031
	-7.0000	1.032	1.200	1.189
	-8.0000	15.182	15.942	15.247
	-8.4815	37.321	38.196	36.427
	-9.0000	66.389	63.552	57.527
	-9.4815	89.482	90.642	96.340
	-10.0000	99.759	98.469	95.345

Table A-1h. Reference Chemical VIII: Chrysin
Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Chrysin]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	15.942	17.173	24.364
	-4.4815	8.731	8.160	6.864
	-5.0000	23.273	22.105	14.472
	-6.0000	68.650	67.029	68.069
	-7.0000	83.084	83.010	81.962
	-8.0000	87.121	89.109	85.152
	-9.0000	77.235	75.743	74.010
	-10.0000	84.117	84.636	84.749
3	-4.4815	18.087	19.756	11.349
	-5.0000	30.058	28.986	23.754
	-5.4815	56.577	53.281	54.730
	-6.0000	78.775	59.471	73.504
	-7.0000	100.752	97.252	98.167
	-8.0000	102.056	100.224	92.303
	-9.0000	98.478	90.120	95.560
	-10.0000	102.674	103.247	101.770
4	-4.4815	30.180	30.147	29.897
	-5.0000	27.100	28.639	28.294
	-5.4815	79.665	82.388	79.256
	-6.0000	72.510	77.171	78.157
	-7.0000	90.705	87.593	88.362
	-8.0000	90.680	93.309	89.514
	-9.0000	93.024	91.427	86.316
	-10.0000	87.110	85.784	86.091

Table A-1i. Reference Chemical IX: Dicofol
Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Dicofol]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	45.819	43.996	42.635
	-4.4815	65.301	52.542	58.212
	-5.0000	62.914	54.363	53.185
	-6.0000	91.100	85.342	90.916
	-7.0000	88.060	93.552	91.045
	-8.0000	94.030	87.535	83.495
	-9.0000	77.235	93.751	91.308
	-10.0000	91.804	95.343	93.060
2	-4.0000	37.232	45.836	41.640
	-4.4815	57.633	44.465	52.044
	-5.0000	53.008	52.680	49.240
	-6.0000	83.371	86.167	86.662
	-7.0000	91.961	81.384	85.326
	-8.0000	79.190	75.132	78.875
	-9.0000	89.971	91.437	88.462
	-10.0000	89.614	90.256	82.671
3	-4.0000	31.111	28.474	26.196
	-4.4815	32.655	31.723	29.340
	-5.0000	63.218	64.540	58.999
	-6.0000	89.150	87.753	91.365
	-7.0000	94.842	96.377	87.203
	-8.0000	91.404	89.965	89.505
	-9.0000	91.996	89.407	91.516
	-10.0000	94.534	93.089	93.843

Table A-1j. Reference Chemical X: Atrazine
Percent of Control Activity in Recombinant Assay by Replicate, Chemical Concentration within Replicate, and Repetition within Concentration.

Replicate	Log [Atrazine]	Percent of Control		
		Repetition 1	Repetition 2	Repetition 3
1	-4.0000	88.119	87.613	91.949
	-4.4815	93.204	93.486	95.412
	-5.0000	95.323	92.047	91.669
	-6.0000	87.663	90.999	90.529
	-7.0000	92.797	91.318	92.483
	-8.0000	87.880	86.992	84.934
	-9.0000	94.946	91.988	94.950
	-10.0000	95.734	96.623	96.937
2	-4.0000	86.050	81.350	85.117
	-4.4815	90.895	93.482	93.255
	-5.0000	90.994	90.911	87.955
	-6.0000	80.764	85.127	86.863
	-7.0000	92.053	93.171	91.097
	-8.0000	88.357	90.781	90.347
	-9.0000	88.960	87.898	86.953
	-10.0000	84.596	89.786	92.059
3	-4.0000	89.486	90.833	90.217
	-4.4815	92.557	86.609	90.317
	-5.0000	94.598	94.274	96.563
	-6.0000	96.071	91.205	91.123
	-7.0000	93.435	90.992	94.385
	-8.0000	91.766	94.009	93.803
	-9.0000	96.103	95.746	89.433
	-10.0000	88.249	90.501	87.781

Table A-2a. Reference Chemical I: Aminoglutethimide

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	Percent of Control[a]
Full Activity Control	1	Beginning	5.71636	104.235
		Beginning	5.42720	98.962
		End	5.32475	97.094
		End	5.46812	99.708
	2	Beginning	0.30395	93.035
		Beginning	0.33752	103.310
		End	0.32470	99.386
		End	0.34065	104.269
	3	Beginning	0.31233	104.112
		Beginning	0.30236	100.787
		End	0.29242	97.474
		End	0.29288	97.627
Background Control	1	Beginning	0.03964	0.723
		Beginning	0.00112	0.020
		End	-0.01953	-0.356
		End	-0.02122	-0.387
	2	Beginning	-0.00031	-0.095
		Beginning	-0.00026	-0.078
		End	0.00121	0.369
		End	-0.00064	-0.196
	3	Beginning	-0.00017	-0.057
		Beginning	-0.00027	-0.090
		End	0.00045	0.151
		End	-0.00001	-0.004
Positive Control	1	Beginning	3.26139	59.470
		Beginning	2.69371	49.119
		End	2.69107	49.070
		End	2.84105	51.805
	2	Beginning	0.17926	54.868
		Beginning	0.14577	44.618
		End	0.16792	51.400
		End	0.15596	47.739
	3	Beginning	0.11365	37.882
		Beginning	0.10001	33.338
		End	0.15659	52.199
		End	0.15382	51.274
Negative Control	1	Beginning	4.16588	75.963
		Beginning	3.58968	65.456
		End	5.61082	102.311
		End	5.51602	100.582
	2	Beginning	0.27850	85.245
		Beginning	0.21221	64.956
		End	0.30659	93.844
		End	0.33240	101.743
	3	Beginning	0.11517	38.391
		Beginning	0.12720	42.401
		End	0.25559	85.198
		End	0.22154	73.848

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

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	5.71636	104.235
		Beginning	5.42720	98.962
		End	5.32475	97.094
		End	5.46812	99.708
	2	Beginning	0.30395	93.035
		Beginning	0.33752	103.310
		End	0.32470	99.386
		End	0.34065	104.269
	4	Beginning	0.28976	98.611
		Beginning	0.28107	95.654
		End	0.29619	100.799
		End	0.30835	104.936
Background Control	1	Beginning	0.03964	0.723
		Beginning	0.00112	0.020
		End	-0.01953	-0.356
		End	-0.02122	-0.387
	2	Beginning	-0.00031	-0.095
		Beginning	-0.00026	-0.078
		End	0.00121	0.369
		End	-0.00064	-0.196
	4	Beginning	-0.00033	-0.111
		Beginning	-0.00015	-0.051
		End	-0.00006	-0.019
		End	0.00053	0.181
Positive Control	1	Beginning	3.26139	59.470
		Beginning	2.69371	49.119
		End	2.69107	49.070
		End	2.84105	51.805
	2	Beginning	0.17926	54.868
		Beginning	0.14577	44.618
		End	0.16792	51.400
		End	0.15596	47.739
	4	Beginning	0.16029	54.550
		Beginning	0.16073	54.701
		End	0.15195	51.712
		End	0.15201	51.732
Negative Control	1	Beginning	4.16588	75.963
		Beginning	3.58968	65.456
		End	5.61082	102.311
		End	5.51602	100.582
	2	Beginning	0.27850	85.245
		Beginning	0.21221	64.956
		End	0.30659	93.844
		End	0.33240	101.743
	4	Beginning	0.23716	80.710
		Beginning	0.26900	91.546
		End	0.30097	102.426
		End	0.28207	95.994

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

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	2	Beginning	0.41685	103.026
		Beginning	0.41434	102.407
		End	0.39497	97.619
		End	0.39226	96.948
	3	Beginning	7.94778	122.503
		Beginning	7.86462	121.221
		End	3.28043	50.563
		End	6.85846	105.713
	4	Beginning	0.50472	101.393
		Beginning	0.50925	102.304
		End	0.48216	96.860
		End	0.49502	99.443
Background Control	2	Beginning	-0.00008	-0.019
		Beginning	-0.00063	-0.155
		End	-0.00035	-0.086
		End	0.00105	0.260
	3	Beginning	-0.00471	-0.073
		Beginning	-0.00955	-0.147
		End	0.00580	0.089
		End	0.00846	0.130
	4	Beginning	0.00060	0.121
		Beginning	0.00056	0.112
		End	-0.00057	-0.114
		End	-0.00059	-0.119
Positive Control	2	Beginning	0.21204	52.406
		Beginning	0.20663	51.071
		End	0.17966	44.403
		End	0.19471	48.123
	3	Beginning	4.23843	65.329
		Beginning	4.24724	65.465
		End	3.87691	59.757
		End	4.02844	62.092
	4	Beginning	0.24348	48.913
		Beginning	0.23088	46.381
		End	0.24235	48.685
		End	0.24042	48.298
Negative Control	2	Beginning	0.35335	87.332
		Beginning	0.34454	85.156
		End	0.33875	83.724
		End	0.33718	83.336
	3	Beginning	7.63695	117.712
		Beginning	7.10067	109.446
		End	6.73929	103.876
		End	5.92360	91.303
	4	Beginning	0.43339	87.064
		Beginning	0.42134	84.644
		End	0.41068	82.501
		End	0.40915	82.194

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

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.38817	104.205
		Beginning	0.39213	105.268
		End	0.35240	94.602
		End	0.35733	95.925
	2	Beginning	0.40079	99.460
		Beginning	0.40717	101.045
		End	0.40315	100.046
		End	0.40075	99.450
	3	Beginning	0.57163	110.689
		Beginning	0.55320	107.122
		End	0.46959	90.931
		End	0.47127	91.257
Background Control	1	Beginning	0.00013	0.036
		Beginning	0.00025	0.068
		End	-0.00060	-0.161
		End	0.00021	0.057
	2	Beginning	-0.00008	-0.019
		Beginning	0.00039	0.097
		End	0.00002	0.004
		End	-0.00033	-0.082
	3	Beginning	0.00050	0.096
		Beginning	0.00003	0.005
		End	-0.00044	-0.086
		End	-0.00008	-0.016
Positive Control	1	Beginning	0.20382	54.717
		Beginning	0.17725	47.583
		End	0.18900	50.738
		End	0.18778	50.411
	2	Beginning	0.23513	58.350
		Beginning	0.24029	59.632
		End	0.22079	54.791
		End	0.21610	53.628
	3	Beginning	0.28553	55.291
		Beginning	0.28378	54.951
		End	0.23799	46.083
		End	0.25857	50.070
Negative Control	1	Beginning	0.30518	81.926
		Beginning	0.28270	75.892
		End	0.32704	87.794
		End	0.31159	83.646
	2	Beginning	0.37611	93.337
		Beginning	0.36480	90.530
		End	0.32104	79.669
		End	0.32470	80.578
	3	Beginning	0.43936	85.077
		Beginning	0.42829	82.933
		End	0.40956	79.307
		End	0.37093	71.826

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

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.38817	104.205
		Beginning	0.39213	105.268
		End	0.35240	94.602
		End	0.35733	95.925
	2	Beginning	0.40079	99.460
		Beginning	0.40717	101.045
		End	0.40315	100.046
		End	0.40075	99.450
	3	Beginning	0.57163	110.689
		Beginning	0.55320	107.122
		End	0.46959	90.931
		End	0.47127	91.257
Background Control	1	Beginning	0.00013	0.036
		Beginning	0.00025	0.068
		End	-0.00060	-0.161
		End	0.00021	0.057
	2	Beginning	-0.00008	-0.019
		Beginning	0.00039	0.097
		End	0.00002	0.004
		End	-0.00033	-0.082
	3	Beginning	0.00050	0.096
		Beginning	0.00003	0.005
		End	-0.00044	-0.086
		End	-0.00008	-0.016
Positive Control	1	Beginning	0.20382	54.717
		Beginning	0.17725	47.583
		End	0.18900	50.738
		End	0.18778	50.411
	2	Beginning	0.23513	58.350
		Beginning	0.24029	59.632
		End	0.22079	54.791
		End	0.21610	53.628
	3	Beginning	0.28553	55.291
		Beginning	0.28378	54.951
		End	0.23799	46.083
		End	0.25857	50.070
Negative Control	1	Beginning	0.30518	81.926
		Beginning	0.28270	75.892
		End	0.32704	87.794
		End	0.31159	83.646
	2	Beginning	0.37611	93.337
		Beginning	0.36480	90.530
		End	0.32104	79.669
		End	0.32470	80.578
	3	Beginning	0.43936	85.077
		Beginning	0.42829	82.933
		End	0.40956	79.307
		End	0.37093	71.826

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

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	2.84293	99.735
		Beginning	3.15321	110.620
		End	2.68519	94.201
		End	2.72061	95.444
	2	Beginning	0.41685	103.026
		Beginning	0.41434	102.407
		End	0.39497	97.619
		End	0.39226	96.948
	3	Beginning	7.94778	122.503
		Beginning	7.86462	121.221
		End	3.28043	50.563
		End	6.85846	105.713
Background Control	1	Beginning	0.01195	0.419
		Beginning	0.00730	0.256
		End	-0.01197	-0.420
		End	-0.00728	-0.256
	2	Beginning	-0.00008	-0.019
		Beginning	-0.00063	-0.155
		End	-0.00035	-0.086
		End	0.00105	0.260
	3	Beginning	-0.00471	-0.073
		Beginning	-0.00955	-0.147
		End	0.00580	0.089
		End	0.00846	0.130
Positive Control	1	Beginning	1.08608	38.102
		Beginning	1.02230	35.864
		End	1.34304	47.116
		End	1.31566	46.156
	2	Beginning	0.21204	52.406
		Beginning	0.20663	51.071
		End	0.17966	44.403
		End	0.19471	48.123
	3	Beginning	4.23843	65.329
		Beginning	4.24724	65.465
		End	3.87691	59.757
		End	4.02844	62.092
Negative Control	1	Beginning	1.70923	59.963
		Beginning	1.65551	58.078
		End	2.39659	84.077
		End	2.62427	92.064
	2	Beginning	0.35335	87.332
		Beginning	0.34454	85.156
		End	0.33875	83.724
		End	0.33718	83.336
	3	Beginning	7.63695	117.712
		Beginning	7.10067	109.446
		End	6.73929	103.876
		End	5.92360	91.303

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

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.57190	103.852
		Beginning	0.61447	111.582
		End	0.51835	94.129
		End	0.49802	90.437
	2	Beginning	0.61153	105.872
		Beginning	0.58118	100.619
		End	0.54432	94.237
		End	0.57341	99.273
	3	Beginning	0.71551	92.841
		Beginning	0.78514	101.875
		End	0.78477	101.827
		End	0.79734	103.458
Background Control	1	Beginning	0.00047	0.086
		Beginning	-0.00024	-0.043
		End	-0.00033	-0.059
		End	0.00009	0.017
	2	Beginning	0.00021	0.036
		Beginning	-0.00111	-0.192
		End	0.00162	0.281
		End	-0.00072	-0.125
	3	Beginning	-0.00067	-0.087
		Beginning	-0.00044	-0.058
		End	0.00073	0.094
		End	0.00039	0.051
Positive Control	1	Beginning	0.23319	42.346
		Beginning	0.24621	44.710
		End	0.22494	40.848
		End	0.22336	40.561
	2	Beginning	0.26833	46.456
		Beginning	0.23254	40.259
		End	0.23819	41.237
		End	0.23603	40.864
	3	Beginning	0.31547	40.933
		Beginning	0.26798	34.772
		End	0.35450	45.998
		End	0.35365	45.887
Negative Control	1	Beginning	0.49820	90.469
		Beginning	0.52544	95.416
		End	0.40199	72.999
		End	0.39492	71.715
	2	Beginning	0.42137	72.951
		Beginning	0.32172	55.698
		End	0.48443	83.868
		End	0.47124	81.584
	3	Beginning	0.43725	56.734
		Beginning	0.39910	51.785
		End	0.70393	91.337
		End	0.70053	90.896

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

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.57190	103.852
		Beginning	0.61447	111.582
		End	0.51835	94.129
		End	0.49802	90.437
	3	Beginning	0.71551	92.841
		Beginning	0.78514	101.875
		End	0.78477	101.827
		End	0.79734	103.458
	4	Beginning	0.51313	101.393
		Beginning	0.51774	102.304
		End	0.49019	96.860
		End	0.50327	99.443
Background Control	1	Beginning	0.00047	0.086
		Beginning	-0.00024	-0.043
		End	-0.00033	-0.059
		End	0.00009	0.017
	3	Beginning	-0.00067	-0.087
		Beginning	-0.00044	-0.058
		End	0.00073	0.094
		End	0.00039	0.051
	4	Beginning	0.00061	0.121
		Beginning	0.00057	0.112
		End	-0.00058	-0.114
		End	-0.00060	-0.119
Positive Control	1	Beginning	0.23319	42.346
		Beginning	0.24621	44.710
		End	0.22494	40.848
		End	0.22336	40.561
	3	Beginning	0.31547	40.933
		Beginning	0.26798	34.772
		End	0.35450	45.998
		End	0.35365	45.887
	4	Beginning	0.24754	48.913
		Beginning	0.23472	46.381
		End	0.24639	48.685
		End	0.24443	48.298
Negative Control	1	Beginning	0.49820	90.469
		Beginning	0.52544	95.416
		End	0.40199	72.999
		End	0.39492	71.715
	3	Beginning	0.43725	56.734
		Beginning	0.39910	51.785
		End	0.70393	91.337
		End	0.70053	90.896
	4	Beginning	0.44062	87.064
		Beginning	0.42837	84.644
		End	0.41752	82.501
		End	0.41597	82.194

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

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.59093	100.439
		Beginning	0.58774	99.897
		End	0.57073	97.007
		End	0.60398	102.657
	2	Beginning	0.58638	102.418
		Beginning	0.55772	97.413
		End	0.58374	101.959
		End	0.56228	98.209
	3	Beginning	0.68948	102.077
		Beginning	0.69973	103.594
		End	0.65715	97.290
		End	0.65546	97.040
Background Control	1	Beginning	0.00180	0.306
		Beginning	0.00137	0.234
		End	-0.00186	-0.317
		End	-0.00131	-0.223
	2	Beginning	-0.00202	-0.353
		Beginning	-0.00139	-0.242
		End	0.00048	0.084
		End	0.00293	0.511
	3	Beginning	-0.00033	-0.049
		Beginning	-0.00001	-0.001
		End	-0.00064	-0.095
		End	0.00098	0.144
Positive Control	1	Beginning	0.30051	51.077
		Beginning	0.21433	36.429
		End	0.30844	52.425
		End	0.31522	53.577
	2	Beginning	0.26823	46.851
		Beginning	0.23210	40.539
		End	0.29664	51.812
		End	0.30389	53.079
	3	Beginning	0.32661	48.354
		Beginning	0.32541	48.176
		End	0.30290	44.844
		End	0.26163	38.733
Negative Control	1	Beginning	0.43348	73.678
		Beginning	0.39465	67.078
		End	0.52850	89.828
		End	0.51760	87.976
	2	Beginning	0.40527	70.785
		Beginning	0.32617	56.970
		End	0.48403	84.542
		End	0.44976	78.556
	3	Beginning	0.59560	88.177
		Beginning	0.56428	83.540
		End	0.49721	73.612
		End	0.41539	61.498

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

Full Enzyme Activity Control, Background Activity Control, Positive Control, and Negative Control Corrected Aromatase Activity, and Percent of Control Data by Replicate and Portion (Beginning or End) for Recombinant Aromatase Assay.

Aromatase Activity	Replicate	Portion	Corrected Aromatase Activity	percent of Control[a]
Full Activity Control	1	Beginning	0.59093	100.439
		Beginning	0.58774	99.897
		End	0.57073	97.007
		End	0.60398	102.657
	2	Beginning	0.58638	102.418
		Beginning	0.55772	97.413
		End	0.58374	101.959
		End	0.56228	98.209
	3	Beginning	0.68948	102.077
		Beginning	0.69973	103.594
		End	0.65715	97.290
		End	0.65546	97.040
Background Control	1	Beginning	0.00180	0.306
		Beginning	0.00137	0.234
		End	-0.00186	-0.317
		End	-0.00131	-0.223
	2	Beginning	-0.00202	-0.353
		Beginning	-0.00139	-0.242
		End	0.00048	0.084
		End	0.00293	0.511
	3	Beginning	-0.00033	-0.049
		Beginning	-0.00001	-0.001
		End	-0.00064	-0.095
		End	0.00098	0.144
Positive Control	1	Beginning	0.30051	51.077
		Beginning	0.21433	36.429
		End	0.30844	52.425
		End	0.31521	53.576
	2	Beginning	0.26823	46.851
		Beginning	0.23210	40.539
		End	0.29664	51.812
		End	0.30389	53.079
	3	Beginning	0.32661	48.354
		Beginning	0.32541	48.176
		End	0.30290	44.844
		End	0.26163	38.733
Negative Control	1	Beginning	0.43348	73.678
		Beginning	0.39465	67.078
		End	0.52850	89.828
		End	0.51760	87.976
	2	Beginning	0.40527	70.785
		Beginning	0.32617	56.970
		End	0.48403	84.542
		End	0.44976	78.556
	3	Beginning	0.59560	88.177
		Beginning	0.56428	83.540
		End	0.49721	73.612
		End	0.41539	61.498

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

AUDITED TASK REPORT

VALIDATION OF THE AROMATASE ASSAY FOR ENDOCRINE DISRUPTOR SCREENING USING RECOMBINANT MICROSOMES (WA 4-17, TASK 4)

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

EPA Contract Number 68-W-01-023
Work Assignment 4-17
WIL-431011

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 AROMATASE ASSAY FOR ENDOCRINE DISRUPTOR SCREENING USING RECOMBINANT MICROSOMES (WA 4-17, TASK 4)
Task 4:	Conduct Multiple Chemical Studies with Recombinant Microsomes
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, PhD EDSP Program Manager Battelle Memorial Institute Jerry D. Johnson, PhD Diplomate, ABT Work Assignment Leader/Study Monitor Battelle Memorial Institute
Study Initiation Date:	July 7, 2005
Experimental Start Date:	July 21, 2005
Experimental Termination Date:	October 5, 2005
Audited Task Report Date	January 18, 2006

Quality Assurance Statements

The study was conducted 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 amendment 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.

Intralaboratory 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
05-Jul-2005	Protocol / Protocol Amendment Review / Protocol Review	5-Jul-2005	25-Aug-2005	L.Goodrich
27-Jul-2005	Test & Control Articles & Analytical Chemistry (Aromatase Assay - 27 July 2005)	27-Jul-2005	25-Aug-2005	E.Crawford
02-Nov-2005, 09-Nov-2005, 10-Nov-2005, 17-Nov-2005	Study Records (A-1)	17-Nov-2005	21-Dec-2005	A.Deppe / L.Goodrich
10-Nov-2005, 14-Nov-2005, 15-Nov-2005, 17-Nov-2005	Study Records (A-2)	17-Nov-2005	21-Dec-2005	A.Deppe / L.Goodrich
02-Nov-2005, 17-Nov-2005	Study Records (A-3)	17-Nov-2005	21-Dec-2005	A.Deppe / L.Goodrich
14-Nov-2005, 15-Nov-2005, 17-Nov-2005	Study Records (A-4)	17-Nov-2005	21-Dec-2005	L.Goodrich
1-Dec-2005	Spreadsheet Data for Statistical Analysis	2-Dec-2005	27-Jan-2006	A.Deppe
19-Dec-2005, 20-Dec-2005, 21-Dec-2005, 22-Dec-2005, 23-Dec-2005, 24-Dec-2005, 27-Dec-2005, 03-Jan-2006, 04-Jan-2006	Draft Report (without Statistical Analysis Summary Appendix)	4-Jan-2006	20-Feb-2006	A.Deppe
21-Dec-2005	Statistical Analysis Summary Appendix	4-Jan-2006	20-Feb-2006	A.Deppe

Approval

This study was inspected according to the criteria discussed above.

Report Audited By:

_____ _____
Alan D. Deppe, BA, RQAP-GLP Date
Project Coordinator, Quality Assurance

Report Released By:

_____ _____
Heather L. Osborn, BS, RQAP-GLP Date
Manager, Quality Assurance

Compliance Statement

This study, designated WIL-431011, 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 as approved by the sponsor.

_____ _____

Jennifer Thomas-Wohlever, PhD
Research Scientist, Metabolism
Study Director

Date

Table of Contents

Page

LIST OF FIGURES

Page

Page

LIST OF APPENDICES

1.0 Executive Summary

The recombinant aromatase assay combines human recombinant 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 recombinant microsome aromatase assay at WIL Research Laboratories, LLC (a participating laboratory in the inter-laboratory validation of this method) using the known aromatase inhibitor 4-hydroxyandrostenedione as the Positive control, lindane as a Negative control and ten reference chemicals whose IC_{50} will be determined in the course of this Task (WA 4-17, Task 4).

Complete dose response curves were achieved consistently with the reference chemicals prochloraz, 4-nonylphenol, fenarimol, econazole, dicofol, 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 observed in the presence 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 a 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. The validation assays are currently being administered by Battelle Memorial Institute.

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 utilized 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 have been 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 recombinant microsomal assay system, with IC_{50} values for aromatase inhibition ranging from 0.04 µM to greater than 50 µM (Final Detailed Review Paper on Aromatase, 2005).

The recombinant microsomal aromatase assay was recommended as the *in vitro* aromatase screening assay to be included in the Tier 1 Screening Battery. This assay will detect environmental toxicants that possess the ability to inhibit aromatase activity. Prevalidation studies on 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 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 recombinant 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-431010, WA 4-17, Task 3).

2.2 Task Description and Objectives

WIL Research Laboratories, LLC (as one of the participating laboratories) has demonstrated the responsiveness of the human recombinant 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 recombinant 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 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 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 the intralaboratory report.

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. The following table summarized 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.

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 in a given day. 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 reporting.

Reference Chemical Information								
Chemical name	Chemical code	Mfr. Purity	CAS No.	Molecular formula	M.W.	Stock Solution (WIL ID)	Vehicle (used for chemical 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 # 2969A 46428	~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 chemical 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 # 2969A4 6428	~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 # 2969A4 6428	RT
4-Nonylphenol	Reference Chemical 4	> 98.5%	104-40-5	$C_{15}H_{24}O$	220.4	MET-0319A	DMSO Lot # 2969A4 6428	~5°C
Debenz[a,h]-anthracene	Reference Chemical 5	97%	53-70-3	$C_{22}H_{14}$	278.35	MET-1320A	DMSO Lot # 2969A4 6428	~5°C
Fenarimol	Reference Chemical 6	99%	60168-88-9	$C_{17}H_{12}Cl_2N_2O$	331.2	MET-0309A	DMSO Lot # 2969A4 6428	RT
Econazole	Reference Chemical 7	98%	24169-02-6	$C_{18}H_{15}Cl_3N_2O$-HNO_3	444.7	MET-0313A	DMSO Lot # 2969A4 6428	~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 # 2964A4 6428 and 44155	2-8°C
Dicofol	Reference Chemical 9	96.5%	115-32-2	$C_{14}H_9Cl_5O$	370.47	MET-0317A	DMSO Lot # 2969A4 6428	~5°C
Atrazine	Reference Chemical 10	98%	1912-24-9	$C_8H_{14}ClN_5$	215.69	MET-0318A	DMSO Lot # 2969A4 6428	~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^{-6} 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 dimethylsulfoxide (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 4-17 Task 3, WIL-431010) 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 recombinant microsomes from baculovirus infected insect cells were received as multiple frozen aliquots from RTI International, which acquired the microsomes from GenTest (Human CYP19 + P450 Reductase SUPERSOMES™, Woburn, MA). Upon receipt, the sample code numbers MET-0254A and MET-0307A were assigned. The accompanying information sheets specified storage at -70 to -80°C. On the day of the assay, microsomes were thawed rapidly in a 37 ± 1°C water bath, re-homogenized using a Potter Elvejhem homogenizer and then kept on ice until used. Fresh aliquots of microsomes were used for each assay day. At no time were microsome aliquots thawed, refrozen and thawed again for use. The supplier supplied protein concentration was 4.9 mg/mL. The microsomes were diluted to approximately 0.008 mg/mL for use in the assay.

3.4 Other Assay Components

Assay Reagents – Information			
Chemical	Supplier	WIL ID	Lot Number
NADPH	Sigma-Aldrich	CP#05-77A,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-0315A#1 MET-0316A#1	2969A46428 2969A46428 44155

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 is 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 was 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 (microsome sample, QC 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 interpolation 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 recombinant microsomes supplied by RTI International. 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 reference chemical, 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, respectively. Positive controls utilizing the known aromatase inhibitor 4-hydroxyandrostenedione and 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 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^{-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] = Complete assay includes buffer, propylene glycol, vehicle [used for preparation of reference chemical solutions], 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 approximately 10 second intervals. Each assay was incubated at 37 ± 1°C for 15 minutes. At the conclusion of the reaction time, tubes were quenched with 2.0 mL methylene chloride in the order in which microsomes had been added, one approximately 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 (500 µL) were transferred to 20 mL 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 $^{3}H_2O$ formed. One $^{3}H_2O$ 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 $^{3}H_2O$ (in dpm) formed by the specific activity of the [^{3}H]ASDN substrate (expressed in dpm/nmol). Results are presented as the activity (velocity) of the enzyme reaction normalized per mg protein. The activity of the enzyme reaction is expressed in nmol/mg protein/min. Activity is also presented as percent of Full Enzyme Activity Control.

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	7/21/2005	JG
Aminoglutethimide Rep 2	7/25/2005	JG
Aminoglutethimide Rep 3	7/27/2005	JG
Ketoconazole Rep 1	7/21/2005	JG
Ketoconazole Rep 2	7/25/2005	JG
Ketoconazole Rep 3	7/27/2005	JG
Prochloraz Rep 1	8/4/2005	TNB
Prochloraz Rep 2	8/8/2005	TNB
Prochloraz Rep 3	8/10/2005	TNB
4-Nonylphenol Rep 1	9/20/2005	JG
4-Nonylphenol Rep 2	9/23/2005	JG
4-Nonylphenol Rep 3	9/27/2005	JG
Dibenz[a,h]anthracene Rep 1	9/29/2005	TNB
Dibenz[a,h]anthracene Rep 2	10/3/2005	TNB
Dibenz[a,h]anthracene Rep 3	10/5/2005	TNB
Fenarimol Rep 1	8/4/2005	TNB
Fenarimol Rep 2	8/8/2005	TNB
Fenarimol Rep 3	8/10/2005	TNB
Econazole Rep 1	8/18/2005	JG
Econazole Rep 2	8/29/2005	JG
Econazole Rep 3	8/31/2005	JG
Chrysin Rep 1	8/18/2005	JG
Chrysin Rep 2	8/29/2005	JG
Chrysin Rep 3	8/31/2005	JG
Dicofol Rep 1	9/2/2005	TNB
Dicofol Rep 2	9/7/2005	TNB
Dicofol Rep 3	9/9/2005	TNB
Atrazine Rep 1	9/2/2005	TNB
Atrazine Rep 2	9/7/2005	TNB
Atrazine Rep 3	9/9/2005	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. Each chemical's data was saved as an individual replicate worksheet. 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 identification, number of concentrations tested, replicate, technician, reference chemical and log 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 total 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 yielded 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 was then converted to nmol product formed by dividing by the substrate specific activity (DPM/nmol). The activity of the enzyme reaction was 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 control remaining in the presence of various reference chemical 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 was necessarily equal to 0 within each replicate and the average percent activity across the four Full Enzyme Activity repeat tubes was necessarily equal to 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 sometimes extended 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 Hill slope (ß). The average values and standard errors of T, B, $\log_{10}IC_{50}$, or ß 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, μ and ß 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 were 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 were 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, the portion main effect, the portion by reference chemical interaction, and the portion by replicate within reference chemical interaction were 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	Radiochemical Code	Radiochemical ID	Radiochemical Stock Concentration (µCi/g)	Assay Substrate Final Concentration (µg/g) (ASDN + ^{3}H ASDN)	Substrate solution Specific Activity (dpm/nmol)
Aminoglutethimide /Ketoconazole Rep 1	[^{3}H]-ASDN	CP #05-079	0.662	0.589	714672
Aminoglutethimide /Ketoconazole Rep 2	[^{3}H]-ASDN	CP #05-079	0.616	0.574	681232
Aminoglutethimide /Ketoconazole Rep 3	[^{3}H]-ASDN	CP #05-079	0.696	0.594	744847
Prochloraz/ Fenarimol Rep 1	[^{3}H]-ASDN	CP #05-079	0.737	0.571	820400
Prochloraz/ Fenarimol Rep 2	[^{3}H]-ASDN	CP #05-079	0.723	0.580	793140
Prochloraz/ Fenarimol Rep 3	[^{3}H]-ASDN	CP #05-079	0.749	0.573	831944
4-Nonylphenol Rep 1	[^{3}H]-ASDN	CP #05-079	0.788	0.594	843711
4-Nonylphenol Rep 2	[^{3}H]-ASDN	CP #05-079	0.794	0.589	857260
4-Nonylphenol Rep 3	[^{3}H]-ASDN	CP #05-079	0.835	0.594	894166
Dibenz[a,h]anthracene Rep 1	[^{3}H]-ASDN	CP #05-079	0.887	0.600	941145
Dibenz[a,h]anthracene Rep 2	[^{3}H]-ASDN	CP #05-079	0.863	0.580	946063
Dibenz[a,h]anthracene Rep 3	[^{3}H]-ASDN	CP #05-079	0.858	0.596	914644
Econazole/ Chrysin Rep 1	[^{3}H]-ASDN	CP #05-079	0.743	0.581	812761
Econazole/ Chrysin Rep 2	[^{3}H]-ASDN	CP #05-079	1.494	0.580	1636908
Econazole/ Chrysin Rep 3	[^{3}H]-ASDN	CP #05-079	0.727	0.591	781092
Dicofol/ Atrazine Rep 1	[^{3}H]-ASDN	CP #05-079	0.826	0.574	915025
Dicofol/ Atrazine Rep 2	[^{3}H]-ASDN	CP #05-079	0.638	0.573	707370
Dicofol/ Atrazine Rep 3	[^{3}H]-ASDN	CP #05-079	0.802	0.575	886532

4.2 Stock Formulation Analysis

Stock formulation analysis was performed by the sponsor. 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 intralaboratory report (WA 4-17, Task 4) submitted to the EPA and will include the individual reports from each individual lab.

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 0.01 M	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 0.01 M	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-0320A	2.78 mg/mL 0.01M	10-03-05
Fenarimol	FRM	6	MET-0309A	33.13 mg/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 0.01 M	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 the final microsomal dilution used in the aromatase assay was determined on the day that the microsomes were used in the assay. The results of the daily protein analysis are summarized in the following table.

Protein Analysis Results		
Replicate	Assay Date	Protein stock concentration (mg/mL, measured)
Aminoglutethimide /Ketoconazole Rep 1	7/21/2005	5.043
Aminoglutethimide /Ketoconazole Rep 2	7/25/2005	4.138
Aminoglutethimide /Ketoconazole Rep 3	7/27/2005	4.587
Prochloraz/ Fenarimol Rep 1	8/4/2005	3.764

Replicate	Assay Date	Protein stock concentration (mg/mL, measured)
Prochloraz/ Fenarimol Rep 2	8/8/2005	4.936
Prochloraz/ Fenarimol Rep 3	8/10/2005	5.664
4-Nonylphenol Rep 1	9/20/2005	8.449
4-Nonylphenol Rep 2	9/23/2005	5.677
4-Nonylphenol Rep 3	9/27/2005	6.222
Dibenz[a,h]anthracene Rep 1	9/29/2005	6.322
Dibenz[a,h]anthracene Rep 2	10/3/2005	7.748
Dibenz[a,h]anthracene Rep 3	10/5/2005	9.477
Econazole/ Chrysin Rep 1	8/18/2005	6.432
Econazole/ Chrysin Rep 2	8/29/2005	8.073
Econazole/ Chrysin Rep 3	8/31/2005	7.306
Dicofol/ Atrazine Rep 1	9/2/2005	9.532
Dicofol/ Atrazine Rep 2	9/7/2005	8.813
Dicofol/ Atrazine Rep 3	9/9/2005	7.309

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	11	101
Aminoglutethimide /Ketoconazole Rep 1	9	98
Aminoglutethimide /Ketoconazole Rep 1	9	99
Prochloraz/ Fenarimol Rep 1	8	99
Prochloraz/ Fenarimol Rep 2	8	95
Prochloraz/ Fenarimol Rep 3	11	99
4-Nonylphenol Rep 1	14	101
4-Nonylphenol Rep 2	11	101

Replicate	Experimental µg/mL BSA (10 µg/mL target)	Experimental µg/mL BSA (100 µg/mL target)
4-Nonylphenol Rep 3	11	100
Dibenz[a,h]anthracene Rep 1	10	101
Dibenz[a,h]anthracene Rep 2	12	102
Dibenz[a,h]anthracene Rep 3	13	102
Econazole/ Chrysin Rep 1	13	102
Econazole/ Chrysin Rep 2	13	103
Econazole/ Chrysin Rep 3	15	87
Dicofol/ Atrazine Rep 1	17	98
Dicofol/ Atrazine Rep 2	15	102
Dicofol/ Atrazine Rep 3	10	103
AVERAGE	11.67	99.67
Standard Deviation	2.54	3.79
%CV	21.80	3.80
Percent of Expected Value	116.67	99.67

4.4 Control Aromatase Activity

Figure 1a. Full Enzyme Activity Controls By Portion
Figure 1b. Full Enzyme Activity -Difference In Percent Of Control Activity By Portion
Figure 2a. Background Activity Controls By Portion
Figure 2b. Background Activity -Difference In Percent Of Control Activity By Portion
Figure 3a. Positive Controls By Portion
Figure 3b. Positive Control Activity -Difference In Percent Of Control Activity By Portion
Figure 4a. Negative Controls By Portion
Figure 4b. Negative Control Activity -Difference In Percent Of Control Activity By Portion

Appendix D – Individual Replicate Spreadsheets

The Full Enzyme Activity controls percent of control and the Background Activity controls percent of Full Enzyme Activity control 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). The Negative and Positive controls percent of control values were plotted in a similar manner with reference lines at 100% and 50% of Full Enzyme Activity, 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 within each reference chemical was less than 10%. By contrast, the inter-assay variability within a reference chemical averaged 25%. This value excludes prochloraz and fenarimol, as Replicate 2 for these chemicals had negligible enzyme activity indicating that the enzyme was inactive. Plotting the Full Enzyme Activity controls by portion revealed that the beginning values were frequently higher than the ending enzyme activity values. Plotting the difference between beginning and ending portion (Figure 1b) revealed 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 (nmol/mg protein/min)	End (nmol/mg protein/min)	Within Replicate Mean (± sd, sem)	% CV	Overall Mean (± sd, sem)	Overall % CV
1	0.409	0.381	0.395 (0.017, 0.008)	4.21	0.593 (0.218, 0.063)	36.78
2	0.895	0.864	0.880 (0.030, 0.015)	3.37		
3	0.520	0.488	0.504 (0.023, 0.012)	4.66		
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	0.478	0.430	0.446 (0.030, 0.017)	6.61	0.279 (0.209, 0.063)	74.80
2	0.018	0.017	0.017 (0.001, 0.000)	4.48		
3	0.433	0.397	0.415 (0.024, 0.012)	5.90		
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	0.242	0.243	0.242 (0.009, 0.005)	3.83	0.321 (0.070, 0.020)	21.71
2	0.312	0.324	0.318 (0.009, 0.004)	2.81		
3	0.417	0.390	0.404 (0.017, 0.009)	4.29		
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	0.332	0.336	0.334 (0.011, 0.006)	3.43	0.271 (0.051, 0.015)	18.74
2	0.256	0.256	0.256 (0.004, 0.002)	1.42		
3	0.237	0.207	0.222 (0.022, 0.011)	9.71		

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	0.403	0.383	0.393 (0.013, 0.007)	3.42	0.321 (0.056, 0.016)	17.40
2	0.300	0.261	0.281 (0.023, 0.012)	8.31		
3	0.305	0.274	0.289 (0.018, 0.009)	6.22		
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	0.239	0.237	0.238 (0.002, 0.001)	0.71	0.255 (0.063, 0.018)	24.89
2	0.195	0.185	0.190 (0.006, 0.003)	3.24		
3	0.340	0.330	0.335 (0.013, 0.006)	3.84		

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	0.213	0.175	0.194 (0.023, 0.011)	11.71	0.255 (0.059, 0.017)	23.27
2	0.342	0.303	0.322 (0.024, 0.012)	7.47		
3	0.271	0.225	0.248 (0.027, 0.013)	10.88		
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	0.238	0.213	0.225 (0.017, 0.008)	7.39	0.156 (0.103, 0.030)	66.21
2	0.018	0.017	0.017 (0.001, 0.000)	3.78		
3	0.239	0.212	0.226 (0.018, 0.009)	7.85		

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	0.119	0.114	0.116 (0.007, 0.004)	6.10	0.161 (0.036, 0.011)	22.60
2	0.168	0.165	0.166 (0.002, 0.001)	1.35		
3	0.204	0.196	0.200 (0.006, 0.003)	2.99		
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	0.161	0.165	0.163 (0.014, 0.007)	8.57	0.134 (0.023, 0.007)	17.06
2	0.129	0.121	0.125 (0.005, 0.002)	3.95		
3	0.118	0.112	0.115 (0.004, 0.002)	3.74		
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	0.190	0.175	0.183 (0.009, 0.004)	4.75	0.156 (0.023, 0.007)	14.48
2	0.147	0.125	0.136 (0.014, 0.007)	10.31		
3	0.157	0.143	0.150 (0.008, 0.004)	5.49		
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	0.124	0.113	0.118 (0.007, 0.003)	5.58	0.131 (0.030, 0.009)	22.55
2	0.108	0.102	0.105 (0.003, 0.002)	2.97		
3	0.173	0.166	0.170 (0.004, 0.002)	2.50		

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	0.384	0.341	0.362 (0.027, 0.014)	7.49	0.564 (0.210, 0.061)	37.18
2	0.881	0.782	0.831 (0.063, 0.031)	7.54		
3	0.528	0.468	0.498 (0.035, 0.018)	7.05		
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	0.473	0.418	0.445 (0.033, 0.017)	7.49	0.295 (0.206, 0.060)	69.87
2	0.017	0.017	0.017 (0.000, 0.000)	2.01		
3	0.433	0.412	0.423 (0.013, 0.006)	3.04		
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	0.247	0.223	0.235 (0.014, 0.007)	5.86	0.318 (0.070, 0.020)	22.11
2	0.325	0.317	0.321 (0.006, 0.003)	1.88		
3	0.404	0.394	0.399 (0.009, 0.004)	2.14		
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	0.275	0.308	0.291 (0.062, 0.031)	21.15	0.256 (0.044, 0.013)	17.18
2	0.258	0.245	0.251 (0.012, 0.006)	4.75		
3	0.233	0.216	0.225 (0.011, 0.005)	4.68		

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	0.368	0.350	0.359 (0.013, 0.007)	3.63	0.288 (0.055, 0.016)	19.11
2	0.280	0.249	0.265 (0.019, 0.009)	7.12		
3	0.250	0.231	0.240 (0.012, 0.006)	5.20		
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	0.247	0.223	0.235 (0.016, 0.008)	6.64	0.247 (0.062, 0.018)	25.33
2	0.187	0.176	0.182 (0.006, 0.003)	3.43		
3	0.338	0.309	0.324 (0.018, 0.009)	5.49		

Background Activity controls were conducted in duplicate at the beginning and end of each assay. Figure 2a (Background Activity Controls 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, indicating that there was no background activity (potentially caused by nonspecific turnover of reactant to product, or unintentional NADPH contamination) that might interfere with the interpretation of the results. For all replicates except the prochloraz and fenarimol Replicate 2, dicofol/atrazine Replicate 1 (all) and Replicate 2 (beginning), the Background Activity was only -0.1% to 0.1% of the Full Enzyme Activity. In addition, 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). In addition, there was a negligible difference by portion across replicates, as illustrated by Figure 2b. An equal number of replicates had higher values in the beginning versus the end, and vice versa.

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 ten reference chemicals. The Positive and Negative controls behaved as expected, with an average inhibition in the Positive Controls of 49.1%, excepting the prochloraz and fenarimol data whose controls were affected by compromised enzyme activity. The portion effect was negligible (51.6% of Full Enzyme Activity at the beginning, and 47.2% at the end, again excepting the prochloraz and fenarimol data). The average Negative Control data was 96.09% of the Full Enzyme Activity, closely approaching the expected 100% value. As in the Positive Controls, a negligible portion effect was present (98.7% at the beginning versus 91.9% at the end, excluding prochloraz and fenarimol data).

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 generally inhibited aromatase activity in a dose responsive manner. Low intra-assay variability was generally characterized by a coefficient of variance of less than 10% across triplicate samples (tubes) at all 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 error terms (large %CV), for example the first prochloraz replicate had unusually high variability at the highest concentrations, as did econazole. For all the replicates, outliers were rejected with a 90% confidence level using the Q-test described by Dean and Dixon (1951). For this report, no outliers were detected. Decreasing concentrations of reference chemical resulted in decreased inhibition of aromatase activity characterized by increased percent of control activity for all chemicals shown to be inhibitors of aromatase. 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 8 of the 10 chemicals. Dibenz[a,h]anthracene and atrazine did not inhibit aromatase. The daily fluctuation in Full Enzyme Activity discussed in Section 4.4 did not affect the percent inhibition, or the shape or fit of the dose-response curve, because the data are reported as a percent of control activity.

Reference Chemical Aromatase Activity – Percent of Full Enzyme Activity Control									
Reference Chemical	Rep	Log [reference chemical]	Percent of Control			Overall			
			Tube 1	Tube 2	Tube 3	Mean	sd	sem	%CV
AG	1	-10	101.52	100.83	106.88	103.08	3.31	1.91	3.21
AG	1	-9	102.64	106.12	101.09	103.28	2.58	1.49	2.49
AG	1	-8	104.77	105.08	103.71	104.52	0.72	0.41	0.69
AG	1	-7	99.94	104.98	105.28	103.40	3.00	1.73	2.90
AG	1	-6	101.36	100.82	101.00	101.06	0.27	0.16	0.27
AG	1	-5	88.51	89.96	86.33	88.27	1.83	1.05	2.07
AG	1	-4	43.25	41.47	42.31	42.34	0.89	0.51	2.10
AG	1	-3	5.69	5.71	5.63	5.68	0.04	0.02	0.73
AG	2	-9	99.22	97.13	99.44	98.60	1.27	0.74	1.29
AG	2	-7	103.26	101.40	102.16	102.27	0.94	0.54	0.91
AG	2	-6	99.82	95.84	101.54	99.07	2.92	1.69	2.95
AG	2	-5	81.17	79.95	81.50	80.87	0.82	0.47	1.01
AG	2	-4.3	48.68	47.38	45.94	47.33	1.37	0.79	2.90
AG	2	-4	32.30	32.98	34.67	33.32	1.22	0.70	3.66
AG	2	-3.3	9.01	8.90	9.01	8.97	0.06	0.04	0.71
AG	2	-3	3.77	3.59	3.82	3.73	0.12	0.07	3.25
AG	3	-9	103.31	101.28	103.48	102.69	1.22	0.71	1.19
AG	3	-7	103.25	98.87	106.71	102.94	3.93	2.27	3.82

NA = Not Applicable. Cannot divide by zero to calculate %CV.

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	3	-6	102.18	99.53	101.87	101.19	1.45	0.84	1.43
AG	3	-5	91.17	89.58	90.17	90.31	0.80	0.46	0.89
AG	3	-4.3	62.08	58.51	64.60	61.73	3.06	1.77	4.96
AG	3	-4	45.63	45.67	44.46	45.25	0.69	0.40	1.52
AG	3	-3.3	14.24	14.10	14.49	14.28	0.20	0.11	1.38
AG	3	-3	6.20	6.32	6.07	6.20	0.13	0.07	2.02
KCZ	1	-10	99.04	97.79	96.97	97.93	1.04	0.60	1.06
KCZ	1	-9	98.05	100.37	101.36	99.93	1.70	0.98	1.70
KCZ	1	-8	95.91	99.18	100.00	98.36	2.16	1.25	2.20
KCZ	1	-7	72.70	78.46	82.57	77.91	4.96	2.86	6.36
KCZ	1	-6	30.48	30.19	30.49	30.39	0.17	0.10	0.56
KCZ	1	-5.3	8.29	8.27	8.26	8.27	0.02	0.01	0.18
KCZ	1	-5	4.34	3.90	4.39	4.21	0.27	0.16	6.40
KCZ	1	-4	0.42	0.32	0.36	0.37	0.05	0.03	13.73
KCZ	2	-10	99.52	95.20	94.36	96.36	2.77	1.60	2.87
KCZ	2	-9	101.03	96.53	100.88	99.48	2.56	1.48	2.57
KCZ	2	-8	99.83	78.93	105.51	94.76	14.00	8.08	14.77
KCZ	2	-7	74.48	70.64	74.86	73.33	2.33	1.35	3.18
KCZ	2	-6	23.32	15.27	23.98	20.86	4.85	2.80	23.25
KCZ	2	-5.3	6.05	3.81	5.91	5.26	1.25	0.72	23.87
KCZ	2	-5	3.02	3.08	3.04	3.05	0.03	0.02	1.00
KCZ	2	-4	0.26	0.31	0.28	0.28	0.03	0.01	8.88
KCZ	3	-10	100.50	95.36	99.11	98.32	2.66	1.54	2.70
KCZ	3	-9	102.23	96.84	98.55	99.21	2.75	1.59	2.78
KCZ	3	-8	93.85	97.58	100.22	97.22	3.20	1.85	3.29
KCZ	3	-7	84.64	83.21	84.23	84.03	0.74	0.43	0.88
KCZ	3	-6	37.23	35.58	35.52	36.11	0.97	0.56	2.69
KCZ	3	-5.3	9.98	10.81	10.52	10.44	0.42	0.24	4.04
KCZ	3	-5	5.28	5.43	5.42	5.38	0.08	0.05	1.56
KCZ	3	-4	0.51	0.49	0.48	0.49	0.02	0.01	3.10
PCZ	1	-10	106.47	110.31	109.74	108.84	2.07	1.20	1.90
PCZ	1	-9	101.50	108.31	107.99	105.93	3.84	2.22	3.63
PCZ	1	-8	72.72	79.92	84.03	78.89	5.72	3.31	7.26
PCZ	1	-7	22.91	23.75	24.40	23.69	0.75	0.43	3.15
PCZ	1	-6	3.22	3.41	3.63	3.42	0.21	0.12	6.00
PCZ	1	-5	0.35	0.56	0.30	0.40	0.14	0.08	34.21
PCZ	1	-4	0.13	0.42	0.10	0.22	0.18	0.10	81.57
PCZ	1	-3	0.17	0.05	0.02	0.08	0.08	0.05	99.22
PCZ	2	-10	100.74	99.04	100.01	99.93	0.85	0.49	0.85
PCZ	2	-9	103.27	99.19	106.96	103.14	3.89	2.24	3.77
PCZ	2	-8.52	107.59	100.45	107.33	105.12	4.05	2.34	3.85
PCZ	2	-8	99.06	96.80	111.57	102.48	7.96	4.59	7.76
PCZ	2	-7.52	100.68	97.31	99.40	99.13	1.70	0.98	1.72
PCZ	2	-7	97.58	92.58	103.43	97.86	5.43	3.14	5.55
PCZ	2	-6	53.19	54.83	51.19	53.07	1.82	1.05	3.44

NA = Not Applicable. Cannot divide by zero to calculate %CV.

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	-5	12.70	12.23	9.69	11.54	1.62	0.93	14.03
PCZ	3	-10	108.73	100.24	107.79	105.59	4.65	2.69	4.41
PCZ	3	-9	105.72	106.45	101.67	104.61	2.58	1.49	2.46
PCZ	3	-8.52	96.61	98.07	95.46	96.71	1.31	0.76	1.35
PCZ	3	-8	85.13	76.16	76.90	79.40	4.98	2.87	6.27
PCZ	3	-7.52	53.30	57.29	53.90	54.83	2.15	1.24	3.92
PCZ	3	-7	24.30	22.24	26.10	24.21	1.93	1.12	7.98
PCZ	3	-6	3.93	3.51	3.52	3.65	0.24	0.14	6.56
PCZ	3	-5	0.50	0.40	0.34	0.41	0.08	0.05	19.56
NYP	1	-10	98.35	97.93	96.66	97.65	0.88	0.51	0.90
NYP	1	-9	99.35	98.11	101.68	99.71	1.81	1.05	1.82
NYP	1	-8	99.48	100.89	100.35	100.24	0.71	0.41	0.71
NYP	1	-7	93.69	101.09	95.72	96.83	3.82	2.21	3.95
NYP	1	-6	99.35	99.15	95.06	97.85	2.42	1.40	2.47
NYP	1	-5	74.38	69.62	44.86	62.95	15.85	9.15	25.18
NYP	1	-4	1.51	1.50	1.66	1.56	0.09	0.05	5.76
NYP	1	-3	0.21	0.17	0.16	0.18	0.03	0.02	14.70
NYP	2	-9	104.02	104.03	85.99	98.01	10.41	6.01	10.62
NYP	2	-8	105.33	103.86	101.20	103.46	2.09	1.21	2.02
NYP	2	-7	105.54	107.32	105.75	106.20	0.97	0.56	0.92
NYP	2	-6	100.75	100.47	101.38	100.87	0.47	0.27	0.46
NYP	2	-5	72.13	73.46	69.52	71.70	2.00	1.16	2.80
NYP	2	-4.3	11.20	9.42	10.39	10.34	0.89	0.51	8.62
NYP	2	-4	1.78	1.78	1.96	1.84	0.10	0.06	5.65
NYP	2	-3	0.13	0.09	0.07	0.10	0.03	0.02	31.60
NYP	3	-9	95.18	94.43	94.43	94.68	0.43	0.25	0.46
NYP	3	-8	92.92	91.05	96.29	93.42	2.66	1.53	2.84
NYP	3	-7	96.71	95.60	95.69	96.00	0.62	0.36	0.64
NYP	3	-6	95.59	95.12	95.27	95.33	0.24	0.14	0.25
NYP	3	-5	65.20	71.21	70.00	68.80	3.18	1.84	4.62
NYP	3	-4.3	13.36	14.86	13.66	13.96	0.79	0.46	5.69
NYP	3	-4	1.99	1.97	1.96	1.97	0.02	0.01	0.77
NYP	3	-3	0.17	0.07	0.14	0.13	0.05	0.03	40.51
DBA	1	-10	101.38	101.91	104.25	102.51	1.53	0.88	1.49
DBA	1	-9	104.29	105.43	104.93	104.88	0.57	0.33	0.54
DBA	1	-8	103.12	105.66	103.21	104.00	1.44	0.83	1.39
DBA	1	-7	102.24	95.61	59.63	85.83	22.93	13.24	26.71
DBA	1	-6	104.54	104.08	99.73	102.78	2.65	1.53	2.58
DBA	1	-5.3	104.55	102.40	102.55	103.17	1.20	0.69	1.16
DBA	1	-5	109.92	100.07	97.01	102.33	6.75	3.89	6.59
DBA	1	-4	101.35	92.91	86.25	93.50	7.57	4.37	8.09
DBA	2	-10	108.87	101.74	107.86	106.16	3.86	2.23	3.63
DBA	2	-9	101.39	105.23	103.14	103.25	1.92	1.11	1.86
DBA	2	-8	106.26	105.28	106.26	105.93	0.57	0.33	0.53
DBA	2	-7	99.28	100.97	104.35	101.53	2.58	1.49	2.54

NA = Not Applicable. Cannot divide by zero to calculate %CV.

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	-6	105.20	104.82	101.92	103.98	1.79	1.04	1.73
DBA	2	-5.3	102.46	102.00	106.87	103.78	2.69	1.55	2.59
DBA	2	-5	104.55	105.23	103.55	104.44	0.85	0.49	0.81
DBA	2	-4	94.83	98.41	95.08	96.11	2.00	1.15	2.08
DBA	3	-10	105.85	91.31	108.10	101.75	9.11	5.26	8.96
DBA	3	-9	100.18	105.53	105.26	103.66	3.01	1.74	2.91
DBA	3	-8	107.58	103.15	109.93	106.89	3.44	1.99	3.22
DBA	3	-7	103.38	96.78	93.27	97.81	5.13	2.96	5.25
DBA	3	-6	102.14	108.15	107.80	106.03	3.37	1.95	3.18
DBA	3	-5.3	105.69	105.09	106.93	105.90	0.94	0.54	0.89
DBA	3	-5	101.99	104.32	103.13	103.15	1.17	0.67	1.13
DBA	3	-4	95.67	99.55	97.68	97.63	1.94	1.12	1.99
FRM	1	-10	98.11	98.51	100.23	98.95	1.13	0.65	1.14
FRM	1	-9	98.25	104.47	103.72	102.15	3.40	1.96	3.32
FRM	1	-8	102.41	106.48	102.46	103.78	2.34	1.35	2.25
FRM	1	-7	100.58	100.65	101.70	100.98	0.63	0.36	0.62
FRM	1	-6	86.54	86.58	92.99	88.70	3.71	2.14	4.19
FRM	1	-5	37.77	39.86	38.24	38.62	1.10	0.63	2.84
FRM	1	-4	5.30	5.99	6.43	5.91	0.57	0.33	9.64
FRM	1	-3	0.60	0.95	1.21	0.92	0.31	0.18	33.27
FRM	2	-9	98.42	102.75	96.27	99.15	3.30	1.91	3.33
FRM	2	-7	101.82	102.56	97.25	100.54	2.88	1.66	2.86
FRM	2	-6	93.37	93.61	102.04	96.34	4.94	2.85	5.13
FRM	2	-5.52	96.68	100.88	90.74	96.10	5.09	2.94	5.30
FRM	2	-5	99.63	98.85	102.23	100.24	1.77	1.02	1.77
FRM	2	-4.52	90.44	87.23	95.06	90.91	3.94	2.27	4.33
FRM	2	-4	66.40	72.18	72.80	70.46	3.53	2.04	5.01
FRM	2	-3	23.86	24.17	20.40	22.81	2.09	1.21	9.18
FRM	3	-9	79.89	91.85	91.66	87.80	6.85	3.96	7.80
FRM	3	-7	92.50	96.35	95.76	94.87	2.07	1.20	2.19
FRM	3	-6	85.21	85.28	84.35	84.95	0.52	0.30	0.61
FRM	3	-5.52	67.90	66.12	66.88	66.97	0.89	0.52	1.33
FRM	3	-5	36.91	40.94	38.06	38.64	2.08	1.20	5.37
FRM	3	-4.52	24.22	19.46	17.79	20.49	3.34	1.93	16.28
FRM	3	-4	5.82	4.57	7.07	5.82	1.25	0.72	21.48
FRM	3	-3	0.15	1.02	1.05	0.74	0.51	0.30	69.08
ECZ	1	-10	98.09	97.38	100.30	98.59	1.52	0.88	1.54
ECZ	1	-9	73.62	71.77	73.42	72.94	1.02	0.59	1.39
ECZ	1	-8	16.27	15.40	15.15	15.61	0.59	0.34	3.77
ECZ	1	-7	1.68	1.78	1.66	1.71	0.06	0.04	3.77
ECZ	1	-6	0.20	0.15	0.22	0.19	0.04	0.02	18.98
ECZ	1	-5	0.02	-0.02	-0.01	0.00	0.02	0.01	-624.50
ECZ	1	-4	0.12	-0.09	-0.03	0.00	0.11	0.06	NA
ECZ	1	-3	0.05	-0.02	-0.01	0.01	0.04	0.02	567.89
ECZ	2	-10	98.17	96.72	96.14	97.01	1.05	0.60	1.08

NA = Not Applicable. Cannot divide by zero to calculate %CV.

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	2	-9.52	93.45	92.20	90.68	92.11	1.39	0.80	1.51
ECZ	2	-9	72.84	74.05	73.49	73.46	0.61	0.35	0.82
ECZ	2	-8.52	42.50	38.34	43.52	41.45	2.74	1.58	6.62
ECZ	2	-8	16.40	15.53	16.33	16.09	0.48	0.28	3.00
ECZ	2	-7	1.90	1.90	1.83	1.88	0.04	0.02	2.15
ECZ	2	-6	0.28	0.23	0.19	0.23	0.05	0.03	19.33
ECZ	2	-5	0.11	0.05	-0.02	0.05	0.07	0.04	139.42
ECZ	3	-10	102.18	97.15	101.99	100.44	2.85	1.65	2.84
ECZ	3	-9.52	95.88	96.13	95.85	95.95	0.15	0.09	0.16
ECZ	3	-9	80.39	76.16	77.24	77.93	2.20	1.27	2.82
ECZ	3	-8.52	46.03	45.49	46.66	46.06	0.59	0.34	1.27
ECZ	3	-8	15.94	17.21	17.26	16.80	0.75	0.43	4.45
ECZ	3	-7	1.97	1.91	1.85	1.91	0.06	0.03	3.14
ECZ	3	-6	0.19	0.25	0.16	0.20	0.05	0.03	22.91
ECZ	3	-5	0.13	0.02	0.04	0.06	0.06	0.03	92.52
CYN	1	-10	96.74	98.10	98.14	97.66	0.80	0.46	0.82
CYN	1	-9	99.56	99.65	101.87	100.36	1.31	0.76	1.30
CYN	1	-8	98.04	94.03	98.48	96.85	2.45	1.42	2.53
CYN	1	-7	95.46	95.30	97.69	96.15	1.34	0.77	1.39
CYN	1	-6	77.67	78.70	78.91	78.43	0.66	0.38	0.85
CYN	1	-5.3	45.63	45.14	43.40	44.72	1.17	0.68	2.62
CYN	1	-5	29.21	29.04	29.11	29.12	0.09	0.05	0.29
CYN	1	-4	16.58	14.65	11.86	14.36	2.37	1.37	16.52
CYN	2	-10	87.32	88.24	89.19	88.25	0.94	0.54	1.06
CYN	2	-8	89.37	92.66	92.92	91.65	1.98	1.14	2.16
CYN	2	-7	92.80	92.36	92.60	92.59	0.22	0.13	0.24
CYN	2	-6	75.01	75.49	75.42	75.31	0.26	0.15	0.34
CYN	2	-5.6	60.60	59.88	58.66	59.71	0.98	0.57	1.64
CYN	2	-5.3	43.97	44.19	42.25	43.47	1.06	0.61	2.44
CYN	2	-5	31.73	31.51	30.44	31.23	0.69	0.40	2.21
CYN	2	-4	17.20	9.95	16.04	14.40	3.89	2.25	27.05
CYN	3	-10	79.96	82.28	79.84	80.69	1.38	0.79	1.70
CYN	3	-8	98.95	99.47	97.21	98.54	1.18	0.68	1.20
CYN	3	-7	98.53	101.62	98.51	99.55	1.79	1.03	1.80
CYN	3	-6	80.04	83.38	83.05	82.16	1.84	1.06	2.24
CYN	3	-5.6	62.21	65.57	64.60	64.13	1.73	1.00	2.70
CYN	3	-5.3	47.96	49.45	47.61	48.34	0.98	0.56	2.02
CYN	3	-5	29.22	30.26	31.94	30.47	1.37	0.79	4.50
CYN	3	-4	21.83	13.38	16.99	17.40	4.24	2.45	24.37
DCF	1	-10	103.96	102.87	99.57	102.13	2.29	1.32	2.24
DCF	1	-9	103.28	103.40	102.69	103.12	0.38	0.22	0.37
DCF	1	-8	104.08	99.54	102.82	102.15	2.34	1.35	2.29
DCF	1	-7	95.98	98.11	95.87	96.65	1.26	0.73	1.31
DCF	1	-6	95.57	93.34	92.69	93.87	1.51	0.87	1.61
DCF	1	-5	61.64	71.02	69.57	67.41	5.05	2.92	7.49

NA = Not Applicable. Cannot divide by zero to calculate %CV.

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	-4	23.69	24.92	25.44	24.68	0.90	0.53	3.64
DCF	1	-3	2.27	2.19	3.27	2.58	0.60	0.35	23.35
DCF	2	-8	98.61	97.92	99.64	98.72	0.87	0.50	0.88
DCF	2	-6	96.65	91.70	91.50	93.28	2.92	1.68	3.13
DCF	2	-5.52	83.25	85.60	87.16	85.34	1.97	1.14	2.31
DCF	2	-5	58.71	69.90	77.50	68.70	9.45	5.46	13.76
DCF	2	-4.52	50.14	59.44	62.02	57.20	6.25	3.61	10.92
DCF	2	-4	27.01	30.78	27.55	28.45	2.04	1.18	7.17
DCF	2	-3.52	12.13	12.95	12.00	12.36	0.52	0.30	4.17
DCF	2	-3	4.12	3.21	3.06	3.46	0.57	0.33	16.56
DCF	3	-8	105.86	101.30	102.66	103.27	2.34	1.35	2.27
DCF	3	-6	98.45	98.46	94.11	97.01	2.51	1.45	2.59
DCF	3	-5.52	89.96	85.92	86.19	87.36	2.26	1.30	2.59
DCF	3	-5	71.02	70.57	75.66	72.42	2.82	1.63	3.89
DCF	3	-4.52	57.48	63.43	64.52	61.81	3.79	2.19	6.13
DCF	3	-4	35.35	27.80	28.74	30.63	4.11	2.38	13.43
DCF	3	-3.52	11.68	11.25	12.61	11.85	0.70	0.40	5.87
DCF	3	-3	4.31	3.89	3.26	3.82	0.53	0.31	13.83
ATZ	1	-10	98.78	99.33	95.98	98.03	1.80	1.04	1.83
ATZ	1	-9	98.28	102.19	102.47	100.98	2.34	1.35	2.32
ATZ	1	-8	104.18	102.45	102.21	102.95	1.07	0.62	1.04
ATZ	1	-7	99.92	99.96	102.12	100.67	1.26	0.73	1.25
ATZ	1	-6	103.06	100.14	100.04	101.08	1.72	0.99	1.70
ATZ	1	-5	103.06	101.73	99.27	101.35	1.92	1.11	1.90
ATZ	1	-4	102.91	95.49	98.95	99.12	3.71	2.14	3.75
ATZ	1	-3	79.53	83.05	86.72	83.10	3.60	2.08	4.33
ATZ	2	-10	98.02	98.01	99.35	98.46	0.77	0.45	0.78
ATZ	2	-9	100.14	98.42	98.51	99.02	0.97	0.56	0.98
ATZ	2	-8	97.21	101.59	95.18	97.99	3.28	1.89	3.34
ATZ	2	-7	99.16	98.57	102.28	100.00	1.99	1.15	1.99
ATZ	2	-6	98.53	95.64	97.85	97.34	1.51	0.87	1.55
ATZ	2	-5	99.34	97.24	99.07	98.55	1.14	0.66	1.16
ATZ	2	-4	95.09	92.30	98.22	95.20	2.96	1.71	3.11
ATZ	2	-3	89.47	90.02	86.97	88.82	1.63	0.94	1.83
ATZ	3	-10	102.37	100.34	100.67	101.13	1.09	0.63	1.08
ATZ	3	-9	100.81	103.23	103.40	102.48	1.45	0.84	1.41
ATZ	3	-8	106.70	100.17	101.00	102.62	3.55	2.05	3.46
ATZ	3	-7	102.55	98.44	105.11	102.03	3.36	1.94	3.30
ATZ	3	-6	107.81	103.60	99.40	103.60	4.21	2.43	4.06
ATZ	3	-5	104.61	105.34	102.60	104.18	1.42	0.82	1.36
ATZ	3	-4	98.20	98.83	95.19	97.41	1.95	1.12	2.00
ATZ	3	-3	87.05	87.85	86.38	87.09	0.74	0.42	0.85

NA = Not Applicable. Cannot divide by zero to calculate %CV.

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. The largest coefficient of variation occurs between replicates of assays that include prochloraz or

fenarimol as a result of the low enzyme activity achieved during the second replicate for these chemicals.

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	103.08	*	*	103.08			
Aminoglutethimide	-9	103.28	98.60	102.69	101.52	2.55	1.47	2.51
Aminoglutethimide	-8	104.52	*	*	104.52			
Aminoglutethimide	-7	103.40	102.27	102.94	102.87	0.57	0.33	0.55
Aminoglutethimide	-6	101.06	99.07	101.19	100.44	1.19	0.69	1.19
Aminoglutethimide	-5	88.27	80.87	90.31	86.48	4.96	2.87	5.74
Aminoglutethimide	-4.3	*	47.33	61.73	54.53	10.18	7.20	18.67
Aminoglutethimide	-4	42.34	33.32	45.25	40.30	6.22	3.59	15.44
Aminoglutethimide	-3.3	*	8.97	14.28	11.63	3.75	2.65	32.26
Aminoglutethimide	-3	5.68	3.73	6.20	5.20	1.30	0.75	25.04
Ketoconazole	-10	97.93	96.36	98.32	97.54	1.04	0.60	1.07
Ketoconazole	-9	99.93	99.48	99.21	99.54	0.36	0.21	0.37
Ketoconazole	-8	98.36	94.76	97.22	96.78	1.84	1.06	1.90
Ketoconazole	-7	77.91	73.33	84.03	78.42	5.37	3.10	6.85
Ketoconazole	-6	30.39	20.86	36.11	29.12	7.71	4.45	26.46
Ketoconazole	-5.3	8.27	5.26	10.44	7.99	2.60	1.50	32.57
Ketoconazole	-5	4.21	3.05	5.38	4.21	1.17	0.67	27.66
Ketoconazole	-4	0.37	0.28	0.49	0.38	0.11	0.06	27.75
Prochloraz	-10	108.84	99.93	105.59	104.79	4.51	2.60	4.30
Prochloraz	-9	105.93	103.14	104.61	104.56	1.40	0.81	1.34
Prochloraz	-8.52	*	105.12	96.71	100.92	5.95	4.21	5.89
Prochloraz	-8	78.89	102.48	79.40	86.92	13.47	7.78	15.50
Prochloraz	-7.52	*	99.13	54.83	76.98	31.32	22.15	40.69
Prochloraz	-7	23.69	97.86	24.21	48.59	42.67	24.64	87.83
Prochloraz	-6	3.42	53.07	3.65	20.05	28.60	16.51	142.65
Prochloraz	-5	0.40	11.54	0.41	4.12	6.43	3.71	156.03
Prochloraz	-4	0.22	*	*	0.22			
Prochloraz	-3	0.08	*	*	0.08			
4-nonylphenol	-10	97.65	*	*	97.65			
4-nonylphenol	-9	99.71	98.01	94.68	97.47	2.56	1.48	2.63
4-nonylphenol	-8	100.24	103.46	93.42	99.04	5.13	2.96	5.18
4-nonylphenol	-7	96.83	106.20	96.00	99.68	5.67	3.27	5.68
4-nonylphenol	-6	97.85	100.87	95.33	98.02	2.77	1.60	2.83
4-nonylphenol	-5	62.95	71.70	68.80	67.82	4.46	2.57	6.57
4-nonylphenol	-4.3	*	10.34	13.96	12.15	2.56	1.81	21.09
4-nonylphenol	-4	1.56	1.84	1.97	1.79	0.21	0.12	11.89
4-nonylphenol	-3	0.18	0.10	0.13	0.13	0.04	0.02	31.39
Dibenz[a,h]anthracene	-10	102.51	106.16	101.75	103.47	2.35	1.36	2.27
Dibenz[a,h]anthracene	-9	104.88	103.25	103.66	103.93	0.85	0.49	0.82
Dibenz[a,h]anthracene	-8	104.00	105.93	106.89	105.61	1.47	0.85	1.39

*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]anthracene	-7	85.83	101.53	97.81	95.06	8.21	4.74	8.63
Dibenz[a,h]anthracene	-6	102.78	103.98	106.03	104.26	1.64	0.95	1.57
Dibenz[a,h]anthracene	-5.3	103.17	103.78	105.90	104.28	1.44	0.83	1.38
Dibenz[a,h]anthracene	-5	102.33	104.44	103.15	103.31	1.06	0.61	1.03
Dibenz[a,h]anthracene	-4	93.50	96.11	97.63	95.75	2.09	1.21	2.18
Fenarimol	-10	98.95	*	*	98.95			
Fenarimol	-9	102.15	99.15	87.80	96.36	7.57	4.37	7.85
Fenarimol	-8	103.78	*	*	103.78			
Fenarimol	-7	100.98	100.54	94.87	98.80	3.41	1.97	3.45
Fenarimol	-6	88.70	96.34	84.95	90.00	5.81	3.35	6.45
Fenarimol	-5.52	*	96.10	66.97	81.53	20.60	14.57	25.27
Fenarimol	-5	38.62	100.24	38.64	59.17	35.57	20.54	60.12
Fenarimol	-4.52	*	90.91	20.49	55.70	49.79	35.21	89.40
Fenarimol	-4	5.91	70.46	5.82	27.40	37.29	21.53	136.13
Fenarimol	-3	0.92	22.81	0.74	8.16	12.69	7.33	155.58
Econazole	-10	98.59	97.01	100.44	98.68	1.72	0.99	1.74
Econazole	-9.52	*	92.11	95.95	94.03	2.72	1.92	2.89
Econazole	-9	72.94	73.46	77.93	74.78	2.74	1.58	3.67
Econazole	-8.52	*	41.45	46.06	43.76	3.26	2.30	7.44
Econazole	-8	15.61	16.09	16.80	16.17	0.60	0.35	3.73
Econazole	-7	1.71	1.88	1.91	1.83	0.11	0.06	5.96
Econazole	-6	0.19	0.23	0.20	0.21	0.02	0.01	10.92
Econazole	-5	0.00	0.05	0.06	0.04	0.03	0.02	89.54
Econazole	-4	0.00	*	*	0.00			
Econazole	-3	0.01	*	*	0.01			
Chrysin	-10	97.66	88.25	80.69	88.87	8.50	4.91	9.56
Chrysin	-9	100.36	*	*	100.36			
Chrysin	-8	96.85	91.65	98.54	95.68	3.59	2.07	3.75
Chrysin	-7	96.15	92.59	99.55	96.10	3.48	2.01	3.63
Chrysin	-6	78.43	75.31	82.16	78.63	3.43	1.98	4.36
Chrysin	-5.6	*	59.71	64.13	61.92	3.12	2.21	5.04
Chrysin	-5.3	44.72	43.47	48.34	45.51	2.53	1.46	5.56
Chrysin	-5	29.12	31.23	30.47	30.27	1.07	0.62	3.53
Chrysin	-4	14.36	14.40	17.40	15.39	1.74	1.01	11.33
Dicofol	-10	102.13	*	*	102.13			
Dicofol	-9	103.12	*	*	103.12			
Dicofol	-8	102.15	98.72	103.27	101.38	2.37	1.37	2.34
Dicofol	-7	96.65	*	*	96.65			
Dicofol	-6	93.87	93.28	97.01	94.72	2.00	1.16	2.11
Dicofol	-5.52	*	85.34	87.36	86.35	1.43	1.01	1.65
Dicofol	-5	67.41	68.70	72.42	69.51	2.60	1.50	3.74
Dicofol	-4.52	*	57.20	61.81	59.51	3.26	2.31	5.48
Dicofol	-4	24.68	28.45	30.63	27.92	3.01	1.74	10.77

*Empty cells result from changes in reference chemical concentration following examination of theresults 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	-3.52	*	12.36	11.85	12.10	0.36	0.26	3.00
Dicofol	-3	2.58	3.46	3.82	3.29	0.64	0.37	19.48
Atrazine	-10	98.03	98.46	101.13	99.21	1.68	0.97	1.69
Atrazine	-9	100.98	99.02	102.48	100.83	1.73	1.00	1.72
Atrazine	-8	102.95	97.99	102.62	101.19	2.77	1.60	2.74
Atrazine	-7	100.67	100.00	102.03	100.90	1.04	0.60	1.03
Atrazine	-6	101.08	97.34	103.60	100.67	3.15	1.82	3.13
Atrazine	-5	101.35	98.55	104.18	101.36	2.82	1.63	2.78
Atrazine	-4	99.12	95.20	97.41	97.24	1.96	1.13	2.02
Atrazine	-3	83.10	88.82	87.09	86.34	2.93	1.69	3.40

*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 ß (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 2.4 nM to 67.3 μM (econazole to aminoglutethimide, respectively). The inter-assay variability (%CV) of the arithmetic IC_{50} determination varied according to reference chemical from 6-35%, excluding the prochloraz and fenarimol data.

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} (μM) (± sd, sem)	Overall %CV
AMINOGLUTETHIMIDE									
1	1	-4.138	0.032	72.72	-0.8992	0.0475	Complete	67.30 (18.18, 10.49)	27.01
	2	-4.328	0.022	47.03	-0.9173	0.0429	Complete		
	3	-4.085	0.028	82.15	-0.9315	0.0504	Complete		
KETOCONAZOLE									
2	1	-6.387	0.027	0.4105	-0.9349	0.0422	Complete	0.422 (0.147, 0.085)	34.70
	2	-6.550	0.062	0.2821	-1.0425	0.1149	Complete		
	3	-6.241	0.020	0.5745	-0.9907	0.0377	Complete		
PROCHLORAZ									
3	1	-7.579	0.026	0.0263	-0.9744	0.0444	Complete	0.335 (0.532, 0.307)	158.5
	2	-6.023	0.047	0.9492	-1.2731	0.2475	Complete		
	3	-7.516	0.025	0.0305	-1.0019	0.0543	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} (µM) (± sd, sem)	Overall %CV
4-NONYLPHENOL									
4	1	-4.879	0.079	13.20	-2.0442	1.1580	Complete	15.59 (2.30, 1.33)	14.75
	2	-4.802	0.033	15.79	-1.8805	0.2139	Complete		
	3	-4.750	0.016	17.79	-1.7222	0.0766	Complete		
DIBENZ[a,h]ANTHRACENE									
5	1	NO INHIBITION							
	2								
	3								
FENARIMOL									
6	1	-5.209	0.026	6.178	-1.0523	0.0598	Complete	54.81 (82.90, 47.86)	151.25
	2	-3.822	0.080	150.5	-1.4869	0.3403	Complete		
	3	-5.113	0.039	7.718	-1.0769	0.0878	Complete		
ECONAZOLE									
7	1	-8.644	0.008	0.002268	-1.1489	0.0168	Complete	0.0024 (0.0002, 0.0001)	6.39
	2	-8.636	0.011	0.002314	-1.1875	0.0336	Complete		
	3	-8.593	0.011	0.002551	-1.2174	0.0332	Complete		
CHRYSIN									
8	1	-5.519	0.021	3.030	-1.1072	0.0500	Complete	3.41 (0.35, 0.20)	10.17
	2	-5.459	0.024	3.472	-1.1553	0.0702	Complete		
	3	-5.430	0.056	3.713	-1.4316	0.2350	Complete		
DICOFOL									
9	1	-4.565	0.050	27.22	-0.7327	0.0480	Complete	39.96 (11.35, 6.56)	28.41
	2	-4.360	0.084	43.67	-0.7303	0.0852	Complete		
	3	-4.310	0.074	49.00	-0.7148	0.0697	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-h – Statistical Average Concentration Response Curves
Figure 16. Validation Of The Recombinant Aromatase Assay (WIL-431011) Reference Chemical 1-Aminoglutethimide Response Curve Summary Table
Figure 17. Validation Of The Recombinant Aromatase Assay (WIL-431011) Reference Chemical 2-Ketoconazole Response Curve Summary Table
Figure 18. Validation Of The Recombinant Aromatase Assay (WIL-431011) Reference Chemical 3-Prochloraz Response Curve Summary Table

Figure 19. Validation Of The Recombinant Aromatase Assay (WIL-431011) Reference Chemical 4-4-Nonylphenol Response Curve Summary Table
Figure 20. Validation Of The Recombinant Aromatase Assay (WIL-431011) Reference Chemical 6-Fenarimol Response Curve Summary Table
Figure 21. Validation Of The Recombinant Aromatase Assay (WIL-431011) Reference Chemical 7-Econazole Response Curve Summary Table
Figure 22. Validation Of The Recombinant Aromatase Assay (WIL-431011) Reference Chemical 8-Chrysin Response Curve Summary Table
Figure 23. Validation Of The Recombinant Aromatase Assay (WIL-431011) Reference Chemical 9-Difocol Response Curve Summary Table

The Log IC_{50} (μ) and slope (ß) 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 Determination). Log IC_{50} and slope were also compared across replicates based on random effects analysis of variance using μ_{AVG} and $ß_{AVG}$. 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 (ß), the within-replicate standard error of ß 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 $ß_{AVG}$ was between 2.37nM and 65.3μM for all eight reference chemicals that inhibited aromatase activity (See following table). Statistical Average Concentration Response Curves for each chemical acting as an aromatase inhibitor are shown in Figures
15 a-h. Summaries of the response curve parameters, including the average across replicates for each chemical, are shown in Figures 16-23.

Reference Chemical	Top_{avg}	$Bottom_{avg}$	μ_{avg}	IC_{50} (log at μM)	$ß_{avg}$
Aminoglutethimide	102.574	-2.478	-4.185	65.31	-0.916
Ketoconazole	99.124	-0.137	-6.385	0.4121	-0.970
Prochloraz	106.245	0.329	-7.040	0.09120	-0.991
4-Nonylphenol	98.502	-0.731	-4.782	16.52	-1.874
Fenarimol	97.664	5.732	-4.717	19.19	-1.069
Econazole	101.298	0.160	-8.625	0.002371	-1.177
Chrysin	94.189	12.790	-5.481	3.304	-1.132
Dicofol	101.615	-5.999	-4.421	37.93	-0.728

The Full Enzyme Activity, Background Activity, Positive control and Negative control values among replicates were analyzed by two-way 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 Summary). In addition, estimates for the LSMean, LSMean 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 Positive controls

indicating a small, but reproducible decrease in enzyme activity over time. In all other instances (Chemical, Chemical by Portion, Replicate within Chemical and Portion by Replicate within Chemical) the differences 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 activity 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 EPA, the supposed non-inhibitor 4-nonylphenol was found to inhibit aromatase with an IC_{50} of approximately 16μM.

The Full Enzyme Activity controls demonstrated that the enzyme was active on each assay day. During one replicate the enzyme was found to have less than 5% of the expected activity, hence this data was eliminated from the discussion of the validation results. However, the data was included in the statistical analysis in order to demonstrate the response of the statistical method to questionable data. 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, and that no NADPH contamination of the Background Activity controls occurred. The Positive and Negative Controls responded as expected to the presence of 4-OH ASDN or lindane.

6.0 Conclusion

The human recombinant aromatase assay responds to known aromatase inhibitors and shows no response to non-inhibitors across a 10^{7}-fold concentration range. The assay results in IC_{50} values that correlate well to the inhibition of human placental aromatase (WIL-431009). The data generated for the validation of the human recombinant 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, including the occasional loss of enzyme activity, and 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 7: Conduct Multiple Chemical Studies With Microsomes Prepared In Participating Laboratories. (Study No. WIL-431009). WIL Research Laboratories, LLC, Ashland, OH, Draft

Bowman, C.J., Thomas-Wohlever, J.A. WA 4-17, Task 3. Validation Of The Recombinant Microsomal Aromatase Assay: Positive Control Study. (Study No. WIL 431010). WIL Research Laboratories, LLC, Ashland, OH, Draft

Final Detailed Review Paper on Aromatase, (WA 2-7 and 5-5, Task 2), Battelle Memorial Institute, Columbus, OH, 2005

Dean, R.B., Dixon, W.J. Simplified Statistics for Small Numbers of Observations. Analytical Chemistry v.23 no.4, 636-638 (1951)

Pre-Optimization Experiments for Substrate Characterization for Human Recombinant and Human Placental Microsomes (WA 2-24). Battelle Memorial Institute, Columbus,OH, 2003

Microsomal Aromatase Prevalidation Study Reports (WA 4-10, Tasks 3 through 6), Battelle Memorial Institute, Columbus, OH, 2004.

8.0 Protocol Deviations

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 5.1.2** states that the tritium added to each incubation should be about 0.1 µCi. A 10 µL aliquot of the [^{3}H]-ASDN stock solution was supposed to be diluted to 1 mL in phosphate buffer resulting in approximately 0.1 µCi of tritium being added to each reaction tube. However, on study day 2 for chemicals 7 and 8, a 20 µL aliquot of the ^{3}H]-ASDN stock solution was diluted to 1.01 mL in phosphate buffer resulting in approximately 0.2 µCi of tritium being added to each reaction tube.

- **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 1 and 2, for WTDMS count file 9 (LSC vial numbers: 0161-0192), 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 (0161-0192) 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.

- **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, WTDMS count file 34 (LSC vial numbers 631-632), the post sample sequence negative control replicate 1 was inadvertently placed into a broken vial resulting in the loss of a significant amount of the aqueous layer. As a result, only 150 µL aliquots of the aqueous layer were taken in duplicate and not the required 500 µL.

- **Protocol Section 8.** states that four types of control samples will be included for each replicate day. During the first replicate of Reference Chemicals 3 and 6 (tested concurrently), tube 1 of the Full Enzyme Activity controls showed no enzyme activity. Because the calculation of enzyme inhibition and subsequent fitting of the inhibition curve to determine an IC_{50} for the Reference Chemical being tested is dependent on the accurate identification of Full Enzyme Activity, the results of tube 1 were omitted from the calculation of the daily Full Enzyme Activity.

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

Report Submitted By:

Jennifer A. Thomas-Wohlever, PhD Research Scientist, Metabolism Study Director	Date

Report Reviewed By:

Christopher J. Bowman, PhD Staff Toxicologist, Developmental and Reproductive Toxicology	Date
Terry L. Johnson, PhD Associate Director, Metabolism	Date
Mark D. Nemec, BS, DABT Director, Developmental and Reproductive Toxicology	Date
Daniel W. Sved, PhD Director, Metabolism and Analytical Chemistry	Date
Robert A. Wally, BS, RAC Manager, Reporting and Regulatory Technical Services	Date

FIGURES 1-23

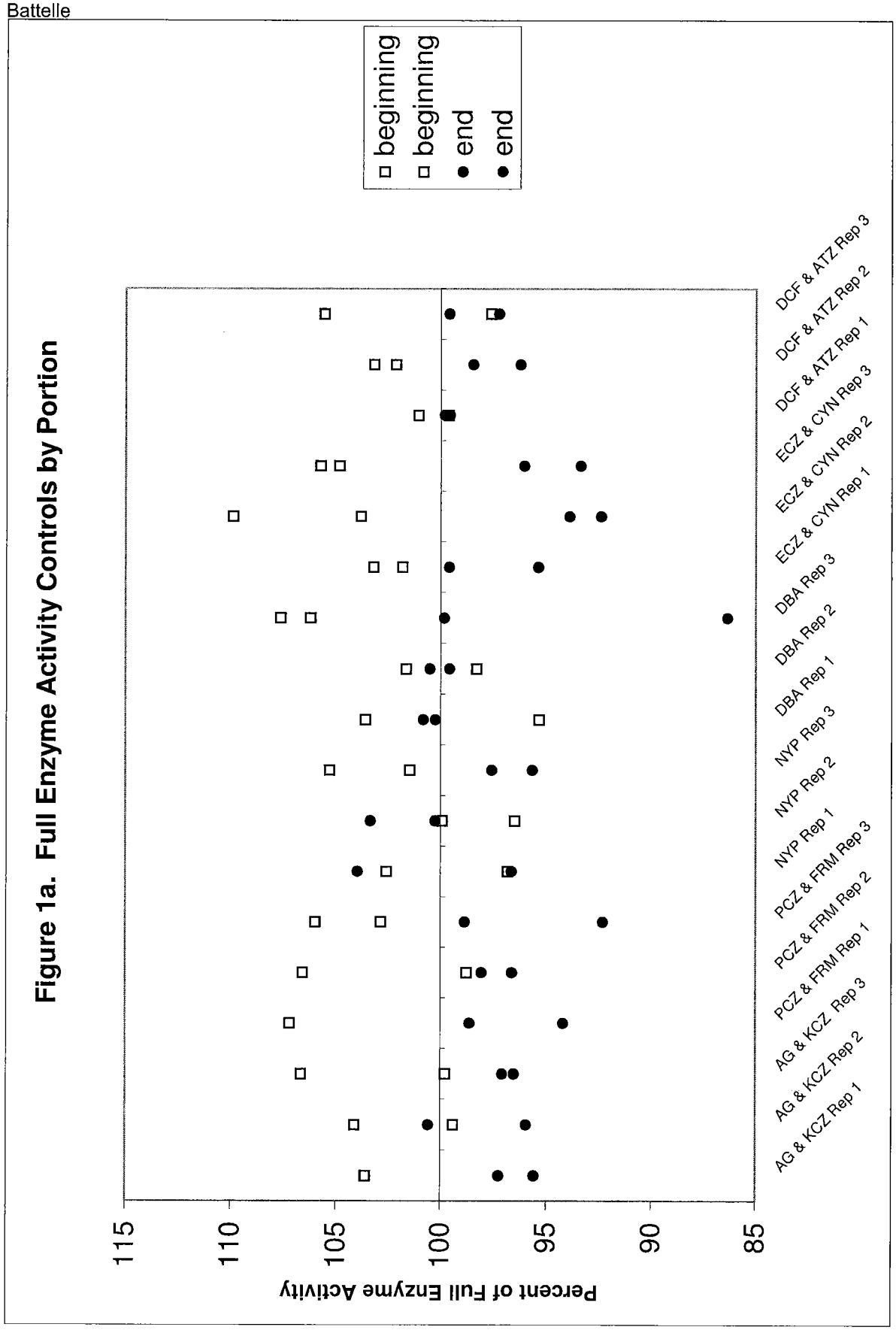


Figure 1a. Full Enzyme Activity Controls by Portion
beginning
beginning
end
end
Percent of Full Enzyme Activity
115
110
105
100
95
90
85
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

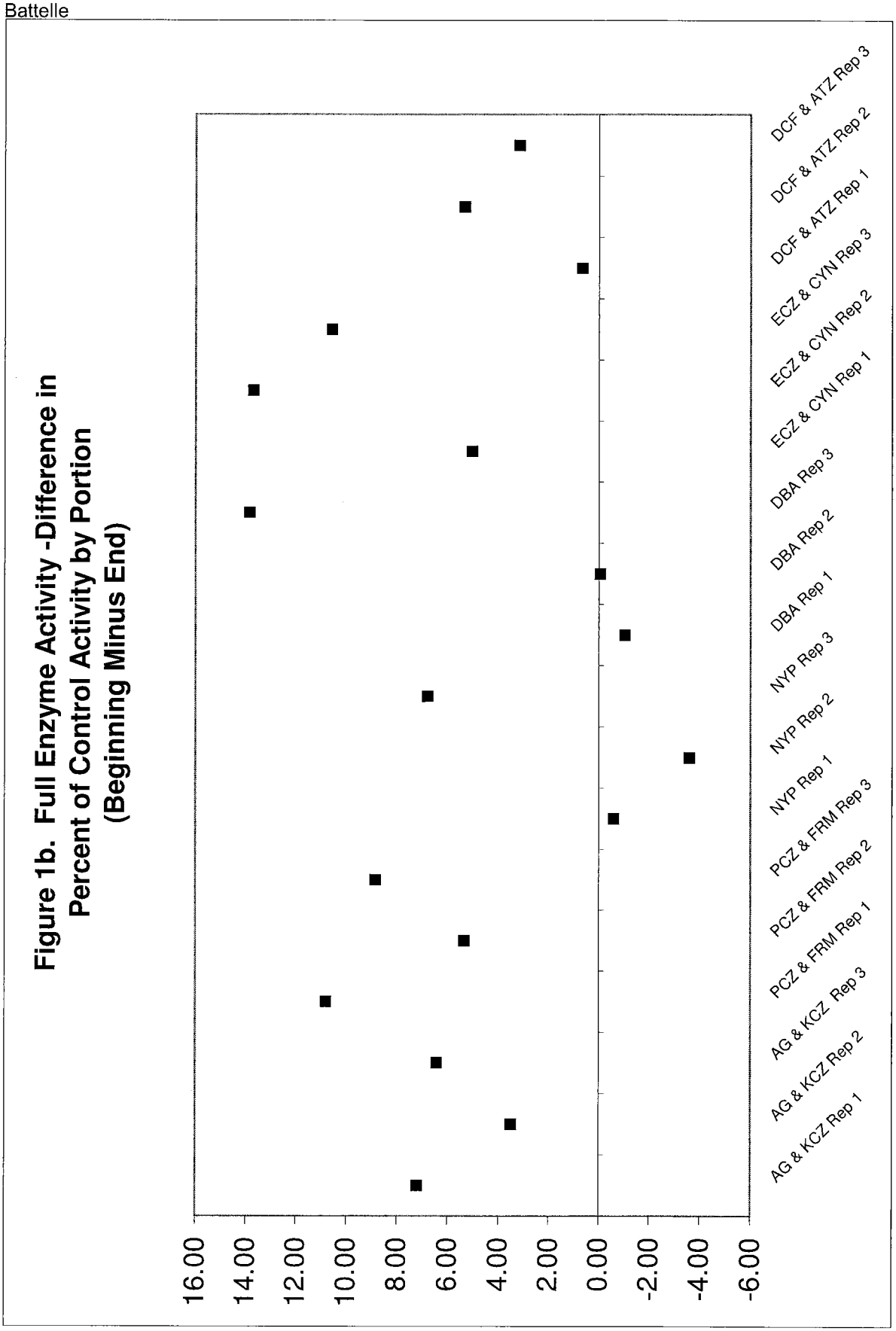


Figure 1b. Full Enzyme Activity -Difference in Percent of Control Activity by Portion (Beginning Minus End)
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP 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

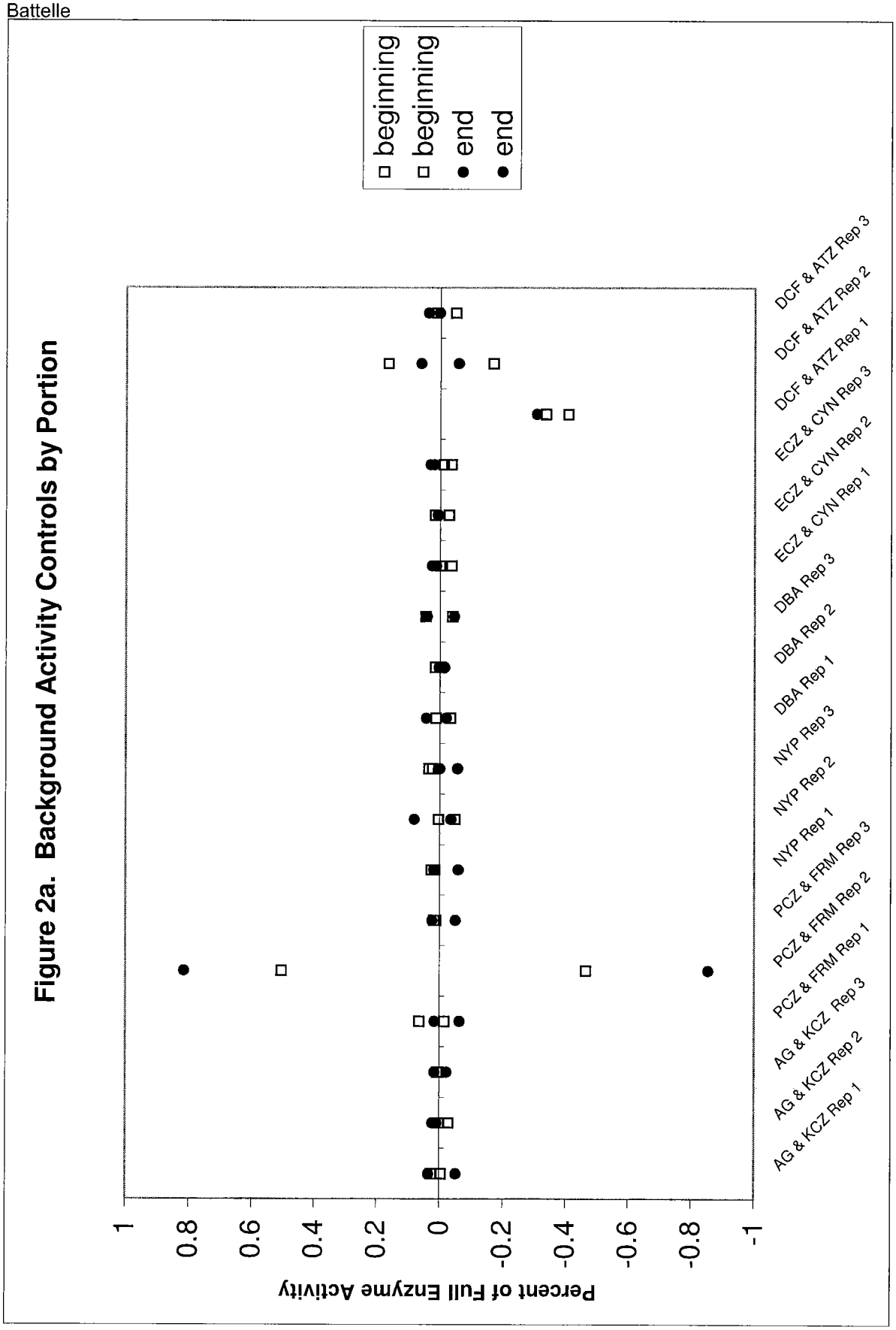


Figure 2a. Background Activity Controls by Portion
beginning
beginning
end
end
Percent of Full Enzyme Activity
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP 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

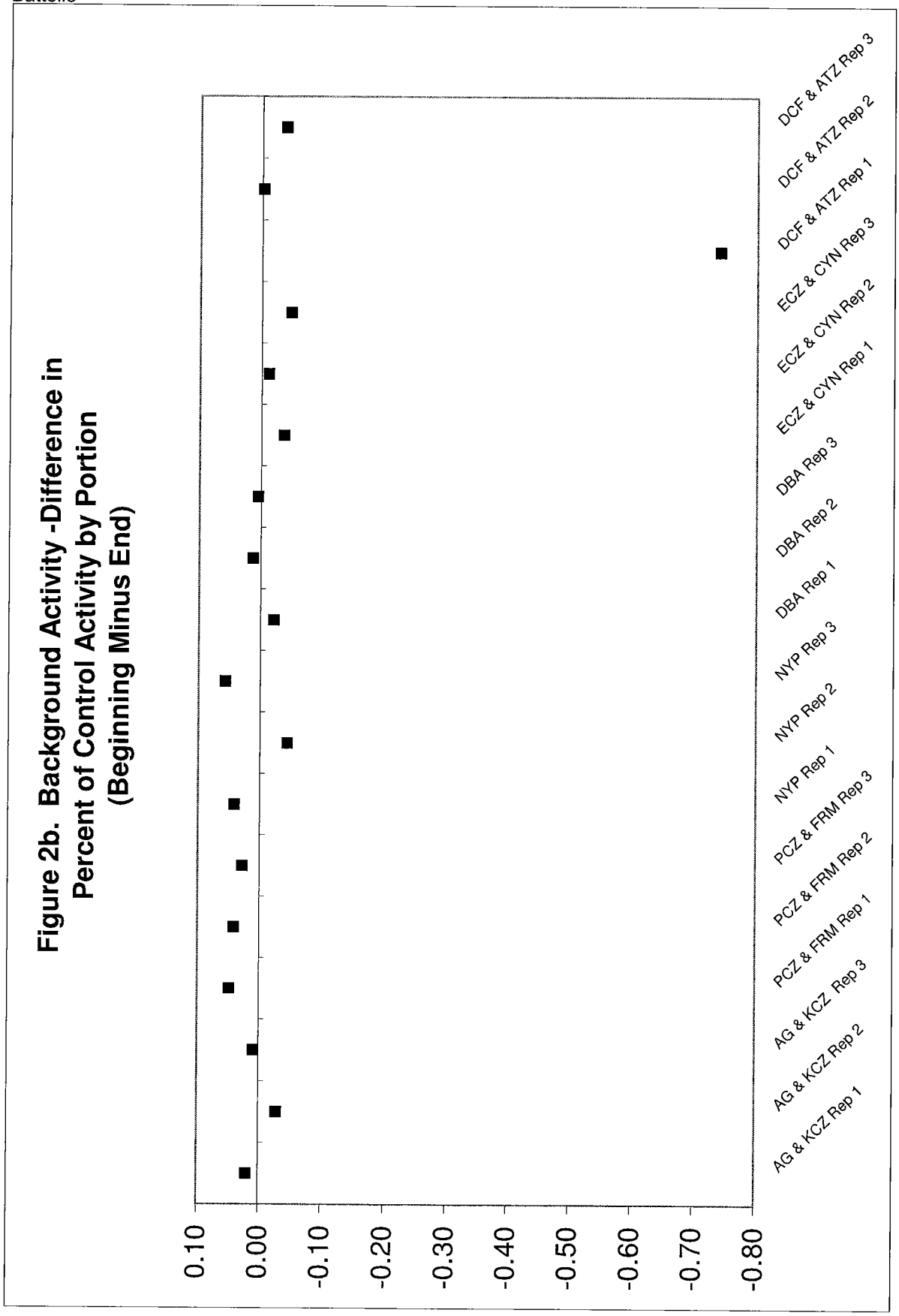


Figure 2b. Background Activity -Difference in Percent of Control Activity by Portion (Beginning Minus End)
0.10
0.00
-0.10
-0.20
-0.30
-0.40
-0.50
-0.60
-0.70
-0.80
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP 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

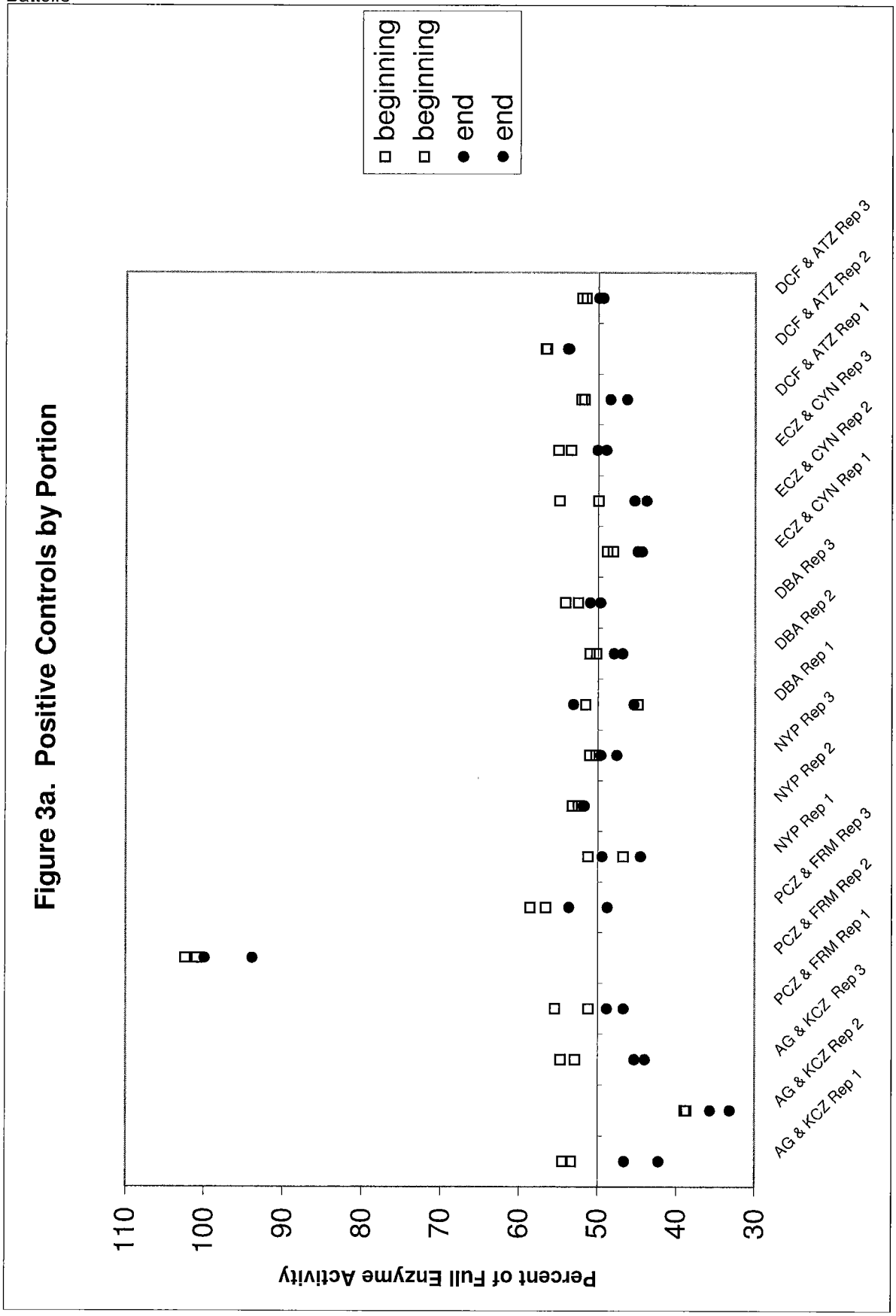


Figure 3a. Positive Controls by Portion
beginning
beginning
end
end
Percent of Full Enzyme Activity
110
100
90
80
70
60
50
40
30
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

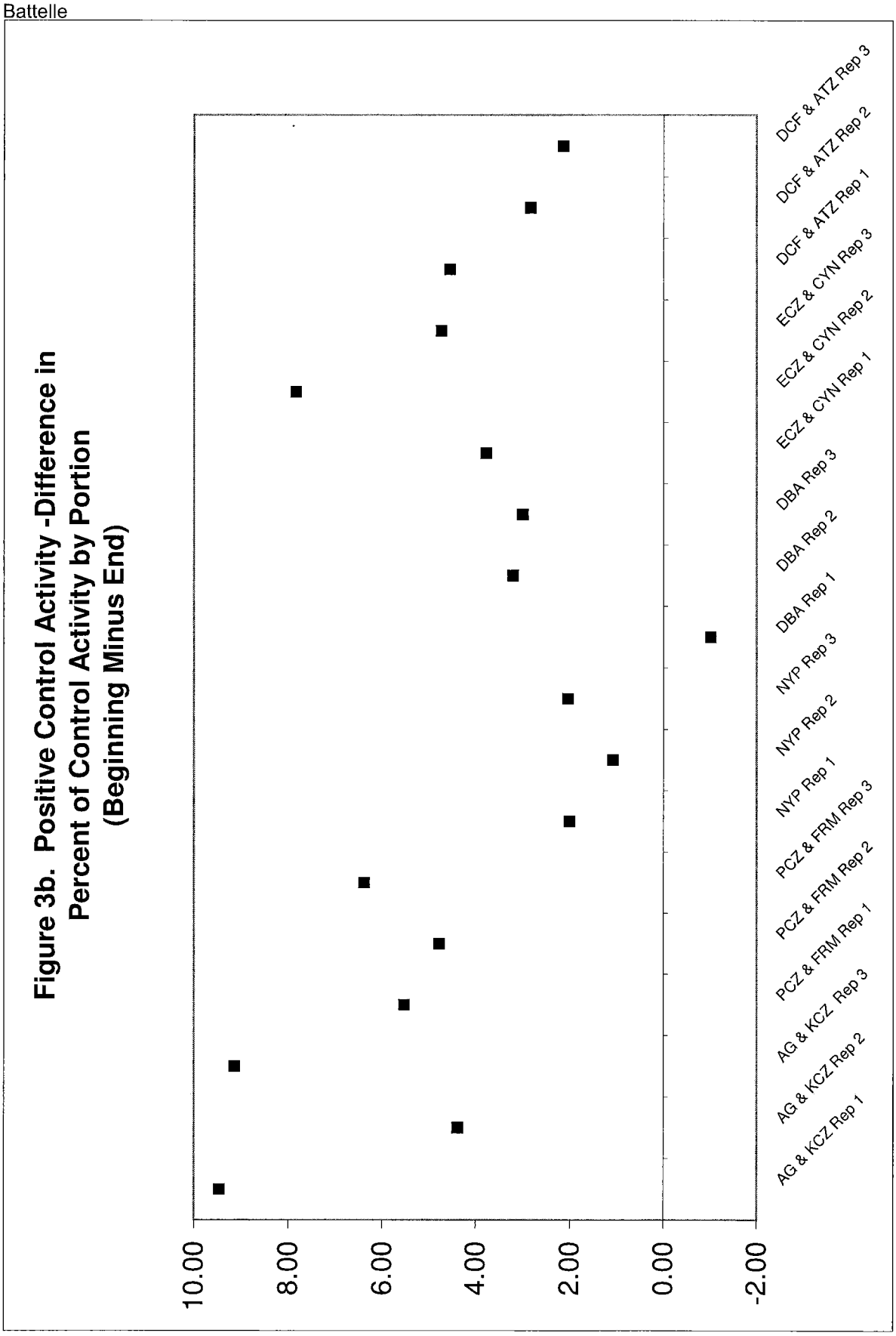


Figure 3b. Positive Control Activity -Difference in Percent of Control Activity by Portion (Beginning Minus End)
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 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

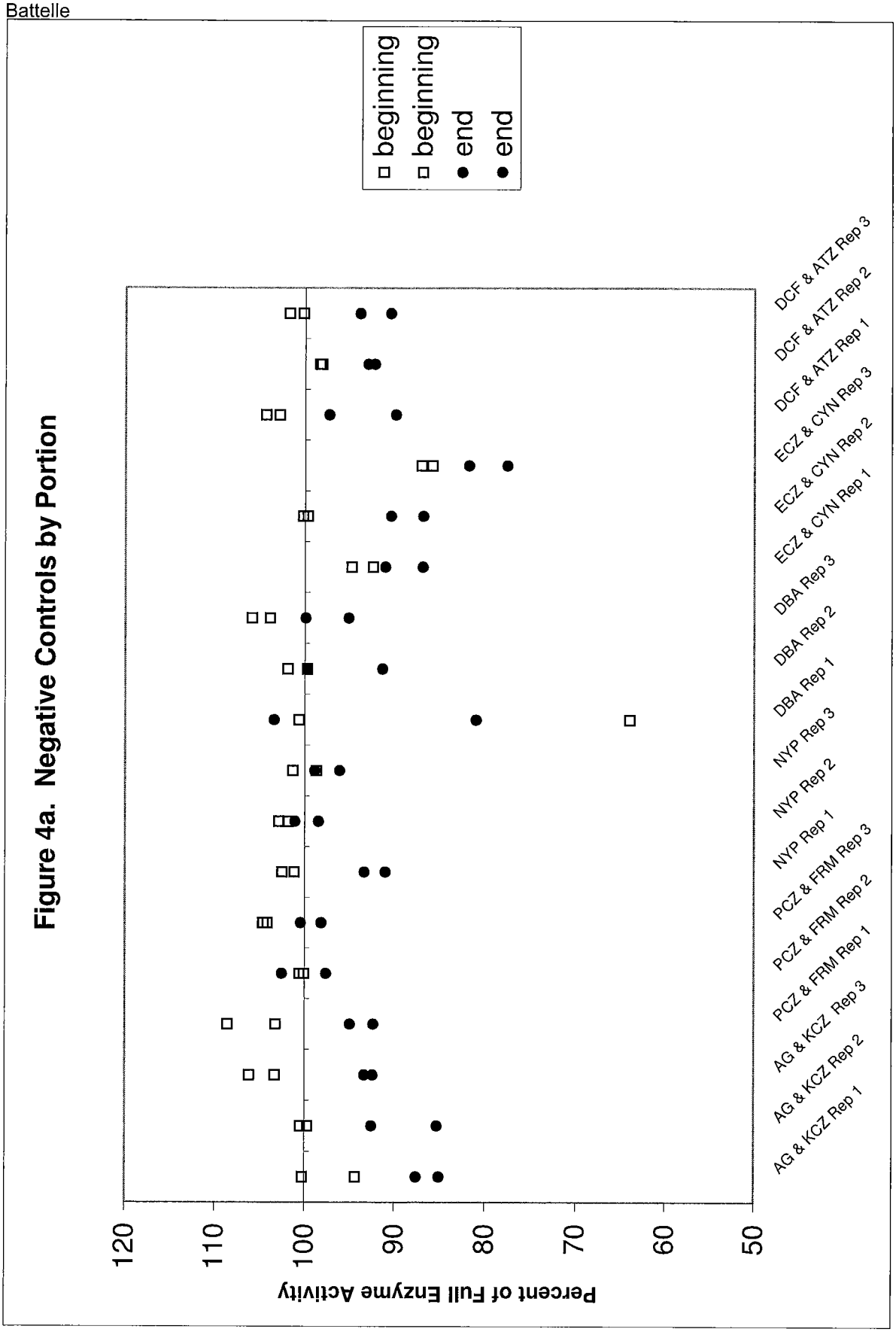


Figure 4a. Negative Controls by Portion
beginning
beginning
end
end
Percent of Full Enzyme Activity
120
110
100
90
80
70
60
50
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP Rep 1
PCZ & FRM Rep 3
PCZ & FRM Rep 2
PCZ & FRM Rep 1
AG & KCZ Rep 3
AG & KCZ Rep 2
AG & KCZ Rep 1

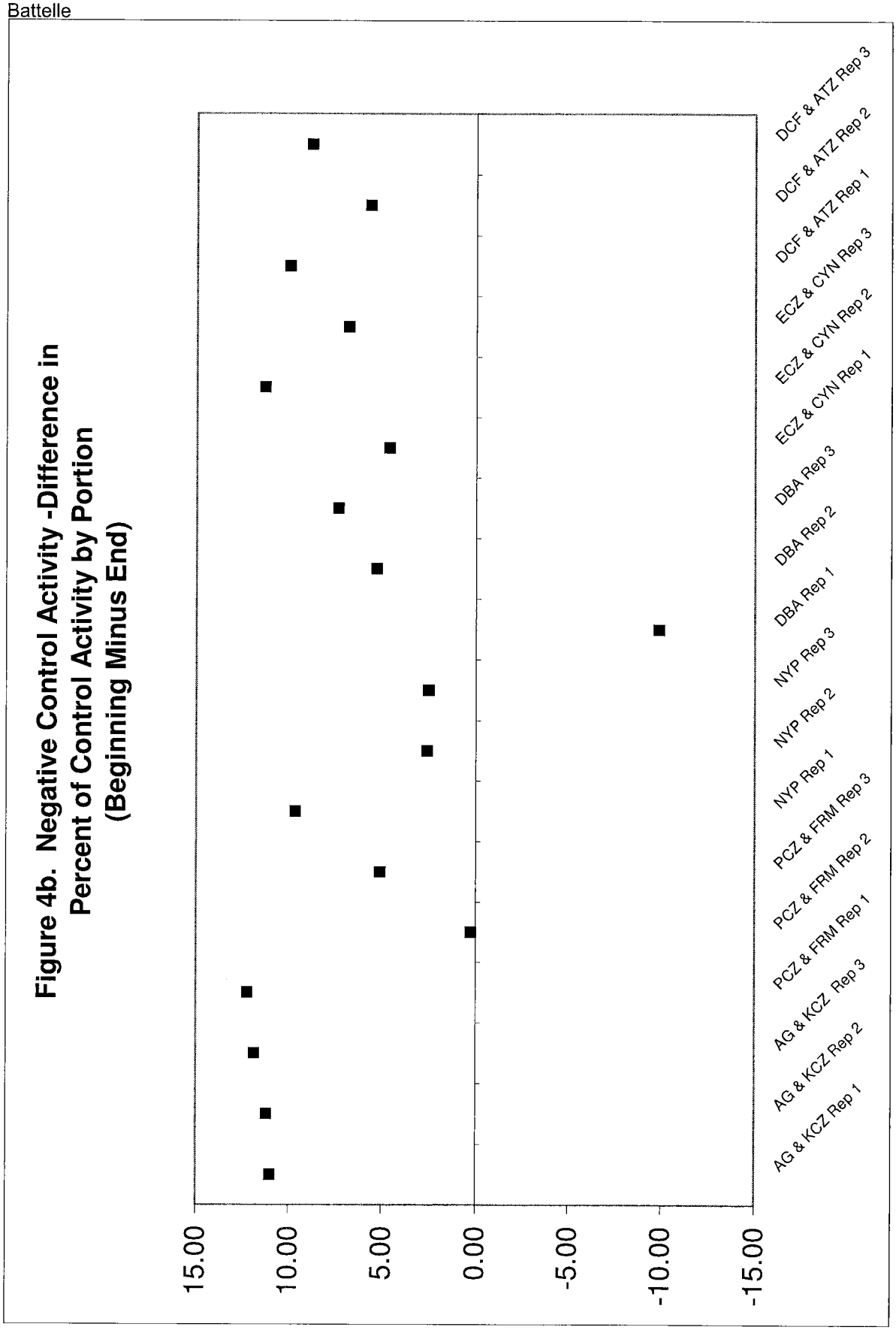
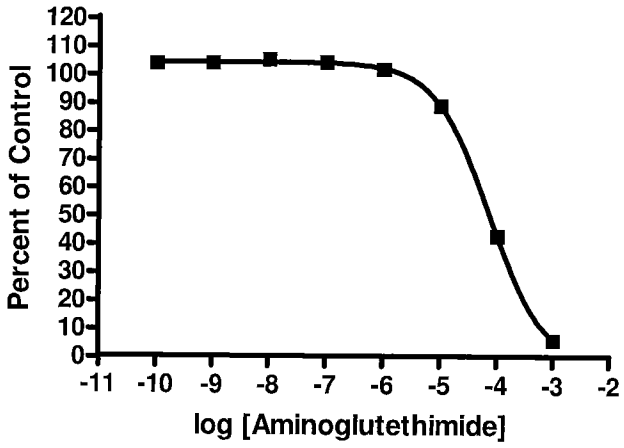


Figure 4b. Negative Control Activity -Difference in Percent of Control Activity by Portion (Beginning Minus End)
15.00
10.00
5.00
0.00
-5.00
-10.00
-15.00
DCF & ATZ Rep 3
DCF & ATZ Rep 2
DCF & ATZ Rep 1
ECZ & CYN Rep 3
ECZ & CYN Rep 2
ECZ & CYN Rep 1
DBA Rep 3
DBA Rep 2
DBA Rep 1
NYP Rep 3
NYP Rep 2
NYP 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 WA417 TK4

Figure 5a.
Aminoglutethimide Response Curve
Replicate 1

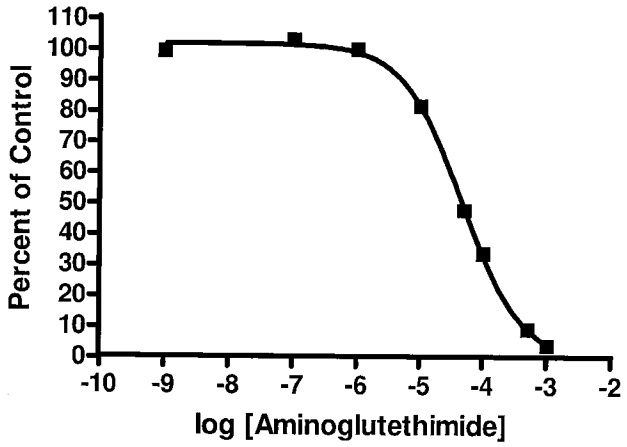


log [RC1]	Replicate 1		
	Y1	Y2	Y3
-10.00	101.52	100.83	106.88
-9.00	102.64	106.12	101.09
-8.00	104.77	105.08	103.71
-7.00	99.94	104.98	105.28
-6.00	101.36	100.82	101.00
-5.00	88.51	89.96	86.33
-4.30			
-4.00	43.25	41.47	42.31
-3.30			
-3.00	5.69	5.71	5.63

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-3.6058
TOP	103.59
LOGEC50	-4.1384
HILLSLOPE	-0.89917
EC50	7.2716e-005
Std. Error	
BOTTOM	2.2388
TOP	0.50971
LOGEC50	0.031673
HILLSLOPE	0.047451
95% Confidence Intervals	
BOTTOM	-8.2758 to 1.0643
TOP	102.53 to 104.66
LOGEC50	-4.2044 to -4.0723
HILLSLOPE	-0.99815 to -0.80018
EC50	6.2454e-005 to 8.4665e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99770
Absolute Sum of Squares	66.859
Sy.x	1.8284
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA417 TK4

Figure 5b.
Aminoglutethimide Response Curve
Replicate 2

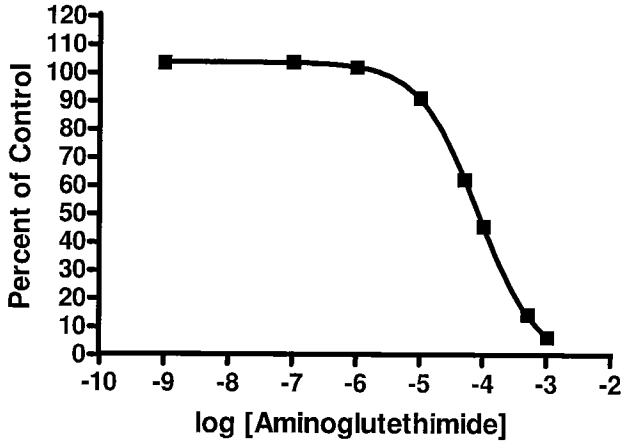


log [RC1]	Replicate 2		
	Y1	Y2	Y3
-10.00			
-9.00	99.22	97.13	99.44
-8.00			
-7.00	103.26	101.40	102.16
-6.00	99.82	95.84	101.54
-5.00	81.17	79.95	81.50
-4.30	48.68	47.38	45.94
-4.00	32.30	32.98	34.67
-3.30	9.01	8.90	9.01
-3.00	3.77	3.59	3.82

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-1.7747
TOP	101.00
LOGEC50	-4.3276
HILLSLOPE	-0.91727
EC50	4.7030e-005
Std. Error	
BOTTOM	1.5628
TOP	0.67205
LOGEC50	0.021967
HILLSLOPE	0.042896
95% Confidence Intervals	
BOTTOM	-5.0348 to 1.4854
TOP	99.594 to 102.40
LOGEC50	-4.3735 to -4.2818
HILLSLOPE	-1.0067 to -0.82779
EC50	4.2320e-005 to 5.2263e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99824
Absolute Sum of Squares	62.773
Sy.x	1.7716
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA417 TK4

Figure 5c.
Aminoglutethimide Response Curve
Replicate 3

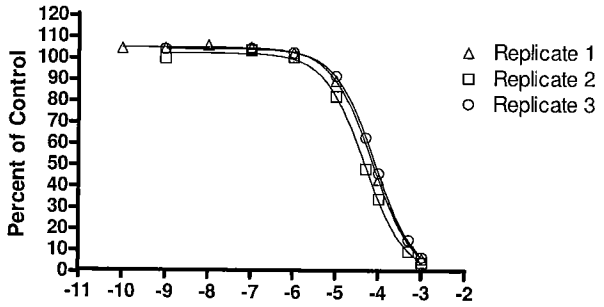


log [RC1]	Replicate 3		
	Y1	Y2	Y3
-10.00			
-9.00	103.31	101.28	103.48
-8.00			
-7.00	103.25	98.87	106.71
-6.00	102.18	99.53	101.87
-5.00	91.17	89.58	90.17
-4.30	62.08	58.51	64.60
-4.00	45.63	45.67	44.46
-3.30	14.24	14.10	14.49
-3.00	6.20	6.32	6.07

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-2.8008
TOP	102.97
LOGEC50	-4.0854
HILLSLOPE	-0.93153
EC50	8.2151e-005
Std. Error	
BOTTOM	2.2668
TOP	0.64160
LOGEC50	0.027805
HILLSLOPE	0.050420
95% Confidence Intervals	
BOTTOM	-7.5293 to 1.9277
TOP	101.64 to 104.31
LOGEC50	-4.1434 to -4.0274
HILLSLOPE	-1.0367 to -0.82635
EC50	7.1880e-005 to 9.3889e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99818
Absolute Sum of Squares	61.505
Sy.x	1.7536
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA417 TK4

Figure 5d.
Aminoglutethimide Response Curve
Replicates 1, 2, 3

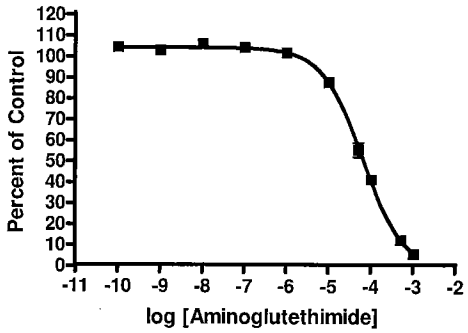


log [RC1]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	101.52	100.83	106.88						
-9.00	102.64	106.12	101.09	99.22	97.13	99.44	103.31	101.28	103.48
-8.00	104.77	105.08	103.71						
-7.00	99.94	104.98	105.28	103.26	101.40	102.16	103.25	98.87	106.71
-6.00	101.36	100.82	101.00	99.82	95.84	101.54	102.18	99.53	101.87
-5.00	88.51	89.96	86.33	81.17	79.95	81.50	91.17	89.58	90.17
-4.30				48.68	47.38	45.94	62.08	58.51	64.60
-4.00	43.25	41.47	42.31	32.30	32.98	34.67	45.63	45.67	44.46
-3.30				9.01	8.90	9.01	14.24	14.10	14.49
-3.00	5.69	5.71	5.63	3.77	3.59	3.82	6.20	6.32	6.07

Sigmoidal dose-response (variable slope)	Replicate 1	Replicate 2	Replicate 3
Best-fit values			
BOTTOM	-3.6058	-1.7747	-2.8008
TOP	103.59	101.00	102.97
LOGEC50	-4.1384	-4.3276	-4.0854
HILLSLOPE	-0.89917	-0.91727	-0.93153
EC50	7.2716e-005	4.7030e-005	8.2151e-005
Std. Error			
BOTTOM	2.2388	1.5628	2.2668
TOP	0.50971	0.67205	0.64160
LOGEC50	0.031673	0.021967	0.027805
HILLSLOPE	0.047451	0.042896	0.050420
95% Confidence Intervals			
BOTTOM	-8.2758 to 1.0643	-5.0348 to 1.4854	-7.5293 to 1.9277
TOP	102.53 to 104.66	99.594 to 102.40	101.64 to 104.31
LOGEC50	-4.2044 to -4.0723	-4.3735 to -4.2818	-4.1434 to -4.0274
HILLSLOPE	-0.99815 to -0.80018	-1.0067 to -0.82779	-1.0367 to -0.82635
EC50	6.2454e-005 to 8.4665e-005	4.2320e-005 to 5.2263e-005	7.1880e-005 to 9.3889e-005
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.99770	0.99824	0.99818
Absolute Sum of Squares	66.859	62.773	61.505
Sy.x	1.8284	1.7716	1.7536
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 WA417 TK4

Figure 5e.
Aminoglutethimide Response Curve
Average of All Replicates

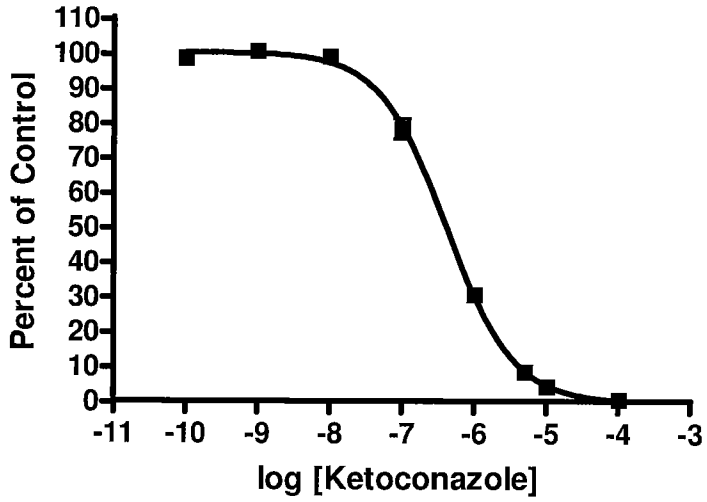


log [RC1]	All Aminoglutethimide Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	101.52	100.83	106.88						
-9.00	102.64	106.12	101.09	99.22	97.13	99.44	103.31	101.28	103.48
-8.00	104.77	105.08	103.71						
-7.00	99.94	104.98	105.28	103.26	101.40	102.16	103.25	98.87	106.71
-6.00	101.36	100.82	101.00	99.82	95.84	101.54	102.18	99.53	101.87
-5.00	88.51	89.96	86.33	81.17	79.95	81.50	91.17	89.58	90.17
-4.30				48.68	47.38	45.94	62.08	58.51	64.60
-4.00	43.25	41.47	42.31	32.30	32.98	34.67	45.63	45.67	44.46
-3.30				9.01	8.90	9.01	14.24	14.10	14.49
-3.00	5.69	5.71	5.63	3.77	3.59	3.82	6.20	6.32	6.07

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-2.926
TOP	102.8
LOGEC50	-4.191
HILLSLOPE	-0.8985
EC50	6.438e-005
Std. Error	
BOTTOM	2.462
TOP	0.7332
LOGEC50	0.03292
HILLSLOPE	0.05551
95% Confidence Intervals	
BOTTOM	-7.843 to 1.990
TOP	101.3 to 104.3
LOGEC50	-4.257 to -4.125
HILLSLOPE	-1.009 to -0.7876
EC50	5.534e-005 to 7.491e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9906
Absolute Sum of Squares	987.0
Sy.x	3.810
Data	
Number of X values	10
Number of Y replicates	9
Total number of values	72
Number of missing values	18

WIL WA417 TK4

Figure 6a.
Ketoconazole Response Curve
Replicate 1

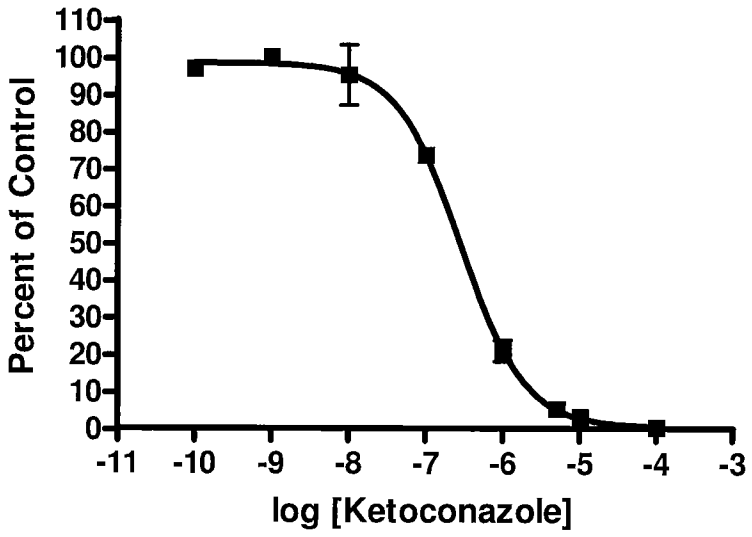


log [RC2]	Replicate 1		
	Y1	Y2	Y3
-10.00	99.04	97.79	96.97
-9.00	98.05	100.37	101.36
-8.00	95.91	99.18	100.00
-7.00	72.70	78.46	82.57
-6.00	30.48	30.19	30.49
-5.30	8.29	8.27	8.26
-5.00	4.34	3.90	4.39
-4.00	0.42	0.32	0.36

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.37625
TOP	99.711
LOGEC50	-6.3867
HILLSLOPE	-0.93491
EC50	4.1053e-007
Std. Error	
BOTTOM	1.0640
TOP	0.78445
LOGEC50	0.026968
HILLSLOPE	0.042217
95% Confidence Intervals	
BOTTOM	-2.5958 to 1.8433
TOP	98.074 to 101.35
LOGEC50	-6.4429 to -6.3304
HILLSLOPE	-1.0230 to -0.84684
EC50	3.6065e-007 to 4.6730e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99800
Absolute Sum of Squares	87.349
Sy.x	2.0898
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA417 TK4

Figure 6b.
Ketoconazole Response Curve
Replicate 2

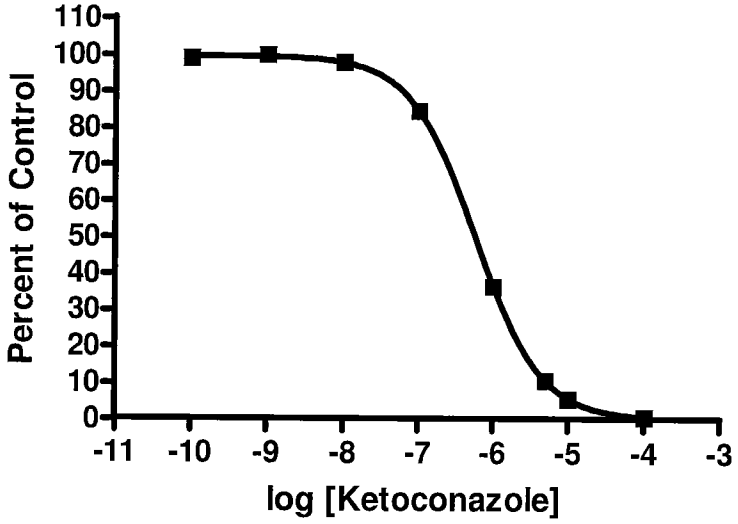


log [RC2]	Replicate 2		
	Y1	Y2	Y3
-10.00	99.52	95.20	94.36
-9.00	101.03	96.53	100.88
-8.00	99.83	78.93	105.51
-7.00	74.48	70.64	74.86
-6.00	23.32	15.27	23.98
-5.30	6.05	3.81	5.91
-5.00	3.02	3.08	3.04
-4.00	0.26	0.31	0.28

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.43900
TOP	97.949
LOGEC50	-6.5496
HILLSLOPE	-1.0425
EC50	2.8212e-007
Std. Error	
BOTTOM	2.2034
TOP	1.8464
LOGEC50	0.061938
HILLSLOPE	0.11487
95% Confidence Intervals	
BOTTOM	-4.1573 to 5.0353
TOP	94.097 to 101.80
LOGEC50	-6.6788 to -6.4204
HILLSLOPE	-1.2821 to -0.80286
EC50	2.0952e-007 to 3.7987e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.98877
Absolute Sum of Squares	499.54
Sy.x	4.9977
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA417 TK4

Figure 6c.
Ketoconazole Response Curve
Replicate 3

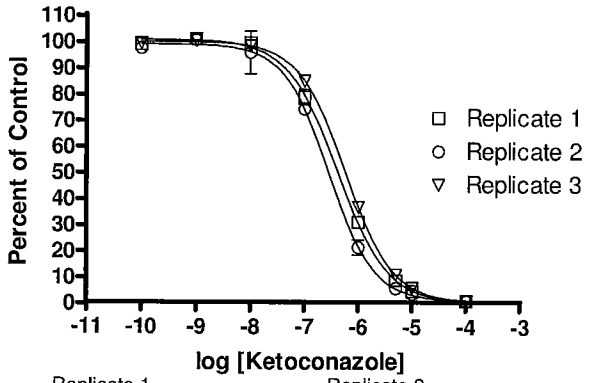


log [RC2]	Replicate 3		
	Y1	Y2	Y3
-10.00	100.50	95.36	99.11
-9.00	102.23	96.84	98.55
-8.00	93.85	97.58	100.22
-7.00	84.64	83.21	84.23
-6.00	37.23	35.58	35.52
-5.30	9.98	10.81	10.52
-5.00	5.28	5.43	5.42
-4.00	0.51	0.49	0.48

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.067544
TOP	98.899
LOGEC50	-6.2407
HILLSLOPE	-0.99074
EC50	5.7452e-007
Std. Error	
BOTTOM	0.86629
TOP	0.59855
LOGEC50	0.020223
HILLSLOPE	0.037652
95% Confidence Intervals	
BOTTOM	-1.8746 to 1.7395
TOP	97.650 to 100.15
LOGEC50	-6.2829 to -6.1985
HILLSLOPE	-1.0693 to -0.91220
EC50	5.2134e-007 to 6.3312e-007
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99872
Absolute Sum of Squares	54.888
Sy.x	1.6566
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA417 TK4

Figure 6d.
Ketoconazole Response Curve
Replicates 1, 2, 3

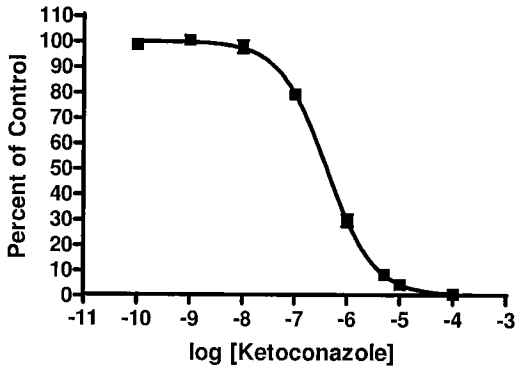


log [RC2]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	99.04	97.79	96.97	99.52	95.20	94.36	100.50	95.36	99.11
-9.00	98.05	100.37	101.36	101.03	96.53	100.88	102.23	96.84	98.55
-8.00	95.91	99.18	100.00	99.83	78.93	105.51	93.85	97.58	100.22
-7.00	72.70	78.46	82.57	74.48	70.64	74.86	84.64	83.21	84.23
-6.00	30.48	30.19	30.49	23.32	15.27	23.98	37.23	35.58	35.52
-5.30	8.29	8.27	8.26	6.05	3.81	5.91	9.98	10.81	10.52
-5.00	4.34	3.90	4.39	3.02	3.08	3.04	5.28	5.43	5.42
-4.00	0.42	0.32	0.36	0.26	0.31	0.28	0.51	0.49	0.48

Sigmoidal dose-response (variable slope)	Replicate 1	Replicate 2	Replicate 3
Best-fit values			
BOTTOM	-0.37625	0.43900	-0.067544
TOP	99.711	97.949	98.899
LOGEC50	-6.3867	-6.5496	-6.2407
HILLSLOPE	-0.93491	-1.0425	-0.99074
EC50	4.1053e-007	2.8212e-007	5.7452e-007
Std. Error			
BOTTOM	1.0640	2.2034	0.86629
TOP	0.78445	1.8464	0.59855
LOGEC50	0.026968	0.061938	0.020223
HILLSLOPE	0.042217	0.11487	0.037652
95% Confidence Intervals			
BOTTOM	-2.5958 to 1.8433	-4.1573 to 5.0353	-1.8746 to 1.7395
TOP	98.074 to 101.35	94.097 to 101.80	97.650 to 100.15
LOGEC50	-6.4429 to -6.3304	-6.6788 to -6.4204	-6.2829 to -6.1985
HILLSLOPE	-1.0230 to -0.84684	-1.2821 to -0.80286	-1.0693 to -0.91220
EC50	3.6065e-007 to 4.6730e-007	2.0952e-007 to 3.7987e-007	5.2134e-007 to 6.3312e-007
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.99800	0.98877	0.99872
Absolute Sum of Squares	87.349	499.54	54.888
Sy.x	2.0898	4.9977	1.6566
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

WIL WA417 TK4

Figure 6e.
Ketoconazole Response Curve
Average of All Replicates

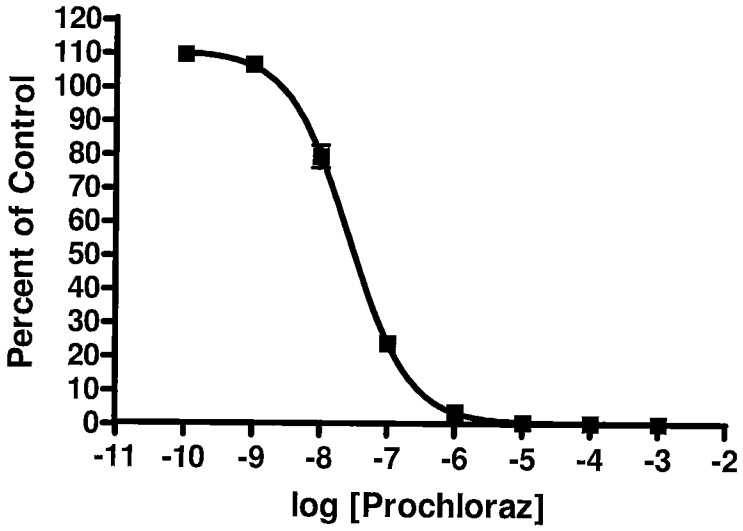


log [RC2]	All Ketoconazole Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	99.04	97.79	96.97	99.52	95.20	94.36	100.50	95.36	99.11
-9.00	98.05	100.37	101.36	101.03	96.53	100.88	102.23	96.84	98.55
-8.00	95.91	99.18	100.00	99.83	78.93	105.51	93.85	97.58	100.22
-7.00	72.70	78.46	82.57	74.48	70.64	74.86	84.64	83.21	84.23
-6.00	30.48	30.19	30.49	23.32	15.27	23.98	37.23	35.58	35.52
-5.30	8.29	8.27	8.26	6.05	3.81	5.91	9.98	10.81	10.52
-5.00	4.34	3.90	4.39	3.02	3.08	3.04	5.28	5.43	5.42
-4.00	0.42	0.32	0.36	0.26	0.31	0.28	0.51	0.49	0.48

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.01857
TOP	98.90
LOGEC50	-6.393
HILLSLOPE	-0.9692
EC50	4.050e-007
Std. Error	
BOTTOM	1.211
TOP	0.9126
LOGEC50	0.03129
HILLSLOPE	0.05186
95% Confidence Intervals	
BOTTOM	-2.438 to 2.401
TOP	97.07 to 100.7
LOGEC50	-6.455 to -6.330
HILLSLOPE	-1.073 to -0.8656
EC50	3.507e-007 to 4.677e-007
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9906
Absolute Sum of Squares	1234
Sy.x	4.259
Data	
Number of X values	8
Number of Y replicates	9
Total number of values	72
Number of missing values	0

WIL WA417 TK4

Figure 7a.
Prochloraz Response Curve
Replicate 1

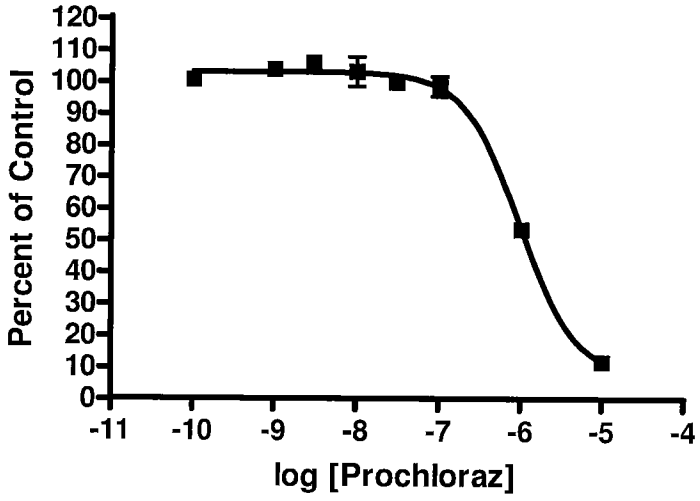


log [RC3]	Replicate 1		
	Y1	Y2	Y3
-10.00	106.47	110.31	109.74
-9.00	101.50	108.31	107.99
-8.52			
-8.00	72.72	79.92	84.03
-7.52			
-7.00	22.91	23.75	24.40
-6.00	3.22	3.41	3.63
-5.00	0.35	0.56	0.30
-4.00	0.13	0.42	0.10
-3.00	0.17	0.05	0.02

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.16937
TOP	109.74
LOGEC50	-7.5793
HILLSLOPE	-0.97444
EC50	2.6342e-008
Std. Error	
BOTTOM	0.72405
TOP	1.1530
LOGEC50	0.025997
HILLSLOPE	0.044352
95% Confidence Intervals	
BOTTOM	-1.3410 to 1.6797
TOP	107.33 to 112.14
LOGEC50	-7.6336 to -7.5251
HILLSLOPE	-1.0670 to -0.88192
EC50	2.3250e-008 to 2.9846e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99791
Absolute Sum of Squares	106.61
Sy.x	2.3088
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA417 TK4

Figure 7b.
Prochloraz Response Curve
Replicate 2

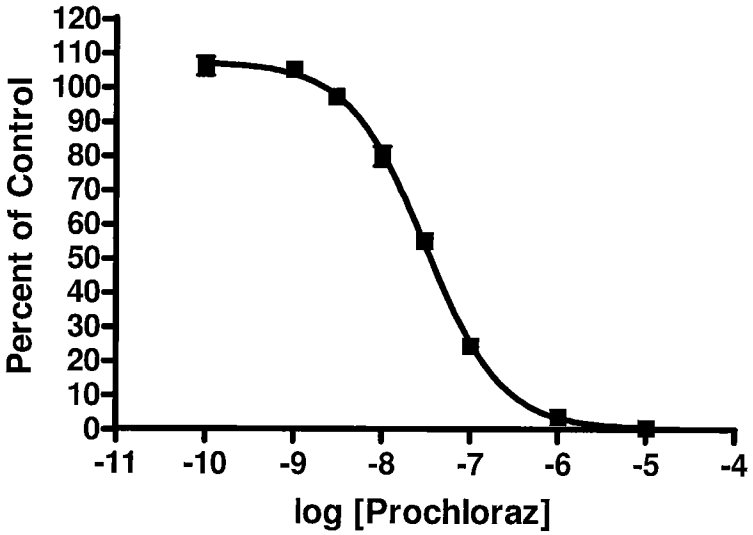


log [RC3]	Replicate 2		
	Y1	Y2	Y3
-10.00	100.74	99.04	100.01
-9.00	103.27	99.19	106.96
-8.52	107.59	100.45	107.33
-8.00	99.06	96.80	111.57
-7.52	100.68	97.31	99.40
-7.00	97.58	92.58	103.43
-6.00	53.19	54.83	51.19
-5.00	12.70	12.23	9.69

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	6.9969
TOP	102.39
LOGEC50	-6.0226
HILLSLOPE	-1.2731
EC50	9.4922e-007
Std. Error	
BOTTOM	4.1244
TOP	1.1646
LOGEC50	0.047282
HILLSLOPE	0.24748
95% Confidence Intervals	
BOTTOM	-1.6067 to 15.600
TOP	99.956 to 104.81
LOGEC50	-6.1213 to -5.9240
HILLSLOPE	-1.7894 to -0.75690
EC50	7.5638e-007 to 1.1912e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.98664
Absolute Sum of Squares	325.75
Sy.x	4.0358
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA417 TK4

Figure 7c.
Prochloraz Response Curve
Replicate 3

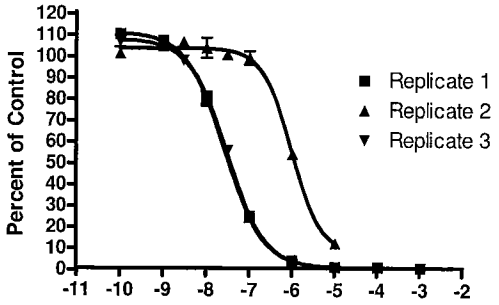


log [RC3]	Replicate 3		
	Y1	Y2	Y3
-10.00	108.73	100.24	107.79
-9.00	105.72	106.45	101.67
-8.52	96.61	98.07	95.46
-8.00	85.13	76.16	76.90
-7.52	53.30	57.29	53.90
-7.00	24.30	22.24	26.10
-6.00	3.93	3.51	3.52
-5.00	0.50	0.40	0.34

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.18878
TOP	106.63
LOGEC50	-7.5163
HILLSLOPE	-1.0019
EC50	3.0458e-008
Std. Error	
BOTTOM	1.2684
TOP	1.2670
LOGEC50	0.024676
HILLSLOPE	0.054342
95% Confidence Intervals	
BOTTOM	-2.4572 to 2.8347
TOP	103.98 to 109.27
LOGEC50	-7.5678 to -7.4648
HILLSLOPE	-1.1152 to -0.88851
EC50	2.7053e-008 to 3.4290e-008
Goodness of Fit	
Degrees of Freedom	20
R^2	0.99656
Absolute Sum of Squares	142.99
Sy.x	2.6738
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA417 TK4

Figure 7d.
Prochloraz Response Curve
Replicates 1, 2, 3

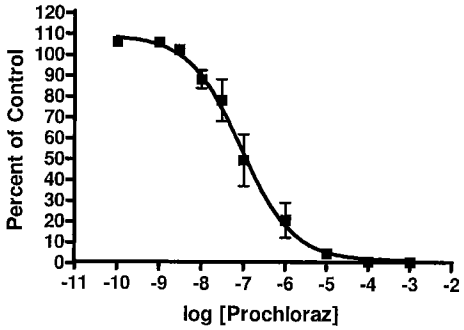


log [RC3]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	106.47	110.31	109.74	100.74	99.04	100.01	108.73	100.24	107.79
-9.00	101.50	108.31	107.99	103.27	99.19	106.96	105.72	106.45	101.67
-8.52				107.59	100.45	107.33	96.61	98.07	95.46
-8.00	72.72	79.92	84.03	99.06	96.80	111.57	85.13	76.16	76.90
-7.52				100.68	97.31	99.40	53.30	57.29	53.90
-7.00	22.91	23.75	24.40	97.58	92.58	103.43	24.30	22.24	26.10
-6.00	3.22	3.41	3.63	53.19	54.83	51.19	3.93	3.51	3.52
-5.00	0.35	0.56	0.30	12.70	12.23	9.69	0.50	0.40	0.34
-4.00	0.13	0.42	0.10						
-3.00	0.17	0.05	0.02						

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	0.16937	6.9969	0.18878
TOP	109.74	102.39	106.63
LOGEC50	-7.5793	-6.0226	-7.5163
HILLSLOPE	-0.97444	-1.2731	-1.0019
EC50	2.6342e-008	9.4922e-007	3.0458e-008
Std. Error			
BOTTOM	0.72405	4.1244	1.2684
TOP	1.1530	1.1646	1.2670
LOGEC50	0.025997	0.047282	0.024676
HILLSLOPE	0.044352	0.24748	0.054342
95% Confidence Intervals			
BOTTOM	-1.3410 to 1.6797	-1.6067 to 15.600	-2.4572 to 2.8347
TOP	107.33 to 112.14	99.956 to 104.81	103.98 to 109.27
LOGEC50	-7.6336 to -7.5251	-6.1213 to -5.9240	-7.5678 to -7.4648
HILLSLOPE	-1.0670 to -0.88192	-1.7894 to -0.75690	-1.1152 to -0.88851
EC50	2.3250e-008 to 2.9846e-008	7.5638e-007 to 1.1912e-006	2.7053e-008 to 3.4290e-008
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.99791	0.98664	0.99656
Absolute Sum of Squares	106.61	325.75	142.99
Sy.x	2.3088	4.0358	2.6738
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 WA417 TK4

Figure 7e.
Prochloraz Response Curve
Average of All Replicates

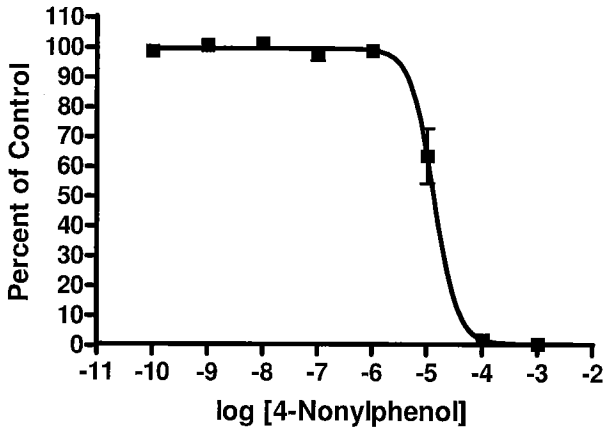


log [RC3]	All Prochloraz Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	106.47	110.31	109.74	100.74	99.04	100.01	108.73	100.24	107.79
-9.00	101.50	108.31	107.99	103.27	99.19	106.96	105.72	106.45	101.67
-8.52				107.59	100.45	107.33	96.61	98.07	95.46
-8.00	72.72	79.92	84.03	99.06	96.80	111.57	85.13	76.16	76.90
-7.52				100.68	97.31	99.40	53.30	57.29	53.90
-7.00	22.91	23.75	24.40	97.58	92.58	103.43	24.30	22.24	26.10
-6.00	3.22	3.41	3.63	53.19	54.83	51.19	3.93	3.51	3.52
-5.00	0.35	0.56	0.30	12.70	12.23	9.69	0.50	0.40	0.34
-4.00	0.13	0.42	0.10						
-3.00	0.17	0.05	0.02						

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.8584
TOP	107.8
LOGEC50	-7.058
HILLSLOPE	-0.6979
EC50	8.747e-008
Std. Error	
BOTTOM	5.713
TOP	5.321
LOGEC50	0.1443
HILLSLOPE	0.1549
95% Confidence Intervals	
BOTTOM	-10.55 to 12.27
TOP	97.13 to 118.4
LOGEC50	-7.346 to -6.770
HILLSLOPE	-1.007 to -0.3886
EC50	4.505e-008 to 1.698e-007
Goodness of Fit	
Degrees of Freedom	68
R^2	0.8486
Absolute Sum of Squares	21217
Sy.x	17.66
Data	
Number of X values	10
Number of Y replicates	9
Total number of values	72
Number of missing values	18

WIL WA417 TK4

Figure 8a.
4-Nonylphenol Response Curve
Replicate 1

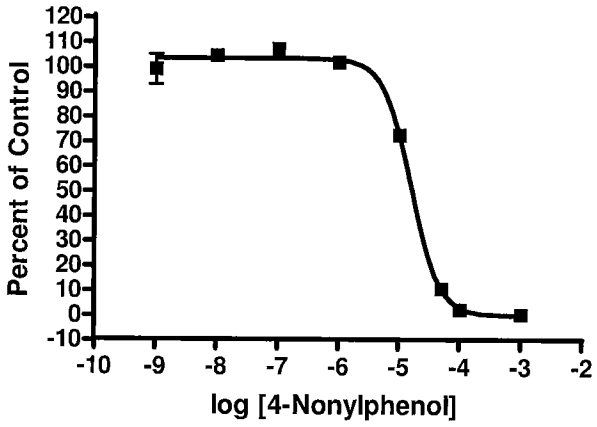


log [RC4]	Replicate 1		
	Y1	Y2	Y3
-10.00	98.35	97.93	96.66
-9.00	99.35	98.11	101.68
-8.00	99.48	100.89	100.35
-7.00	93.69	101.09	95.72
-6.00	99.35	99.15	95.06
-5.00	74.38	69.62	44.86
-4.30			
-4.00	1.51	1.50	1.66
-3.00	0.21	0.17	0.16

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.0907242
TOP	98.5598
LOGEC50	-4.87933
HILLSLOPE	-2.04423
EC50	1.32028e-005
Std. Error	
BOTTOM	3.04242
TOP	1.41918
LOGEC50	0.0791211
HILLSLOPE	1.15803
95% Confidence Intervals	
BOTTOM	-6.25577 to 6.43722
TOP	95.5994 to 101.520
LOGEC50	-5.04438 to -4.71429
HILLSLOPE	-4.45989 to 0.371419
EC50	9.02860e-006 to 1.93070e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.986124
Absolute Sum of Squares	576.514
Sy.x	5.36896
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

WIL WA417 TK4

Figure 8b.
4-Nonylphenol Response Curve
Replicate 2

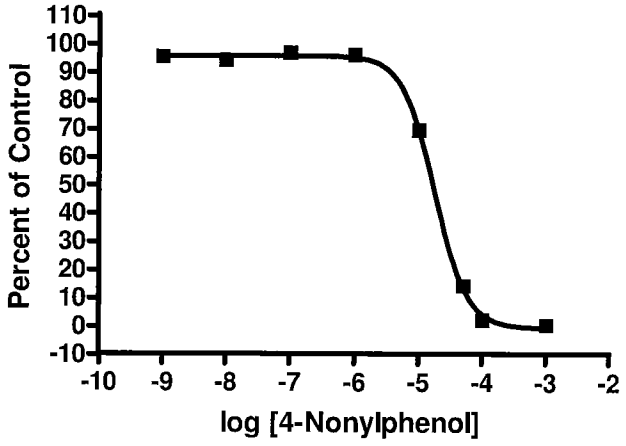


log [RC4]	Replicate 2		
	Y1	Y2	Y3
-10.00			
-9.00	104.02	104.03	85.99
-8.00	105.33	103.86	101.20
-7.00	105.54	107.32	105.75
-6.00	100.75	100.47	101.38
-5.00	72.13	73.46	69.52
-4.30	11.20	9.42	10.39
-4.00	1.78	1.78	1.96
-3.00	0.13	0.09	0.07

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.459328
TOP	102.284
LOGEC50	-4.80176
HILLSLOPE	-1.88051
EC50	1.57850e-005
Std. Error	
BOTTOM	2.14935
TOP	1.21892
LOGEC50	0.0328596
HILLSLOPE	0.213908
95% Confidence Intervals	
BOTTOM	-4.94288 to 4.02422
TOP	99.7416 to 104.827
LOGEC50	-4.87030 to -4.73321
HILLSLOPE	-2.32672 to -1.43430
EC50	1.34803e-005 to 1.84837e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.993087
Absolute Sum of Squares	347.833
Sy.x	4.17033
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

WIL WA417 TK4

Figure 8c.
4-Nonylphenol Response Curve
Replicate 3

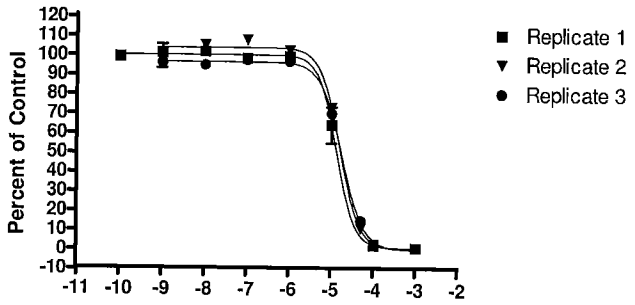


log [RC4]	Replicate 3		
	Y1	Y2	Y3
-10.00			
-9.00	95.18	94.43	94.43
-8.00	92.92	91.05	96.29
-7.00	96.71	95.60	95.69
-6.00	95.59	95.12	95.27
-5.00	65.20	71.21	70.00
-4.30	13.36	14.86	13.66
-4.00	1.99	1.97	1.96
-3.00	0.17	0.07	0.14

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.871458
TOP	94.9930
LOGEC50	-4.74976
HILLSLOPE	-1.72220
EC50	1.77925e-005
Std. Error	
BOTTOM	0.975466
TOP	0.535144
LOGEC50	0.0158023
HILLSLOPE	0.0765877
95% Confidence Intervals	
BOTTOM	-2.90628 to 1.16337
TOP	93.8767 to 96.1093
LOGEC50	-4.78273 to -4.71680
HILLSLOPE	-1.88196 to -1.56244
EC50	1.64920e-005 to 1.91955e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.998416
Absolute Sum of Squares	66.5159
Sy.x	1.82368
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

WIL WA417 TK4

Figure 8d.
4-Nonylphenol Response Curve
Replicates 1, 2, 3

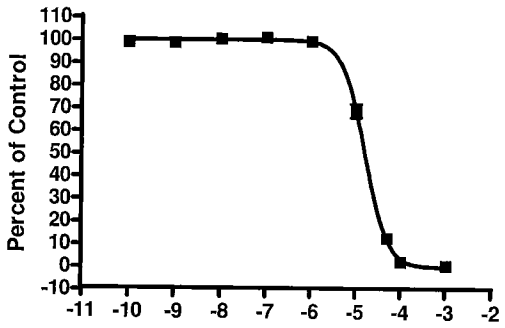


log [RC4]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	98.35	97.93	96.66						
-9.00	99.35	98.11	101.68	104.02	104.03	85.99	95.18	94.43	94.43
-8.00	99.48	100.89	100.35	105.33	103.86	101.20	92.92	91.05	96.29
-7.00	93.69	101.09	95.72	105.54	107.32	105.75	96.71	95.60	95.69
-6.00	99.35	99.15	95.06	100.75	100.47	101.38	95.59	95.12	95.27
-5.00	74.38	69.62	44.86	72.13	73.46	69.52	65.20	71.21	70.00
-4.30				11.20	9.42	10.39	13.36	14.86	13.66
-4.00	1.51	1.50	1.66	1.78	1.78	1.96	1.99	1.97	1.96
-3.00	0.21	0.17	0.16	0.13	0.09	0.07	0.17	0.07	0.14

Sigmoidal dose-response (variable slope)	Replicate 1	Replicate 2	Replicate 3
Best-fit values			
BOTTOM	0.0907242	-0.459328	-0.871458
TOP	98.5598	102.284	94.9930
LOGEC50	-4.87933	-4.80176	-4.74976
HILLSLOPE	-2.04423	-1.88051	-1.72220
EC50	1.32028e-005	1.57850e-005	1.77925e-005
Std. Error			
BOTTOM	3.04242	2.14935	0.975466
TOP	1.41918	1.21892	0.535144
LOGEC50	0.0791211	0.0328596	0.0158023
HILLSLOPE	1.15803	0.213908	0.0765877
95% Confidence Intervals			
BOTTOM	-6.25577 to 6.43722	-4.94288 to 4.02422	-2.90628 to 1.16337
TOP	95.5994 to 101.520	99.7416 to 104.827	93.8767 to 96.1093
LOGEC50	-5.04438 to -4.71429	-4.87030 to -4.73321	-4.78273 to -4.71680
HILLSLOPE	-4.45989 to 0.371419	-2.32672 to -1.43430	-1.88196 to -1.56244
EC50	9.02860e-006 to 1.93070e-005	1.34803e-005 to 1.84837e-005	1.64920e-005 to 1.91955e-005
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.986124	0.993087	0.998416
Absolute Sum of Squares	576.514	347.833	66.5159
Sy.x	5.36896	4.17033	1.82368
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 WA417 TK4

Figure 8e.
4-Nonylphenol Response Curve
Average of All Replicates

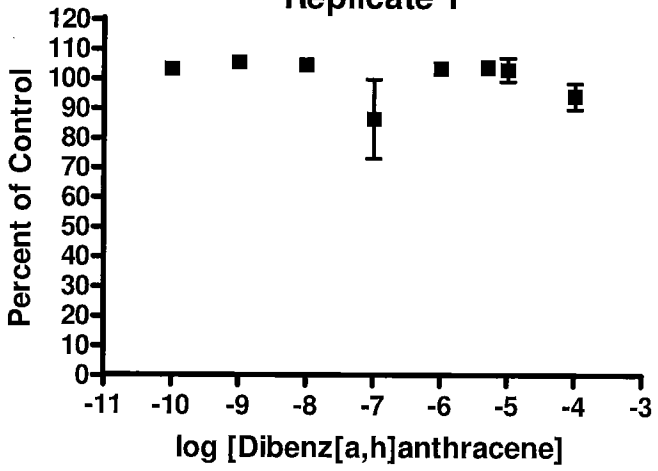


log [RC4]	All 4-Nonylphenol Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	98.35	97.93	96.66						
-9.00	99.35	98.11	101.68	104.02	104.03	85.99	95.18	94.43	94.43
-8.00	99.48	100.89	100.35	105.33	103.86	101.20	92.92	91.05	96.29
-7.00	93.69	101.09	95.72	105.54	107.32	105.75	96.71	95.60	95.69
-6.00	99.35	99.15	95.06	100.75	100.47	101.38	95.59	95.12	95.27
-5.00	74.38	69.62	44.86	72.13	73.46	69.52	65.20	71.21	70.00
-4.30				11.20	9.42	10.39	13.36	14.86	13.66
-4.00	1.51	1.50	1.66	1.78	1.78	1.96	1.99	1.97	1.96
-3.00	0.21	0.17	0.16	0.13	0.09	0.07	0.17	0.07	0.14

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.6737
TOP	98.66
LOGEC50	-4.800
HILLSLOPE	-1.744
EC50	1.585e-005
Std. Error	
BOTTOM	1.427
TOP	0.7539
LOGEC50	0.02325
HILLSLOPE	0.1381
95% Confidence Intervals	
BOTTOM	-3.523 to 2.176
TOP	97.15 to 100.2
LOGEC50	-4.846 to -4.754
HILLSLOPE	-2.020 to -1.468
EC50	1.424e-005 to 1.764e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9892
Absolute Sum of Squares	1458
Sy.x	4.630
Data	
Number of X values	9
Number of Y replicates	9
Total number of values	72
Number of missing values	9

WIL WA417 TK4

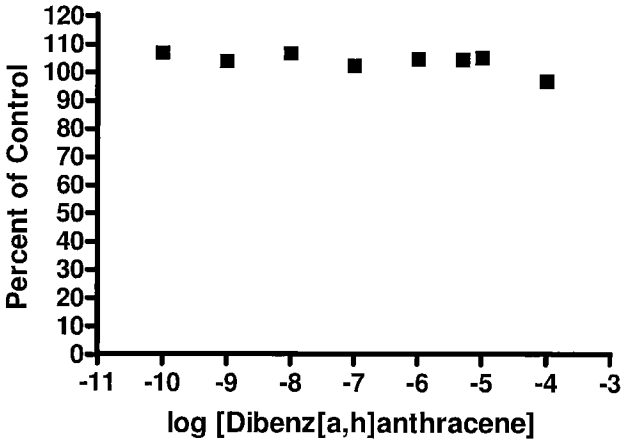
Figure 9a.
Dibenz[a,h]anthracene Response Curve
Replicate 1



log [RC5]	Replicate 1		
	Y1	Y2	Y3
-10.00	101.38	101.91	104.25
-9.00	104.29	105.43	104.93
-8.00	103.12	105.66	103.21
-7.00	102.24	95.61	59.63
-6.00	104.54	104.08	99.73
-5.30	104.55	102.40	102.55
-5.00	109.92	100.07	97.01
-4.00	101.35	92.91	86.25

WIL WA417 TK4

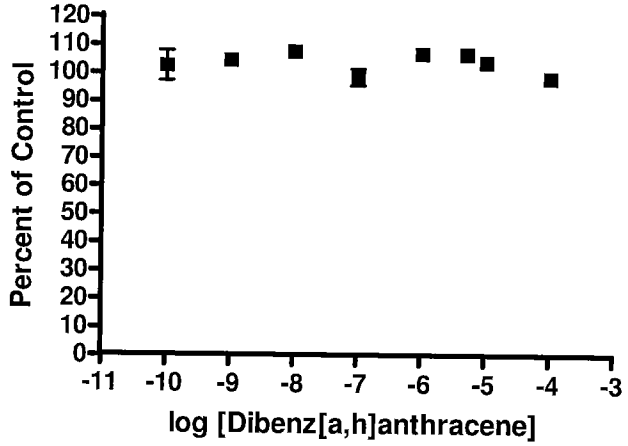
Figure 9b.
Dibenz[a,h]anthracene Response Curve
Replicate 2



log [RC5]	Replicate 2		
	Y1	Y2	Y3
-10.00	108.87	101.74	107.86
-9.00	101.39	105.23	103.14
-8.00	106.26	105.28	106.26
-7.00	99.28	100.97	104.35
-6.00	105.20	104.82	101.92
-5.30	102.46	102.00	106.87
-5.00	104.55	105.23	103.55
-4.00	94.83	98.41	95.08

WIL WA417 TK4

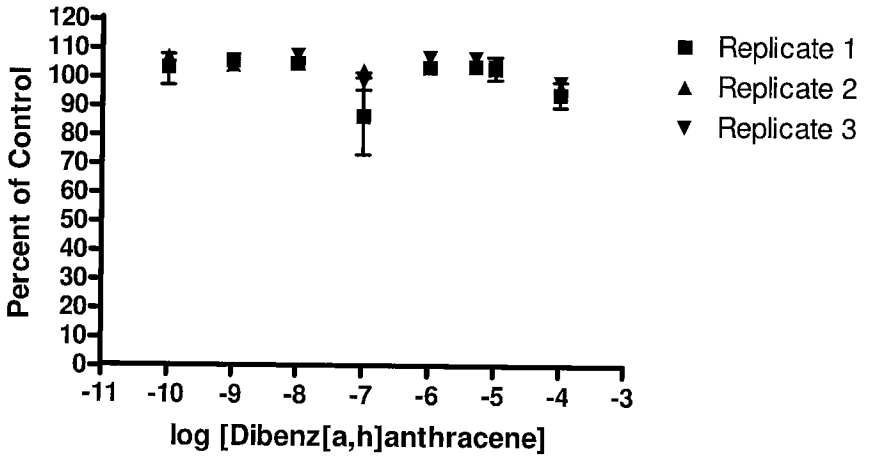
Figure 9c.
Dibenz[a,h]anthracene Response Curve
Replicate 3



log [RC5]	Replicate 3		
	Y1	Y2	Y3
-10.00	105.85	91.31	108.10
-9.00	100.18	105.53	105.26
-8.00	107.58	103.15	109.93
-7.00	103.38	96.78	93.27
-6.00	102.14	108.15	107.80
-5.30	105.69	105.09	106.93
-5.00	101.99	104.32	103.13
-4.00	95.67	99.55	97.68

WIL WA417 TK4

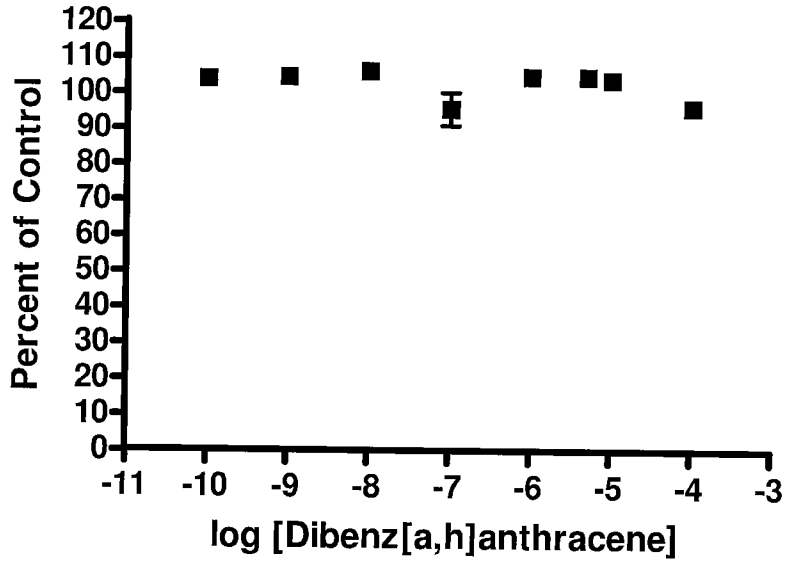
Figure 9d.
Dibenz[a,h]anthracene Response Curve
Replicates 1, 2, 3



log [RC5]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	101.38	101.91	104.25	108.87	101.74	107.86	105.85	91.31	108.10
-9.00	104.29	105.43	104.93	101.39	105.23	103.14	100.18	105.53	105.26
-8.00	103.12	105.66	103.21	106.26	105.28	106.26	107.58	103.15	109.93
-7.00	102.24	95.61	59.63	99.28	100.97	104.35	103.38	96.78	93.27
-6.00	104.54	104.08	99.73	105.20	104.82	101.92	102.14	108.15	107.80
-5.30	104.55	102.40	102.55	102.46	102.00	106.87	105.69	105.09	106.93
-5.00	109.92	100.07	97.01	104.55	105.23	103.55	101.99	104.32	103.13
-4.00	101.35	92.91	86.25	94.83	98.41	95.08	95.67	99.55	97.68

WIL WA417 TK4

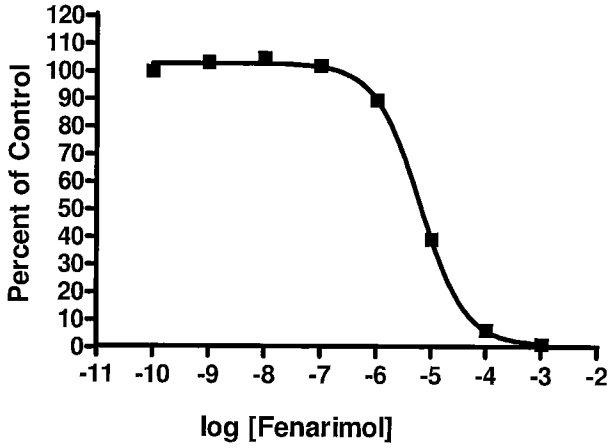
Figure 9e.
Dibenz[a,h]anthracene Response Curve
Average of All Replicates



log [RC5]	All Dibenz[a,h]anthracene Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	101.38	101.91	104.25	108.87	101.74	107.86	105.85	91.31	108.10
-9.00	104.29	105.43	104.93	101.39	105.23	103.14	100.18	105.53	105.26
-8.00	103.12	105.66	103.21	106.26	105.28	106.26	107.58	103.15	109.93
-7.00	102.24	95.61	59.63	99.28	100.97	104.35	103.38	96.78	93.27
-6.00	104.54	104.08	99.73	105.20	104.82	101.92	102.14	108.15	107.80
-5.30	104.55	102.40	102.55	102.46	102.00	106.87	105.69	105.09	106.93
-5.00	109.92	100.07	97.01	104.55	105.23	103.55	101.99	104.32	103.13
-4.00	101.35	92.91	86.25	94.83	98.41	95.08	95.67	99.55	97.68

WIL WA417 TK4

Figure 10a.
Fenarimol Response Curve
Replicate 1

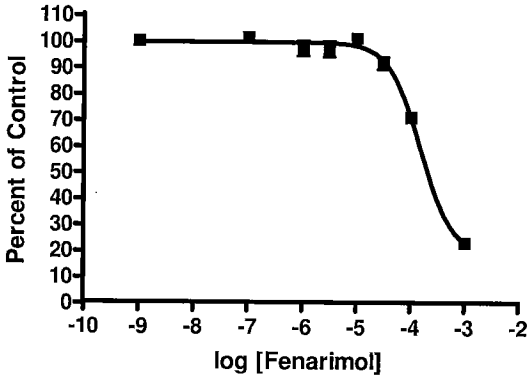


log [RC6]	Replicate 1		
	Y1	Y2	Y3
-10.00	98.11	98.51	100.23
-9.00	98.25	104.47	103.72
-8.00	102.41	106.48	102.46
-7.00	100.58	100.65	101.70
-6.00	86.54	86.58	92.99
-5.52			
-5.00	37.77	39.86	38.24
-4.52			
-4.00	5.30	5.99	6.43
-3.00	0.60	0.95	1.21

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.597763
TOP	101.796
LOGEC50	-5.20919
HILLSLOPE	-1.05236
EC50	6.17754e-006
Std. Error	
BOTTOM	1.18532
TOP	0.703549
LOGEC50	0.0256636
HILLSLOPE	0.0598007
95% Confidence Intervals	
BOTTOM	-1.87481 to 3.07034
TOP	100.329 to 103.264
LOGEC50	-5.26272 to -5.15565
HILLSLOPE	-1.17710 to -0.927613
EC50	5.46112e-006 to 6.98795e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.997482
Absolute Sum of Squares	106.962
Sy.x	2.31259
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA417 TK4

Figure 10b.
Fenarimol Response Curve
Replicate 2

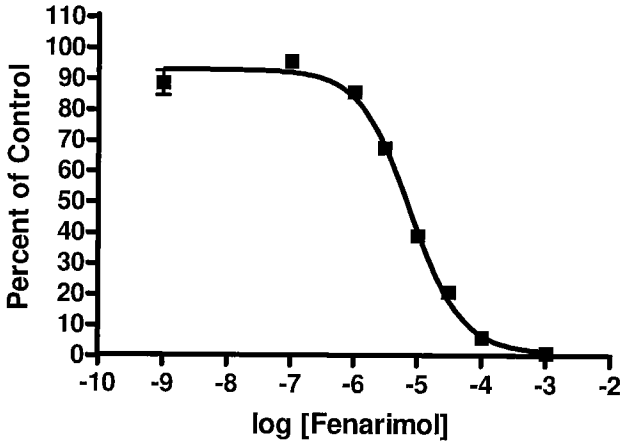


log [RC6]	Replicate 2		
	Y1	Y2	Y3
-10.00			
-9.00	98.42	102.75	96.27
-8.00			
-7.00	101.82	102.56	97.25
-6.00	93.37	93.61	102.04
-5.52	96.68	100.88	90.74
-5.00	99.63	98.85	102.23
-4.52	90.44	87.23	95.06
-4.00	66.40	72.18	72.80
-3.00	23.86	24.17	20.40

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	18.2843
TOP	98.6416
LOGEC50	-3.82238
HILLSLOPE	-1.48692
EC50	0.000150528
Std. Error	
BOTTOM	4.85046
TOP	1.08146
LOGEC50	0.0802117
HILLSLOPE	0.340332
95% Confidence Intervals	
BOTTOM	8.16622 to 28.4023
TOP	96.3857 to 100.898
LOGEC50	-3.98970 to -3.65506
HILLSLOPE	-2.19685 to -0.776987
EC50	0.000102399 to 0.000221278
Goodness of Fit	
Degrees of Freedom	20
R^2	0.981560
Absolute Sum of Squares	282.575
Sy.x	3.75882
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA417 TK4

Figure 10c.
Fenarimol Response Curve
Replicate 3

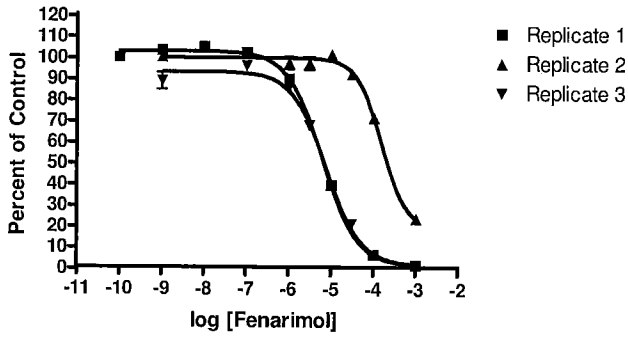


log [RC6]	Replicate 3		
	Y1	Y2	Y3
-10.00			
-9.00	79.89	91.85	91.66
-8.00			
-7.00	92.50	96.35	95.76
-6.00	85.21	85.28	84.35
-5.52	67.90	66.12	66.88
-5.00	36.91	40.94	38.06
-4.52	24.22	19.46	17.79
-4.00	5.82	4.57	7.07
-3.00	0.15	1.02	1.05

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.908680
TOP	92.1601
LOGEC50	-5.11252
HILLSLOPE	-1.07692
EC50	7.71760e-006
Std. Error	
BOTTOM	1.96145
TOP	1.54292
LOGEC50	0.0386292
HILLSLOPE	0.0878494
95% Confidence Intervals	
BOTTOM	-3.18291 to 5.00027
TOP	88.9416 to 95.3786
LOGEC50	-5.19310 to -5.03194
HILLSLOPE	-1.26017 to -0.893664
EC50	6.41064e-006 to 9.29101e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.991166
Absolute Sum of Squares	275.004
Sy.x	3.70812
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA417 TK4

Figure 10d.
Fenarimol Response Curve
Replicates 1, 2, 3

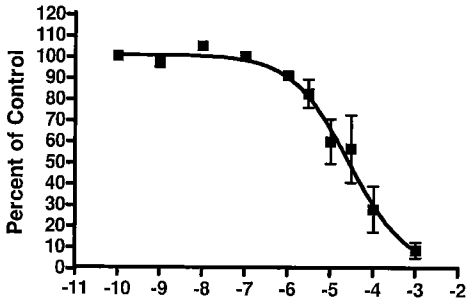


log [RC6]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	98.11	98.51	100.23						
-9.00	98.25	104.47	103.72	98.42	102.75	96.27	79.89	91.85	91.66
-8.00	102.41	106.48	102.46						
-7.00	100.58	100.65	101.70	101.82	102.56	97.25	92.50	96.35	95.76
-6.00	86.54	86.58	92.99	93.37	93.61	102.04	85.21	85.28	84.35
-5.52				96.68	100.88	90.74	67.90	66.12	66.88
-5.00	37.77	39.86	38.24	99.63	98.85	102.23	36.91	40.94	38.06
-4.52				90.44	87.23	95.06	24.22	19.46	17.79
-4.00	5.30	5.99	6.43	66.40	72.18	72.80	5.82	4.57	7.07
-3.00	0.60	0.95	1.21	23.86	24.17	20.40	0.15	1.02	1.05

Sigmoidal dose-response (variable slope)	Replicate 1	Replicate 2	Replicate 3
Best-fit values			
BOTTOM	0.597763	18.2843	0.908680
TOP	101.796	98.6416	92.1601
LOGEC50	-5.20919	-3.82238	-5.11252
HILLSLOPE	-1.05236	-1.48692	-1.07692
EC50	6.17754e-006	0.000150528	7.71760e-006
Std. Error			
BOTTOM	1.18532	4.85046	1.96145
TOP	0.703549	1.08146	1.54292
LOGEC50	0.0256636	0.0802117	0.0386292
HILLSLOPE	0.0598007	0.340332	0.0878494
95% Confidence Intervals			
BOTTOM	-1.87481 to 3.07034	8.16622 to 28.4023	-3.18291 to 5.00027
TOP	100.329 to 103.264	96.3857 to 100.898	88.9416 to 95.3786
LOGEC50	-5.26272 to -5.15565	-3.98970 to -3.65506	-5.19310 to -5.03194
HILLSLOPE	-1.17710 to -0.927613	-2.19685 to -0.776987	-1.26017 to -0.893664
EC50	5.46112e-006 to 6.98795e-006	0.000102399 to 0.000221278	6.41064e-006 to 9.29101e-006
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.997482	0.981560	0.991166
Absolute Sum of Squares	106.962	282.575	275.004
Sy.x	2.31259	3.75882	3.70812
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 WA417 TK4

Figure 10e.
Fenarimol Response Curve
Average of All Replicates

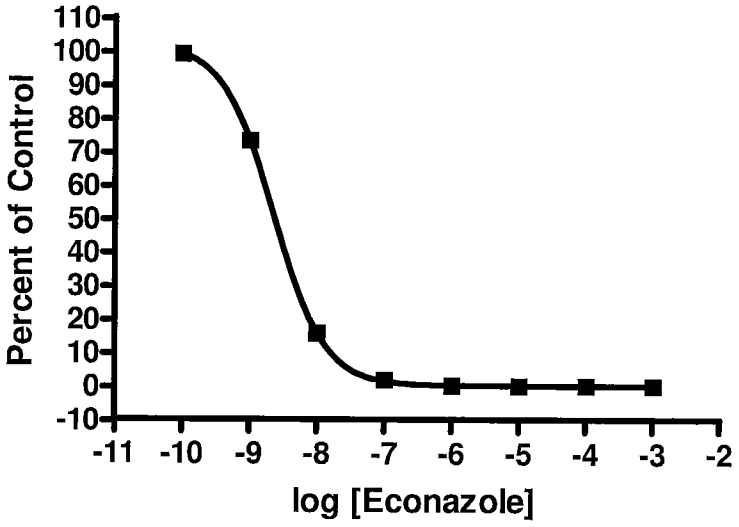


log [RC6]	All Fenarimol Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	98.11	98.51	100.23						
-9.00	98.25	104.47	103.72	98.42	102.75	96.27	79.89	91.85	91.66
-8.00	102.41	106.48	102.46						
-7.00	100.58	100.65	101.70	101.82	102.56	97.25	92.50	96.35	95.76
-6.00	86.54	86.58	92.99	93.37	93.61	102.04	85.21	85.28	84.35
-5.52				96.68	100.88	90.74	67.90	66.12	66.88
-5.00	37.77	39.86	38.24	99.63	98.85	102.23	36.91	40.94	38.06
-4.52				90.44	87.23	95.06	24.22	19.46	17.79
-4.00	5.30	5.99	6.43	66.40	72.18	72.80	5.82	4.57	7.07
-3.00	0.60	0.95	1.21	23.86	24.17	20.40	0.15	1.02	1.05

Sigmoidal dose-response (variable slope)	Average Data Fit
Best-fit values	
BOTTOM	-0.6682
TOP	99.65
LOGEC50	-4.574
HILLSLOPE	-0.6477
EC50	2.666e-005
Std. Error	
BOTTOM	13.96
TOP	4.689
LOGEC50	0.2592
HILLSLOPE	0.1999
95% Confidence Intervals	
BOTTOM	-28.56 to 27.22
TOP	90.29 to 109.0
LOGEC50	-5.092 to -4.056
HILLSLOPE	-1.047 to -0.2485
EC50	8.095e-006 to 8.782e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.7328
Absolute Sum of Squares	27591
Sy.x	20.14
Data	
Number of X values	10
Number of Y replicates	9
Total number of values	72
Number of missing values	18

WIL WA417 TK4

Figure 11a.
Econazole Response Curve
Replicate 1

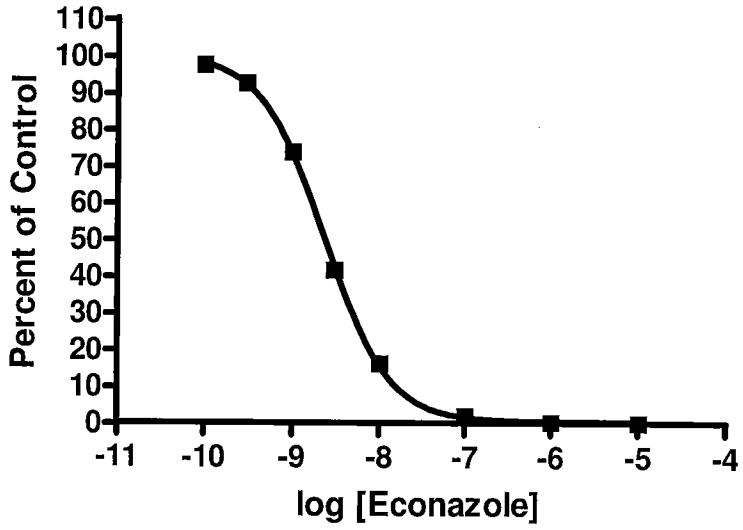


log [RC7]	Replicate 1		
	Y1	Y2	Y3
-10.00	98.09	97.38	100.30
-9.52			
-9.00	73.62	71.77	73.42
-8.52			
-8.00	16.27	15.40	15.15
-7.00	1.68	1.78	1.66
-6.00	0.20	0.15	0.22
-5.00	0.02	-0.02	-0.01
-4.00	0.12	-0.09	-0.03
-3.00	0.05	-0.02	-0.01

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.0914149
TOP	101.329
LOGEC50	-8.64426
HILLSLOPE	-1.14890
EC50	2.26849e-009
Std. Error	
BOTTOM	0.168107
TOP	0.465846
LOGEC50	0.00780145
HILLSLOPE	0.0167686
95% Confidence Intervals	
BOTTOM	-0.259257 to 0.442087
TOP	100.358 to 102.301
LOGEC50	-8.66054 to -8.62799
HILLSLOPE	-1.18388 to -1.11392
EC50	2.18506e-009 to 2.35511e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.999759
Absolute Sum of Squares	7.83843
Sy.x	0.626036
Data	
Number of X values	10
Number of Y replicates	3
Total number of values	24
Number of missing values	6

WIL WA417 TK4

Figure 11b.
Econazole Response Curve
Replicate 2

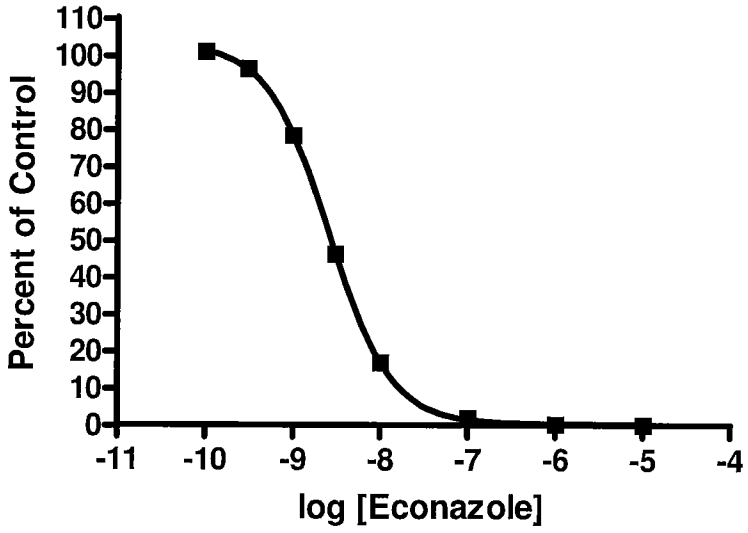


log [RC7]	Replicate 2		
	Y1	Y2	Y3
-10.00	98.17	96.72	96.14
-9.52	93.45	92.20	90.68
-9.00	72.84	74.05	73.49
-8.52	42.50	38.34	43.52
-8.00	16.40	15.53	16.33
-7.00	1.90	1.90	1.83
-6.00	0.28	0.23	0.19
-5.00	0.11	0.05	-0.02

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.422291
TOP	99.8173
LOGEC50	-8.63562
HILLSLOPE	-1.18745
EC50	2.31411e-009
Std. Error	
BOTTOM	0.420613
TOP	0.804894
LOGEC50	0.0114377
HILLSLOPE	0.0336323
95% Confidence Intervals	
BOTTOM	-0.455109 to 1.29969
TOP	98.1383 to 101.496
LOGEC50	-8.65948 to -8.61176
HILLSLOPE	-1.25761 to -1.11729
EC50	2.19041e-009 to 2.44480e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.999205
Absolute Sum of Squares	29.3168
Sy.x	1.21072
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA417 TK4

Figure 11c.
Econazole Response Curve
Replicate 3

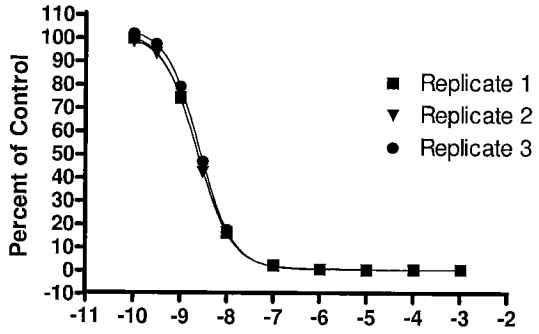


log [RC7]	Replicate 3		
	Y1	Y2	Y3
-10.00	102.18	97.15	101.99
-9.52	95.88	96.13	95.85
-9.00	80.39	76.16	77.24
-8.52	46.03	45.49	46.66
-8.00	15.94	17.21	17.26
-7.00	1.97	1.91	1.85
-6.00	0.19	0.25	0.16
-5.00	0.13	0.02	0.04

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.327527
TOP	102.692
LOGEC50	-8.59332
HILLSLOPE	-1.21736
EC50	2.55083e-009
Std. Error	
BOTTOM	0.422026
TOP	0.758403
LOGEC50	0.0106432
HILLSLOPE	0.0331876
95% Confidence Intervals	
BOTTOM	-0.552820 to 1.20787
TOP	101.110 to 104.275
LOGEC50	-8.61552 to -8.57112
HILLSLOPE	-1.28659 to -1.14813
EC50	2.42371e-009 to 2.68463e-009
Goodness of Fit	
Degrees of Freedom	20
R^2	0.999265
Absolute Sum of Squares	29.5228
Sy.x	1.21496
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

WIL WA417 TK4

Figure 11d.
Econazole Response Curve
Replicates 1, 2, 3

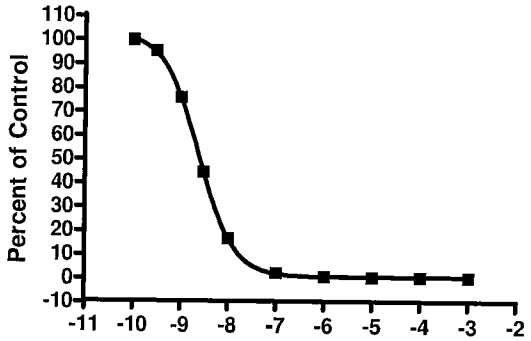


log [RC7]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	98.09	97.38	100.30	98.17	96.72	96.14	102.18	97.15	101.99
-9.52				93.45	92.20	90.68	95.88	96.13	95.85
-9.00	73.62	71.77	73.42	72.84	74.05	73.49	80.39	76.16	77.24
-8.52				42.50	38.34	43.52	46.03	45.49	46.66
-8.00	16.27	15.40	15.15	16.40	15.53	16.33	15.94	17.21	17.26
-7.00	1.68	1.78	1.66	1.90	1.90	1.83	1.97	1.91	1.85
-6.00	0.20	0.15	0.22	0.28	0.23	0.19	0.19	0.25	0.16
-5.00	0.02	-0.02	-0.01	0.11	0.05	-0.02	0.13	0.02	0.04
-4.00	0.12	-0.09	-0.03						
-3.00	0.05	-0.02	-0.01						

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	0.0914149	0.422291	0.327527
TOP	101.329	99.8173	102.692
LOGEC50	-8.64426	-8.63562	-8.59332
HILLSLOPE	-1.14890	-1.18745	-1.21736
EC50	2.26849e-009	2.31411e-009	2.55083e-009
Std. Error			
BOTTOM	0.168107	0.420613	0.422026
TOP	0.465846	0.804894	0.758403
LOGEC50	0.00780145	0.0114377	0.0106432
HILLSLOPE	0.0167686	0.0336323	0.0331876
95% Confidence Intervals			
BOTTOM	-0.259257 to 0.442087	-0.455109 to 1.29969	-0.552820 to 1.20787
TOP	100.358 to 102.301	98.1383 to 101.496	101.110 to 104.275
LOGEC50	-8.66054 to -8.62799	-8.65948 to -8.61176	-8.61552 to -8.57112
HILLSLOPE	-1.18388 to -1.11392	-1.25761 to -1.11729	-1.28659 to -1.14813
EC50	2.18506e-009 to 2.35511e-009	2.19041e-009 to 2.44480e-009	2.42371e-009 to 2.68463e-009
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.999759	0.999205	0.999265
Absolute Sum of Squares	7.83843	29.3168	29.5228
Sy.x	0.626036	1.21072	1.21496
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 WA417 TK4

Figure 11e.
Econazole Response Curve
Average of All Replicates

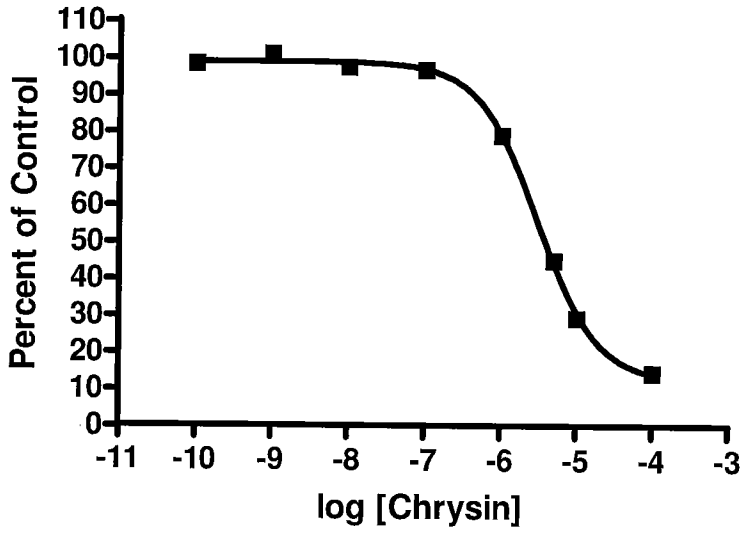


log [RC7]	All Econazole Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	98.09	97.38	100.30	98.17	96.72	96.14	102.18	97.15	101.99
-9.52				93.45	92.20	90.68	95.88	96.13	95.85
-9.00	73.62	71.77	73.42	72.84	74.05	73.49	80.39	76.16	77.24
-8.52				42.50	38.34	43.52	46.03	45.49	46.66
-8.00	16.27	15.40	15.15	16.40	15.53	16.33	15.94	17.21	17.26
-7.00	1.68	1.78	1.66	1.90	1.90	1.83	1.97	1.91	1.85
-6.00	0.20	0.15	0.22	0.28	0.23	0.19	0.19	0.25	0.16
-5.00	0.02	-0.02	-0.01	0.11	0.05	-0.02	0.13	0.02	0.04
-4.00	0.12	-0.09	-0.03						
-3.00	0.05	-0.02	-0.01						

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.2219
TOP	101.3
LOGEC50	-8.620
HILLSLOPE	-1.182
EC50	2.401e-009
Std. Error	
BOTTOM	0.2972
TOP	0.6372
LOGEC50	0.009363
HILLSLOPE	0.02553
95% Confidence Intervals	
BOTTOM	-0.3718 to 0.8156
TOP	100.1 to 102.6
LOGEC50	-8.638 to -8.601
HILLSLOPE	-1.233 to -1.131
EC50	2.300e-009 to 2.507e-009
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9984
Absolute Sum of Squares	183.5
Sy.x	1.643
Data	
Number of X values	10
Number of Y replicates	9
Total number of values	72
Number of missing values	18

WIL WA417 TK4

Figure 12a.
Chrysin Response Curve
Replicate 1

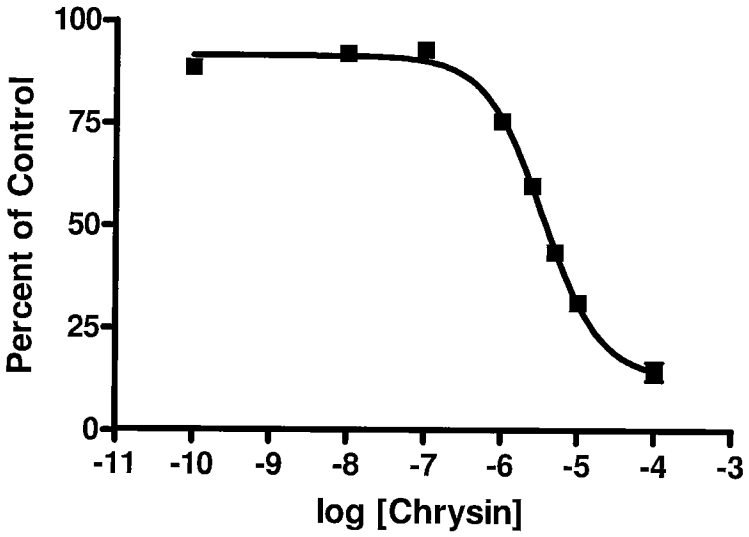


log [RC8]	Replicate 1		
	Y1	Y2	Y3
-10.00	96.74	98.10	98.14
-9.00	99.56	99.65	101.87
-8.00	98.04	94.03	98.48
-7.00	95.46	95.30	97.69
-6.00	77.67	78.70	78.91
-5.60			
-5.30	45.63	45.14	43.40
-5.00	29.21	29.04	29.11
-4.00	16.58	14.65	11.86

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	12.2648
TOP	98.2546
LOGEC50	-5.51860
HILLSLOPE	-1.10717
EC50	3.02971e-006
Std. Error	
BOTTOM	1.24102
TOP	0.549135
LOGEC50	0.0212645
HILLSLOPE	0.0500451
95% Confidence Intervals	
BOTTOM	9.67602 to 14.8535
TOP	97.1091 to 99.4001
LOGEC50	-5.56296 to -5.47424
HILLSLOPE	-1.21156 to -1.00278
EC50	2.73554e-006 to 3.35551e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.997523
Absolute Sum of Squares	63.7474
Sy.x	1.78532
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

WIL WA417 TK4

Figure 12b.
Chrysin Response Curve
Replicate 2

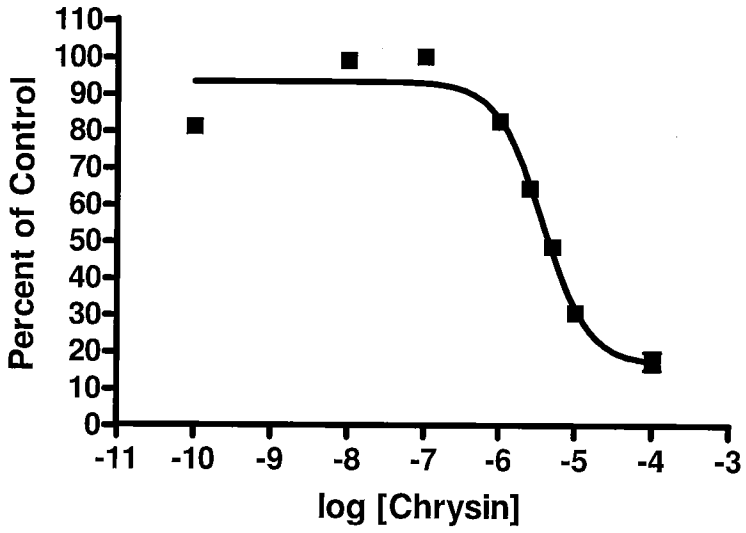


log [RC8]	Replicate 2		
	Y1	Y2	Y3
-10.00	87.32	88.24	89.19
-9.00			
-8.00	89.37	92.66	92.92
-7.00	92.80	92.36	92.60
-6.00	75.01	75.49	75.42
-5.60	60.60	59.88	58.66
-5.30	43.97	44.19	42.25
-5.00	31.73	31.51	30.44
-4.00	17.20	9.95	16.04

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	12.9885
TOP	91.1130
LOGEC50	-5.45936
HILLSLOPE	-1.15530
EC50	3.47245e-006
Std. Error	
BOTTOM	1.52542
TOP	0.780122
LOGEC50	0.0242470
HILLSLOPE	0.0702070
95% Confidence Intervals	
BOTTOM	9.80649 to 16.1706
TOP	89.4857 to 92.7404
LOGEC50	-5.50994 to -5.40878
HILLSLOPE	-1.30175 to -1.00885
EC50	3.09070e-006 to 3.90136e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.994803
Absolute Sum of Squares	97.5658
Sy.x	2.20869
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

WIL WA417 TK4

Figure 12c.
Chrysin Response Curve
Replicate 3

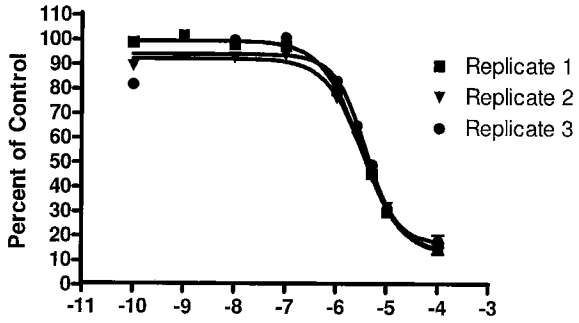


log [RC8]	Replicate 3		
	Y1	Y2	Y3
-10.00	79.96	82.28	79.84
-9.00			
-8.00	98.95	99.47	97.21
-7.00	98.53	101.62	98.51
-6.00	80.04	83.38	83.05
-5.60	62.21	65.57	64.60
-5.30	47.96	49.45	47.61
-5.00	29.22	30.26	31.94
-4.00	21.83	13.38	16.99

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	16.5720
TOP	92.8486
LOGEC50	-5.43022
HILLSLOPE	-1.43155
EC50	3.71344e-006
Std. Error	
BOTTOM	3.85083
TOP	2.09768
LOGEC50	0.0562916
HILLSLOPE	0.234999
95% Confidence Intervals	
BOTTOM	8.53913 to 24.6048
TOP	88.4728 to 97.2243
LOGEC50	-5.54765 to -5.31280
HILLSLOPE	-1.92176 to -0.941344
EC50	2.83369e-006 to 4.86632e-006
Goodness of Fit	
Degrees of Freedom	20
R^2	0.960889
Absolute Sum of Squares	776.530
Sy.x	6.23109
Data	
Number of X values	9
Number of Y replicates	3
Total number of values	24
Number of missing values	3

WIL WA417 TK4

Figure 12d.
Chrysin Response Curve
Replicates 1, 2, 3

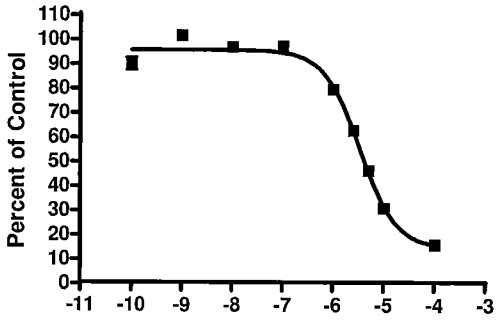


log [RC8]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	96.74	98.10	98.14	87.32	88.24	89.19	79.96	82.28	79.84
-9.00	99.56	99.65	101.87						
-8.00	98.04	94.03	98.48	89.37	92.66	92.92	98.95	99.47	97.21
-7.00	95.46	95.30	97.69	92.80	92.36	92.60	98.53	101.62	98.51
-6.00	77.67	78.70	78.91	75.01	75.49	75.42	80.04	83.38	83.05
-5.60				60.60	59.88	58.66	62.21	65.57	64.60
-5.30	45.63	45.14	43.40	43.97	44.19	42.25	47.96	49.45	47.61
-5.00	29.21	29.04	29.11	31.73	31.51	30.44	29.22	30.26	31.94
-4.00	16.58	14.65	11.86	17.20	9.95	16.04	21.83	13.38	16.99

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	12.2648	12.9885	16.5720
TOP	98.2546	91.1130	92.8486
LOGEC50	-5.51860	-5.45936	-5.43022
HILLSLOPE	-1.10717	-1.15530	-1.43155
EC50	3.02971e-006	3.47245e-006	3.71344e-006
Std. Error			
BOTTOM	1.24102	1.52542	3.85083
TOP	0.549135	0.780122	2.09768
LOGEC50	0.0212645	0.0242470	0.0562916
HILLSLOPE	0.0500451	0.0702070	0.234999
95% Confidence Intervals			
BOTTOM	9.67602 to 14.8535	9.80649 to 16.1706	8.53913 to 24.6048
TOP	97.1091 to 99.4001	89.4857 to 92.7404	88.4728 to 97.2243
LOGEC50	-5.56296 to -5.47424	-5.50994 to -5.40878	-5.54765 to -5.31280
HILLSLOPE	-1.21156 to -1.00278	-1.30175 to -1.00885	-1.92176 to -0.941344
EC50	2.73554e-006 to 3.35551e-006	3.09070e-006 to 3.90136e-006	2.83369e-006 to 4.86632e-006
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.997523	0.994803	0.960889
Absolute Sum of Squares	63.7474	97.5658	776.530
Sy.x	1.78532	2.20869	6.23109
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 WA417 TK4

Figure 12e.
Chrysin Response Curve
Average of All Replicates

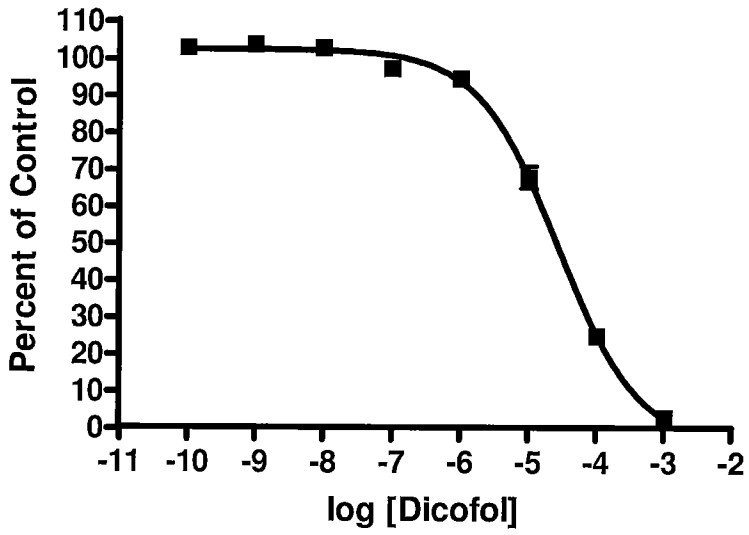


log [RC8]	All Chrysin Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	96.74	98.10	98.14	87.32	88.24	89.19	79.96	82.28	79.84
-9.00	99.56	99.65	101.87						
-8.00	98.04	94.03	98.48	89.37	92.66	92.92	98.95	99.47	97.21
-7.00	95.46	95.30	97.69	92.80	92.36	92.60	98.53	101.62	98.51
-6.00	77.67	78.70	78.91	75.01	75.49	75.42	80.04	83.38	83.05
-5.60				60.60	59.88	58.66	62.21	65.57	64.60
-5.30	45.63	45.14	43.40	43.97	44.19	42.25	47.96	49.45	47.61
-5.00	29.21	29.04	29.11	31.73	31.51	30.44	29.22	30.26	31.94
-4.00	16.58	14.65	11.86	17.20	9.95	16.04	21.83	13.38	16.99

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	14.00
TOP	94.38
LOGEC50	-5.469
HILLSLOPE	-1.225
EC50	3.398e-006
Std. Error	
BOTTOM	1.720
TOP	0.8583
LOGEC50	0.02688
HILLSLOPE	0.08329
95% Confidence Intervals	
BOTTOM	10.57 to 17.44
TOP	92.66 to 96.09
LOGEC50	-5.522 to -5.415
HILLSLOPE	-1.392 to -1.059
EC50	3.003e-006 to 3.846e-006
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9788
Absolute Sum of Squares	1381
Sy.x	4.507
Data	
Number of X values	9
Number of Y replicates	9
Total number of values	72
Number of missing values	9

WIL WA417 TK4

Figure 13a.
Dicofol Response Curve
Replicate 1

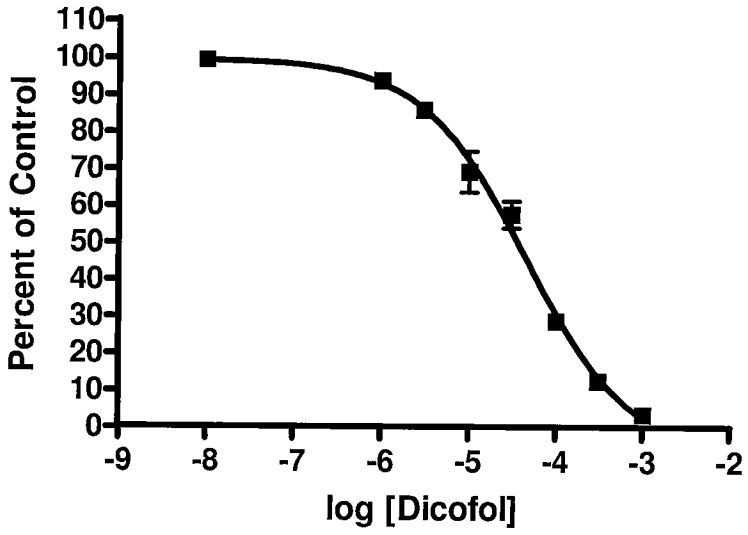


log [RC9]	Replicate 1		
	Y1	Y2	Y3
-10.00	103.96	102.87	99.57
-9.00	103.28	103.40	102.69
-8.00	104.08	99.54	102.82
-7.00	95.98	98.11	95.87
-6.00	95.57	93.34	92.69
-5.52			
-5.00	61.64	71.02	69.57
-4.52			
-4.00	23.69	24.92	25.44
-3.52			
-3.00	2.27	2.19	3.27

	Replicate 1
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-4.64593
TOP	101.751
LOGEC50	-4.56504
HILLSLOPE	-0.732681
EC50	2.72243e-005
Std. Error	
BOTTOM	2.75677
TOP	0.773999
LOGEC50	0.0502174
HILLSLOPE	0.0479683
95% Confidence Intervals	
BOTTOM	-10.3965 to 1.10469
TOP	100.136 to 103.365
LOGEC50	-4.66980 to -4.46029
HILLSLOPE	-0.832742 to -0.632619
EC50	2.13896e-005 to 3.46505e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.996118
Absolute Sum of Squares	127.452
Sy.x	2.52441
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

WIL WA417 TK4

Figure 13b.
Dicofol Response Curve
Replicate 2

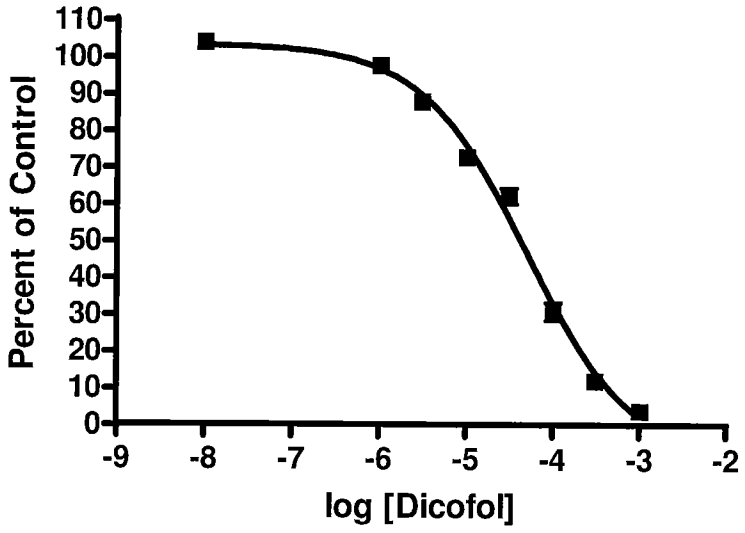


log [RC9]	Replicate 2		
	Y1	Y2	Y3
-10.00			
-9.00			
-8.00	98.61	97.92	99.64
-7.00			
-6.00	96.65	91.70	91.50
-5.52	83.25	85.60	87.16
-5.00	58.71	69.90	77.50
-4.52	50.14	59.44	62.02
-4.00	27.01	30.78	27.55
-3.52	12.13	12.95	12.00
-3.00	4.12	3.21	3.06

	Replicate 2
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-7.32928
TOP	98.8290
LOGEC50	-4.35977
HILLSLOPE	-0.730324
EC50	4.36747e-005
Std. Error	
BOTTOM	5.59453
TOP	2.39036
LOGEC50	0.0844486
HILLSLOPE	0.0852312
95% Confidence Intervals	
BOTTOM	-18.9995 to 4.34091
TOP	93.8427 to 103.815
LOGEC50	-4.53593 to -4.18361
HILLSLOPE	-0.908117 to -0.552532
EC50	2.91119e-005 to 6.55223e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.986459
Absolute Sum of Squares	396.472
Sy.x	4.45237
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

WIL WA417 TK4

Figure 13c.
Dicofol Response Curve
Replicate 3

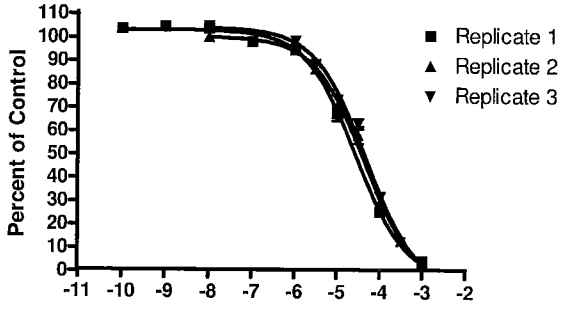


log [RC9]	Replicate 3		
	Y1	Y2	Y3
-10.00			
-9.00			
-8.00	105.86	101.30	102.66
-7.00			
-6.00	98.45	98.46	94.11
-5.52	89.96	85.92	86.19
-5.00	71.02	70.57	75.66
-4.52	57.48	63.43	64.52
-4.00	35.35	27.80	28.74
-3.52	11.68	11.25	12.61
-3.00	4.31	3.89	3.26

	Replicate 3
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-9.71800
TOP	102.721
LOGEC50	-4.30980
HILLSLOPE	-0.714759
EC50	4.90009e-005
Std. Error	
BOTTOM	5.23921
TOP	2.05526
LOGEC50	0.0741200
HILLSLOPE	0.0697207
95% Confidence Intervals	
BOTTOM	-20.6470 to 1.21099
TOP	98.4340 to 107.009
LOGEC50	-4.46441 to -4.15518
HILLSLOPE	-0.860197 to -0.569322
EC50	3.43233e-005 to 6.99549e-005
Goodness of Fit	
Degrees of Freedom	20
R^2	0.990760
Absolute Sum of Squares	291.229
Sy.x	3.81595
Data	
Number of X values	11
Number of Y replicates	3
Total number of values	24
Number of missing values	9

WIL WA417 TK4

Figure 13d.
Dicofol Response Curve
Replicates 1, 2, 3

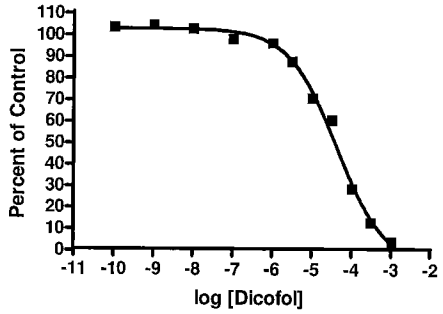


log [RC9]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	103.96	102.87	99.57						
-9.00	103.28	103.40	102.69						
-8.00	104.08	99.54	102.82	98.61	97.92	99.64	105.86	101.30	102.66
-7.00	95.98	98.11	95.87						
-6.00	95.57	93.34	92.69	96.65	91.70	91.50	98.45	98.46	94.11
-5.52				83.25	85.60	87.16	89.96	85.92	86.19
-5.00	61.64	71.02	69.57	58.71	69.90	77.50	71.02	70.57	75.66
-4.52				50.14	59.44	62.02	57.48	63.43	64.52
-4.00	23.69	24.92	25.44	27.01	30.78	27.55	35.35	27.80	28.74
-3.52				12.13	12.95	12.00	11.68	11.25	12.61
-3.00	2.27	2.19	3.27	4.12	3.21	3.06	4.31	3.89	3.26

	Replicate 1	Replicate 2	Replicate 3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	-4.64593	-7.32928	-9.71800
TOP	101.751	98.8290	102.721
LOGEC50	-4.56504	-4.35977	-4.30980
HILLSLOPE	-0.732681	-0.730324	-0.714759
EC50	2.72243e-005	4.36747e-005	4.90009e-005
Std. Error			
BOTTOM	2.75677	5.59453	5.23921
TOP	0.773999	2.39036	2.05526
LOGEC50	0.0502174	0.0844486	0.0741200
HILLSLOPE	0.0479683	0.0852312	0.0697207
95% Confidence Intervals			
BOTTOM	-10.3965 to 1.10469	-18.9995 to 4.34091	-20.6470 to 1.21099
TOP	100.136 to 103.365	93.8427 to 103.815	98.4340 to 107.009
LOGEC50	-4.66980 to -4.46029	-4.53593 to -4.18361	-4.46441 to -4.15518
HILLSLOPE	-0.832742 to -0.632619	-0.908117 to -0.552532	-0.860197 to -0.569322
EC50	2.13896e-005 to 3.46505e-005	2.91119e-005 to 6.55223e-005	3.43233e-005 to 6.99549e-005
Goodness of Fit			
Degrees of Freedom	20	20	20
R^2	0.996118	0.986459	0.990760
Absolute Sum of Squares	127.452	396.472	291.229
Sy.x	2.52441	4.45237	3.81595
Data			
Number of X values	11	11	11
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	9	9	9

WIL WA417 TK4

Figure 13e.
Dicofol Response Curve
Average of All Replicates

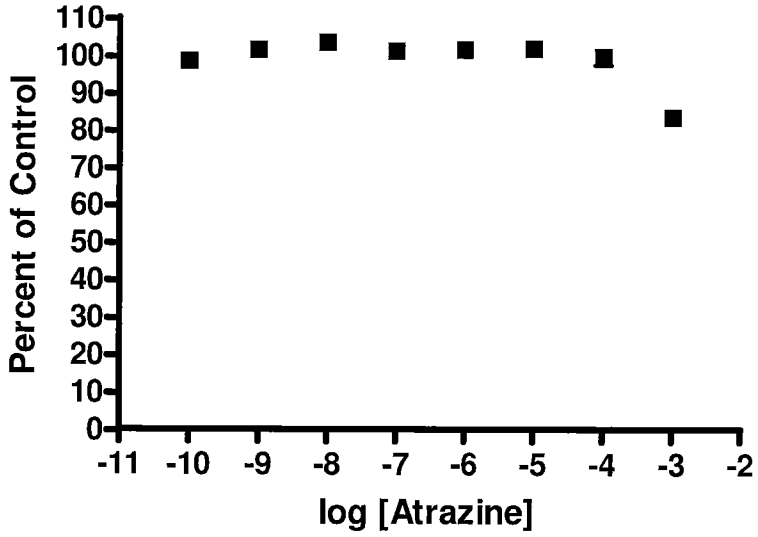


log [RC9]	All Dicofol Data								
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
-10.00	103.96	102.87	99.57						
-9.00	103.28	103.40	102.69						
-8.00	104.08	99.54	102.82	98.61	97.92	99.64	105.86	101.30	102.66
-7.00	95.98	98.11	95.87						
-6.00	95.57	93.34	92.69	96.65	91.70	91.50	98.45	98.46	94.11
-5.52				83.25	85.60	87.16	89.96	85.92	86.19
-5.00	61.64	71.02	69.57	58.71	69.90	77.50	71.02	70.57	75.66
-4.52				50.14	59.44	62.02	57.48	63.43	64.52
-4.00	23.69	24.92	25.44	27.01	30.78	27.55	35.35	27.80	28.74
-3.52				12.13	12.95	12.00	11.68	11.25	12.61
-3.00	2.27	2.19	3.27	4.12	3.21	3.06	4.31	3.89	3.26

	Average Data Fit
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-8.005
TOP	101.4
LOGEC50	-4.382
HILLSLOPE	-0.7119
EC50	4.153e-005
Std. Error	
BOTTOM	2.973
TOP	0.9695
LOGEC50	0.04654
HILLSLOPE	0.04112
95% Confidence Intervals	
BOTTOM	-13.94 to -2.068
TOP	99.44 to 103.3
LOGEC50	-4.475 to -4.289
HILLSLOPE	-0.7940 to -0.6298
EC50	3.352e-005 to 5.144e-005
Goodness of Fit	
Degrees of Freedom	68
R^2	0.9885
Absolute Sum of Squares	1125
Sy.x	4.068
Data	
Number of X values	11
Number of Y replicates	9
Total number of values	72
Number of missing values	27

WIL WA417 TK4

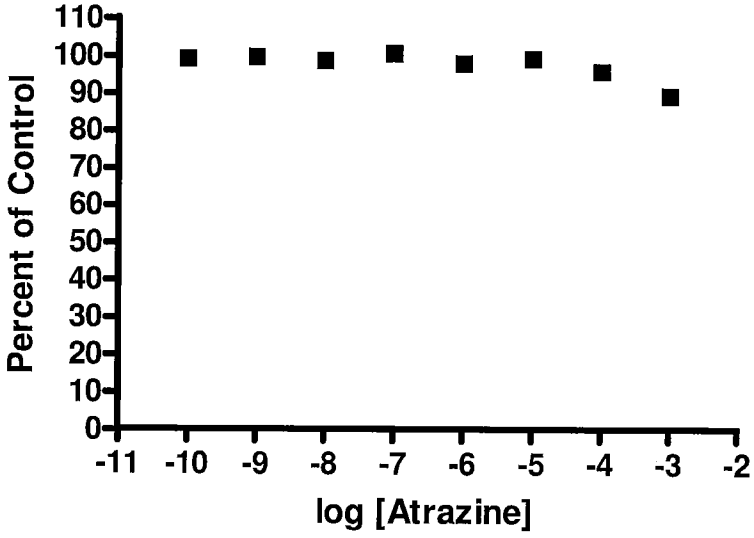
Figure 14a.
Atrazine Response Curve
Replicate 1



log [RC10]	Replicate 1		
	Y1	Y2	Y3
-10.00	98.78	99.33	95.98
-9.00	98.28	102.19	102.47
-8.00	104.18	102.45	102.21
-7.00	99.92	99.96	102.12
-6.00	103.06	100.14	100.04
-5.00	103.06	101.73	99.27
-4.00	102.91	95.49	98.95
-3.00	79.53	83.05	86.72

WIL WA417 TK4

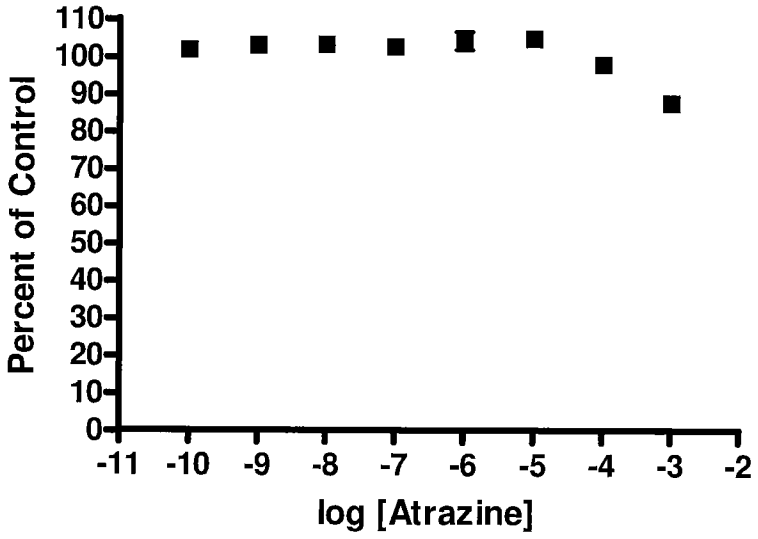
Figure 14b.
Atrazine Response Curve
Replicate 2



log [RC10]	Replicate 2		
	Y1	Y2	Y3
-10.00	98.02	98.01	99.35
-9.00	100.14	98.42	98.51
-8.00	97.21	101.59	95.18
-7.00	99.16	98.57	102.28
-6.00	98.53	95.64	97.85
-5.00	99.34	97.24	99.07
-4.00	95.09	92.30	98.22
-3.00	89.47	90.02	86.97

WIL WA417 TK4

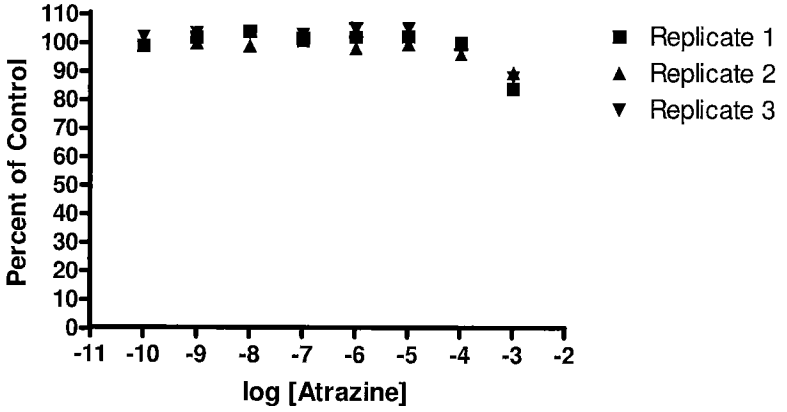
Figure 14c.
Atrazine Response Curve
Replicate 3



log [RC10]	Replicate 3		
	Y1	Y2	Y3
-10.00	102.37	100.34	100.67
-9.00	100.81	103.23	103.40
-8.00	106.70	100.17	101.00
-7.00	102.55	98.44	105.11
-6.00	107.81	103.60	99.40
-5.00	104.61	105.34	102.60
-4.00	98.20	98.83	95.19
-3.00	87.05	87.85	86.38

WIL WA417 TK4

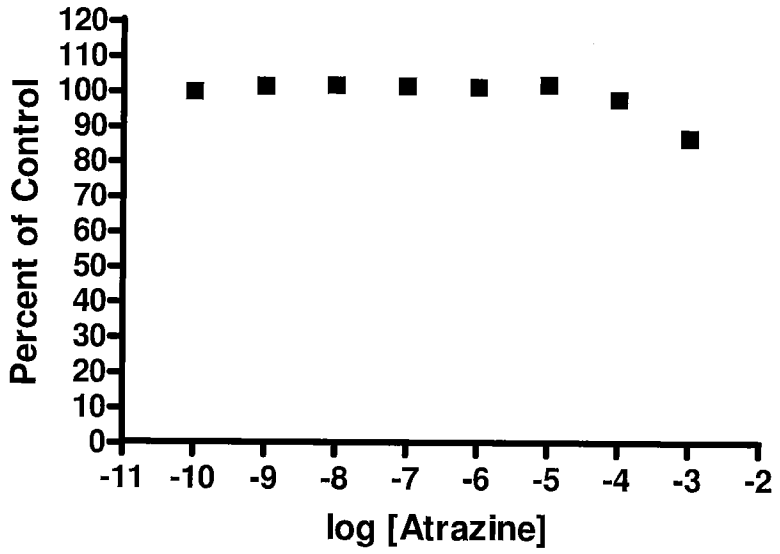
Figure 14d.
Atrazine Response Curve
Replicates 1, 2, 3



log [RC10]	Replicate 1			Replicate 2			Replicate 3		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
-10.00	98.78	99.33	95.98	98.02	98.01	99.35	102.37	100.34	100.67
-9.00	98.28	102.19	102.47	100.14	98.42	98.51	100.81	103.23	103.40
-8.00	104.18	102.45	102.21	97.21	101.59	95.18	106.70	100.17	101.00
-7.00	99.92	99.96	102.12	99.16	98.57	102.28	102.55	98.44	105.11
-6.00	103.06	100.14	100.04	98.53	95.64	97.85	107.81	103.60	99.40
-5.00	103.06	101.73	99.27	99.34	97.24	99.07	104.61	105.34	102.60
-4.00	102.91	95.49	98.95	95.09	92.30	98.22	98.20	98.83	95.19
-3.00	79.53	83.05	86.72	89.47	90.02	86.97	87.05	87.85	86.38

WIL WA417 TK4

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	98.78	99.33	95.98	98.02	98.01	99.35	102.37	100.34	100.67
-9.00	98.28	102.19	102.47	100.14	98.42	98.51	100.81	103.23	103.40
-8.00	104.18	102.45	102.21	97.21	101.59	95.18	106.70	100.17	101.00
-7.00	99.92	99.96	102.12	99.16	98.57	102.28	102.55	98.44	105.11
-6.00	103.06	100.14	100.04	98.53	95.64	97.85	107.81	103.60	99.40
-5.00	103.06	101.73	99.27	99.34	97.24	99.07	104.61	105.34	102.60
-4.00	102.91	95.49	98.95	95.09	92.30	98.22	98.20	98.83	95.19
-3.00	79.53	83.05	86.72	89.47	90.02	86.97	87.05	87.85	86.38

Figure 15a. Aminoglutethimide Statistical Average Concentration Response Curve

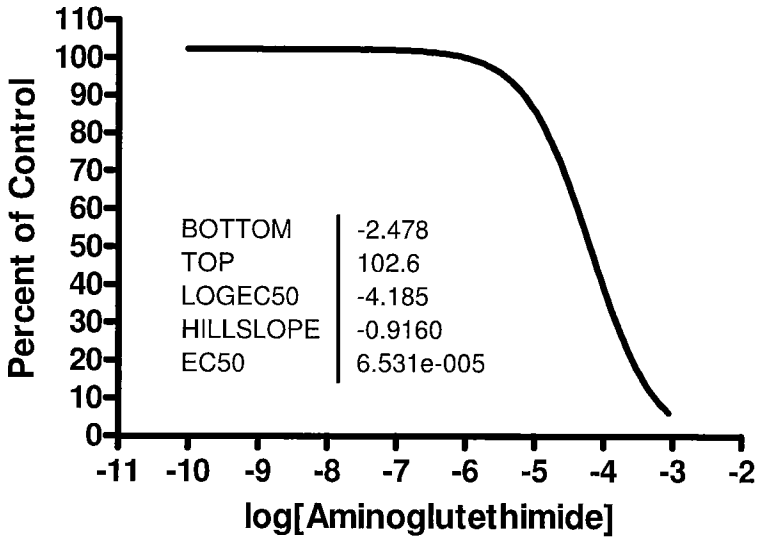


Figure 15b. Ketoconazole Statistical Average Concentration Response Curve

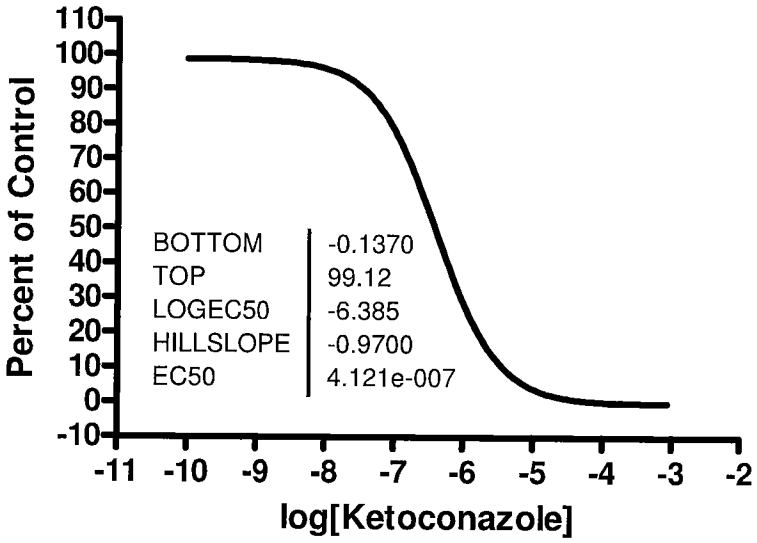


Figure 15c. Prochloraz Statistical Average Concentration Response Curve

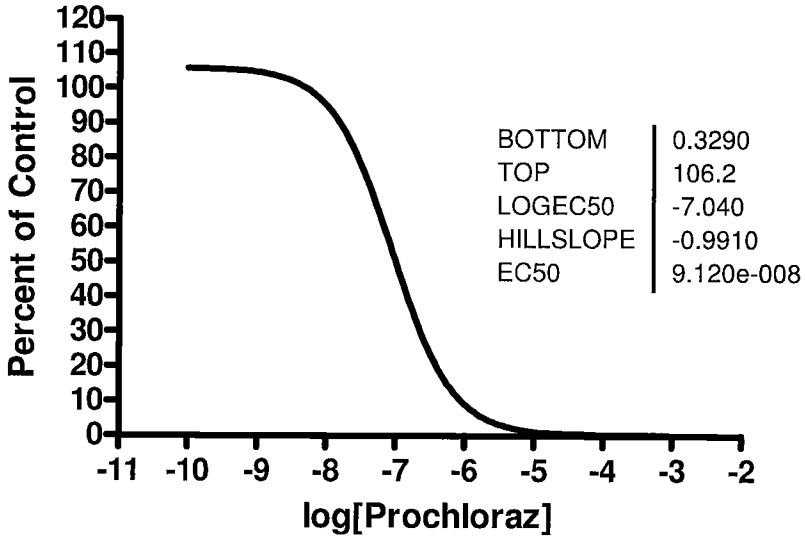


Figure 15d. 4-Nonylphenol Statistical Average Concentration Response Curve

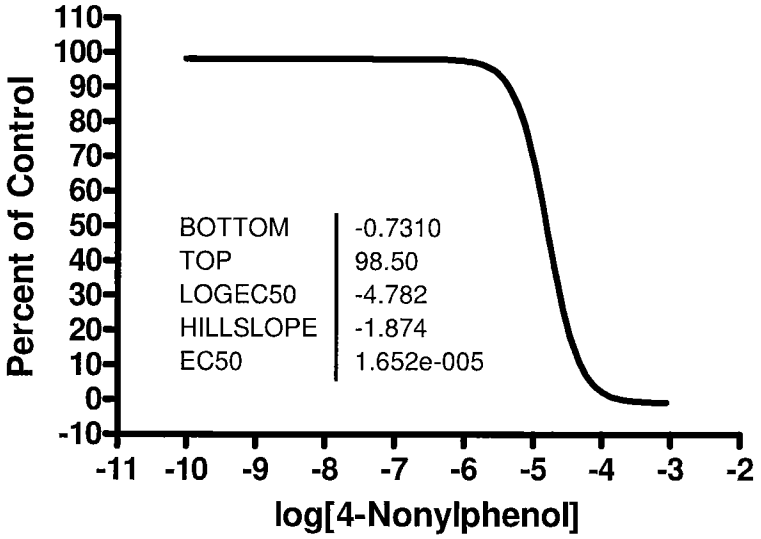


**Figure 15e. Fenarimol
Statistical Average
Concentration Response Curve**

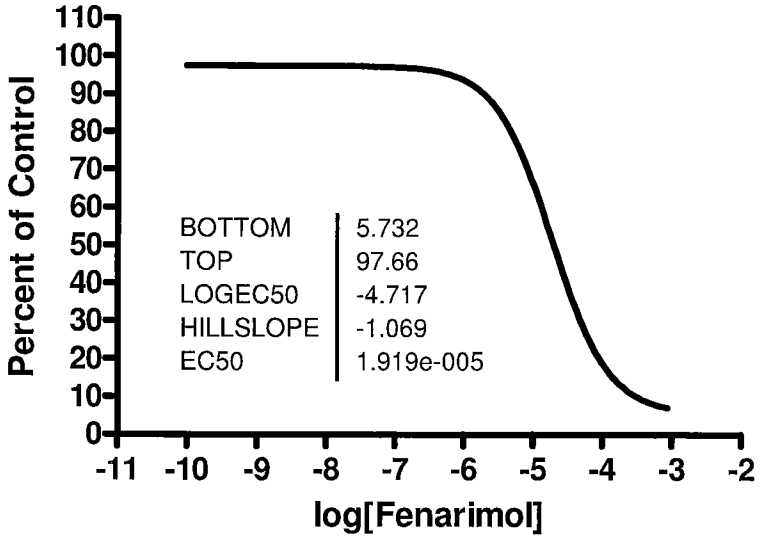


Figure 15f. Econazole Statistical Average Concentration Response Curve

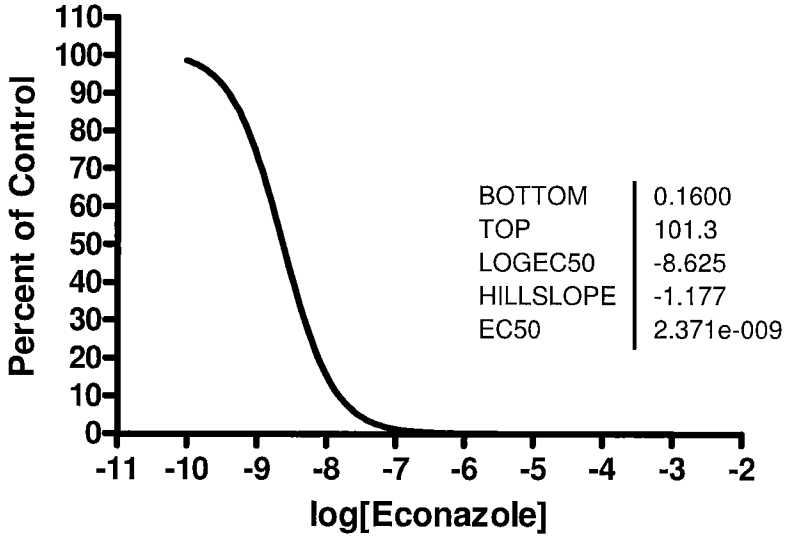


**Figure 15g. Chrysin
Statistical Average
Concentration Response Curve**

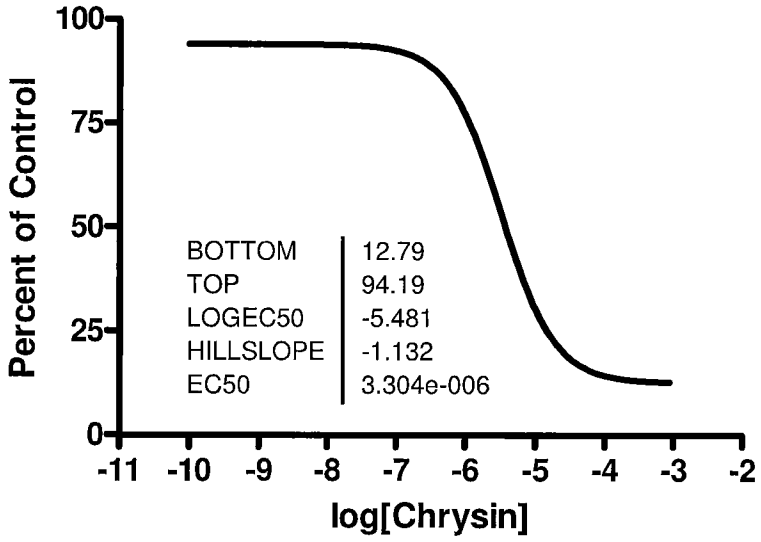


**Figure 15h. Dicofol
Statistical Average
Concentration Response Curve**

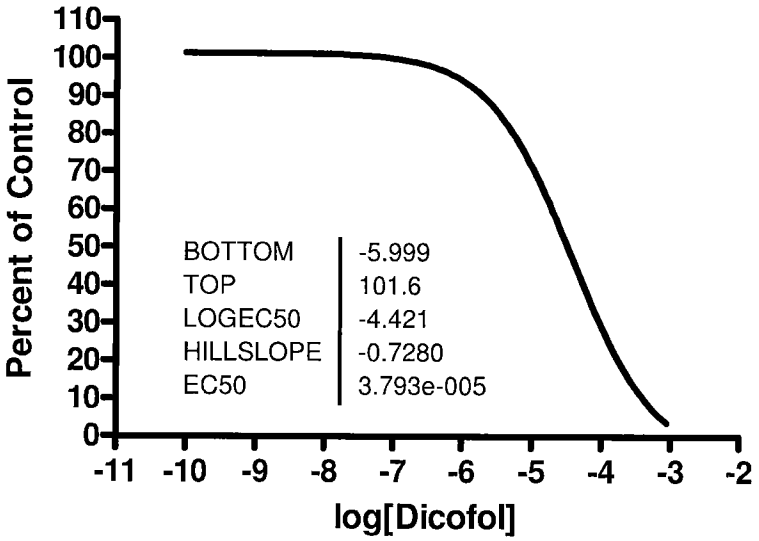


Figure 16. Validation of the Recombinant Aromatase Assay (WIL-431011)
Reference Chemical 1- Aminoglutethimide

Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	-3.606	2.239	-8.276	1.064
T	Top	1	103.6	0.5	102.5	104.7
μ	LOGEC50	1	-4.138	0.032	-4.204	-4.072
β	HILLSLOPE	1	-0.899	0.047	-0.998	-0.800
EC50	EC50 (μM)	1	72.72		62.45	84.67
B	Bottom	2	-1.775	1.563	-5.035	1.485
T	Top	2	101.0	0.7	99.6	102.4
μ	LOGEC50	2	-4.328	0.022	-4.373	-4.282
β	HILLSLOPE	2	-0.917	0.043	-1.007	-0.828
EC50	EC50 (μM)	2	47.03		42.32	52.26
B	Bottom	3	-2.801	2.267	-7.529	1.928
T	Top	3	103.0	0.6	101.6	104.3
μ	LOGEC50	3	-4.085	0.028	-4.143	-4.027
β	HILLSLOPE	3	-0.932	0.050	-1.037	-0.826
EC50	EC50 (μM)	3	82.15		71.88	93.89

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	-2.478	1.116	-7.278	2.322
Top	Overall	102.6	0.8	99.2	105.9
LOGEC50	Overall	-4.185	0.074	-4.505	-3.864
HILLSLOPE	Overall	-0.916	0.027	-1.031	-0.800

Mathematical Averages

AVG LOGEC50	-4.184			
AVG HILLSLOPE	-0.916			
AVG T	102.5			
AVG B	-2.727			
		sd	sem	%CV
Avg EC50	67.30	18.18	10.49	27.01

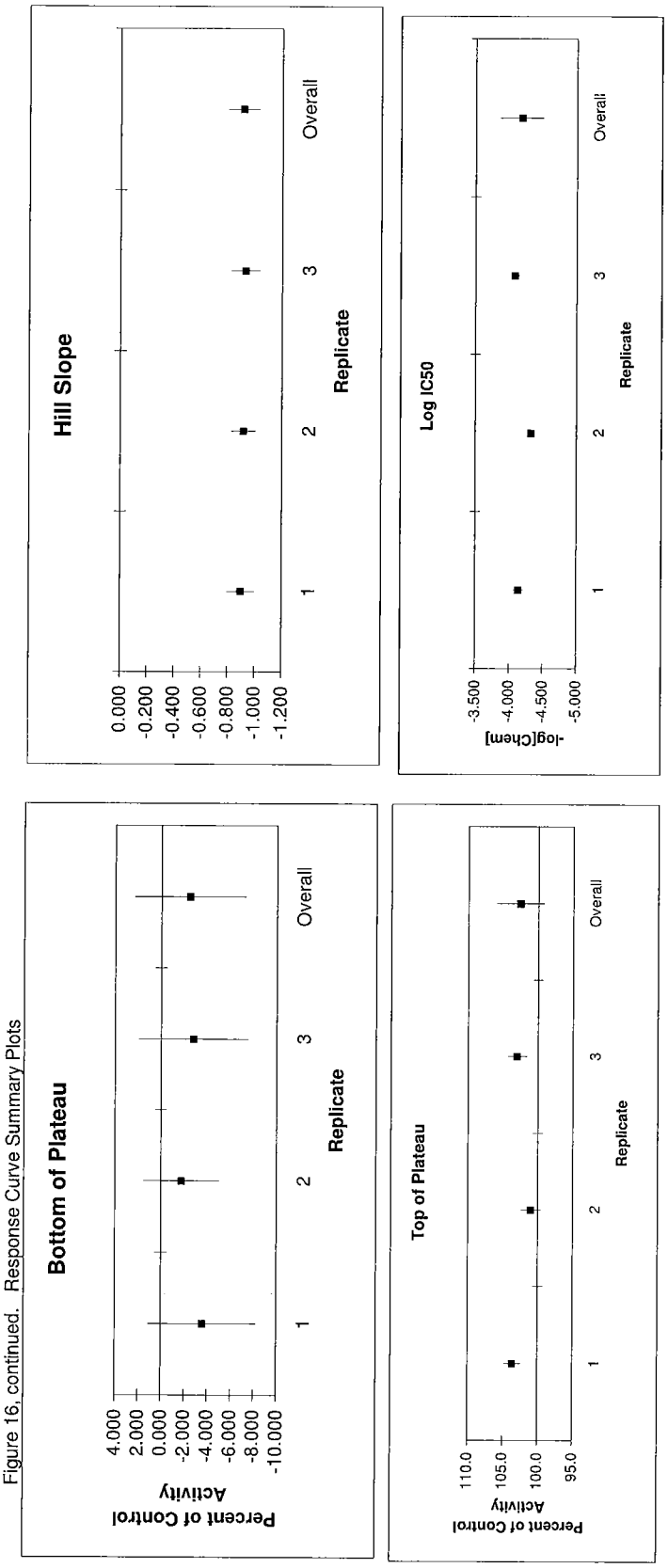


Figure 16, continued. Response Curve Summary Plots

Figure 17. Validation of the Recombinant Aromatase Assay (WIL-431011)
Reference Chemical 2-Ketoconazole
Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	-0.376	1.064	-2.596	1.843
T	Top	1	99.7	0.8	98.1	101.3
μ	LOGEC50	1	-6.387	0.027	-6.443	-6.330
β	HILLSLOPE	1	-0.935	0.042	-1.023	-0.847
EC50	EC50 (μM)	1	0.411		0.361	0.467
B	Bottom	2	0.439	2.203	-4.157	5.035
T	Top	2	98.0	1.8	94.1	101.8
μ	LOGEC50	2	-6.550	0.062	-6.679	-6.420
β	HILLSLOPE	2	-1.042	0.115	-1.282	-0.803
EC50	EC50 (μM)	2	0.282		0.210	0.380
B	Bottom	3	-0.068	0.866	-1.875	1.740
T	Top	3	98.9	0.6	97.7	100.1
μ	LOGEC50	3	-6.241	0.020	-6.283	-6.199
β	HILLSLOPE	3	-0.991	0.038	-1.069	-0.912
EC50	EC50 (μM)	3	0.575		0.521	0.633

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	-0.137	0.642	-2.901	2.627
Top	Overall	99.124	0.461	97.142	101.105
LOGEC50	Overall	-6.385	0.087	-6.76	-6.01
HILLSLOPE	Overall	-0.970	0.027	-1.088	-0.853

Mathematical Averages

AVG LOGEC50	-6.393			
AVG HILLSLOPE	-0.989			
AVG T	98.9			
AVG B	-0.002			
		sd	sem	%CV
Avg EC50	0.422	0.147	0.085	34.700

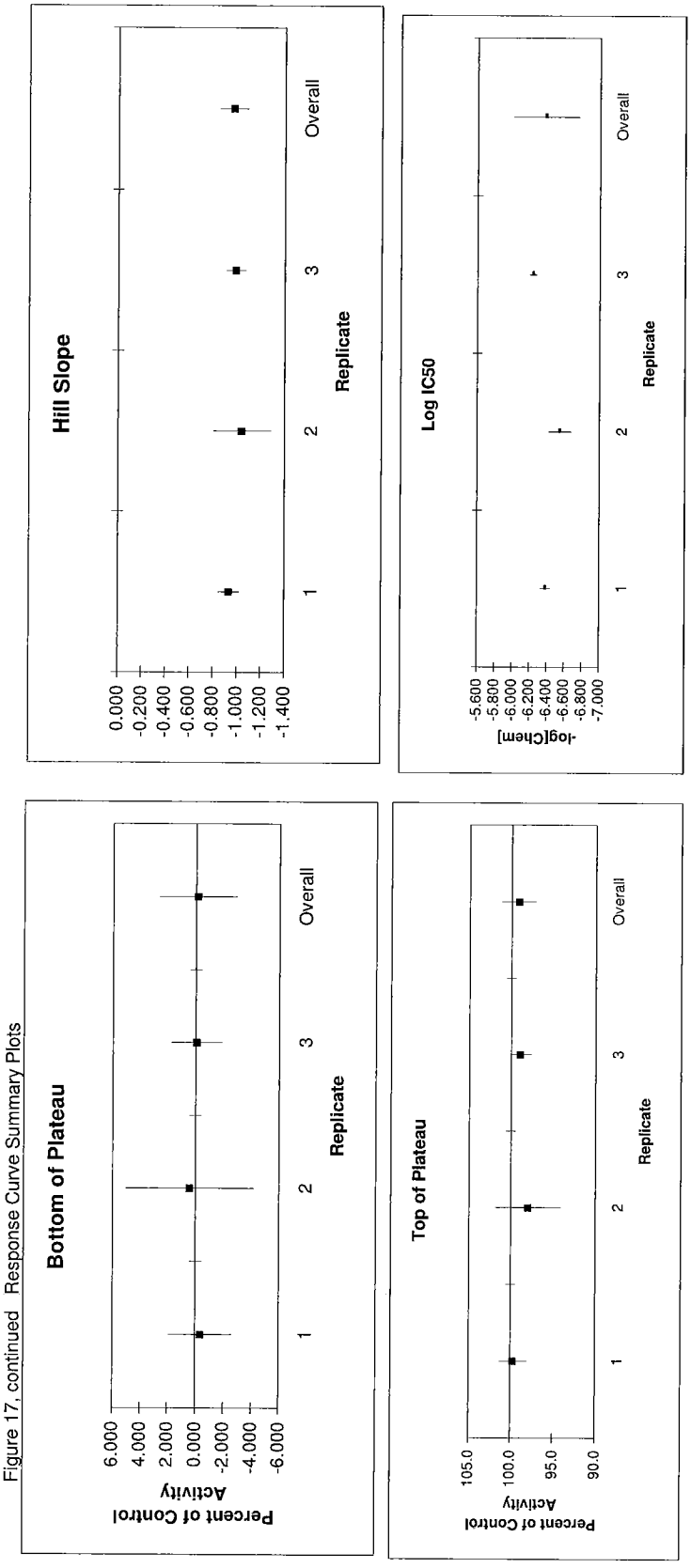


Figure 17, continued Response Curve Summary Plots

Figure 18. Validation of the Recombinant Aromatase Assay (WIL-431011)
Reference Chemical 3-Prochloraz
Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	0.169	0.724	-1.341	1.680
T	Top	1	109.7	1.2	107.3	112.1
μ	LOGEC50	1	-7.579	0.026	-7.634	-7.525
β	HILLSLOPE	1	-0.974	0.044	-1.067	-0.882
EC50	EC50 (μM)	1	0.026		0.023	0.030
B	Bottom	2	6.997	4.124	-1.607	15.600
T	Top	2	102.4	1.2	100.0	104.8
μ	LOGEC50	2	-6.023	0.047	-6.121	-5.924
β	HILLSLOPE	2	-1.273	0.248	-1.789	-0.757
EC50	EC50 (μM)	2	0.949		0.756	1.191
B	Bottom	3	0.189	1.268	-2.457	2.835
T	Top	3	106.6	1.3	104.0	109.3
μ	LOGEC50	3	-7.516	0.025	-7.568	-7.465
β	HILLSLOPE	3	-1.002	0.054	-1.115	-0.889
EC50	EC50 (μM)	3	0.030		0.027	0.034

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	0.329	0.622	-2.345	3.003
Top	Overall	106.245	2.142	97.027	115.463
LOGEC50	Overall	-7.040	0.508	-9.226	-4.854
HILLSLOPE	Overall	-0.991	0.034	-1.137	-0.844

Mathematical Averages

AVG LOGEC50	-7.039			
AVG HILLSLOPE	-1.083			
AVG T	106.233			
AVG B	2.452			
		sd	sem	%CV
Avg EC50	0.335	0.532	0.307	158.537

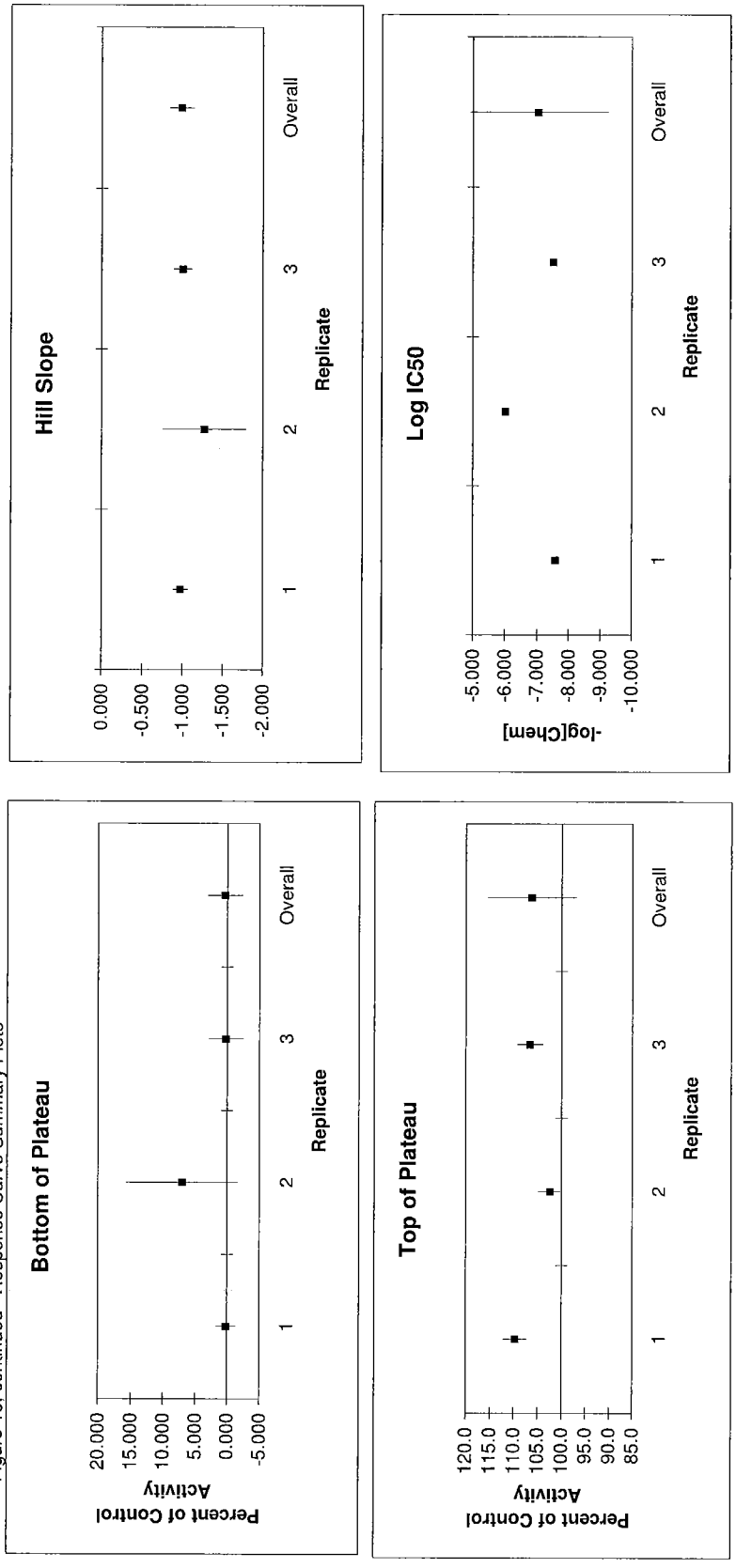


Figure 18, continued Response Curve Summary Plots

Figure 19. Validation of the Recombinant Aromatase Assay (WIL-431011)
Reference Chemical 4-4-Nonylphenol
Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	0.091	3.042	-6.256	6.437
T	Top	1	98.6	1.4	95.6	101.5
μ	LOGEC50	1	-4.879	0.079	-5.044	-4.714
β	HILLSLOPE	1	-2.044	1.158	-4.460	0.371
EC50	EC50 (μM)	1	13.20		9.03	19.31
B	Bottom	2	-0.459	2.149	-4.943	4.024
T	Top	2	102.3	1.2	99.7	104.8
μ	LOGEC50	2	-4.802	0.033	-4.870	-4.733
β	HILLSLOPE	2	-1.881	0.214	-2.327	-1.434
EC50	EC50 (μM)	2	15.79		13.480	18.480
B	Bottom	3	-0.871	0.975	-2.906	1.163
T	Top	3	95.0	0.5	93.9	96.1
μ	LOGEC50	3	-4.750	0.016	-4.783	-4.717
β	HILLSLOPE	3	-1.722	0.766	-1.882	-1.562
EC50	EC50 (μM)	3	17.79		16.49	19.20

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	-0.731	0.852	-4.398	2.937
Top	Overall	98.502	2.159	89.213	107.791
LOGEC50	Overall	-4.782	0.028	-4.904	-4.66
HILLSLOPE	Overall	-1.874	0.203	-2.747	-1.002

Mathematical Averages

AVG LOGEC50	-4.810			
AVG HILLSLOPE	-1.882			
AVG T	98.612			
AVG B	-0.413			
		sd	sem	%CV
Avg EC50	15.59	2.30	1.33	14.75

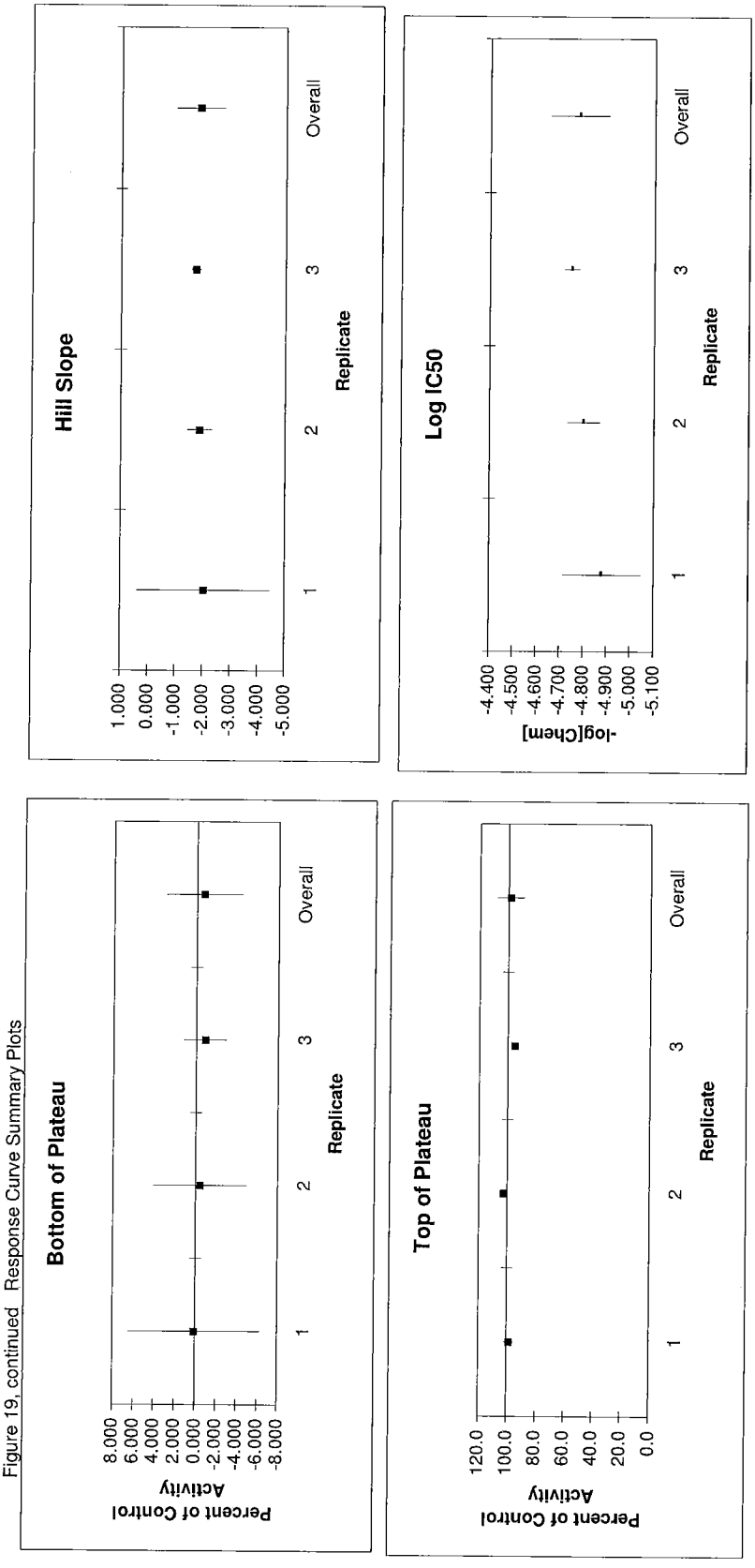


Figure 19, continued Response Curve Summary Plots

Figure 20. Validation of the Recombinant Aromatase Assay (WIL-431011)
Reference Chemical 6-Fenarimol
Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	0.598	1.185	-1.875	3.070
T	Top	1	101.8	0.7	100.3	103.3
μ	LOGEC50	1	-5.209	0.026	-5.263	-5.156
β	HILLSLOPE	1	-1.052	0.060	-1.177	-0.928
EC50	EC50 (μM)	1	6.178		5.461	6.988
B	Bottom	2	18.284	4.850	8.166	28.402
T	Top	2	98.6	1.1	96.4	100.9
μ	LOGEC50	2	-3.822	0.080	-3.990	-3.655
β	HILLSLOPE	2	-1.487	0.340	-2.197	-0.777
EC50	EC50 (μM)	2	150.528		102.399	221.278
B	Bottom	3	0.909	1.961	-3.183	5.000
T	Top	3	92.2	1.5	88.9	95.4
μ	LOGEC50	3	-5.113	0.039	-5.193	-5.032
β	HILLSLOPE	3	-1.077	0.088	-1.260	-0.894
EC50	EC50 (μM)	3	7.718		6.411	9.291

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	5.732	5.365	-17.351	28.815
Top	Overall	97.664	2.802	85.607	109.722
LOGEC50	Overall	-4.717	0.446	-6.636	-2.799
HILLSLOPE	Overall	-1.069	0.049	-1.279	-0.859

Mathematical Averages

AVG LOGEC50	-4.715			
AVG HILLSLOPE	-1.205			
AVG T	97.533			
AVG B	6.597			
		sd	sem	%CV
Avg EC50	54.808	82.900	47.862	151.254

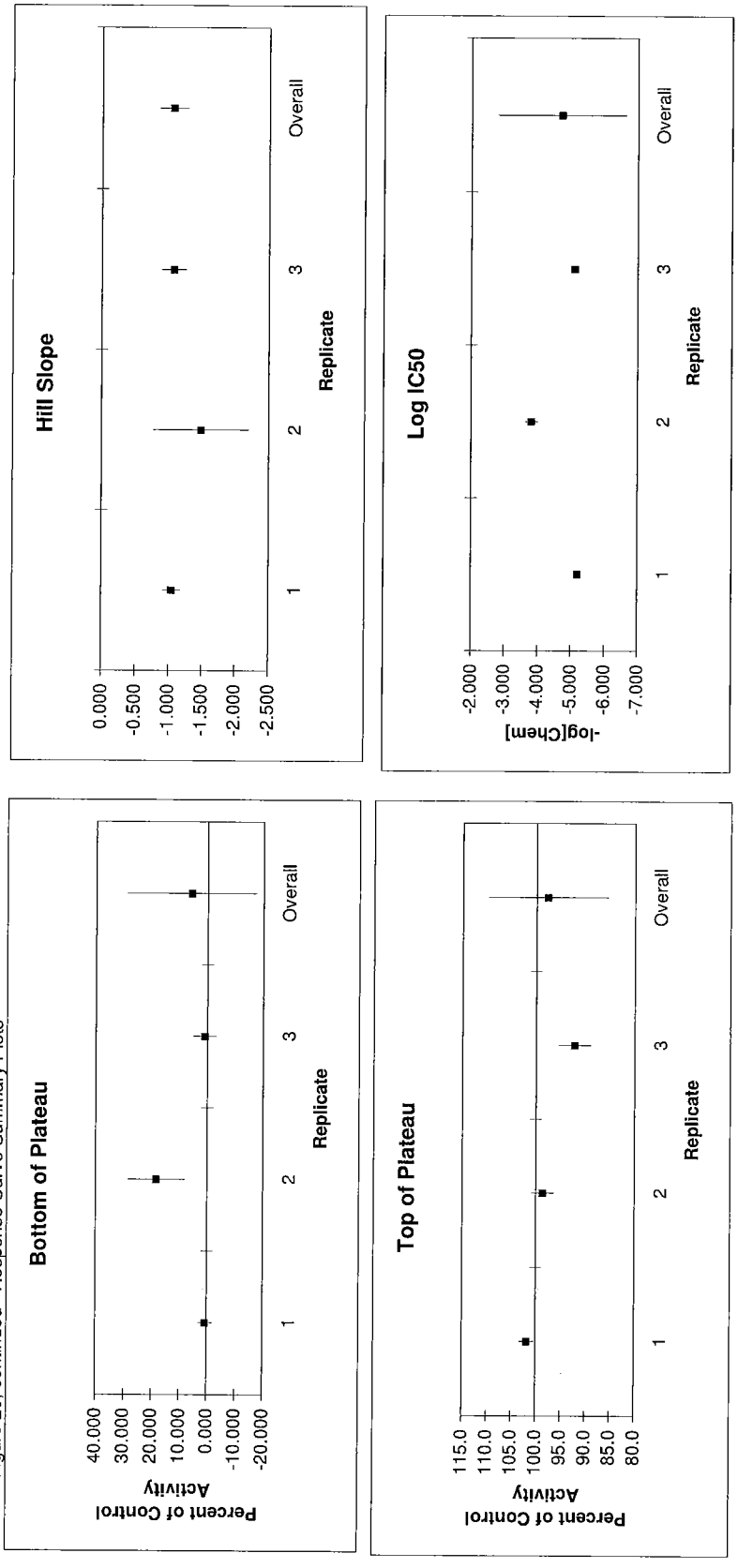


Figure 20, continued Response Curve Summary Plots

Figure 21. Validation of the Recombinant Aromatase Assay (WIL-431011)
Reference Chemical 7-Econazole
Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	0.091	0.168	-0.259	0.442
T	Top	1	101.3	0.5	100.4	102.3
μ	LOGEC50	1	-8.644	0.008	-8.661	-8.628
β	HILLSLOPE	1	-1.149	0.017	-1.184	-1.114
EC50	EC50 (μM)	1	0.0023		0.0022	0.0024
B	Bottom	2	0.422	0.421	-0.455	1.300
T	Top	2	99.8	0.8	98.1	101.5
μ	LOGEC50	2	-8.636	0.011	-8.659	-8.612
β	HILLSLOPE	2	-1.187	0.034	-1.258	-1.117
EC50	EC50 (μM)	2	0.0023		0.0022	0.0024
B	Bottom	3	0.328	0.422	-0.553	1.208
T	Top	3	102.7	0.8	101.1	104.3
μ	LOGEC50	3	-8.593	0.011	-8.616	-8.571
β	HILLSLOPE	3	-1.217	0.033	-1.287	-1.148
EC50	EC50 (μM)	3	0.0026		0.0024	0.0027

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	0.16	0.146	-0.47	0.789
Top	Overall	101.298	0.766	98.003	104.594
LOGEC50	Overall	-8.625	0.016	-8.693	-8.557
HILLSLOPE	Overall	-1.177	0.022	-1.27	-1.083

Mathematical Averages

AVG LOGEC50	-8.624			
AVG HILLSLOPE	-1.185			
AVG T	101.279			
AVG B	0.280			
		sd	sem	%CV
Avg EC50	0.0024	0.00015186	8.77E-05	6.387055

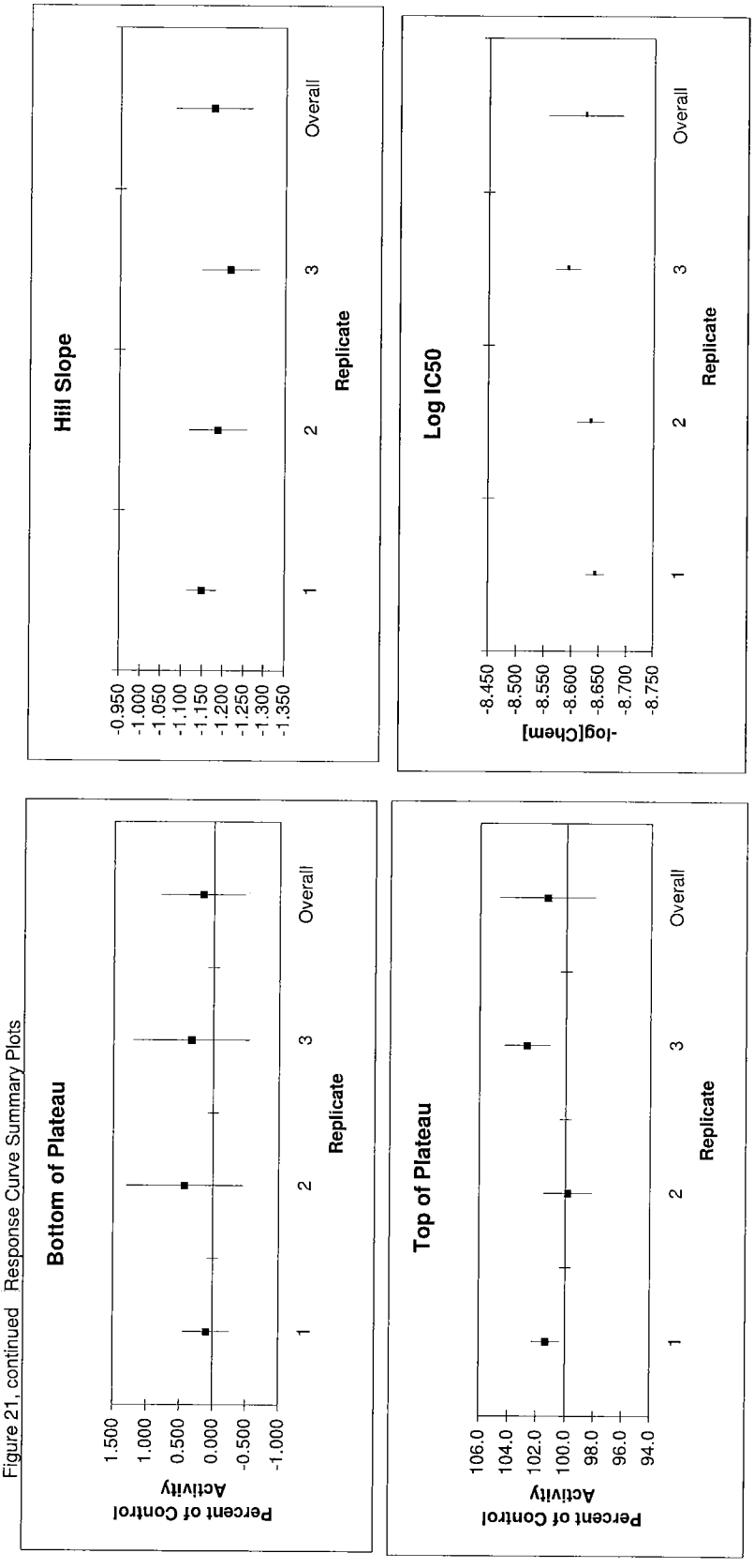


Figure 21, continued Response Curve Summary Plots

Figure 22. Validation of the Recombinant Aromatase Assay (WIL-431011)
Reference Chemical 8-Chrysin
Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	12.265	1.241	9.676	14.854
T	Top	1	98.3	0.5	97.1	99.4
μ	LOGEC50	1	-5.519	0.021	-5.563	-5.474
β	HILLSLOPE	1	-1.107	0.050	-1.212	-1.003
EC50	EC50 (μM)	1	3.030		2.736	3.356
B	Bottom	2	12.989	1.525	9.806	16.171
T	Top	2	91.1	0.8	89.5	92.7
μ	LOGEC50	2	-5.459	0.024	-5.510	-5.409
β	HILLSLOPE	2	-1.155	0.070	-1.302	-1.009
EC50	EC50 (μM)	2	3.472		3.091	3.901
B	Bottom	3	16.572	3.851	8.539	24.605
T	Top	3	92.8	2.1	88.5	97.2
μ	LOGEC50	3	-5.430	0.056	-5.548	-5.313
β	HILLSLOPE	3	-1.432	0.235	-1.922	-0.941
EC50	EC50 (μM)	3	3.713		2.834	4.866

Summary of Statistical Analysis Results

	Replicate	Values	Std. Error	95% Confidence Level	
Bottom	Overall	12.79	0.934	8.772	16.808
Top	Overall	94.189	2.299	84.298	104.081
LOGEC50	Overall	-5.481	0.026	-5.593	-5.368
HILLSLOPE	Overall	-1.132	0.04	-1.305	-0.96

Mathematical Averages

AVG LOGEC50	-5.469			
AVG HILLSLOPE	-1.231			
AVG T	94.072			
AVG B	13.942			
		sd	sem	%CV
Avg EC50	3.405	0.346	0.200	10.173

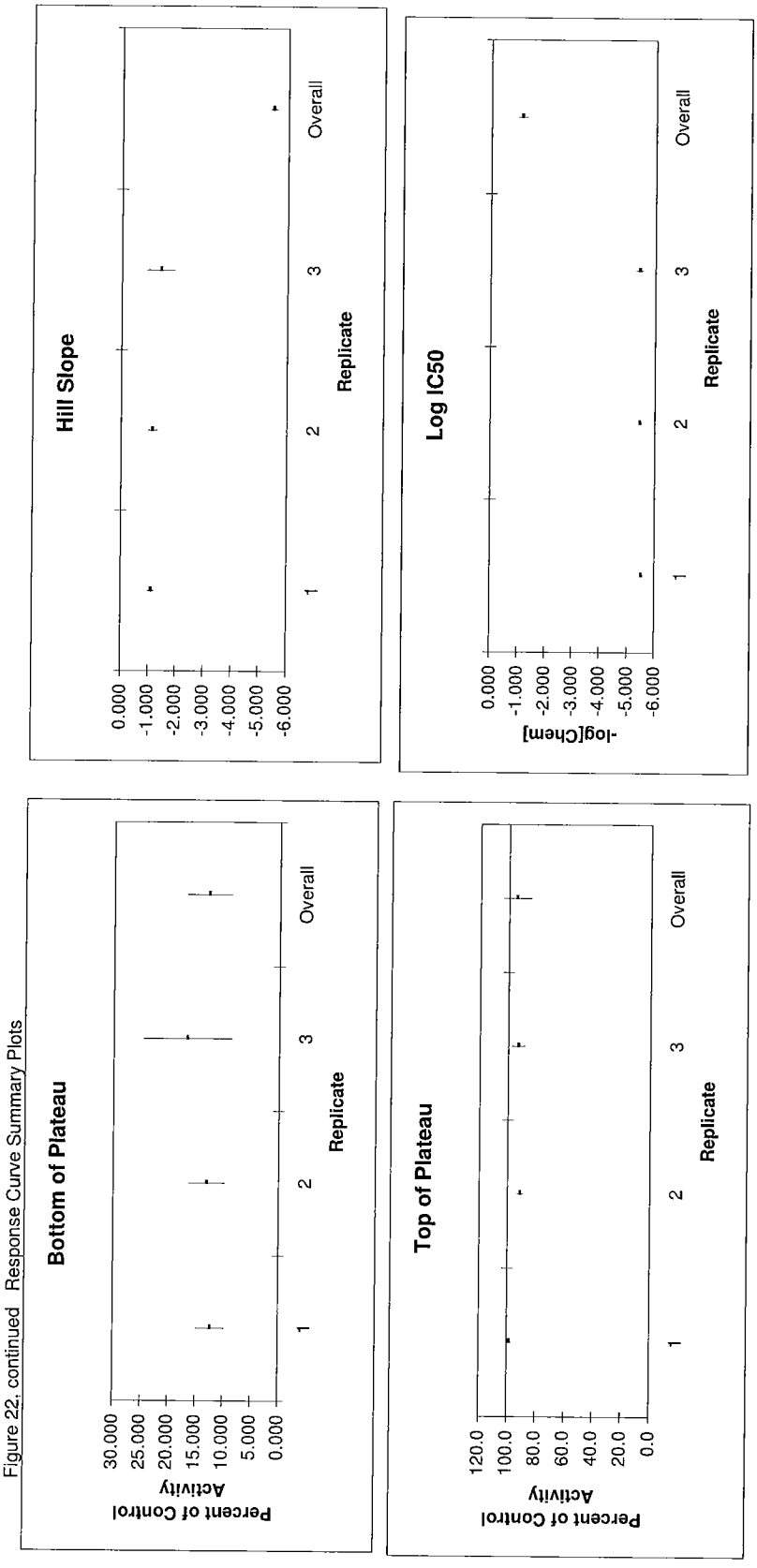


Figure 22, continued Response Curve Summary Plots

Figure 23. Validation of the Recombinant Aromatase Assay (WIL-431011)
Reference Chemical 9-Dicofol

Response Curve Summary Table

		Replicate	Values	Std. Error	95% Confidence Level	
B	Bottom	1	-4.646	2.757	-10.397	1.105
T	Top	1	101.8	0.8	100.1	103.4
μ	LOGEC50	1	-4.565	0.050	-4.670	-4.460
β	HILLSLOPE	1	-0.733	0.048	-0.833	-0.633
EC50	EC50 (μM)	1	27.22		21.39	34.65
B	Bottom	2	-7.329	5.595	-19.000	4.341
T	Top	2	98.829	2.390	93.843	103.815
μ	LOGEC50	2	-4.360	0.084	-4.536	-4.184
β	HILLSLOPE	2	-0.730	0.085	-0.908	-0.553
EC50	EC50 (μM)	2	43.67		29.11	65.52
B	Bottom	3	-9.718	5.239	-20.647	1.211
T	Top	3	102.721	2.055	98.434	107.009
μ	LOGEC50	3	-4.310	0.074	-4.464	-4.155
β	HILLSLOPE	3	-0.715	0.070	-0.860	-0.569
EC50	EC50 (μM)	3	49.00		34.32	69.95

Summary of Statistical Analysis Results

		Values	Std. Error	95% Confidence Level	
Bottom	Overall	-5.999	2.236	-15.621	3.624
Top	Overall	101.615	0.691	98.644	104.586
LOGEC50	Overall	-4.421	0.083	-4.777	-4.064
HILLSLOPE	Overall	-0.728	0.036	-0.882	-0.573

Mathematical Averages

AVG LOGEC50	-4.412			
AVG HILLSLOPE	-0.726			
AVG T	101.100			
AVG B	-7.231			
		sd	sem	%CV
Avg EC50	39.96	11.35	6.56	28.41

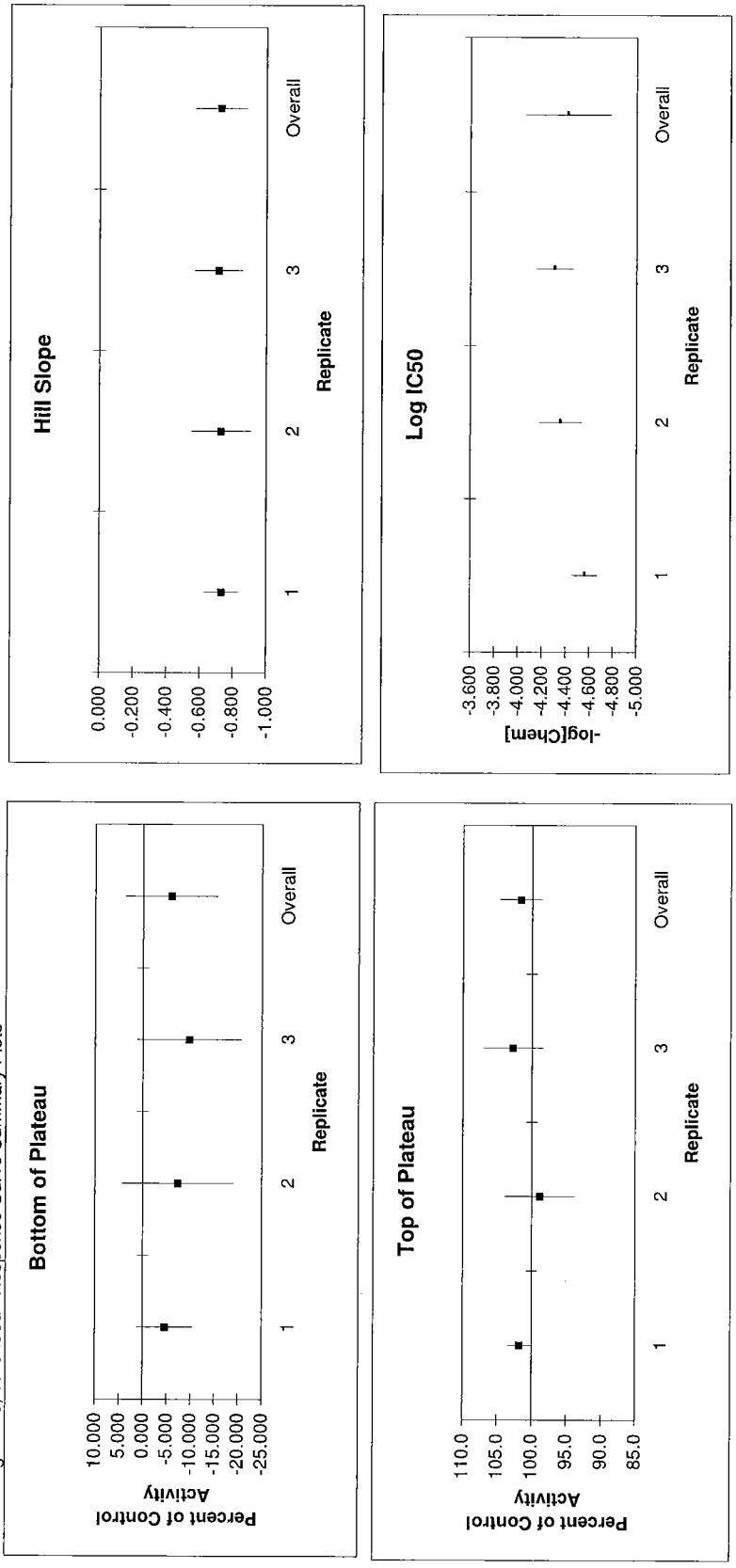


Figure 23, continued Response Curve Summary Plots

Appendix A

Study Protocol (with Amendments)

Study Number: WIL-431011

PROTOCOL AMENDMENT I

Sponsor: Battelle Memorial Institute

EPA Contract No.: 68-W-01-023

A. Title of Study:

Conduct Multiple Chemical Studies with Recombinant Microsomes (WA 4-17, Task 4)

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-431011

PROTOCOL AMENDMENT II

Sponsor: Battelle Memorial Institute

EPA Contract No.: 68-W-01-023

A. Title of Study:

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

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:

$$Y = B + \frac{T - B}{1 + 10^{(X-\mu)*H}}$$

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:

$$Y = B + \frac{T - B}{1 + 10^{(\mu-X)*H}}$$

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 4-17, TASK 4: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES

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 4: The objectives of this protocol are to test 10 reference chemicals using human recombinant microsomes obtained from insect cells, and to determine the intra- and interlaboratory variability in the test results.

The test system for this study is human recombinant 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 a human microsome 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 reference 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:

Jerry D. Johnson, PhD, DABT
Work Assignment Leader/Study Monitor
Endocrine Disruptor Screening Program
Battelle Memorial Institute
Tel: (614) 424-4499
Fax: (614) 424-5221
Email: johnsojd@battelle.org

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:

Jennifer Thomas-Wohlever, PhD
Research Scientist
Tel: (419) 289-8700
Fax: (419) 289-3650
Email: jwohlever@wilresearch.com

2.4 WIL Deputy Director:

Justin Godsey, BS, LATG
Biologist, Metabolism

2.5 WIL Staff Involved with Study:

Joseph F. Holson, PhD
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 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 dimethylsulfoxide (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 4, 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 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 to 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.

5 ASSAY MATERIALS RECEIPT AND/OR PREPARATION:

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

Recombinant microsomes will be supplied to each laboratory by the lead laboratory (RTI International). The recombinant microsomes are a product of Gentest™ (Woburn, MA). The product name is Human CYP19 (Aromatase) Supersomes™ and the catalog number is 456260. The Supersomes™ package size is 0.5 nmoles cytochrome P450 in 0.5 mL. These are the same recombinant microsomes used in a previous positive control study (WIL-431010). Supplier-provided values for protein concentration, Cytochrome c reductase activity, and aromatase activity will be found on the data sheet accompanying each shipment and will be included in the report. 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.

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 recombinant microsomes are supplied in aliquots in excess of what is required to conduct a single experiment, they will be thawed, pooled, homogenized, divided into appropriate aliquots for conduct of a single experiment and refrozen as described below in order to minimize and standardize the number of freeze/thaw cycles each preparation undergoes. Microsomes will be thawed quickly in a 37 ± 1 °C water bath and then will be immediately transferred to an ice bath. The microsomes will be pooled and rehomogenized using a Potter-Elvejhem homogenizer (about 5–10 passes). The pooled sample will be aliquoted into portions appropriate for use in a single 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 0.008 mg/mL. The addition of 1 mL of that microsome dilution will result in a final approximate protein concentration of 0.004 mg/mL in the assay tubes. All microsome samples must be kept on ice until they are placed in the water bath just prior to their addition to the aromatase assay. Microsomes are not to be left on ice for longer than approximately 1 h before proceeding with the assay. Appropriate documentation of time from thaw to use must be maintained.

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

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, 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. Recombinant Microsomal Aromatase Assay-Optimized Conditions

Microsomal Protein	0.004 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 mL, and the tubes will be incubated for 15 minutes. 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 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:

Ten inhibitor compounds will be tested, including the four inhibitor compounds in WA 4-16, Task 5 (WIL-431007).

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 4.

Table 4. 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 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 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 intralaboratory 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**.

Bowman, C.J. Validation of the Recombinant Microsomal Aromatase Assay: Positive Control Study (WA 4-17, Task 3) (Study No. WIL-431010). 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**.

16 PROTOCOL APPROVAL:

Sponsor approval received via email on 7/6/05
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. Syed, 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

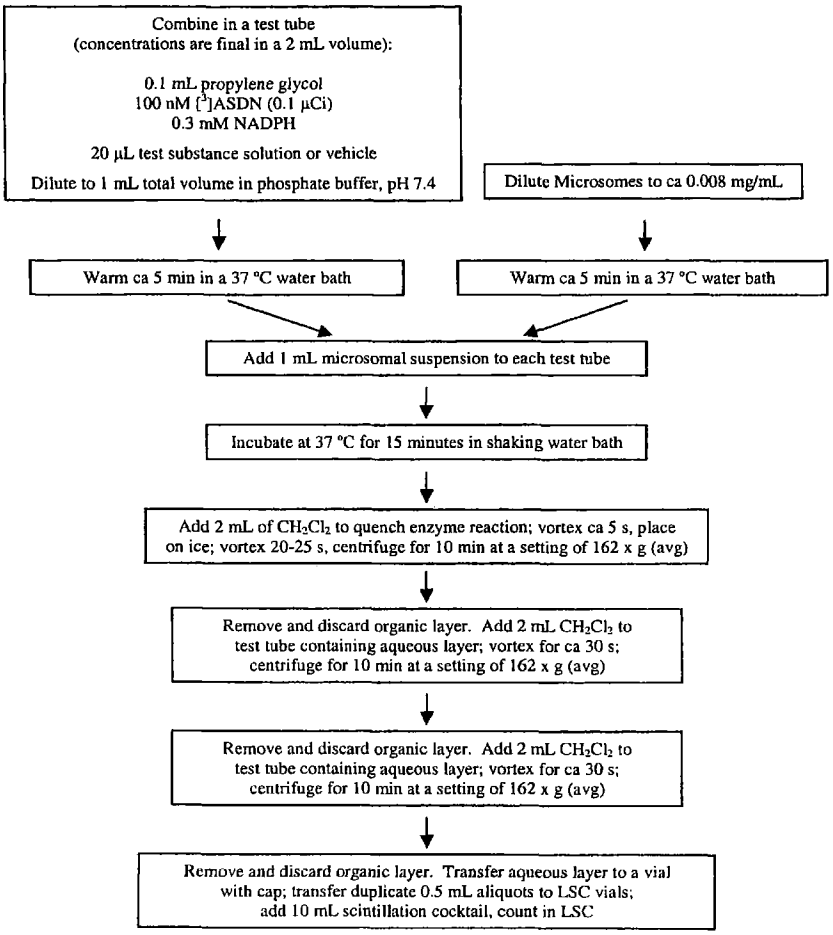
David P. Houchens 7/11/05
David P. Houchens, PhD Date
Program Manager
Endocrine Disruptor Screening Program
Battelle Memorial Institute

17 PROTOCOL REVIEW:

Heather L Osborn 7/12/05
Heather L. Osborn, BS, RQAP-GLP Date
Manager, Quality Assurance
WIL Research Laboratories, LLC

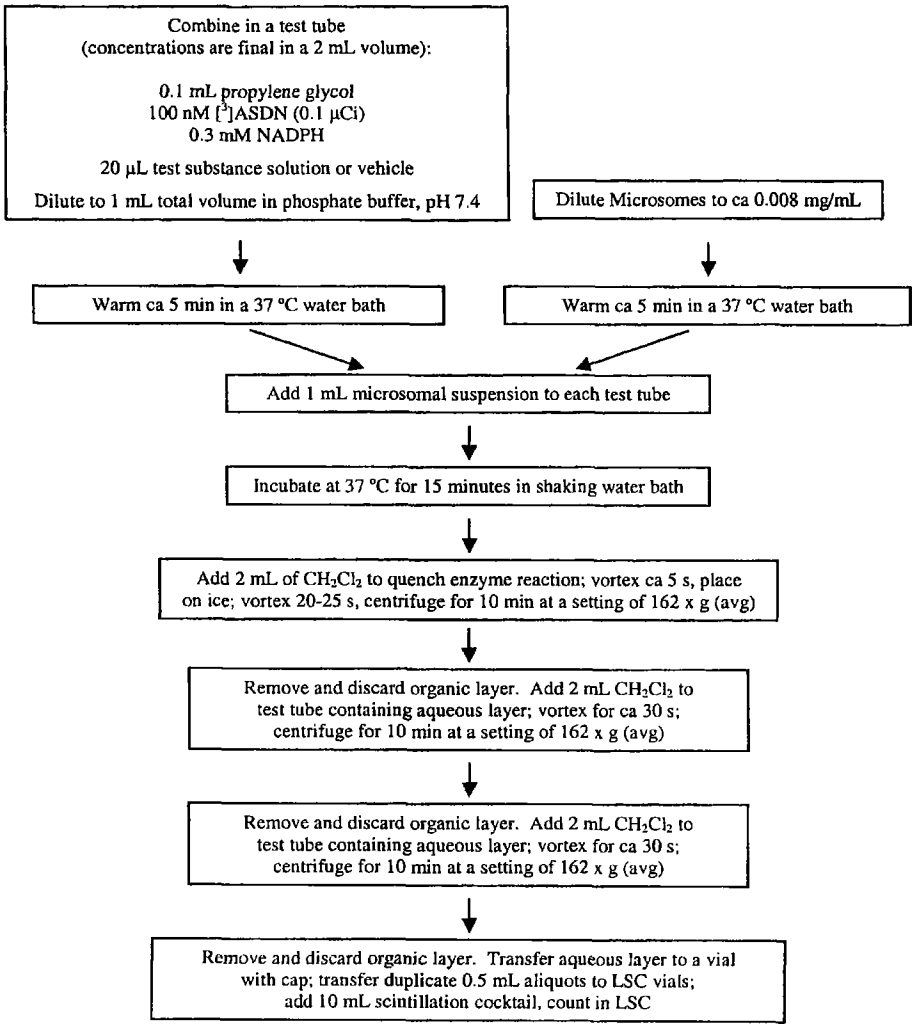
Terri L Pollock 7-11-05
Terri L. Pollock, BA Date
Quality Assurance Manager
Endocrine Disruptor Screening Program
Battelle Memorial Institute

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 0.008 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 CH_2Cl_2 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 CH_2Cl_2 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 CH_2Cl_2 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 0.008 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:

J Mathews 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).

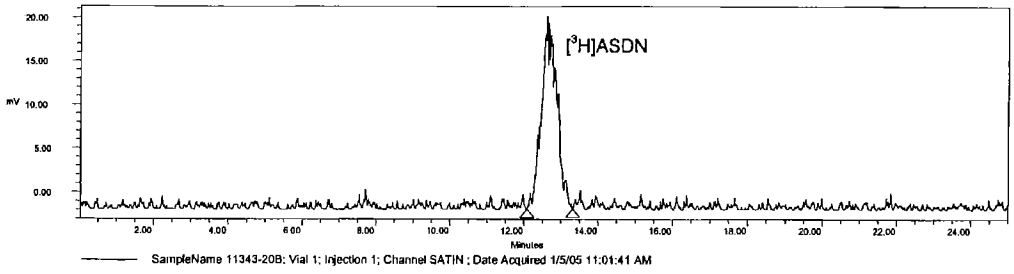
The radiochemical purity of the [^{3}H]ASDN (1:100 dilution in ethanol) was determined using high performance liquid chromatography (HPLC) and liquid scintillation counting. The HPLC system consists of a Waters 2690 Separations Module, a Waters 2487 Dual λ Absorbance Detector and a β-RAM Model 3 flow-through 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	7/21/2005	Test Chemical ID	Chem 1	# Concentrations tested	8
ID	JG	Replicate #	Rep 1	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	23477.06	1179752	
2	0.0201	30715.31	1528125	
3	0.0200	30749.59	1537480	
4	0.0200	30928.34	1546417	
5	0.0201	31292.56	1556844	
			Average DPM/g soln	1469723
			SD	162448
			CV	11.05
			µCi/g soln	0.662

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	8.1486 g
Mass of dilution B used in substrate prep	4.6003 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.581487 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00749 µg/g soln.
µg/g soln.

a. µCi/g soln 0.662
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.

= 0.581487 + 0.00749
= 0.588982 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.124 µCi/µg ASDN

714672 dpm/nmol

Assay Date 7/21/2005 Test Chemical ID Chem 1 tested 8

ID JG Replicate # Rep 1 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.555	0.325	0.163	0.096	0.053	0.037	0.033	25	100	
	0.562	0.342	0.161	0.097	0.053	0.039	0.031			
	0.565	0.346	0.165	0.098	0.054	0.041	0.028			
Samples:	10	100	microsomes							
	0.057	0.278	0.052							
	0.058	0.283	0.051							
			0.052							

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	0.561	0.530	0.0483
0.125	100	200	0.00013	200	0.0250	0.338	0.307	0.0275
0.05	200	1000	0.00005	200	0.0100	0.163	0.133	0.0113
0.025	100	1000	0.00003	200	0.0050	0.097	0.067	0.0051
0.01	40	1000	0.00001	200	0.0020	0.054	0.023	0.0011
0.005	20	1000	0.00001	200	0.0010	0.039	0.008	-0.0003

Blank 0.030

r^2= 0.993
m= 0.093
b= -0.001

Variables	Regression results	
m, b	0.093	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	536	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	0.057	0.027	0.001	200	1	1	0.000	0.000	0.007
10	0.058	0.028	0.002	200	1	1	0.000		
10					1	1			
100	0.278	0.248	0.022	200	1	1	0.000	0.000	0.111
100	0.283	0.253	0.022	200	1	1	0.000		
100									
	0.052	0.022	0.001	200	114	69814	0.003	0.003	2.833
	0.051	0.021	0.001	200	114	69814	0.003		
	0.052	0.022	0.001	200	114	69814	0.003		

Test

Assay Date 7/21/2005 Chemical ID Chem 1 tested 8

ID JG Replicate # Rep 1 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.555	0.325	0.163	0.096	0.053	0.037	0.033	25	100
	0.562	0.342	0.161	0.097	0.053	0.039	0.031		
	0.565	0.346	0.165	0.098	0.054	0.041	0.028		

Protein stock ID

Samples:	10	100	microsomes
	0.057	0.278	0.052
	0.058	0.283	0.051
			0.052

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	0.561	0.530	0.0427
0.125	100	200	0.00013	200	0.0250	0.338	0.307	0.0247
0.05	200	1000	0.00005	200	0.0100	0.163	0.133	0.0106
0.025	100	1000	0.00003	200	0.0050	0.097	0.067	0.0053
0.01	40	1000	0.00001	200	0.0020	0.054	0.023	0.0018
0.005	20	1000	0.00001	200	0.0010	0.039	0.008	0.0006

Blank 0.030

r^2= 0.998
m= 0.081
b= 0.000

Variables	Regression results	
m, b	0.081	0.000
se_m, se_b	0.002	0.000
r , se_y	0.998	0.001
F, df	1529	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	0.027	0.002	200	1	1	0.000	0.000	0.011
10	0.058	0.028	0.002	200	1	1	0.000		
10					1	1			
100	0.278	0.248	0.020	200	1	1	0.000	0.000	0.101
100	0.283	0.253	0.020	200	1	1	0.000		
100									
	0.052	0.022	0.002	200	114	69814	0.005	0.005	5.043
	0.051	0.021	0.002	200	114	69814	0.005		
	0.052	0.022	0.002	200	114	69814	0.005		

Assay Date	7/21/2005	Test Chemical ID	Chem 1	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

								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	7/21/2005	Chemical ID	Chem 1	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.40351	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
	612.40351	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.043
Protein Concentration (dilution added to assay, mg/mL):	0.008235

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	7/21/2005	Test Chemical ID	Chem 1	# Concentrations tested	8	Microsome type		Microsome ID		0 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	9031.54	18063.08	18118.48	36236.96	0.1	146972	24.66	36107	0.0505	1	0.004	15	0.4090
			0.5	2	9086.94	18173.88			0.1					1		15	
	2	2	0.5	1	9080.67	18161.34	18121.75	36243.5	0.1	146972	24.66	36113	0.0505	1	0.004	15	0.4091
			0.5	2	9041.08	18082.16			0.1					1		15	
	3	2	0.5	1	8504.73	17009.46	17010.35	34020.7	0.1	146972	23.15	33890	0.0474	1	0.004	15	0.3839
			0.5	2	8505.62	17011.24			0.1					1		15	
	4	2	0.5	1	8362.88	16725.76	16720.89	33441.78	0.1	146972	22.75	33312	0.0466	1	0.004	15	0.3774
			0.5	2	8358.01	16716.02			0.1					1		15	
Background control	1	2	0.5	1	33.32	66.64	64.06	128.12	0.1	146972	0.09	-2	0.0000	1	0.004	15	0.0000
			0.5	2	30.74	61.48			0.1					1		15	
	2	2	0.5	1	33.60	67.2	69.53	139.06	0.1	146972	0.09	9	0.0000	1	0.004	15	0.0001
			0.5	2	35.93	71.86			0.1					1		15	
	3	2	0.5	1	32.45	64.9	70.91	141.82	0.1	146972	0.10	12	0.0000	1	0.004	15	0.0001
			0.5	2	36.46	76.92			0.1					1		15	
	4	2	0.5	1	24.81	49.62	55.96	111.96	0.1	146972	0.08	-18	0.0000	1	0.004	15	-0.0002
			0.5	2	31.17	62.34			0.1					1		15	
Positive control	1	2	0.5	1	4802.04	9604.08	9544.22	19088.44	0.1	146972	12.99	18958	0.0265	1	0.004	15	0.2148
			0.5	2	4742.18	9484.36			0.1					1		15	
	2	2	0.5	1	4705.09	9410.18	9355.96	18711.92	0.1	146972	12.73	18582	0.0260	1	0.004	15	0.2105
			0.5	2	4650.87	9301.74			0.1					1		15	
	3	2	0.5	1	4070.73	8141.46	8180.24	16360.48	0.1	146972	11.13	16230	0.0227	1	0.004	15	0.1839
			0.5	2	4109.51	8219.02			0.1					1		15	
	4	2	0.5	1	3721.24	7442.48	7425.12	14850.24	0.1	146972	10.10	14720	0.0206	1	0.004	15	0.1667
			0.5	2	3703.88	7407.76			0.1					1		15	
Negative Control	1	2	0.5	1	8834.52	17669.04	17539.07	35078.14	0.1	146972	23.87	34948	0.0489	1	0.004	15	0.3959
			0.5	2	8704.55	17409.1			0.1					1		15	
	2	2	0.5	1	8240.04	16480.08	16508.72	33017.44	0.1	146972	22.47	32887	0.0460	1	0.004	15	0.3725
			0.5	2	8268.68	16537.36			0.1					1		15	
	3	2	0.5	1	7667.03	15334.06	15327.91	30655.82	0.1	146972	20.86	30526	0.0427	1	0.004	15	0.3458
			0.5	2	7660.88	15321.76			0.1					1		15	
	4	2	0.5	1	7558.64	15117.28	14883.55	29767.1	0.1	146972	20.25	29637	0.0415	1	0.004	15	0.3357
			0.5	2	7324.91	14649.82			0.1					1		15	
Chem 1	1-1	2	0.5	1	513.43	1026.86	1055.96	2111.92	0.1	146972	1.44	1982	0.0028	1	0.004	15	0.0224
			0.5	2	542.53	1085.06			0.1					1		15	
	1-2	2	0.5	1	535.34	1070.68	1060.22	2120.44	0.1	146972	1.44	1990	0.0028	1	0.004	15	0.0225
			0.5	2	524.88	1049.76			0.1					1		15	
	1-3	2	0.5	1	509.80	1019.6	1046.73	2093.46	0.1	146972	1.42	1963	0.0027	1	0.004	15	0.0222
			0.5	2	536.93	1073.86			0.1					1		15	
	2-1	2	0.5	1	3825.88	7651.76	7602	15204	0.1	146972	10.34	15074	0.0211	1	0.004	15	0.1708
			0.5	2	3776.12	7552.24			0.1					1		15	
	2-2	2	0.5	1	3677.44	7354.88	7292.76	14585.52	0.1	146972	9.92	14455	0.0202	1	0.004	15	0.1637
			0.5	2	3615.32	7230.64			0.1					1		15	
	2-3	2	0.5	1	3741.23	7482.46	7437.93	14875.86	0.1	146972	10.12	14746	0.0206	1	0.004	15	0.1670
			0.5	2	3696.70	7393.4			0.1					1		15	
	3-1	2	0.5	1	7748.95	15497.9	15490.95	30981.9	0.1	146972	21.08	30852	0.0432	1	0.004	15	0.3495
			0.5	2	7742.00	15484			0.1					1		15	
	3-2	2	0.5	1	7870.72	15741.44	15742.86	31485.72	0.1	146972	21.42	31355	0.0439	1	0.004	15	0.3552
			0.5	2	7872.14	15744.28			0.1					1		15	
	3-3	2	0.5	1	7518.43	15036.86	15110.75	30221.5	0.1	146972	20.56	30091	0.0421	1	0.004	15	0.3409
			0.5	2	7592.32	15184.64			0.1					1		15	
	4-1	2	0.5	1	8799.18	17598.36	17730.14	35460.28	0.1	146972	24.13	35330	0.0494	1	0.004	15	0.4002
			0.5	2	8930.96	17861.92			0.1					1		15	
	4-2	2	0.5	1	8815.32	17630.64	17635.94	35271.88	0.1	146972	24.00	35142	0.0492	1	0.004	15	0.3981
			0.5	2	8820.62	17641.24			0.1					1		15	
	4-3	2	0.5	1	8888.39	17776.78	17666.47	35332.94	0.1	146972	24.04	35203	0.0493	1	0.004	15	0.3988
			0.5	2	8777.08	17554.16			0.1					1		15	
	5-1	2	0.5	1	8663.52	17339.04	17482.92	34965.84	0.1	146972	23.79	34836	0.0487	1	0.004	15	0.3946
			0.5	2	8813.40	17626.8			0.1					1		15	
	5-2	2	0.5	1	9160.04	18320.08	18360.86	36721.72	0.1	146972	24.99	36591	0.0512	1	0.004	15	0.4145
			0.5	2	9200.82	18401.64			0.1					1		15	
	5-3	2	0.5	1	9212.39	18424.78	18412.6	36825.2	0.1	146972	25.06	36695	0.0513	1	0.004	15	0.4157
			0.5	2	9200.21	18400.42			0.1					1		15	
	6-1	2	0.5	1	9137.25	18274.5	18323.65	36647.3	0.1	146972	24.93	36517	0.0511	1	0.004	15	0.4137
			0.5	2	9186.40	18372.8			0.1					1		15	
	6-2	2	0.5	1	9161.46	18322.92	18378.88	36757.76	0.1	146972	25.01	36628	0.0513	1	0.004	15	0.4149
			0.5	2	9217.42	18434.84			0.1					1		15	
	6-3	2	0.5	1	9068.53	18137.06	18140.15	36280.3	0.1	146972	24.69	36150	0.0506	1	0.004	15	0.4095
			0.5	2	9071.62	18143.24			0.1					1		15	
	7-1	2	0.5	1	8960.93	17921.86	17953.71	35907.42	0.1	146972	24.43	35777	0.0501	1	0.004	15	0.4053
			0.5	2	8992.78	17985.56			0.1					1		15	
	7-2	2	0.5	1	9266.40	18532.8	18558.76	37117.52	0.1	146972	25.25	36987	0.0518	1	0.004	15	0.4190
			0.5	2	9292.36	18584.72			0.1					1		15	
	7-3	2	0.5	1	8767.11	17534.22	17682.31	35364.62	0.1	146972	24.06	35234	0.0493	1	0.004	15	0.3991
			0.5	2	8915.20	17830.4			0.1					1		15	
	8-1	2	0.5	1	8867.81	17735.62	17757.38	35514.76	0.1	146972	24.16	35385	0.0495	1	0.004	15	0.4008
			0.5	2	8889.57	17779.14			0.1					1		15	
	8-2	2	0.5	1	8861.49	17722.98	17637.85	35275.7	0.1	146972	24.00	35145	0.0492	1	0.004	15	0.3981
			0.5	2	8776.36	17552.72			0.1					1		15	
	8-3	2	0.5	1	9316.06	18632.12	18692.64	37385.28	0.1	146972	25.44	37255	0.0521	1	0.004	15	0.4220
			0.5	2	9376.58	18753.16			0.1					1		15	

Assay Date	7/21/2005	ID	Chem 1	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4091	0.0001
Full activity	End	0.3806	0.0046
Full activity	Overall	0.3948	0.0166
Background	Beginning	0.0000	8.76301E-05
Background	End	0.0000	0.00023918
Background	Overall	0.0000	0.000153456
Positive	Beginning	0.2126	0.0030
Positive	End	0.1753	0.0121
Positive	Overall	0.1940	0.0227
Negative	Beginning	0.3842	0.0165
Negative	End	0.3408	0.0071
Negative	Overall	0.3625	0.0272

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Chem 1	1	1	1.00E-03	-3.00	0.0224
Chem 1	1	2	1.00E-03	-3.00	0.0225
Chem 1	1	3	1.00E-03	-3.00	0.0222
Chem 1	2	1	1.00E-04	-4.00	0.1708
Chem 1	2	2	1.00E-04	-4.00	0.1637
Chem 1	2	3	1.00E-04	-4.00	0.1670
Chem 1	3	1	1.00E-05	-5.00	0.3495
Chem 1	3	2	1.00E-05	-5.00	0.3552
Chem 1	3	3	1.00E-05	-5.00	0.3409
Chem 1	4	1	1.00E-06	-6.00	0.4002
Chem 1	4	2	1.00E-06	-6.00	0.3981
Chem 1	4	3	1.00E-06	-6.00	0.3988
Chem 1	5	1	1.00E-07	-7.00	0.3946
Chem 1	5	2	1.00E-07	-7.00	0.4145
Chem 1	5	3	1.00E-07	-7.00	0.4157
Chem 1	6	1	1.00E-08	-8.00	0.4137
Chem 1	6	2	1.00E-08	-8.00	0.4149
Chem 1	6	3	1.00E-08	-8.00	0.4095
Chem 1	7	1	1.00E-09	-9.00	0.4053
Chem 1	7	2	1.00E-09	-9.00	0.4190
Chem 1	7	3	1.00E-09	-9.00	0.3991
Chem 1	8	1	1.00E-10	-10.00	0.4008
Chem 1	8	2	1.00E-10	-10.00	0.3981
Chem 1	8	3	1.00E-10	-10.00	0.4220

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	5.69	5.71	5.63
2	-4.00	43.25	41.47	42.31
3	-5.00	88.51	89.96	86.33
4	-6.00	101.36	100.82	101.00
5	-7.00	99.94	104.98	105.28
6	-8.00	104.77	105.08	103.71
7	-9.00	102.64	106.12	101.09
8	-10.00	101.52	100.83	106.88

Aromatase Assay Spreadsheet

Assay Date	7/25/2005	Test Chemical ID	RC1	# Concentrations tested	8
ID	JG	Replicate #	Rep 2	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	25877.9	1300397	
2	0.0201	27083.59	1347442	
3	0.0200	27592.72	1379636	
4	0.0200	28221.74	1411087	
5	0.0200	27874.29	1393715	
			Average DPM/g soln	1366455
			SD	43686
			CV	3.20
			µCi/g soln	0.616

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	8.1393 g
Mass of dilution B used in substrate prep	4.5734 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56751 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00697 µg/g soln.
µg/g soln.

a. µCi/g soln	0.616
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.

= 0.567510 + 0.00697
= 0.574478 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.071 µCi/µg ASDN

681232 dpm/nmol

Test

Assay Date 7/25/2005 Chemical ID RC1 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.557	0.335	0.157	0.089	0.055	0.038	0.027	25	100	
	0.569	0.344	0.164	0.091	0.051	0.040	0.027			
	0.568	0.338	0.159	0.098	0.050	0.044	0.027			

Samples:	10	100	microsomes
	0.054	0.281	0.047
	0.051	0.268	0.048
			0.045

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	0.565	0.538	0.0484
0.125	100	200	0.00013	200	0.0250	0.339	0.312	0.0275
0.05	200	1000	0.00005	200	0.0100	0.160	0.133	0.0111
0.025	100	1000	0.00003	200	0.0050	0.092	0.065	0.0048
0.01	40	1000	0.00001	200	0.0020	0.052	0.025	0.0011
0.005	20	1000	0.00001	200	0.0010	0.041	0.014	0.0001

Blank 0.027

Variables	Regression results	
m, b	0.092	-0.001
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

r^2= 0.993
m= 0.092
b= -0.001

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	0.054	0.027	0.001	200	1	1	0.000	0.000	0.006
10	0.051	0.024	0.001	200	1	1	0.000		
10					1	1			
100	0.281	0.253	0.022	200	1	1	0.000	0.000	0.108
100	0.268	0.241	0.021	200	1	1	0.000		
100									
	0.047	0.019	0.001	200	114	69814	0.002	0.002	1.882
	0.048	0.021	0.001	200	114	69814	0.002		
	0.045	0.018	0.000	200	114	69814	0.001		

Test

Assay Date 7/25/2005 Chemical ID RC1 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.557	0.335	0.157	0.089	0.055	0.038	0.027	25	100	
	0.569	0.344	0.164	0.091	0.051	0.040	0.027			
	0.568	0.338	0.159	0.098	0.050	0.044	0.027			

Samples:	10	100	microsomes
	0.054	0.281	0.047
	0.051	0.268	0.048
			0.045

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	0.565	0.538	0.0430	m, b	0.080	0.000
0.125	100	200	0.00013	200	0.0250	0.339	0.312	0.0248	se_m, se_b	0.001	0.000
0.05	200	1000	0.00005	200	0.0100	0.160	0.133	0.0105	r , se_y	0.999	0.000
0.025	100	1000	0.00003	200	0.0050	0.092	0.065	0.0050	F, df	3851	3
0.01	40	1000	0.00001	200	0.0020	0.052	0.025	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.041	0.014	0.0009			

Regression results are calculated using the function LINEST

Blank 0.027

r^2= 0.999
m= 0.080
b= 0.000

	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	0.054	0.027	0.002	200	1	1	0.000	0.000	0.009
10	0.051	0.024	0.002	200	1	1	0.000		
10					1	1			
100	0.281	0.253	0.020	200	1	1	0.000	0.000	0.098
100	0.268	0.241	0.019	200	1	1	0.000		
100									
	0.047	0.019	0.001	200	114	69814	0.004	0.004	4.138
	0.048	0.021	0.001	200	114	69814	0.004		
	0.045	0.018	0.001	200	114	69814	0.004		

Assay Date 7/25/2005 Test Chemical ID RC1 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

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	7/25/2005	Chemical ID	RC1	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.403509	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
	612.403509	total dilution factor

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

Protein Concentration (stock microsomes, mg/mL):	4.138
Protein Concentration (dilution added to assay, mg/mL):	0.006757

Assay Date	7/25/2005	Test Chemical ID	RC1	# Concentrations tested	8	Microsome type		Microsome ID		0 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	14453.62	28907.24	30224.43	60448.86	0.1	136646	44.24	60365	0.0886	1	0.003	15	0.8743
			0.5	2	15770.81	31541.62			0.1					1		15	
	2	2	0.5	1	15867.74	31735.48	31663.74	63327.48	0.1	136646	46.34	63244	0.0928	1	0.003	15	0.9160
			0.5	2	15796	31592			0.1					1		15	
	3	2	0.5	1	15629.07	31258.14	30584.52	61168.04	0.1	136646	44.76	61086	0.0897	1	0.003	15	0.8847
			0.5	2	14955.45	29910.9			0.1					1		15	
	4	2	0.5	1	14411.27	28822.54	29161.69	58363.38	0.1	136646	42.71	58280	0.0856	1	0.003	15	0.8441
			0.5	2	14770.42	29540.84			0.1					1		15	
Background control	1	2	0.5	1	19.59	39.18	41.84	83.68	0.1	136646	0.06	0	0.0000	1	0.003	15	0.0000
			0.5	2	22.25	44.5			0.1					1		15	
	2	2	0.5	1	23.78	47.56	32.95	65.9	0.1	136646	0.05	-18	0.0000	1	0.003	15	-0.0003
			0.5	2	9.17	18.34			0.1					1		15	
	3	2	0.5	1	22.09	44.18	48.06	96.12	0.1	136646	0.07	13	0.0000	1	0.003	15	0.0002
			0.5	2	25.97	51.94			0.1					1		15	
	4	2	0.5	1	24.35	48.7	44.11	88.22	0.1	136646	0.06	5	0.0000	1	0.003	15	0.0001
			0.5	2	19.76	39.52			0.1					1		15	
Positive control	1	2	0.5	1	5809.5	11619	11864.27	23728.54	0.1	136646	17.37	23645	0.0347	1	0.003	15	0.3425
			0.5	2	6054.77	12109.54			0.1					1		15	
	2	2	0.5	1	5841.33	11682.66	11799.87	23599.74	0.1	136646	17.27	23516	0.0345	1	0.003	15	0.3406
			0.5	2	5958.54	11917.08			0.1					1		15	
	3	2	0.5	1	5320.27	10640.54	10887.43	21774.86	0.1	136646	15.94	21691	0.0318	1	0.003	15	0.3142
			0.5	2	5567.16	11134.32			0.1					1		15	
	4	2	0.5	1	5055.07	10110.14	10115.62	20231.24	0.1	136646	14.81	20148	0.0296	1	0.003	15	0.2918
			0.5	2	5060.55	10121.1			0.1					1		15	
Negative Control	1	2	0.5	1	15179.1	30358.2	30566.02	61132.04	0.1	136646	44.74	61049	0.0896	1	0.003	15	0.8842
			0.5	2	15386.92	30773.84			0.1					1		15	
	2	2	0.5	1	15325.2	30650.4	30322.84	60645.68	0.1	136646	44.38	60562	0.0889	1	0.003	15	0.8771
			0.5	2	14897.64	29995.29			0.1					1		15	
	3	2	0.5	1	14442.74	28885.48	28145.49	56290.98	0.1	136646	41.19	56208	0.0825	1	0.003	15	0.8141
			0.5	2	13702.75	27405.5			0.1					1		15	
	4	2	0.5	1	12858.37	25716.74	25936.62	51873.24	0.1	136646	37.96	51790	0.0760	1	0.003	15	0.7501
			0.5	2	13078.25	26156.5			0.1					1		15	
RC1	1-1	2	0.5	1	613.28	1226.56	1186.95	2373.9	0.1	136646	1.74	2290	0.0034	1	0.003	15	0.0332
			0.5	2	573.67	1147.34			0.1					1		15	
	1-2	2	0.2	1	217.76	1088.8	1133.55	2267.1	0.1	136646	1.66	2184	0.0032	1	0.003	15	0.0316
			0.2	2	235.66	1178.3			0.1					1		15	
	1-3	2	0.5	1	591	1182	1200.52	2401.04	0.1	136646	1.76	2318	0.0034	1	0.003	15	0.0336
			0.5	2	609.52	1219.04			0.1					1		15	
	2-1	2	0.5	1	1393.51	2787.02	2777.09	5554.18	0.1	136646	4.06	5471	0.0080	1	0.003	15	0.0792
			0.5	2	1383.58	2767.16			0.1					1		15	
	2-2	2	0.2	1	548.85	2744.25	2744.6	5489.2	0.1	136646	4.02	5406	0.0079	1	0.003	15	0.0783
			0.2	2	548.99	2744.95			0.1					1		15	
	2-3	2	0.5	1	1374.37	2748.74	2779	5558	0.1	136646	4.07	5475	0.0080	1	0.003	15	0.0793
			0.5	2	1404.63	2809.26			0.1					1		15	
	3-1	2	0.5	1	4918.31	9832.62	9851.19	19702.38	0.1	136646	14.42	19619	0.0288	1	0.003	15	0.2841
			0.5	2	4934.88	9869.76			0.1					1		15	
	3-2	2	0.2	1	2005.01	10025.05	10058.425	20116.85	0.1	136646	14.72	20033	0.0294	1	0.003	15	0.2901
			0.2	2	2018.36	10091.8			0.1					1		15	
	3-3	2	0.5	1	5296.97	10593.94	10570.67	21141.34	0.1	136646	15.47	21058	0.0309	1	0.003	15	0.3050
			0.5	2	5273.7	10547.4			0.1					1		15	
	4-1	2	0.5	1	7519.31	15038.62	14826.08	29652.16	0.1	136646	21.70	29569	0.0434	1	0.003	15	0.4282
			0.5	2	7306.77	14613.54			0.1					1		15	
	4-2	2	0.2	1	2914.99	14574.95	14430.8	28861.6	0.1	136646	21.12	28778	0.0422	1	0.003	15	0.4168
			0.2	2	2857.33	14286.65			0.1					1		15	
	4-3	2	0.5	1	6962.72	13925.44	13994.67	27989.34	0.1	136646	20.48	27906	0.0410	1	0.003	15	0.4042
			0.5	2	7031.95	14063.9			0.1					1		15	
	5-1	2	0.5	1	12359.96	24719.92	24694.21	49388.42	0.1	136646	36.14	49305	0.0724	1	0.003	15	0.7141
			0.5	2	12334.25	24668.5			0.1					1		15	
	5-2	2	0.2	1	4896.74	24483.7	24324.5	48649	0.1	136646	35.60	48566	0.0713	1	0.003	15	0.7034
			0.2	2	4833.06	24165.3			0.1					1		15	
	5-3	2	0.5	1	12380.65	24761.3	24794.57	49588.14	0.1	136646	36.29	49506	0.0727	1	0.003	15	0.7170
			0.5	2	12413.92	24827.84			0.1					1		15	
	6-1	2	0.5	1	15296.05	30596.1	30357.56	60715.12	0.1	136646	44.43	60632	0.0890	1	0.003	15	0.8781
			0.5	2	15059.51	30119.02			0.1					1		15	
	6-2	2	0.2	1	5842.04	29210.2	29149.275	58298.55	0.1	136646	42.66	58215	0.0855	1	0.003	15	0.8431
			0.2	2	5817.67	29088.35			0.1					1		15	
	6-3	2	0.5	1	15350.54	30701.08	30882.08	61764.16	0.1	136646	45.20	61681	0.0905	1	0.003	15	0.8933
			0.5	2	15531.54	31063.08			0.1					1		15	
	7-1	2	0.5	1	15562.6	31125.2	31404.72	62809.44	0.1	136646	45.97	62726	0.0921	1	0.003	15	0.9085
			0.5	2	15842.12	31684.24			0.1					1		15	
	7-2	2	0.2	1	6175.15	30875.75	30838.925	61677.85	0.1	136646	45.14	61594	0.0904	1	0.003	15	0.8921
			0.2	2	6160.42	30802.1			0.1					1		15	
	7-3	2	0.5	1	15443.29	30886.58	31068.12	62136.24	0.1	136646	45.47	62053	0.0911	1	0.003	15	0.8987
			0.5	2	15624.83	31249.66			0.1					1		15	
	8-1	2	0.5	1	15006.61	30013.22	30176.33	60352.66	0.1	136646	44.17	60269	0.0885	1	0.003	15	0.8729
			0.5	2	15169.72	30339.44			0.1					1		15	
	8-2	2	0.2	1	5748.27	28741.35	29541.925	59083.85	0.1	136646	43.24	59000	0.0866	1	0.003	15	0.8545
			0.2	2	6068.5	30342.5			0.1					1		15	
	8-3	2	0.5	1	14878.26	29756.52	30242.87	60485.74	0.1	136646	44.26	60402	0.0887	1	0.003	15	0.8748
			0.5	2	15364.61	30729.22			0.1					1		15	

Assay Date	7/25/2005	ID	RC1	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.8951	0.0295
Full activity	End	0.8644	0.0287
Full activity	Overall	0.8798	0.0297
Background	Beginning	-0.0001	0.000182086
Background	End	0.0001	8.09045E-05
Background	Overall	0.0000	0.000185348
Positive	Beginning	0.3415	0.0013
Positive	End	0.3030	0.0158
Positive	Overall	0.3223	0.0241
Negative	Beginning	0.8806	0.0050
Negative	End	0.7821	0.0452
Negative	Overall	0.8314	0.0627

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC1	1	1	1.00E-03	-3.00	0.0332
RC1	1	2	1.00E-03	-3.00	0.0316
RC1	1	3	1.00E-03	-3.00	0.0336
RC1	2	1	5.00E-04	-3.30	0.0792
RC1	2	2	5.00E-04	-3.30	0.0783
RC1	2	3	5.00E-04	-3.30	0.0793
RC1	3	1	1.00E-04	-4.00	0.2841
RC1	3	2	1.00E-04	-4.00	0.2901
RC1	3	3	1.00E-04	-4.00	0.3050
RC1	4	1	5.00E-05	-4.30	0.4282
RC1	4	2	5.00E-05	-4.30	0.4168
RC1	4	3	5.00E-05	-4.30	0.4042
RC1	5	1	1.00E-05	-5.00	0.7141
RC1	5	2	1.00E-05	-5.00	0.7034
RC1	5	3	1.00E-05	-5.00	0.7170
RC1	6	1	1.00E-06	-6.00	0.8781
RC1	6	2	1.00E-06	-6.00	0.8431
RC1	6	3	1.00E-06	-6.00	0.8933
RC1	7	1	1.00E-07	-7.00	0.9085
RC1	7	2	1.00E-07	-7.00	0.8921
RC1	7	3	1.00E-07	-7.00	0.8987
RC1	8	1	1.00E-09	-9.00	0.8729
RC1	8	2	1.00E-09	-9.00	0.8545
RC1	8	3	1.00E-09	-9.00	0.8748

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	3.77	3.59	3.82
2	-3.30	9.01	8.90	9.01
3	-4.00	32.30	32.98	34.67
4	-4.30	48.68	47.38	45.94
5	-5.00	81.17	79.95	81.50
6	-6.00	99.82	95.84	101.54
7	-7.00	103.26	101.40	102.16
8	-9.00	99.22	97.13	99.44

Aromatase Assay Spreadsheet

Assay Date	7/27/2005	Test Chemical ID	1	# Concentrations tested	8
ID	JG	Replicate #	Rep 3	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	28938.48	1461539	
2	0.0199	29839.25	1499460	
3	0.0199	31503.05	1583068	
4	0.0202	31810.71	1574788	
5	0.0199	31915.46	1603792	
			Average DPM/g soln	1544529
			SD	60892
			CV	3.94
			µCi/g soln	0.696

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	8.2283 g
Mass of dilution B used in substrate prep	4.6364 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.586009 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00788 µg/g soln.
µg/g soln.

a. µCi/g soln 0.696
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.

= 0.586009 + 0.00788
= 0.593885 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.171 µCi/µg ASDN

744847 dpm/nmol

Assay Date 7/27/2005 Test Chemical ID 1 tested 8

ID JG Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.577	0.346	0.164	0.094	0.053	0.041	0.037	25		
	0.586	0.346	0.164	0.098	0.053	0.042	0.027			
	0.590	0.365	0.162	0.098	0.057	0.041	0.030			
Samples:	10	100	microsomes							
	0.053	0.286	0.050							
	0.053	0.285	0.050							
			0.048							

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	0.584	0.553	0.0483
0.125	100	200	0.00013	200	0.0250	0.352	0.321	0.0277
0.05	200	1000	0.00005	200	0.0100	0.163	0.132	0.0108
0.025	100	1000	0.00003	200	0.0050	0.097	0.065	0.0049
0.01	40	1000	0.00001	200	0.0020	0.054	0.023	0.0012
0.005	20	1000	0.00001	200	0.0010	0.041	0.010	0.0000

Blank 0.031

r^2= 0.993
m= 0.089
b= -0.001

Variables	Regression results	
m, b	0.089	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.993	0.002
F, df	586	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	0.053	0.021	0.001	200	1	1	0.000	0.000	0.005
10	0.053	0.022	0.001	200	1	1	0.000		
10					1	1			
100	0.286	0.255	0.022	200	1	1	0.000	0.000	0.109
100	0.285	0.253	0.022	200	1	1	0.000		
100									
	0.050	0.019	0.001	200	114	69814	0.003	0.002	2.351
	0.050	0.019	0.001	200	114	69814	0.002		
	0.048	0.017	0.001	200	114	69814	0.002		

Assay Date 7/27/2005 Test Chemical ID 1 tested 8

ID JG Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.577	0.346	0.164	0.094	0.053	0.041	0.037	25	100	
	0.586	0.346	0.164	0.098	0.053	0.042	0.027			
	0.590	0.365	0.162	0.098	0.057	0.041	0.030			
Samples:	10	100	microsomes							
	0.053	0.286	0.050							
	0.053	0.285	0.050							
			0.048							

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	0.584	0.553	0.0428
0.125	100	200	0.00013	200	0.0250	0.352	0.321	0.0249
0.05	200	1000	0.00005	200	0.0100	0.163	0.132	0.0103
0.025	100	1000	0.00003	200	0.0050	0.097	0.065	0.0051
0.01	40	1000	0.00001	200	0.0020	0.054	0.023	0.0019
0.005	20	1000	0.00001	200	0.0010	0.041	0.010	0.0009

Blank 0.031

r^2= 1.000
m= 0.077
b= 0.000

Variables	Regression results	
m, b	0.077	0.000
se_m, se_b	0.001	0.000
r , se_y	1.000	0.000
F, df	8527	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	0.053	0.021	0.002	200	1	1	0.000	0.000	0.009
10	0.053	0.022	0.002	200	1	1	0.000		
10					1	1			
100	0.286	0.255	0.020	200	1	1	0.000	0.000	0.099
100	0.285	0.253	0.020	200	1	1	0.000		
100									
	0.050	0.019	0.002	200	114	69814	0.005	0.005	4.587
	0.050	0.019	0.002	200	114	69814	0.005		
	0.048	0.017	0.001	200	114	69814	0.004		

Assay Date	7/27/2005	Test Chemical ID	1	tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

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	7/27/2005	Chemical ID	1	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 3

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

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

Protein Concentration (stock microsomes, mg/mL):	4.587
Protein Concentration (dilution added to assay, mg/mL):	0.00749

Assay Date	7/27/2005	Test Chemical ID	1	# Concentrations tested	8	Microsome type	Microsome ID	0	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	11130.01	22260.02	22540.96	45081.92	0.1	154453	29.19	44971	0.0804	1	0.004	15	0.5374
			0.5	2	11410.95	22821.9			0.1					1		15	
	2	2	0.5	1	10576.17	21152.34	21086.78	42171.56	0.1	154453	27.30	42060	0.0565	1	0.004	15	0.5026
			0.5	2	10509.61	21019.22			0.1					1		15	
	3	2	0.5	1	10170.25	20340.5	20401.94	40803.88	0.1	154453	26.42	40693	0.0546	1	0.004	15	0.4863
			0.5	2	10231.69	20463.38			0.1					1		15	
	4	2	0.5	1	10295.1	20590.2	20516.8	41033.6	0.1	154453	26.57	40922	0.0549	1	0.004	15	0.4890
			0.5	2	10221.7	20443.4			0.1					1		15	
Background control	1	2	0.5	1	28.08	56.16	57.14	114.28	0.1	154453	0.07	3	0.0000	1	0.004	15	0.0000
			0.5	2	28.05	56.1			0.1					1		15	
	2	2	0.5	1	24.83	49.66	55.94	111.88	0.1	154453	0.07	1	0.0000	1	0.004	15	0.0000
			0.5	2	31.11	62.22			0.1					1		15	
	3	2	0.5	1	22.53	45.06	58.82	117.64	0.1	154453	0.08	6	0.0000	1	0.004	15	0.0001
			0.5	2	36.29	72.58			0.1					1		15	
	4	2	0.5	1	29.12	58.24	50.76	101.52	0.1	154453	0.07	-10	0.0000	1	0.004	15	-0.0001
			0.5	2	21.64	43.28			0.1					1		15	
Positive control	1	2	0.5	1	5565.51	11131.02	11200.04	22400.08	0.1	154453	14.50	22289	0.0299	1	0.004	15	0.2663
			0.5	2	5634.53	11269.06			0.1					1		15	
	2	2	0.5	1	5762.28	11524.56	11586.38	23172.76	0.1	154453	15.00	23061	0.0310	1	0.004	15	0.2756
			0.5	2	5824.1	11648.2			0.1					1		15	
	3	2	0.5	1	4669.17	9338.34	9329.45	18658.9	0.1	154453	12.08	18548	0.0249	1	0.004	15	0.2216
			0.5	2	4660.28	9320.56			0.1					1		15	
	4	2	0.5	1	4809.17	9618.34	9605.64	19211.28	0.1	154453	12.44	19100	0.0256	1	0.004	15	0.2282
			0.5	2	4796.47	9592.94			0.1					1		15	
Negative Control	1	2	0.5	1	10847.69	21695.38	21636.12	43672.24	0.1	154453	28.28	43561	0.0565	1	0.004	15	0.5205
			0.5	2	10988.43	21976.86			0.1					1		15	
	2	2	0.5	1	11268.79	22537.58	22436.59	44873.18	0.1	154453	29.05	44762	0.0601	1	0.004	15	0.5349
			0.5	2	11167.8	22335.6			0.1					1		15	
	3	2	0.5	1	9861.42	19722.84	19733.56	39467.12	0.1	154453	25.55	39356	0.0528	1	0.004	15	0.4703
			0.5	2	9872.14	19744.28			0.1					1		15	
	4	2	0.5	1	9735.34	19470.68	19534.14	39068.28	0.1	154453	25.29	38957	0.0523	1	0.004	15	0.4655
			0.5	2	9798.8	19597.6			0.1					1		15	
1	1-1	2	0.5	1	660.14	1320.28	1361.94	2723.88	0.1	154453	1.76	2613	0.0035	1	0.004	15	0.0312
			0.5	2	701.8	1403.6			0.1					1		15	
	1-2	2	0.5	1	701.29	1402.58	1387.49	2774.98	0.1	154453	1.80	2664	0.0036	1	0.004	15	0.0318
			0.5	2	686.2	1372.4			0.1					1		15	
	1-3	2	0.5	1	670.49	1340.98	1335.78	2671.56	0.1	154453	1.73	2560	0.0034	1	0.004	15	0.0306
			0.5	2	665.29	1330.58			0.1					1		15	
	2-1	2	0.5	1	1506.87	3013.74	3056.65	6113.3	0.1	154453	3.96	6002	0.0081	1	0.004	15	0.0717
			0.5	2	1549.78	3099.56			0.1					1		15	
	2-2	2	0.5	1	1525.47	3050.94	3029.08	6058.16	0.1	154453	3.92	5947	0.0080	1	0.004	15	0.0711
			0.5	2	1503.61	3007.22			0.1					1		15	
	2-3	2	0.5	1	1553.1	3106.2	3109.61	6219.22	0.1	154453	4.03	6108	0.0082	1	0.004	15	0.0730
			0.5	2	1556.51	3113.02			0.1					1		15	
	3-1	2	0.5	1	4848.75	9699.5	9674.31	19348.62	0.1	154453	12.53	19237	0.0258	1	0.004	15	0.2299
			0.5	2	4824.56	9649.12			0.1					1		15	
	3-2	2	0.5	1	4827.81	9655.62	9683.42	19366.84	0.1	154453	12.54	19256	0.0259	1	0.004	15	0.2301
			0.5	2	4855.61	9711.22			0.1					1		15	
	3-3	2	0.5	1	4694.79	9389.58	9427.35	18854.7	0.1	154453	12.21	18743	0.0252	1	0.004	15	0.2240
			0.5	2	4732.56	9465.12			0.1					1		15	
	4-1	2	0.5	1	6542.96	13085.92	13142.28	26284.56	0.1	154453	17.02	26173	0.0351	1	0.004	15	0.3128
			0.5	2	6599.32	13198.64			0.1					1		15	
	4-2	2	0.5	1	6056.44	12112.88	12390.66	24781.32	0.1	154453	16.04	24670	0.0331	1	0.004	15	0.2948
			0.5	2	6334.22	12668.44			0.1					1		15	
	4-3	2	0.5	1	6843.55	13687.1	13673.5	27347	0.1	154453	17.71	27236	0.0366	1	0.004	15	0.3255
			0.5	2	6829.95	13659.9			0.1					1		15	
	5-1	2	0.5	1	9567.96	19135.92	19274.12	38548.24	0.1	154453	24.96	38437	0.0516	1	0.004	15	0.4593
			0.5	2	9706.16	19412.32			0.1					1		15	
	5-2	2	0.5	1	9429.59	18859.18	18939.67	37879.34	0.1	154453	24.52	37768	0.0507	1	0.004	15	0.4513
			0.5	2	9510.08	19020.16			0.1					1		15	
	5-3	2	0.5	1	9548.53	19097.06	19064.9	38129.8	0.1	154453	24.69	38018	0.0510	1	0.004	15	0.4543
			0.5	2	9516.37	19032.74			0.1					1		15	
	6-1	2	0.5	1	10865.22	21730.44	21596.31	43192.62	0.1	154453	27.96	43081	0.0578	1	0.004	15	0.5146
			0.5	2	10731.09	21462.18			0.1					1		15	
	6-2	2	0.5	1	10539.89	21079.78	21037.99	42075.98	0.1	154453	27.24	41965	0.0563	1	0.004	15	0.5015
			0.5	2	10496.1	20996.2			0.1					1		15	
	6-3	2	0.5	1	10763.98	21527.96	21531.06	43062.12	0.1	154453	27.88	42951	0.0577	1	0.004	15	0.5132
			0.5	2	10767.08	21534.16			0.1					1		15	
	7-1	2	0.5	1	10941.67	21883.34	21820.8	43641.6	0.1	154453	28.26	43530	0.0584	1	0.004	15	0.5202
			0.5	2	10879.13	21758.26			0.1					1		15	
	7-2	2	0.5	1	10584.23	21168.46	20898.96	41797.92	0.1	154453	27.06	41687	0.0560	1	0.004	15	0.4981
			0.5	2	10314.73	20629.46			0.1					1		15	
	7-3	2	0.5	1	11158.48	22316.96	22548.89	45099.78	0.1	154453	29.20	44988	0.0604	1	0.004	15	0.5376
			0.5	2	11391.41	22782.82			0.1					1		15	
	8-1	2	0.5	1	10923.86	21847.72	21833.35	43666.7	0.1	154453	28.27	43555	0.0585	1	0.004	15	0.5205
			0.5	2	10909.49	21818.98			0.1					1		15	
	8-2	2	0.5	1	10646.13	21292.26	21406.86	42813.72	0.1	154453	27.72	42702	0.0573	1	0.004	15	0.5103
			0.5	2	10760.73	21521.46			0.1					1		15	
	8-3	2	0.5	1	10895.55	21791.1	21870.22	43740.44	0.1	154453	28.32	43629	0.0586	1	0.004	15	0.5213
			0.5	2	10974.67	21949.34			0.1					1		15	

Assay Date	7/27/2005	ID	1	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.5200	0.0246
Full activity	End	0.4876	0.0019
Full activity	Overall	0.5038	0.0235
Background	Beginning	0.0000	2.0279E-05
Background	End	0.0000	0.000136207
Background	Overall	0.0000	8.30921E-05
Positive	Beginning	0.2710	0.0065
Positive	End	0.2249	0.0047
Positive	Overall	0.2479	0.0270
Negative	Beginning	0.5277	0.0101
Negative	End	0.4679	0.0034
Negative	Overall	0.4978	0.0351

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
1	1	1	1.00E-03	-3.00	0.0312
1	1	2	1.00E-03	-3.00	0.0318
1	1	3	1.00E-03	-3.00	0.0306
1	2	1	5.00E-04	-3.30	0.0717
1	2	2	5.00E-04	-3.30	0.0711
1	2	3	5.00E-04	-3.30	0.0730
1	3	1	1.00E-04	-4.00	0.2299
1	3	2	1.00E-04	-4.00	0.2301
1	3	3	1.00E-04	-4.00	0.2240
1	4	1	5.00E-05	-4.30	0.3128
1	4	2	5.00E-05	-4.30	0.2948
1	4	3	5.00E-05	-4.30	0.3255
1	5	1	1.00E-05	-5.00	0.4593
1	5	2	1.00E-05	-5.00	0.4513
1	5	3	1.00E-05	-5.00	0.4543
1	6	1	1.00E-06	-6.00	0.5148
1	6	2	1.00E-06	-6.00	0.5015
1	6	3	1.00E-06	-6.00	0.5132
1	7	1	1.00E-07	-7.00	0.5202
1	7	2	1.00E-07	-7.00	0.4981
1	7	3	1.00E-07	-7.00	0.5376
1	8	1	1.00E-09	-9.00	0.5205
1	8	2	1.00E-09	-9.00	0.5103
1	8	3	1.00E-09	-9.00	0.5213

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	6.20	6.32	6.07
2	-3.30	14.24	14.10	14.49
3	-4.00	45.63	45.67	44.46
4	-4.30	62.08	58.51	64.60
5	-5.00	91.17	89.58	90.17
6	-6.00	102.18	99.53	101.87
7	-7.00	103.25	98.87	106.71
8	-9.00	103.31	101.28	103.48

Aromatase Assay Spreadsheet

Assay Date	7/21/2005	Test Chemical ID	Chem 2	# Concentrations tested	8
ID	JG	Replicate #	Rep 1	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	23477.06	1179752	
2	0.0201	30715.31	1528125	
3	0.0200	30749.59	1537480	
4	0.0200	30928.34	1546417	
5	0.0201	31292.56	1556844	
			Average DPM/g soln	1469723
			SD	162448
			CV	11.05
			µCi/g soln	0.662

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	8.1486 g
Mass of dilution B used in substrate prep	4.6003 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.581487 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00749 µg/g soln.
µg/g soln.

a. µCi/g soln 0.662
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.

= 0.581487 + 0.00749
= 0.588982 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.124 µCi/µg ASDN

714672 dpm/nmol

Assay Date	7/21/2005	Test Chemical ID	Chem 2	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.555	0.325	0.163	0.096	0.053	0.037	0.033	25	100	
	0.562	0.342	0.161	0.097	0.053	0.039	0.031			
	0.565	0.346	0.165	0.098	0.054	0.041	0.028			

Samples:	10	100	microsomes
	0.057	0.278	0.052
	0.058	0.283	0.051
			0.052

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	0.561	0.530	0.0483	m, b	0.093	-0.001
0.125	100	200	0.00013	200	0.0250	0.338	0.307	0.0275	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.163	0.133	0.0113	r , se_y	0.993	0.002
0.025	100	1000	0.00003	200	0.0050	0.097	0.067	0.0051	F, df	536	4
0.01	40	1000	0.00001	200	0.0020	0.054	0.023	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.039	0.008	-0.0003			

Regression results are calculated using the function LINEST

Blank 0.030

r^2= 0.993
m= 0.093
b= -0.001

	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	0.027	0.001	200	1	1	0.000	0.000	0.007
10	0.058	0.028	0.002	200	1	1	0.000		
10					1	1			
100	0.278	0.248	0.022	200	1	1	0.000	0.000	0.111
100	0.283	0.253	0.022	200	1	1	0.000		
100									
	0.052	0.022	0.001	200	114	69814	0.003	0.003	2.833
	0.051	0.021	0.001	200	114	69814	0.003		
	0.052	0.022	0.001	200	114	69814	0.003		

Assay Date 7/21/2005 Test Chemical ID Chem 2 tested 8

ID JG Replicate # Rep 1 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.555	0.325	0.163	0.096	0.053	0.037	0.033	25	100	
	0.562	0.342	0.161	0.097	0.053	0.039	0.031			
	0.565	0.346	0.165	0.098	0.054	0.041	0.028			

Samples:	10	100	microsomes
	0.057	0.278	0.052
	0.058	0.283	0.051
			0.052

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	0.561	0.530	0.0427	m, b	0.081	0.000
0.125	100	200	0.00013	200	0.0250	0.338	0.307	0.0247	se_m, se_b	0.002	0.000
0.05	200	1000	0.00005	200	0.0100	0.163	0.133	0.0106	r , se_y	0.998	0.001
0.025	100	1000	0.00003	200	0.0050	0.097	0.067	0.0053	F, df	1529	3
0.01	40	1000	0.00001	200	0.0020	0.054	0.023	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.039	0.008	0.0006			

Regression results are calculated using the function LINEST

Blank 0.030

r^2= 0.998
m= 0.081
b= 0.000

	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	0.027	0.002	200	1	1	0.000	0.000	0.011
10	0.058	0.028	0.002	200	1	1	0.000		
10					1	1			
100	0.278	0.248	0.020	200	1	1	0.000	0.000	0.101
100	0.283	0.253	0.020	200	1	1	0.000		
100									
	0.052	0.022	0.002	200	114	69814	0.005	0.005	5.043
	0.051	0.021	0.002	200	114	69814	0.005		
	0.052	0.022	0.002	200	114	69814	0.005		

Assay Date	7/21/2005	Test Chemical ID	Chem 2	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

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	7/21/2005	Chemical ID	Chem 2	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.40351	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
	612.40351	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):	5.043
Protein Concentration (dilution added to assay, mg/mL):	0.008235

Assay Date	7/21/2005	Test Chemical ID	Chem 2	# Concentrations tested	8	Microsome type		Microsome ID		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 $^{18}H_2O$ 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	9031.54	18063.08	18118.48	36236.96	0.1	146972	24.66	36107	0.0505	1	0.004	15	0.4090
			0.5	2	9086.94	18173.88			0.1					1		15	
	2	2	0.5	1	9080.67	18161.34	18121.75	36243.5	0.1	146972	24.66	36113	0.0505	1	0.004	15	0.4091
			0.5	2	9041.08	18082.16			0.1					1		15	
	3	2	0.5	1	8504.73	17009.46	17010.35	34020.7	0.1	146972	23.15	33890	0.0474	1	0.004	15	0.3839
			0.5	2	8505.62	17011.24			0.1					1		15	
	4	2	0.5	1	8362.88	16725.76	16720.89	33441.78	0.1	146972	22.75	33312	0.0466	1	0.004	15	0.3774
			0.5	2	8358.01	16716.02			0.1					1		15	
Background control	1	2	0.5	1	33.32	66.64	64.06	128.12	0.1	146972	0.09	-2	0.0000	1	0.004	15	0.0000
			0.5	2	30.74	61.48			0.1					1		15	
	2	2	0.5	1	33.60	67.2	69.53	139.06	0.1	146972	0.09	9	0.0000	1	0.004	15	0.0001
			0.5	2	35.93	71.86			0.1					1		15	
	3	2	0.5	1	32.45	64.9	70.91	141.82	0.1	146972	0.10	12	0.0000	1	0.004	15	0.0001
			0.5	2	38.46	76.92			0.1					1		15	
	4	2	0.5	1	24.81	49.62	55.98	111.96	0.1	146972	0.08	-18	0.0000	1	0.004	15	-0.0002
			0.5	2	31.17	62.34			0.1					1		15	
Positive control	1	2	0.5	1	4802.04	9604.08	9544.22	19088.44	0.1	146972	12.99	18958	0.0255	1	0.004	15	0.2148
			0.5	2	4742.18	9484.36			0.1					1		15	
	2	2	0.5	1	4705.09	9410.18	9355.96	18711.92	0.1	146972	12.73	18582	0.0250	1	0.004	15	0.2106
			0.5	2	4650.87	9301.74			0.1					1		15	
	3	2	0.5	1	4070.73	8141.46	8180.24	16360.48	0.1	146972	11.13	16230	0.0227	1	0.004	15	0.1839
			0.5	2	4109.51	8219.02			0.1					1		15	
	4	2	0.5	1	3721.24	7442.48	7425.12	14850.24	0.1	146972	10.10	14720	0.0206	1	0.004	15	0.1667
			0.5	2	3703.88	7407.76			0.1					1		15	
Negative Control	1	2	0.5	1	8834.52	17669.04	17539.07	35078.14	0.1	146972	23.87	34948	0.0489	1	0.004	15	0.3958
			0.5	2	8704.55	17409.1			0.1					1		15	
	2	2	0.5	1	8240.04	16480.08	16508.72	33017.44	0.1	146972	22.47	32887	0.0460	1	0.004	15	0.3725
			0.5	2	8268.68	16537.36			0.1					1		15	
	3	2	0.5	1	7667.03	15334.06	15327.91	30655.82	0.1	146972	20.86	30526	0.0427	1	0.004	15	0.3458
			0.5	2	7660.88	15321.76			0.1					1		15	
	4	2	0.5	1	7556.64	15117.28	14883.55	29767.1	0.1	146972	20.25	29637	0.0415	1	0.004	15	0.3357
			0.5	2	7324.91	14649.82			0.1					1		15	
Chem 2	1-1	2	0.5	1	64.48	128.96	139.13	278.26	0.1	146972	0.18	148	0.0002	1	0.004	15	0.0017
			0.5	2	74.65	149.3			0.1					1		15	
	1-2	2	0.5	1	55.54	111.08	121.04	242.08	0.1	146972	0.16	112	0.0002	1	0.004	15	0.0013
			0.5	2	65.50	131			0.1					1		15	
	1-3	2	0.5	1	53.16	106.32	127.24	254.48	0.1	146972	0.17	124	0.0002	1	0.004	15	0.0014
			0.5	2	74.08	148.16			0.1					1		15	
	2-1	2	0.5	1	418.73	837.46	821.06	1642.12	0.1	146972	1.12	1512	0.0021	1	0.004	15	0.0171
			0.5	2	402.33	804.66			0.1					1		15	
	2-2	2	0.5	1	374.82	749.64	745.53	1491.06	0.1	146972	1.01	1361	0.0019	1	0.004	15	0.0154
			0.5	2	370.71	741.42			0.1					1		15	
	2-3	2	0.5	1	406.49	812.98	830.07	1660.14	0.1	146972	1.13	1530	0.0021	1	0.004	15	0.0173
			0.5	2	423.58	847.16			0.1					1		15	
	3-1	2	0.5	1	768.85	1533.7	1510.11	3020.22	0.1	146972	2.05	2890	0.0040	1	0.004	15	0.0327
			0.5	2	743.26	1486.52			0.1					1		15	
	3-2	2	0.5	1	753.44	1506.88	1507.05	3014.1	0.1	146972	2.05	2884	0.0040	1	0.004	15	0.0327
			0.5	2	753.61	1507.22			0.1					1		15	
	3-3	2	0.5	1	762.10	1524.2	1504.35	3008.7	0.1	146972	2.05	2878	0.0040	1	0.004	15	0.0326
			0.5	2	742.25	1484.5			0.1					1		15	
	4-1	2	0.5	1	2632.98	5265.96	5376.44	10752.88	0.1	146972	7.32	10623	0.0149	1	0.004	15	0.1203
			0.5	2	2743.46	5486.92			0.1					1		15	
	4-2	2	0.5	1	2673.74	5347.48	5325.7	10651.4	0.1	146972	7.25	10521	0.0147	1	0.004	15	0.1192
			0.5	2	2651.96	5303.92			0.1					1		15	
	4-3	2	0.5	1	2743.06	5486.12	5379.35	10758.7	0.1	146972	7.32	10628	0.0149	1	0.004	15	0.1204
			0.5	2	2636.29	5272.58			0.1					1		15	
	5-1	2	0.5	1	6347.61	12695.22	12734.48	25468.96	0.1	146972	17.33	25339	0.0355	1	0.004	15	0.2870
			0.5	2	6386.87	12773.74			0.1					1		15	
	5-2	2	0.5	1	6892.60	13785.2	13736.5	27477	0.1	146972	18.70	27347	0.0383	1	0.004	15	0.3096
			0.5	2	6845.90	13691.8			0.1					1		15	
	5-3	2	0.5	1	7378.75	14757.5	14455.59	28911.18	0.1	146972	19.67	28781	0.0403	1	0.004	15	0.3260
			0.5	2	7076.84	14153.68			0.1					1		15	
	6-1	2	0.5	1	8041.63	16083.26	16779.35	33558.7	0.1	146972	22.83	33428	0.0468	1	0.004	15	0.3787
			0.5	2	8737.72	17475.44			0.1					1		15	
	6-2	2	0.5	1	8750.82	17501.64	17350.18	34700.36	0.1	146972	23.61	34570	0.0484	1	0.004	15	0.3916
			0.5	2	8599.36	17198.72			0.1					1		15	
	6-3	2	0.5	1	8824.94	17649.88	17492.64	34985.28	0.1	146972	23.80	34855	0.0488	1	0.004	15	0.3948
			0.5	2	8667.70	17335.4			0.1					1		15	
	7-1	2	0.5	1	8554.10	17108.2	17152.32	34304.64	0.1	146972	23.34	34174	0.0478	1	0.004	15	0.3871
			0.5	2	8598.22	17196.44			0.1					1		15	
	7-2	2	0.5	1	8729.12	17458.24	17557.92	35115.84	0.1	146972	23.89	34986	0.0490	1	0.004	15	0.3983
			0.5	2	8826.80	17657.6			0.1					1		15	
	7-3	2	0.5	1	8900.11	17800.22	17729.51	35459.02	0.1	146972	24.13	35329	0.0494	1	0.004	15	0.4002
			0.5	2	8829.40	17658.8			0.1					1		15	
	8-1	2	0.5	1	8666.59	17333.18	17325.07	34650.14	0.1	146972	23.58	34520	0.0483	1	0.004	15	0.3910
			0.5	2	8658.48	17316.96			0.1					1		15	
	8-2	2	0.5	1	8577.03	17154.06	17107.73	34215.46	0.1	146972	23.28	34085	0.0477	1	0.004	15	0.3861
			0.5	2	8530.70	17061.4			0.1					1		15	
	8-3	2	0.5	1	8421.30	16842.6	16964.89	33929.78	0.1	146972	23.09	33800	0.0473	1	0.004	15	0.3829
			0.5	2	8543.59	17087.18			0.1					1		15	

Assay Date	7/21/2005	ID	Chem 2	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0 Technician ID	JG	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4091	0.0001
Full activity	End	0.3806	0.0046
Full activity	Overall	0.3948	0.0166
Background	Beginning	0.0000	8.76301E-05
Background	End	0.0000	0.00023918
Background	Overall	0.0000	0.000153456
Positive	Beginning	0.2126	0.0030
Positive	End	0.1753	0.0121
Positive	Overall	0.1940	0.0227
Negative	Beginning	0.3842	0.0165
Negative	End	0.3408	0.0071
Negative	Overall	0.3625	0.0272

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Chem 2	1	1	1.00E-04	-4.00	0.0017
Chem 2	1	2	1.00E-04	-4.00	0.0013
Chem 2	1	3	1.00E-04	-4.00	0.0014
Chem 2	2	1	1.00E-05	-5.00	0.0171
Chem 2	2	2	1.00E-05	-5.00	0.0154
Chem 2	2	3	1.00E-05	-5.00	0.0173
Chem 2	3	1	5.00E-06	-5.30	0.0327
Chem 2	3	2	5.00E-06	-5.30	0.0327
Chem 2	3	3	5.00E-06	-5.30	0.0326
Chem 2	4	1	1.00E-06	-6.00	0.1203
Chem 2	4	2	1.00E-06	-6.00	0.1192
Chem 2	4	3	1.00E-06	-6.00	0.1204
Chem 2	5	1	1.00E-07	-7.00	0.2870
Chem 2	5	2	1.00E-07	-7.00	0.3098
Chem 2	5	3	1.00E-07	-7.00	0.3260
Chem 2	6	1	1.00E-08	-8.00	0.3787
Chem 2	6	2	1.00E-08	-8.00	0.3916
Chem 2	6	3	1.00E-08	-8.00	0.3948
Chem 2	7	1	1.00E-09	-9.00	0.3871
Chem 2	7	2	1.00E-09	-9.00	0.3963
Chem 2	7	3	1.00E-09	-9.00	0.4002
Chem 2	8	1	1.00E-10	-10.00	0.3910
Chem 2	8	2	1.00E-10	-10.00	0.3861
Chem 2	8	3	1.00E-10	-10.00	0.3829

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-4.00	0.42	0.32	0.36
2	-5.00	4.34	3.90	4.39
3	-5.30	8.29	8.27	8.26
4	-6.00	30.48	30.19	30.49
5	-7.00	72.70	78.46	82.57
6	-8.00	95.91	99.18	100.00
7	-9.00	98.05	100.37	101.36
8	-10.00	99.04	97.79	96.97

Aromatase Assay Spreadsheet

Assay Date	7/25/2005	Test Chemical ID	RC2	# Concentrations tested	8
ID	JG	Replicate #	Rep 2	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	25877.9	1300397	
2	0.0201	27083.59	1347442	
3	0.0200	27592.72	1379636	
4	0.0200	28221.74	1411087	
5	0.0200	27874.29	1393715	
			Average DPM/g soln	1366455
			SD	43686
			CV	3.20
			µCi/g soln	0.616

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	8.1393 g
Mass of dilution B used in substrate prep	4.5734 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56751 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00697 µg/g soln.
µg/g soln.

a. µCi/g soln	0.616
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.

= 0.567510 + 0.00697
= 0.574478 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.071 µCi/µg ASDN

681232 dpm/nmol

Test

Assay Date 7/25/2005 Chemical ID RC2 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.557	0.335	0.157	0.089	0.055	0.038	0.027	25	100	
	0.569	0.344	0.164	0.091	0.051	0.040	0.027			
	0.568	0.338	0.159	0.098	0.050	0.044	0.027			

Samples:	10	100	microsomes
	0.054	0.281	0.047
	0.051	0.268	0.048
			0.045

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	0.565	0.538	0.0484	m, b	0.092	-0.001
0.125	100	200	0.00013	200	0.0250	0.339	0.312	0.0275	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.160	0.133	0.0111	r , se_y	0.993	0.002
0.025	100	1000	0.00003	200	0.0050	0.092	0.065	0.0048	F, df	606	4
0.01	40	1000	0.00001	200	0.0020	0.052	0.025	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.041	0.014	0.0001			

Regression results are calculated using the function LINEST

Blank 0.027

r^2= 0.993
m= 0.092
b= -0.001

	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	0.054	0.027	0.001	200	1	1	0.000	0.000	0.006
10	0.051	0.024	0.001	200	1	1	0.000		
10					1	1			
100	0.281	0.253	0.022	200	1	1	0.000	0.000	0.108
100	0.268	0.241	0.021	200	1	1	0.000		
100									
	0.047	0.019	0.001	200	114	69814	0.002	0.002	1.882
	0.048	0.021	0.001	200	114	69814	0.002		
	0.045	0.018	0.000	200	114	69814	0.001		

Test

Assay Date 7/25/2005 Chemical ID RC2 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.557	0.335	0.157	0.089	0.055	0.038	0.027	25		
	0.569	0.344	0.164	0.091	0.051	0.040	0.027			
	0.568	0.338	0.159	0.098	0.050	0.044	0.027			

Samples:	10	100	microsomes
	0.054	0.281	0.047
	0.051	0.268	0.048
			0.045

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	0.565	0.538	0.0430
0.125	100	200	0.00013	200	0.0250	0.339	0.312	0.0248
0.05	200	1000	0.00005	200	0.0100	0.160	0.133	0.0105
0.025	100	1000	0.00003	200	0.0050	0.092	0.065	0.0050
0.01	40	1000	0.00001	200	0.0020	0.052	0.025	0.0018
0.005	20	1000	0.00001	200	0.0010	0.041	0.014	0.0009

Blank 0.027

r^2= 0.999
m= 0.080
b= 0.000

Variables	Regression results	
m, b	0.080	0.000
se_m, se_b	0.001	0.000
r , se_y	0.999	0.000
F, df	3851	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	0.054	0.027	0.002	200	1	1	0.000	0.000	0.009
10	0.051	0.024	0.002	200	1	1	0.000		
10					1	1			
100	0.281	0.253	0.020	200	1	1	0.000	0.000	0.098
100	0.268	0.241	0.019	200	1	1	0.000		
100									
	0.047	0.019	0.001	200	114	69814	0.004	0.004	4.138
	0.048	0.021	0.001	200	114	69814	0.004		
	0.045	0.018	0.001	200	114	69814	0.004		

Assay Date 7/25/2005 Test Chemical ID RC2 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

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	7/25/2005	Chemical ID	RC2	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 2

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.403509	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
	612.403509	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.138
Protein Concentration (dilution added to assay, mg/mL):	0.006757

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	7/25/2005	Test Chemical ID	RC2	# Concentrations tested	8	Microsome type		Microsome ID		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	14453.62	28907.24	30224.43	60448.86	0.1	136646	44.24	60365	0.0886	1	0.003	15	0.8743
			0.5	2	15770.81	31541.62			0.1					1		15	
	2	2	0.5	1	15867.74	31735.48	31663.74	63327.48	0.1	136646	46.34	63244	0.0928	1	0.003	15	0.9160
			0.5	2	15796	31592			0.1					1		15	
	3	2	0.5	1	15629.07	31258.14	30584.52	61169.04	0.1	136646	44.76	61086	0.0897	1	0.003	15	0.8847
			0.5	2	14955.45	29910.9			0.1					1		15	
	4	2	0.5	1	14411.27	28822.54	29181.69	58363.38	0.1	136646	42.71	58280	0.0856	1	0.003	15	0.8441
			0.5	2	14770.42	29540.84			0.1					1		15	
Background control	1	2	0.5	1	19.59	39.18	41.84	83.68	0.1	136646	0.06	0	0.0000	1	0.003	15	0.0000
			0.5	2	22.25	44.5			0.1					1		15	
	2	2	0.5	1	23.78	47.56	32.95	65.9	0.1	136646	0.05	-18	0.0000	1	0.003	15	-0.0003
			0.5	2	9.17	18.34			0.1					1		15	
	3	2	0.5	1	22.09	44.18	48.06	96.12	0.1	136646	0.07	13	0.0000	1	0.003	15	0.0002
			0.5	2	25.97	51.94			0.1					1		15	
	4	2	0.5	1	24.35	48.7	44.11	88.22	0.1	136646	0.06	5	0.0000	1	0.003	15	0.0001
			0.5	2	19.76	39.52			0.1					1		15	
Positive control	1	2	0.5	1	5809.5	11619	11864.27	23728.54	0.1	136646	17.37	23645	0.0347	1	0.003	15	0.3425
			0.5	2	6054.77	12109.54			0.1					1		15	
	2	2	0.5	1	5841.33	11682.66	11799.87	23599.74	0.1	136646	17.27	23516	0.0345	1	0.003	15	0.3406
			0.5	2	5958.54	11917.08			0.1					1		15	
	3	2	0.5	1	5320.27	10640.54	10887.43	21774.86	0.1	136646	15.94	21691	0.0318	1	0.003	15	0.3142
			0.5	2	5567.16	11134.32			0.1					1		15	
	4	2	0.5	1	5055.07	10110.14	10115.62	20231.24	0.1	136646	14.81	20148	0.0296	1	0.003	15	0.2918
			0.5	2	5060.55	10121.1			0.1					1		15	
Negative Control	1	2	0.5	1	15179.1	30358.2	30588.02	61132.04	0.1	136646	44.74	61049	0.0896	1	0.003	15	0.8842
			0.5	2	15386.92	30773.84			0.1					1		15	
	2	2	0.5	1	15325.2	30650.4	30322.84	60645.68	0.1	136646	44.38	60562	0.0889	1	0.003	15	0.8771
			0.5	2	14997.64	29995.28			0.1					1		15	
	3	2	0.5	1	14442.74	28885.48	28145.49	56290.98	0.1	136646	41.19	56208	0.0825	1	0.003	15	0.8141
			0.5	2	13702.75	27405.5			0.1					1		15	
	4	2	0.5	1	12858.37	25716.74	25936.62	51873.24	0.1	136646	37.96	51790	0.0760	1	0.003	15	0.7501
			0.5	2	13078.25	26156.5			0.1					1		15	
RC2	1-1	2	0.5	1	68.2	136.4	120.59	241.18	0.1	136646	0.18	158	0.0002	1	0.003	15	0.0023
			0.5	2	52.39	104.78			0.1					1		15	
	1-2	2	0.2	1	29.3	146.5	134.925	269.85	0.1	136646	0.20	186	0.0003	1	0.003	15	0.0027
			0.2	2	24.67	123.35			0.1					1		15	
	1-3	2	0.5	1	66.33	132.66	126.04	252.08	0.1	136646	0.18	169	0.0002	1	0.003	15	0.0024
			0.5	2	59.71	119.42			0.1					1		15	
	2-1	2	0.5	1	474.8	949.6	959.81	1919.62	0.1	136646	1.40	1836	0.0027	1	0.003	15	0.0266
			0.5	2	485.01	970.02			0.1					1		15	
	2-2	2	0.2	1	185.21	926.05	978.675	1957.35	0.1	136646	1.43	1874	0.0028	1	0.003	15	0.0271
			0.2	2	206.26	1031.3			0.1					1		15	
	2-3	2	0.5	1	487.27	974.54	966.35	1932.7	0.1	136646	1.41	1849	0.0027	1	0.003	15	0.0268
			0.5	2	479.08	958.16			0.1					1		15	
	3-1	2	0.5	1	922.11	1844.22	1878.7	3757.4	0.1	136646	2.75	3674	0.0054	1	0.003	15	0.0532
			0.5	2	956.59	1913.18			0.1					1		15	
	3-2	2	0.2	1	169.18	845.9	1197.625	2395.25	0.1	136646	1.75	2312	0.0034	1	0.003	15	0.0335
			0.2	2	309.87	1549.35			0.1					1		15	
	3-3	2	0.5	1	888.72	1777.44	1835.37	3670.74	0.1	136646	2.69	3587	0.0053	1	0.003	15	0.0520
			0.5	2	946.65	1893.3			0.1					1		15	
	4-1	2	0.5	1	3516.72	7033.44	7123.45	14246.9	0.1	136646	10.43	14163	0.0208	1	0.003	15	0.2051
			0.5	2	3606.73	7213.46			0.1					1		15	
	4-2	2	0.2	1	652.15	3260.75	4678.175	9356.35	0.1	136646	6.85	9273	0.0136	1	0.003	15	0.1343
			0.2	2	1219.12	6095.6			0.1					1		15	
	4-3	2	0.5	1	3664.32	7328.64	7324.93	14649.86	0.1	136646	10.72	14566	0.0214	1	0.003	15	0.2110
			0.5	2	3660.61	7321.22			0.1					1		15	
	5-1	2	0.5	1	11344.13	22688.26	22663.8	45327.6	0.1	136646	33.17	45244	0.0664	1	0.003	15	0.6553
			0.5	2	11319.67	22639.34			0.1					1		15	
	5-2	2	0.2	1	4392.38	21961.9	21497.2	42994.4	0.1	136646	31.46	42911	0.0630	1	0.003	15	0.6215
			0.2	2	4206.5	21032.5			0.1					1		15	
	5-3	2	0.5	1	11324.77	22649.54	22779.2	45558.4	0.1	136646	33.34	45475	0.0668	1	0.003	15	0.6586
			0.5	2	11454.43	22908.86			0.1					1		15	
	6-1	2	0.5	1	14996.73	29993.46	30360.58	60721.16	0.1	136646	44.44	60638	0.0890	1	0.003	15	0.8782
			0.5	2	15363.85	30727.7			0.1					1		15	
	6-2	2	0.2	1	3962.67	19813.35	24014.225	48028.45	0.1	136646	35.15	47945	0.0704	1	0.003	15	0.6944
			0.2	2	5643.02	28215.1			0.1					1		15	
	6-3	2	0.5	1	16025.58	32051.16	32087.84	64175.68	0.1	136646	46.97	64092	0.0941	1	0.003	15	0.9283
			0.5	2	16062.26	32124.52			0.1					1		15	
	7-1	2	0.5	1	15356.68	30713.36	30725.14	61450.28	0.1	136646	44.97	61367	0.0901	1	0.003	15	0.8888
			0.5	2	15368.46	30736.92			0.1					1		15	
	7-2	2	0.2	1	5741.23	28706.15	29360.3	58720.6	0.1	136646	42.97	58637	0.0861	1	0.003	15	0.8492
			0.2	2	6002.89	30014.45			0.1					1		15	
	7-3	2	0.5	1	15134.14	30268.28	30679.75	61359.5	0.1	136646	44.90	61276	0.0899	1	0.003	15	0.8875
			0.5	2	15545.61	31091.22			0.1					1		15	
	8-1	2	0.5	1	15242.87	30485.74	30266.74	60533.48	0.1	136646	44.30	60450	0.0887	1	0.003	15	0.8755
			0.5	2	15023.87	30047.74			0.1					1		15	
	8-2	2	0.2	1	5822.47	29112.35	28956.575	57913.15	0.1	136646	42.38	57830	0.0849	1	0.003	15	0.8376
			0.2	2	5760.16	28800.8			0.1					1		15	
	8-3	2	0.5	1	14674.93	29349.86	28700.67	57401.34	0.1	136646	42.01	57318	0.0841	1	0.003	15	0.8301
			0.5	2	14025.74	28051.48			0.1					1		15	

Assay Date	7/25/2005	ID	RC2	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.8951	0.0295
Full activity	End	0.8644	0.0287
Full activity	Overall	0.8798	0.0297
Background	Beginning	-0.0001	0.000182086
Background	End	0.0001	8.09045E-05
Background	Overall	0.0000	0.000185348
Positive	Beginning	0.3415	0.0013
Positive	End	0.3030	0.0158
Positive	Overall	0.3223	0.0241
Negative	Beginning	0.8806	0.0050
Negative	End	0.7821	0.0452
Negative	Overall	0.8314	0.0627

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC2	1	1	1.00E-04	-4.00	0.0023
RC2	1	2	1.00E-04	-4.00	0.0027
RC2	1	3	1.00E-04	-4.00	0.0024
RC2	2	1	1.00E-05	-5.00	0.0266
RC2	2	2	1.00E-05	-5.00	0.0271
RC2	2	3	1.00E-05	-5.00	0.0268
RC2	3	1	5.00E-06	-5.30	0.0532
RC2	3	2	5.00E-06	-5.30	0.0335
RC2	3	3	5.00E-06	-5.30	0.0520
RC2	4	1	1.00E-06	-6.00	0.2051
RC2	4	2	1.00E-06	-6.00	0.1343
RC2	4	3	1.00E-06	-6.00	0.2110
RC2	5	1	1.00E-07	-7.00	0.6553
RC2	5	2	1.00E-07	-7.00	0.6215
RC2	5	3	1.00E-07	-7.00	0.6586
RC2	6	1	1.00E-08	-8.00	0.8782
RC2	6	2	1.00E-08	-8.00	0.6944
RC2	6	3	1.00E-08	-8.00	0.9283
RC2	7	1	1.00E-09	-9.00	0.8888
RC2	7	2	1.00E-09	-9.00	0.8492
RC2	7	3	1.00E-09	-9.00	0.8875
RC2	8	1	1.00E-10	-10.00	0.8755
RC2	8	2	1.00E-10	-10.00	0.8376
RC2	8	3	1.00E-10	-10.00	0.8301

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-4.00	0.26	0.31	0.28
2	-5.00	3.02	3.08	3.04
3	-5.30	6.05	3.81	5.91
4	-6.00	23.32	15.27	23.98
5	-7.00	74.48	70.64	74.86
6	-8.00	99.83	78.93	105.51
7	-9.00	101.03	96.53	100.88
8	-10.00	99.52	95.20	94.36

Aromatase Assay Spreadsheet

Assay Date	7/27/2005	Test Chemical ID	2	# Concentrations tested	8
ID	JG	Replicate #	Rep 3	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	28938.48	1461539	
2	0.0199	29839.25	1499460	
3	0.0199	31503.05	1583068	
4	0.0202	31810.71	1574788	
5	0.0199	31915.46	1603792	
			Average DPM/g soln	1544529
			SD	60892
			CV	3.94
			µCi/g soln	0.696

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	8.2283 g
Mass of dilution B used in substrate prep	4.6364 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.586009 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00788 µg/g soln.
µg/g soln.

a. µCi/g soln 0.696
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.

= 0.586009 + 0.00788
= 0.593885 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.171 µCi/µg ASDN

744847 dpm/nmol

Test

Assay Date 7/27/2005 Chemical ID 2 tested 8

ID JG Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.577	0.346	0.164	0.094	0.053	0.041	0.037	25	100	
	0.586	0.346	0.164	0.098	0.053	0.042	0.027			
	0.590	0.365	0.162	0.098	0.057	0.041	0.030			

Samples:	10	100	microsomes
	0.053	0.286	0.050
	0.053	0.285	0.050
			0.048

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	0.584	0.553	0.0483	m, b	0.089	-0.001
0.125	100	200	0.00013	200	0.0250	0.352	0.321	0.0277	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.163	0.132	0.0108	r , se_y	0.993	0.002
0.025	100	1000	0.00003	200	0.0050	0.097	0.065	0.0049	F, df	586	4
0.01	40	1000	0.00001	200	0.0020	0.054	0.023	0.0012	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.041	0.010	0.0000			

Regression results are calculated using the function LINEST

Blank 0.031

r^2= 0.993
m= 0.089
b= -0.001

	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	0.053	0.021	0.001	200	1	1	0.000	0.000	0.005
10	0.053	0.022	0.001	200	1	1	0.000		
10					1	1			
100	0.286	0.255	0.022	200	1	1	0.000	0.000	0.109
100	0.285	0.253	0.022	200	1	1	0.000		
100									
	0.050	0.019	0.001	200	114	69814	0.003	0.002	2.351
	0.050	0.019	0.001	200	114	69814	0.002		
	0.048	0.017	0.001	200	114	69814	0.002		

Assay Date 7/27/2005 Test Chemical ID 2 tested 8

ID JG Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.577	0.346	0.164	0.094	0.053	0.041	0.037	25		
	0.586	0.346	0.164	0.098	0.053	0.042	0.027			
	0.590	0.365	0.162	0.098	0.057	0.041	0.030			
Samples:	10	100	microsomes							
	0.053	0.286	0.050							
	0.053	0.285	0.050							
			0.048							

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	0.584	0.553	0.0428	m, b	0.077	0.000
0.125	100	200	0.00013	200	0.0250	0.352	0.321	0.0249	se_m, se_b	0.001	0.000
0.05	200	1000	0.00005	200	0.0100	0.163	0.132	0.0103	r , se_y	1.000	0.000
0.025	100	1000	0.00003	200	0.0050	0.097	0.065	0.0051	F, df	8527	3
0.01	40	1000	0.00001	200	0.0020	0.054	0.023	0.0019	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.041	0.010	0.0009			

Regression results are calculated using the function LINEST

Blank 0.031

r^2= 1.000
m= 0.077
b= 0.000

	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	0.053	0.021	0.002	200	1	1	0.000	0.000	0.009
10	0.053	0.022	0.002	200	1	1	0.000		
10					1	1			
100	0.286	0.255	0.020	200	1	1	0.000	0.000	0.099
100	0.285	0.253	0.020	200	1	1	0.000		
100									
	0.050	0.019	0.002	200	114	69814	0.005	0.005	4.587
	0.050	0.019	0.002	200	114	69814	0.005		
	0.048	0.017	0.001	200	114	69814	0.004		

Assay Date	7/27/2005	Test Chemical ID	2	tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

								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	7/27/2005	Chemical ID	2	# Concentrations tested	8	Microsome type	Microsome ID	0	Technician ID	JG	Replicate #	Rep 3

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.587
Protein Concentration (dilution added to assay, mg/mL):	0.00749

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	7/27/2005	Test Chemical ID	2	# Concentrations tested	8	Microsome type		Microsome ID	0	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	11130.01	22260.02	22540.96	45081.92	0.1	154453	29.19	44871	0.0604	1	0.004	15	0.5374
			0.5	2	11410.95	22821.9			0.1					1		15	
	2	2	0.5	1	10576.17	21152.34	21085.78	42171.56	0.1	154453	27.30	42060	0.0565	1	0.004	15	0.5026
			0.5	2	10509.61	21019.22			0.1					1		15	
	3	2	0.5	1	10170.25	20340.5	20401.94	40803.88	0.1	154453	26.42	40693	0.0546	1	0.004	15	0.4863
			0.5	2	10231.69	20463.38			0.1					1		15	
	4	2	0.5	1	10295.1	20590.2	20516.8	41033.6	0.1	154453	26.57	40922	0.0549	1	0.004	15	0.4890
			0.5	2	10221.7	20443.4			0.1					1		15	
Background control	1	2	0.5	1	29.09	58.18	57.14	114.28	0.1	154453	0.07	3	0.0000	1	0.004	15	0.0000
			0.5	2	28.05	56.1			0.1					1		15	
	2	2	0.5	1	24.83	49.66	55.94	111.88	0.1	154453	0.07	1	0.0000	1	0.004	15	0.0000
			0.5	2	31.11	62.22			0.1					1		15	
	3	2	0.5	1	22.53	45.06	58.82	117.64	0.1	154453	0.08	6	0.0000	1	0.004	15	0.0001
			0.5	2	36.28	72.56			0.1					1		15	
	4	2	0.5	1	29.12	58.24	50.76	101.52	0.1	154453	0.07	-10	0.0000	1	0.004	15	-0.0001
			0.5	2	21.64	43.28			0.1					1		15	
Positive control	1	2	0.5	1	5565.51	11131.02	11200.04	22400.08	0.1	154453	14.50	22288	0.0299	1	0.004	15	0.2663
			0.5	2	5634.53	11269.06			0.1					1		15	
	2	2	0.5	1	5762.28	11524.56	11586.38	23172.76	0.1	154453	15.00	23061	0.0310	1	0.004	15	0.2756
			0.5	2	5824.1	11648.2			0.1					1		15	
	3	2	0.5	1	4669.17	9338.34	9329.45	18658.9	0.1	154453	12.08	18548	0.0249	1	0.004	15	0.2216
			0.5	2	4660.28	9320.56			0.1					1		15	
	4	2	0.5	1	4809.17	9618.34	9605.64	19211.28	0.1	154453	12.44	19100	0.0256	1	0.004	15	0.2282
			0.5	2	4796.47	9592.94			0.1					1		15	
Negative Control	1	2	0.5	1	10847.69	21695.38	21636.12	43272.24	0.1	154453	28.28	43561	0.0565	1	0.004	15	0.5205
			0.5	2	10988.43	21976.86			0.1					1		15	
	2	2	0.5	1	11268.79	22537.58	22436.59	44873.18	0.1	154453	29.05	44762	0.0601	1	0.004	15	0.5349
			0.5	2	11167.8	22335.6			0.1					1		15	
	3	2	0.5	1	9861.42	19722.84	19733.56	39467.12	0.1	154453	25.55	39356	0.0528	1	0.004	15	0.4703
			0.5	2	9872.14	19744.28			0.1					1		15	
	4	2	0.5	1	9735.34	19470.68	19534.14	39068.28	0.1	154453	25.29	38857	0.0523	1	0.004	15	0.4655
			0.5	2	9798.8	19597.6			0.1					1		15	
2	1-1	2	0.5	1	84.22	168.44	162.86	325.72	0.1	154453	0.21	214	0.0003	1	0.004	15	0.0026
			0.5	2	78.64	157.28			0.1					1		15	
	1-2	2	0.5	1	81.88	163.76	158.53	317.06	0.1	154453	0.21	206	0.0003	1	0.004	15	0.0025
			0.5	2	76.65	153.3			0.1					1		15	
	1-3	2	0.5	1	78.94	157.88	157.79	315.58	0.1	154453	0.20	204	0.0003	1	0.004	15	0.0024
			0.5	2	78.65	157.7			0.1					1		15	
	2-1	2	0.5	1	583.59	1167.18	1168.27	2336.54	0.1	154453	1.51	2225	0.0030	1	0.004	15	0.0266
			0.5	2	584.68	1169.36			0.1					1		15	
	2-2	2	0.5	1	583.78	1167.56	1201.25	2402.5	0.1	154453	1.56	2281	0.0031	1	0.004	15	0.0274
			0.5	2	607.47	1214.94			0.1					1		15	
	2-3	2	0.5	1	591.35	1182.7	1198.7	2397.4	0.1	154453	1.55	2266	0.0031	1	0.004	15	0.0273
			0.5	2	607.35	1214.7			0.1					1		15	
	3-1	2	0.5	1	1066.58	2133.16	2159.22	4318.44	0.1	154453	2.80	4207	0.0056	1	0.004	15	0.0503
			0.5	2	1092.64	2185.28			0.1					1		15	
	3-2	2	0.5	1	1179.9	2359.8	2334.44	4668.88	0.1	154453	3.02	4558	0.0061	1	0.004	15	0.0545
			0.5	2	1154.54	2309.08			0.1					1		15	
	3-3	2	0.5	1	1138.39	2276.78	2273.29	4546.58	0.1	154453	2.94	4435	0.0060	1	0.004	15	0.0530
			0.5	2	1134.9	2269.8			0.1					1		15	
	4-1	2	0.5	1	3967.59	7935.18	7904.68	15809.36	0.1	154453	10.24	15698	0.0211	1	0.004	15	0.1876
			0.5	2	3937.09	7874.18			0.1					1		15	
	4-2	2	0.5	1	3749.49	7498.98	7556.5	15113	0.1	154453	9.78	15002	0.0201	1	0.004	15	0.1793
			0.5	2	3807.01	7614.02			0.1					1		15	
	4-3	2	0.5	1	3790.81	7581.62	7542.94	15085.88	0.1	154453	9.77	14975	0.0201	1	0.004	15	0.1789
			0.5	2	3752.13	7504.26			0.1					1		15	
	5-1	2	0.5	1	8891.99	17783.98	17897.59	35795.18	0.1	154453	23.16	35984	0.0479	1	0.004	15	0.4264
			0.5	2	9005.6	18011.2			0.1					1		15	
	5-2	2	0.5	1	8845.44	17690.88	17595.96	35191.92	0.1	154453	22.78	35081	0.0471	1	0.004	15	0.4192
			0.5	2	8750.52	17501.04			0.1					1		15	
	5-3	2	0.5	1	8878.41	17756.82	17811.7	35623.4	0.1	154453	23.06	35512	0.0477	1	0.004	15	0.4244
			0.5	2	8933.29	17866.58			0.1					1		15	
	6-1	2	0.5	1	10000.13	20040.26	19840.45	39680.9	0.1	154453	25.69	39570	0.0531	1	0.004	15	0.4728
			0.5	2	9820.32	19640.64			0.1					1		15	
	6-2	2	0.5	1	10316.59	20633.18	20626.58	41253.16	0.1	154453	26.71	41142	0.0552	1	0.004	15	0.4916
			0.5	2	10309.99	20619.98			0.1					1		15	
	6-3	2	0.5	1	10536.36	21072.72	21183.79	42367.58	0.1	154453	27.43	42256	0.0567	1	0.004	15	0.5049
			0.5	2	10647.43	21294.86			0.1					1		15	
	7-1	2	0.5	1	10754.41	21508.82	21607.29	43214.58	0.1	154453	27.98	43103	0.0579	1	0.004	15	0.5151
			0.5	2	10852.88	21705.76			0.1					1		15	
	7-2	2	0.5	1	10016.7	20033.4	20470.71	40941.42	0.1	154453	26.51	40830	0.0548	1	0.004	15	0.4879
			0.5	2	10454.01	20908.02			0.1					1		15	
	7-3	2	0.5	1	10291.37	20582.74	20831.03	41662.06	0.1	154453	26.97	41551	0.0558	1	0.004	15	0.4965
			0.5	2	10539.66	21079.32			0.1					1		15	
	8-1	2	0.5	1	10588.01	21176.02	21242.17	42484.34	0.1	154453	27.51	42373	0.0569	1	0.004	15	0.5063
			0.5	2	10654.16	21308.32			0.1					1		15	
	8-2	2	0.5	1	10143.19	20286.38	20159.14	40318.28	0.1	154453	26.10	40207	0.0540	1	0.004	15	0.4805
			0.5	2	10015.95	20031.9			0.1					1		15	
	8-3	2	0.5	1	10451.48	20902.96	20948.05	41896.1	0.1	154453	27.13	41787	0.0561	1	0.004	15	0.4993
			0.5	2	10497.56	20995.12			0.1					1		15	

Assay Date	7/27/2005	ID	2	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.5200	0.0246
Full activity	End	0.4876	0.0019
Full activity	Overall	0.5038	0.0235
Background	Beginning	0.0000	2.0279E-05
Background	End	0.0000	0.000136207
Background	Overall	0.0000	8.30921E-05
Positive	Beginning	0.2710	0.0065
Positive	End	0.2249	0.0047
Positive	Overall	0.2479	0.0270
Negative	Beginning	0.5277	0.0101
Negative	End	0.4679	0.0034
Negative	Overall	0.4978	0.0351

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
2	1	1	1.00E-04	-4.00	0.0026
2	1	2	1.00E-04	-4.00	0.0025
2	1	3	1.00E-04	-4.00	0.0024
2	2	1	1.00E-05	-5.00	0.0266
2	2	2	1.00E-05	-5.00	0.0274
2	2	3	1.00E-05	-5.00	0.0273
2	3	1	5.00E-06	-5.30	0.0503
2	3	2	5.00E-06	-5.30	0.0545
2	3	3	5.00E-06	-5.30	0.0530
2	4	1	1.00E-06	-6.00	0.1876
2	4	2	1.00E-06	-6.00	0.1793
2	4	3	1.00E-06	-6.00	0.1789
2	5	1	1.00E-07	-7.00	0.4264
2	5	2	1.00E-07	-7.00	0.4192
2	5	3	1.00E-07	-7.00	0.4244
2	6	1	1.00E-08	-8.00	0.4728
2	6	2	1.00E-08	-8.00	0.4916
2	6	3	1.00E-08	-8.00	0.5049
2	7	1	1.00E-09	-9.00	0.5151
2	7	2	1.00E-09	-9.00	0.4879
2	7	3	1.00E-09	-9.00	0.4965
2	8	1	1.00E-10	-10.00	0.5063
2	8	2	1.00E-10	-10.00	0.4805
2	8	3	1.00E-10	-10.00	0.4993

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-4.00	0.51	0.49	0.48
2	-5.00	5.28	5.43	5.42
3	-5.30	9.98	10.81	10.52
4	-6.00	37.23	35.58	35.52
5	-7.00	84.64	83.21	84.23
6	-8.00	93.85	97.58	100.22
7	-9.00	102.23	96.84	98.55
8	-10.00	100.50	95.36	99.11

Aromatase Assay Spreadsheet

Assay Date	8/4/2005	Test Chemical ID	RC3	# Concentrations tested	8
ID	TNB	Replicate #	1	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	32446.93	1638734	
2	0.0202	32884.73	1627957	
3	0.0202	32927.29	1630064	
4	0.0202	32969.04	1632131	
5	0.0202	33300.85	1648557	
			Average DPM/g soln	1635488
			SD	8348
			CV	0.51
			µCi/g soln	0.737

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	8.1661 g
Mass of dilution B used in substrate prep	4.5943 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562606 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00834 µg/g soln.
µg/g soln.

a. µCi/g soln 0.737
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.

= 0.562606 + 0.00834
= 0.570946 µg ASDN/g soln.

3) Calculate Solution Specific Activity

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

820400 dpm/nmol

Test

Assay Date 8/4/2005 Chemical ID RC3 tested 8

ID TNB Replicate # 1 Microsome type Recombinant Microsome ID 0

									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	0.336	0.159	0.094	0.049	0.036	0.027	25		
	0.567	0.342	0.163	0.096	0.051	0.036	0.027			
	0.562	0.341	0.160	0.101	0.050	0.039	0.025			

Samples:	10	100	microsomes
	0.048	0.277	0.044
	0.050	0.278	0.045
			0.043

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	0.564	0.538	0.0482	m, b	0.092	-0.001
0.125	100	200	0.00013	200	0.0250	0.339	0.313	0.0276	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.160	0.134	0.0111	r , se_y	0.992	0.002
0.025	100	1000	0.00003	200	0.0050	0.097	0.070	0.0053	F, df	527	4
0.01	40	1000	0.00001	200	0.0020	0.050	0.024	0.0010	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.037	0.010	-0.0002			

Regression results are calculated using the function LINEST

Blank 0.026

r^2= 0.992
m= 0.092
b= -0.001

	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	0.048	0.021	0.001	200	1	1	0.000	0.000	0.004
10	0.050	0.023	0.001	200	1	1	0.000		
10					1	1			
100	0.277	0.251	0.022	200	1	1	0.000	0.000	0.109
100	0.278	0.251	0.022	200	1	1	0.000		
100									
	0.044	0.018	0.000	200	114	69814	0.001	0.001	1.336
	0.045	0.019	0.001	200	114	69814	0.002		
	0.043	0.016	0.000	200	114	69814	0.001		

Test

Assay Date 8/4/2005 Chemical ID RC3 tested 8

ID TNB Replicate # 1 Microsome type Recombinant Microsome ID 0

									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	0.336	0.159	0.094	0.049	0.036	0.027	25		
	0.567	0.342	0.163	0.096	0.051	0.036	0.027			
	0.562	0.341	0.160	0.101	0.050	0.039	0.025			

Samples:	10	100	microsomes
	0.048	0.277	0.044
	0.050	0.278	0.045
			0.043

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	0.564	0.538	0.0426	m, b	0.080	0.000
0.125	100	200	0.00013	200	0.0250	0.339	0.313	0.0247	se_m, se_b	0.002	0.000
0.05	200	1000	0.00005	200	0.0100	0.160	0.134	0.0105	r , se_y	0.998	0.000
0.025	100	1000	0.00003	200	0.0050	0.097	0.070	0.0054	F, df	1622	3
0.01	40	1000	0.00001	200	0.0020	0.050	0.024	0.0017	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.037	0.010	0.0007			

Regression results are calculated using the function LINEST

Blank 0.026

r^2= 0.998
m= 0.080
b= 0.000

	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	0.048	0.021	0.002	200	1	1	0.000	0.000	0.008
10	0.050	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.277	0.251	0.020	200	1	1	0.000	0.000	0.099
100	0.278	0.251	0.020	200	1	1	0.000		
100									
	0.044	0.018	0.001	200	114	69814	0.004	0.004	3.764
	0.045	0.019	0.001	200	114	69814	0.004		
	0.043	0.016	0.001	200	114	69814	0.003		

Assay Date	8/4/2005	Test Chemical ID	RC3	tested	8		
ID	TNB	Replicate #	1	Microsome type	Recombinant	Microsome ID	0

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	8/4/2005	Chemical ID	RC3	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	1

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.764
Protein Concentration (dilution added to assay, mg/mL):	0.006146

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	8/4/2005	Test Chemical ID	RC3	# Concentrations tested	8	Microsome type		Microsome ID		0 Technician ID	TNB	Replicate #	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					0.1	163549		#VALUE!	#VALUE!	1	0.003	15	#VALUE!
			0.5	2					0.1					1		15	
	2	2	0.5	1	8857.85	17715.3	18164.3	36328.6	0.1	163549	22.21	36184	0.0441	1	0.003	15	0.4784
			0.5	2	9306.65	18613.3			0.1					1		15	
	3	2	0.5	1	8353.77	16687.54	16714.37	33428.74	0.1	163549	20.44	33284	0.0406	1	0.003	15	0.4401
			0.5	2	8380.6	16761.2			0.1					1		15	
	4	2	0.5	1	8053.29	16108.58	15970.46	31940.92	0.1	163549	19.53	31796	0.0388	1	0.003	15	0.4204
			0.5	2	7917.17	15834.34			0.1					1		15	
Background control	1	2	0.5	1	37.64	75.28	69.65	139.3	0.1	163549	0.09	-5	0.0000	1	0.003	15	-0.0001
			0.5	2	32.01	64.02			0.1					1		15	
	2	2	0.5	1	40.68	81.36	82.98	165.96	0.1	163549	0.10	21	0.0000	1	0.003	15	0.0003
			0.5	2	42.3	84.6			0.1					1		15	
	3	2	0.5	1	35.77	71.54	74.85	149.7	0.1	163549	0.09	5	0.0000	1	0.003	15	0.0001
			0.5	2	39.08	78.16			0.1					1		15	
	4	2	0.5	1	32.79	65.58	61.55	123.1	0.1	163549	0.08	-21	0.0000	1	0.003	15	-0.0003
			0.5	2	28.76	57.52			0.1					1		15	
Positive control	1	2	0.5	1	4627.73	9255.46	9425.16	18850.32	0.1	163549	11.53	18706	0.0228	1	0.003	15	0.2473
			0.5	2	4797.43	9594.86			0.1					1		15	
	2	2	0.5	1	4291.49	8582.98	8707.44	17414.88	0.1	163549	10.65	17270	0.0211	1	0.003	15	0.2263
			0.5	2	4415.95	8831.9			0.1					1		15	
	3	2	0.5	1	3968.76	7937.52	7954.66	15909.32	0.1	163549	9.73	15765	0.0192	1	0.003	15	0.2084
			0.5	2	3985.9	7971.8			0.1					1		15	
	4	2	0.5	1	4127.92	8255.84	8311.73	16623.46	0.1	163549	10.16	16479	0.0201	1	0.003	15	0.2179
			0.5	2	4183.81	8367.62			0.1					1		15	
Negative Control	1	2	0.5	1	9151.12	18302.24	18390.11	36780.22	0.1	163549	22.49	36636	0.0447	1	0.003	15	0.4844
			0.5	2	9238.99	18477.98			0.1					1		15	
	2	2	0.5	1	8617.53	17235.06	17495.45	34990.9	0.1	163549	21.39	34846	0.0425	1	0.003	15	0.4607
			0.5	2	8877.92	17755.84			0.1					1		15	
	3	2	0.5	1	8148.7	16297.4	16099.32	32198.64	0.1	163549	19.69	32054	0.0391	1	0.003	15	0.4238
			0.5	2	7950.62	15901.24			0.1					1		15	
	4	2	0.5	1	7751.94	15503.88	15654.46	31308.92	0.1	163549	19.14	31164	0.0380	1	0.003	15	0.4120
			0.5	2	7902.52	15805.04			0.1					1		15	
RC3	1-1	2	0.5	1	49.15	98.3	100.65	201.3	0.1	163549	0.12	57	0.0001	1	0.003	15	0.0008
			0.5	2	51.5	103			0.1					1		15	
	1-2	2	0.5	1	39.7	79.4	80.92	161.84	0.1	163549	0.10	17	0.0000	1	0.003	15	0.0002
			0.5	2	41.22	82.44			0.1					1		15	
	1-3	2	0.5	1	30.58	61.16	75.56	151.12	0.1	163549	0.09	7	0.0000	1	0.003	15	0.0001
			0.5	2	44.96	89.96			0.1					1		15	
	2-1	2	0.5	1	44.94	89.88	94.2	188.4	0.1	163549	0.12	44	0.0001	1	0.003	15	0.0006
			0.5	2	49.26	98.52			0.1					1		15	
	2-2	2	0.5	1	64.57	129.14	143.22	286.44	0.1	163549	0.18	142	0.0002	1	0.003	15	0.0019
			0.5	2	78.65	157.3			0.1					1		15	
	2-3	2	0.5	1	38.27	76.54	89.05	178.1	0.1	163549	0.11	34	0.0000	1	0.003	15	0.0004
			0.5	2	50.76	101.56			0.1					1		15	
	3-1	2	0.5	1	62.26	124.52	130.92	261.84	0.1	163549	0.16	117	0.0001	1	0.003	15	0.0016
			0.5	2	68.66	137.32			0.1					1		15	
	3-2	2	0.5	1	91.01	182.02	167.27	334.54	0.1	163549	0.20	190	0.0002	1	0.003	15	0.0025
			0.5	2	76.26	152.52			0.1					1		15	
	3-3	2	0.5	1	60.03	120.06	123.36	246.76	0.1	163549	0.15	102	0.0001	1	0.003	15	0.0014
			0.5	2	63.35	126.7			0.1					1		15	
	4-1	2	0.5	1	309.19	618.38	615.64	1231.28	0.1	163549	0.75	1087	0.0013	1	0.003	15	0.0144
			0.5	2	306.45	612.9			0.1					1		15	
	4-2	2	0.5	1	336.71	673.42	648.55	1297.1	0.1	163549	0.79	1153	0.0014	1	0.003	15	0.0152
			0.5	2	311.84	623.68			0.1					1		15	
	4-3	2	0.5	1	346.65	693.3	685.24	1370.48	0.1	163549	0.84	1226	0.0015	1	0.003	15	0.0162
			0.5	2	338.59	677.18			0.1					1		15	
	5-1	2	0.5	1	1806.12	3612.24	3938.3	7876.6	0.1	163549	4.82	7732	0.0094	1	0.003	15	0.1022
			0.5	2	2132.18	4264.36			0.1					1		15	
	5-2	2	0.5	1	2050.7	4101.4	4081.07	8162.14	0.1	163549	4.99	8018	0.0098	1	0.003	15	0.1060
			0.5	2	2030.37	4060.74			0.1					1		15	
	5-3	2	0.5	1	2097.41	4194.82	4190.89	8381.78	0.1	163549	5.12	8237	0.0100	1	0.003	15	0.1089
			0.5	2	2093.48	4186.96			0.1					1		15	
	6-1	2	0.5	1	6134.84	12269.68	12345.07	24690.14	0.1	163549	15.10	24546	0.0299	1	0.003	15	0.3245
			0.5	2	6210.23	12420.46			0.1					1		15	
	6-2	2	0.5	1	6604.08	13208.16	13560.78	27121.56	0.1	163549	16.58	26977	0.0329	1	0.003	15	0.3567
			0.5	2	6956.7	13913.4			0.1					1		15	
	6-3	2	0.5	1	7141.5	14283	14254.12	28508.24	0.1	163549	17.43	28364	0.0346	1	0.003	15	0.3750
			0.5	2	7112.62	14225.24			0.1					1		15	
	7-1	2	0.5	1	8724.65	17449.3	17203.04	34406.08	0.1	163549	21.04	34262	0.0418	1	0.003	15	0.4530
			0.5	2	8478.39	16956.78			0.1					1		15	
	7-2	2	0.5	1	9185.92	18371.84	18352.19	36704.38	0.1	163549	22.44	36560	0.0446	1	0.003	15	0.4834
			0.5	2	9166.27	18332.54			0.1					1		15	
	7-3	2	0.5	1	9076.51	18153.02	18298.37	36596.74	0.1	163549	22.38	36452	0.0444	1	0.003	15	0.4819
			0.5	2	9221.86	18443.72			0.1					1		15	
	8-1	2	0.5	1	8936.97	17873.94	18041.91	36083.82	0.1	163549	22.06	35939	0.0438	1	0.003	15	0.4752
			0.5	2	9104.94	18209.88			0.1					1		15	
	8-2	2	0.5	1	9741.67	19483.34	18689.68	37379.36	0.1	163549	22.86	37235	0.0454	1	0.003	15	0.4923
			0.5	2	8948.01	17896.02			0.1					1		15	
	8-3	2	0.5	1	9225.98	18451.96	18593.43	37186.86	0.1	163549	22.74	37042	0.0452	1	0.003	15	0.4897
			0.5	2	9367.45	18734.9			0.1					1		15	

Assay Date	8/4/2005	ID	RC3	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	TNB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4784	#DIV/0!
Full activity	End	0.4302	0.0139
Full activity	Overall	0.4463	0.0295
Background	Beginning	0.0001	0.000249239
Background	End	-0.0001	0.000248678
Background	Overall	0.0000	0.000238052
Positive	Beginning	0.2378	0.0134
Positive	End	0.2132	0.0067
Positive	Overall	0.2255	0.0167
Negative	Beginning	0.4725	0.0167
Negative	End	0.4179	0.0083
Negative	Overall	0.4452	0.0333

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
RC3	1	1	1.00E-03	-3.00	0.0008
RC3	1	2	1.00E-03	-3.00	0.0002
RC3	1	3	1.00E-03	-3.00	0.0001
RC3	2	1	1.00E-04	-4.00	0.0006
RC3	2	2	1.00E-04	-4.00	0.0019
RC3	2	3	1.00E-04	-4.00	0.0004
RC3	3	1	1.00E-05	-5.00	0.0016
RC3	3	2	1.00E-05	-5.00	0.0025
RC3	3	3	1.00E-05	-5.00	0.0014
RC3	4	1	1.00E-06	-6.00	0.0144
RC3	4	2	1.00E-06	-6.00	0.0152
RC3	4	3	1.00E-06	-6.00	0.0162
RC3	5	1	1.00E-07	-7.00	0.1022
RC3	5	2	1.00E-07	-7.00	0.1060
RC3	5	3	1.00E-07	-7.00	0.1089
RC3	6	1	1.00E-08	-8.00	0.3245
RC3	6	2	1.00E-08	-8.00	0.3567
RC3	6	3	1.00E-08	-8.00	0.3750
RC3	7	1	1.00E-09	-9.00	0.4530
RC3	7	2	1.00E-09	-9.00	0.4834
RC3	7	3	1.00E-09	-9.00	0.4819
RC3	8	1	1.00E-10	-10.00	0.4752
RC3	8	2	1.00E-10	-10.00	0.4923
RC3	8	3	1.00E-10	-10.00	0.4897

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	0.17	0.05	0.02
2	-4.00	0.13	0.42	0.10
3	-5.00	0.35	0.56	0.30
4	-6.00	3.22	3.41	3.63
5	-7.00	22.91	23.75	24.40
6	-8.00	72.72	79.92	84.03
7	-9.00	101.50	108.31	107.99
8	-10.00	106.47	110.31	109.74

Aromatase Assay Spreadsheet

Assay Date	8/8/2005	Test Chemical ID	Chem 3	# Concentrations tested	8
ID	TNB	Replicate #	Rep 2	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0202	31831.28	1575806	
2	0.0205	33482.48	1633292	
3	0.0201	32909.91	1637309	
4	0.0201	31529.07	1568610	
5	0.0203	32792.16	1615377	
			Average DPM/g soln	1606079
			SD	32104
			CV	2.00
			µCi/g soln	0.723

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	8.0555 g
Mass of dilution B used in substrate prep	4.5155 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.57176 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00819 µg/g soln.
µg/g soln.

a. µCi/g soln	0.723
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.

= 0.571760 + 0.00819
= 0.579949 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.247 µCi/µg ASDN

793140 dpm/nmol

Assay Date 8/8/2005 Test Chemical ID Chem 3 tested 8

ID TNB Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.581	0.340	0.166	0.101	0.053	0.043	0.034	25	100	
	0.587	0.351	0.160	0.092	0.050	0.044	0.041			
	0.585	0.358	0.160	0.105	0.062	0.042	0.031			

Samples:	10	100	microsomes
	0.052	0.274	0.061
	0.051	0.274	0.044
			0.053

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	0.584	0.549	0.0484	m, b	0.089	-0.001
0.125	100	200	0.00013	200	0.0250	0.349	0.314	0.0275	se_m, se_b	0.003	0.001
0.05	200	1000	0.00005	200	0.0100	0.162	0.126	0.0107	r , se_y	0.994	0.002
0.025	100	1000	0.00003	200	0.0050	0.099	0.064	0.0051	F, df	681	4
0.01	40	1000	0.00001	200	0.0020	0.055	0.019	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.043	0.008	0.0001			

Regression results are calculated using the function LINEST

Blank 0.036

r^2= 0.994
m= 0.089
b= -0.001

	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	0.052	0.017	0.001	200	1	1	0.000	0.000	0.004
10	0.051	0.016	0.001	200	1	1	0.000		
10					1	1			
100	0.274	0.239	0.021	200	1	1	0.000	0.000	0.104
100	0.274	0.238	0.021	200	1	1	0.000		
100									
	0.061	0.025	0.002	200	114	69814	0.005	0.003	2.896
	0.044	0.009	0.000	200	114	69814	0.001		
	0.053	0.017	0.001	200	114	69814	0.003		

Test

Assay Date 8/8/2005 Chemical ID Chem 3 tested 8

ID TNB Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.581	0.340	0.166	0.101	0.053	0.043	0.034	25	100	
	0.587	0.351	0.160	0.092	0.050	0.044	0.041			
	0.585	0.358	0.160	0.105	0.062	0.042	0.031			

Samples:	10	100	microsomes
	0.052	0.274	0.061
	0.051	0.274	0.044
			0.053

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	0.584	0.549	0.0433	m, b	0.078	0.000
0.125	100	200	0.00013	200	0.0250	0.349	0.314	0.0249	se_m, se_b	0.001	0.000
0.05	200	1000	0.00005	200	0.0100	0.162	0.126	0.0102	r , se_y	1.000	0.000
0.025	100	1000	0.00003	200	0.0050	0.099	0.064	0.0053	F, df	6323	3
0.01	40	1000	0.00001	200	0.0020	0.055	0.019	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.043	0.008	0.0009			

Blank 0.036

r^2= 1.000
m= 0.078
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	0.052	0.017	0.002	200	1	1	0.000	0.000	0.008
10	0.051	0.016	0.001	200	1	1	0.000		
10					1	1			
100	0.274	0.239	0.019	200	1	1	0.000	0.000	0.095
100	0.274	0.238	0.019	200	1	1	0.000		
100									
	0.061	0.025	0.002	200	114	69814	0.007	0.005	4.936
	0.044	0.009	0.001	200	114	69814	0.003		
	0.053	0.017	0.002	200	114	69814	0.005		

Assay Date	8/8/2005	Test Chemical ID	Chem 3	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

								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	8/8/2005	Chemical ID	Chem 3	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.40351	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
	612.40351	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.936
Protein Concentration (dilution added to assay, mg/mL):	0.00806

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	8/8/2005	Test Chemical ID	Chem 3	# Concentrations tested	8	Microsome type		Microsome ID		0	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	465.91	931.82	845.64	1691.28	0.1	160608	1.18	1774	0.0022	1	0.004	15	0.0185
			0.5	2	479.73	959.46			0.1					1		15	
	2	2	0.5	1	440.55	881.1	880.51	1761.02	0.1	160608	1.10	1643	0.0021	1	0.004	15	0.0171
			0.5	2	439.96	879.92			0.1					1		15	
	3	2	0.5	1	429.05	858.1	862.67	1725.34	0.1	160608	1.07	1606	0.0020	1	0.004	15	0.0168
			0.5	2	433.62	867.24			0.1					1		15	
	4	2	0.5	1	426.12	852.24	874.58	1749.16	0.1	160608	1.09	1632	0.0021	1	0.004	15	0.0170
			0.5	2	448.46	896.92			0.1					1		15	
Background control	1	2	0.5	1	19.28	38.56	54.91	109.82	0.1	160608	0.07	-8	0.0000	1	0.004	15	-0.0001
			0.5	2	35.63	71.26			0.1					1		15	
	2	2	0.5	1	31.97	63.94	62.97	125.94	0.1	160608	0.08	8	0.0000	1	0.004	15	0.0001
			0.5	2	31	62			0.1					1		15	
	3	2	0.5	1	25.15	50.3	51.66	103.32	0.1	160608	0.06	-14	0.0000	1	0.004	15	-0.0001
			0.5	2	26.51	53.02			0.1					1		15	
	4	2	0.5	1	36.33	72.66	65.54	131.08	0.1	160608	0.08	14	0.0000	1	0.004	15	0.0001
			0.5	2	29.21	58.42			0.1					1		15	
Positive control	1	2	0.5	1	448.33	896.66	910.7	1821.4	0.1	160608	1.13	1704	0.0021	1	0.004	15	0.0178
			0.5	2	462.37	924.74			0.1					1		15	
	2	2	0.5	1	460.24	920.48	898.59	1797.18	0.1	160608	1.12	1680	0.0021	1	0.004	15	0.0175
			0.5	2	438.36	876.7			0.1					1		15	
	3	2	0.5	1	447.12	894.24	890.22	1780.44	0.1	160608	1.11	1663	0.0021	1	0.004	15	0.0173
			0.5	2	443.1	886.2			0.1					1		15	
	4	2	0.5	1	422.6	845.2	839.64	1679.28	0.1	160608	1.05	1582	0.0020	1	0.004	15	0.0163
			0.5	2	417.04	834.08			0.1					1		15	
Negative Control	1	2	0.5	1	447.5	895	895.94	1791.88	0.1	160608	1.12	1674	0.0021	1	0.004	15	0.0175
			0.5	2	448.44	896.88			0.1					1		15	
	2	2	0.5	1	446.84	893.68	891.51	1783.02	0.1	160608	1.11	1665	0.0021	1	0.004	15	0.0174
			0.5	2	444.67	889.34			0.1					1		15	
	3	2	0.15	1	136.82	912.133333	911.8333333	1823.6667	0.1	160608	1.14	1706	0.0022	1	0.004	15	0.0178
			0.15	2	136.73	911.533333			0.1					1		15	
	4	2	0.5	1	435.76	871.52	871.23	1742.46	0.1	160608	1.08	1625	0.0020	1	0.004	15	0.0169
			0.5	2	435.47	870.94			0.1					1		15	
Chem 3	1-1	2	0.5	1	91.47	182.94	164.44	328.88	0.1	160608	0.20	211	0.0003	1	0.004	15	0.0022
			0.5	2	72.97	145.94			0.1					1		15	
	1-2	2	0.5	1	79	158	160.51	321.02	0.1	160608	0.20	203	0.0003	1	0.004	15	0.0021
			0.5	2	81.51	163.02			0.1					1		15	
	1-3	2	0.5	1	69.35	138.7	139.39	278.78	0.1	160608	0.17	161	0.0002	1	0.004	15	0.0017
			0.5	2	70.04	140.08			0.1					1		15	
	2-1	2	0.5	1	253.39	506.78	501.33	1002.66	0.1	160608	0.62	885	0.0011	1	0.004	15	0.0092
			0.5	2	247.94	495.88			0.1					1		15	
	2-2	2	0.5	1	256.07	512.14	515.04	1030.08	0.1	160608	0.64	913	0.0012	1	0.004	15	0.0095
			0.5	2	258.97	517.94			0.1					1		15	
	2-3	2	0.5	1	237.39	474.78	484.75	969.5	0.1	160608	0.60	852	0.0011	1	0.004	15	0.0089
			0.5	2	247.36	494.72			0.1					1		15	
	3-1	2	0.5	1	444.8	889.6	870.68	1741.36	0.1	160608	1.08	1624	0.0020	1	0.004	15	0.0169
			0.5	2	425.88	851.76			0.1					1		15	
	3-2	2	0.5	1	399.58	799.16	829.14	1658.28	0.1	160608	1.03	1541	0.0019	1	0.004	15	0.0161
			0.5	2	429.56	859.12			0.1					1		15	
	3-3	2	0.5	1	463.09	926.18	919.41	1838.82	0.1	160608	1.14	1721	0.0022	1	0.004	15	0.0180
			0.5	2	456.32	912.64			0.1					1		15	
	4-1	2	0.5	1	447.03	894.06	896.52	1793.04	0.1	160608	1.12	1676	0.0021	1	0.004	15	0.0175
			0.5	2	449.49	898.98			0.1					1		15	
	4-2	2	0.5	1	437.41	874.82	868.5	1737	0.1	160608	1.08	1619	0.0020	1	0.004	15	0.0169
			0.5	2	431.09	862.18			0.1					1		15	
	4-3	2	0.5	1	454.78	909.56	885.89	1771.78	0.1	160608	1.10	1654	0.0021	1	0.004	15	0.0173
			0.5	2	431.11	862.22			0.1					1		15	
	5-1	2	0.5	1	434.49	868.98	883.04	1766.08	0.1	160608	1.10	1649	0.0021	1	0.004	15	0.0172
			0.5	2	448.55	897.1			0.1					1		15	
	5-2	2	0.5	1	419.06	838.12	864.25	1728.5	0.1	160608	1.08	1611	0.0020	1	0.004	15	0.0168
			0.5	2	445.19	890.38			0.1					1		15	
	5-3	2	0.5	1	483.59	967.06	987.16	1974.32	0.1	160608	1.23	1857	0.0023	1	0.004	15	0.0194
			0.5	2	503.63	1007.26			0.1					1		15	
	6-1	2	0.5	1	469.82	933.64	954.01	1908.02	0.1	160608	1.19	1790	0.0023	1	0.004	15	0.0187
			0.5	2	487.19	974.38			0.1					1		15	
	6-2	2	0.5	1	432.05	864.1	894.58	1789.16	0.1	160608	1.11	1672	0.0021	1	0.004	15	0.0174
			0.5	2	462.53	925.06			0.1					1		15	
	6-3	2	0.5	1	445.65	891.3	951.84	1903.68	0.1	160608	1.19	1786	0.0023	1	0.004	15	0.0186
			0.5	2	506.19	1012.38			0.1					1		15	
	7-1	2	0.5	1	466.94	933.88	918.02	1836.04	0.1	160608	1.14	1719	0.0022	1	0.004	15	0.0179
			0.5	2	451.08	902.16			0.1					1		15	
	7-2	2	0.5	1	439.02	878.04	884.08	1768.16	0.1	160608	1.10	1651	0.0021	1	0.004	15	0.0172
			0.5	2	445.06	890.12			0.1					1		15	
	7-3	2	0.5	1	471.65	943.3	948.73	1897.46	0.1	160608	1.18	1780	0.0022	1	0.004	15	0.0186
			0.5	2	477.08	954.16			0.1					1		15	
	8-1	2	0.5	1	450.97	901.94	897	1794	0.1	160608	1.12	1676	0.0021	1	0.004	15	0.0175
			0.5	2	446.03	892.06			0.1					1		15	
	8-2	2	0.5	1	435.44	870.88	882.87	1765.74	0.1	160608	1.10	1648	0.0021	1	0.004	15	0.0172
			0.5	2	447.43	894.86			0.1					1		15	
	8-3	2	0.5	1	438.73	877.46	890.96	1781.92	0.1	160608	1.11	1664	0.0021	1	0.004	15	0.0174
			0.5	2	452.23	904.46			0.1					1		15	

Assay Date	8/8/2005	ID	Chem 3	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0	Technician ID	TNB	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0178	0.0010
Full activity	End	0.0169	0.0002
Full activity	Overall	0.0174	0.0008
Background	Beginning	0.0000	0.00011887
Background	End	0.0000	0.000204704
Background	Overall	0.0000	0.000136728
Positive	Beginning	0.0176	0.0002
Positive	End	0.0168	0.0007
Positive	Overall	0.0172	0.0007
Negative	Beginning	0.0174	0.0001
Negative	End	0.0174	0.0006
Negative	Overall	0.0174	0.0003

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Chem 3	1	1	1.00E-05	-5.00	0.0022
Chem 3	1	2	1.00E-05	-5.00	0.0021
Chem 3	1	3	1.00E-05	-5.00	0.0017
Chem 3	2	1	1.00E-06	-6.00	0.0092
Chem 3	2	2	1.00E-06	-6.00	0.0095
Chem 3	2	3	1.00E-06	-6.00	0.0089
Chem 3	3	1	1.00E-07	-7.00	0.0169
Chem 3	3	2	1.00E-07	-7.00	0.0161
Chem 3	3	3	1.00E-07	-7.00	0.0180
Chem 3	4	1	3.00E-08	-7.52	0.0175
Chem 3	4	2	3.00E-08	-7.52	0.0169
Chem 3	4	3	3.00E-08	-7.52	0.0173
Chem 3	5	1	1.00E-08	-8.00	0.0172
Chem 3	5	2	1.00E-08	-8.00	0.0168
Chem 3	5	3	1.00E-08	-8.00	0.0194
Chem 3	6	1	3.00E-09	-8.52	0.0187
Chem 3	6	2	3.00E-09	-8.52	0.0174
Chem 3	6	3	3.00E-09	-8.52	0.0186
Chem 3	7	1	1.00E-09	-9.00	0.0179
Chem 3	7	2	1.00E-09	-9.00	0.0172
Chem 3	7	3	1.00E-09	-9.00	0.0186
Chem 3	8	1	1.00E-10	-10.00	0.0175
Chem 3	8	2	1.00E-10	-10.00	0.0172
Chem 3	8	3	1.00E-10	-10.00	0.0174

	Percent of control values			
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-5.00	12.70	12.23	9.69
2	-6.00	53.19	54.83	51.19
3	-7.00	97.58	92.58	103.43
4	-7.52	100.68	97.31	99.40
5	-8.00	99.06	96.80	111.57
6	-8.52	107.59	100.45	107.33
7	-9.00	103.27	99.19	106.96
8	-10.00	100.74	99.04	100.01

Aromatase Assay Spreadsheet

Assay Date	8/10/2005	Test Chemical ID	RC3	# Concentrations tested	8
ID	TNB	Replicate #	Rep 3	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0203	33657.08	1657984	
2	0.0200	33179.37	1658969	
3	0.0200	33143.17	1657159	
4	0.0201	33186.38	1651064	
5	0.0201	34001.11	1691598	
			Average DPM/g soln	1663354
			SD	16087
			CV	0.97
			µCi/g soln	0.749

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	8.1165 g
Mass of dilution B used in substrate prep	4.5788 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.564135 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00848 µg/g soln.
µg/g soln.

a. µCi/g soln 0.749
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.

= 0.564135 + 0.00848
= 0.572617 µg ASDN/g soln.

3) Calculate Solution Specific Activity

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

831944 dpm/nmol

Assay Date	8/10/2005	Test Chemical ID	RC3	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.576	0.371	0.165	0.097	0.055	0.041	0.038	25	100	
	0.573	0.354	0.163	0.102	0.056	0.050	0.033			
	0.570	0.366	0.163	0.101	0.059	0.046	0.031			

Samples:	10	100	microsomes
	0.059	0.292	0.060
	0.061	0.298	0.055
			0.054

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	0.573	0.539	0.0476
0.125	100	200	0.00013	200	0.0250	0.364	0.330	0.0287
0.05	200	1000	0.00005	200	0.0100	0.163	0.129	0.0107
0.025	100	1000	0.00003	200	0.0050	0.100	0.066	0.0049
0.01	40	1000	0.00001	200	0.0020	0.057	0.023	0.0010
0.005	20	1000	0.00001	200	0.0010	0.046	0.012	0.0000

Variables		Regression results	
m, b		0.090	-0.001
se_m, se_b		0.005	0.001
r , se_y		0.988	0.002
F, df		325	4
ss_{reg}, ss_{resid}		0.002	0.000

Regression results are calculated using the function LINEST

Blank 0.034

r^2= 0.988
m= 0.090
b= -0.001

	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	0.059	0.025	0.001	200	1	1	0.000	0.000	0.007
10	0.061	0.027	0.001	200	1	1	0.000		
10					1	1			
100	0.292	0.258	0.022	200	1	1	0.000	0.000	0.113
100	0.298	0.264	0.023	200	1	1	0.000		
100									
	0.060	0.026	0.001	200	114	69814	0.004	0.003	3.015
	0.055	0.021	0.001	200	114	69814	0.003		
	0.054	0.020	0.001	200	114	69814	0.002		

Test
Assay Date 8/10/2005 Chemical ID RC3 tested 8

ID TNB Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.576	0.371	0.165	0.097	0.055	0.041	0.038	25	100	
	0.573	0.354	0.163	0.102	0.056	0.050	0.033			
	0.570	0.366	0.163	0.101	0.059	0.046	0.031			

Samples:	10	100	microsomes
	0.059	0.292	0.060
	0.061	0.298	0.055
			0.054

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	0.573	0.539	0.0408
0.125	100	200	0.00013	200	0.0250	0.364	0.330	0.0250
0.05	200	1000	0.00005	200	0.0100	0.163	0.129	0.0099
0.025	100	1000	0.00003	200	0.0050	0.100	0.066	0.0051
0.01	40	1000	0.00001	200	0.0020	0.057	0.023	0.0019
0.005	20	1000	0.00001	200	0.0010	0.046	0.012	0.0011

Blank 0.034

r^2= 1.000
m= 0.075
b= 0.000

Variables	Regression results	
m, b	0.075	0.000
se_m, se_b	0.000	0.000
r , se_y	1.000	0.000
F, df	23843	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	0.059	0.025	0.002	200	1	1	0.000	0.000	0.011
10	0.061	0.027	0.002	200	1	1	0.000		
10					1	1			
100	0.292	0.258	0.020	200	1	1	0.000	0.000	0.099
100	0.298	0.264	0.020	200	1	1	0.000		
100									
	0.060	0.026	0.002	200	114	69814	0.006	0.006	5.664
	0.055	0.021	0.002	200	114	69814	0.005		
	0.054	0.020	0.002	200	114	69814	0.005		

Assay Date	8/10/2005	Test Chemical ID	RC3	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

								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	8/10/2005	Chemical ID	RC3	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 3

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.664
Protein Concentration (dilution added to assay, mg/mL):	0.009249

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	8/10/2005	Test Chemical ID	RC3	# Concentrations tested	8	Microsome type		Microsome ID		0 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	12255.02	24510.04	24703.84	49407.68	0.1	166335	29.70	49270	0.0592	1	0.005	15	0.4269
			0.5	2	12448.82	24897.64			0.1					1		15	
	2	2	0.5	1	12613.56	25227.12	25454.84	50909.68	0.1	166335	30.61	50772	0.0610	1	0.005	15	0.4399
			0.5	2	12841.28	25682.56			0.1					1		15	
	3	2	0.5	1	11055.52	22111.04	22179.99	44359.98	0.1	166335	26.67	44223	0.0532	1	0.005	15	0.3832
			0.5	2	11124.47	22248.94			0.1					1		15	
	4	2	0.5	1	11880.86	23761.92	23741.34	47482.68	0.1	166335	28.55	47345	0.0569	1	0.005	15	0.4102
			0.5	2	11860.38	23720.76			0.1					1		15	
Background control	1	2	0.5	1	32.15	64.3	71.1	142.2	0.1	166335	0.09	5	0.0000	1	0.005	15	0.0000
			0.5	2	38.95	77.9			0.1					1		15	
	2	2	0.5	1	34.33	68.66	72.67	145.34	0.1	166335	0.09	8	0.0000	1	0.005	15	0.0001
			0.5	2	38.34	76.68			0.1					1		15	
	3	2	0.5	1	28.71	57.42	56.54	113.08	0.1	166335	0.07	-24	0.0000	1	0.005	15	-0.0002
			0.5	2	27.83	55.66			0.1					1		15	
	4	2	0.5	1	33.72	67.44	74.07	148.14	0.1	166335	0.09	11	0.0000	1	0.005	15	0.0001
			0.5	2	40.35	80.7			0.1					1		15	
Positive control	1	2	0.5	1	7024.25	14048.5	14096.28	28192.56	0.1	166335	16.95	28055	0.0337	1	0.005	15	0.2431
			0.5	2	7072.03	14144.06			0.1					1		15	
	2	2	0.5	1	6791.42	13582.84	13622.28	27244.56	0.1	166335	16.38	27107	0.0326	1	0.005	15	0.2349
			0.5	2	6830.86	13661.72			0.1					1		15	
	3	2	0.5	1	5848.77	11697.54	11745.35	23490.7	0.1	166335	14.12	23354	0.0281	1	0.005	15	0.2023
			0.5	2	5896.58	11793.16			0.1					1		15	
	4	2	0.5	1	6401.56	12803.16	12913.51	25827.02	0.1	166335	15.53	25690	0.0309	1	0.005	15	0.2226
			0.5	2	6511.93	13023.86			0.1					1		15	
Negative Control	1	2	0.5	1	12482	24964	25124.71	50248.42	0.1	166335	30.21	50112	0.0602	1	0.005	15	0.4342
			0.5	2	12642.71	25285.42			0.1					1		15	
	2	2	0.5	1	12438.21	24876.42	25015.41	50030.82	0.1	166335	30.08	49894	0.0600	1	0.005	15	0.4323
			0.5	2	12577.2	25154.4			0.1					1		15	
	3	2	0.5	1	11948.37	23896.74	24127.8	48255.6	0.1	166335	29.01	48118	0.0578	1	0.005	15	0.4169
			0.5	2	12176.43	24352.86			0.1					1		15	
	4	2	0.5	1	11818.71	23637.42	23574.5	47149	0.1	166335	28.35	47012	0.0565	1	0.005	15	0.4073
			0.5	2	11655.79	23311.58			0.1					1		15	
RC3	1-1	2	0.5	1	100.77	201.54	189.47	378.94	0.1	166335	0.23	242	0.0003	1	0.005	15	0.0021
			0.5	2	88.7	177.4			0.1					1		15	
	1-2	2	0.5	1	79.35	158.7	165.13	330.26	0.1	166335	0.20	193	0.0002	1	0.005	15	0.0017
			0.5	2	85.78	171.56			0.1					1		15	
	1-3	2	0.5	1	79.07	158.14	151.09	302.18	0.1	166335	0.18	165	0.0002	1	0.005	15	0.0014
			0.5	2	72.02	144.04			0.1					1		15	
	2-1	2	0.5	1	500.24	1000.48	1009.44	2018.88	0.1	166335	1.21	1882	0.0023	1	0.005	15	0.0163
			0.5	2	509.2	1018.4			0.1					1		15	
	2-2	2	0.5	1	459.65	919.3	908.42	1816.84	0.1	166335	1.09	1680	0.0020	1	0.005	15	0.0146
			0.5	2	448.77	897.54			0.1					1		15	
	2-3	2	0.5	1	458.53	917.06	912.77	1825.54	0.1	166335	1.10	1688	0.0020	1	0.005	15	0.0146
			0.5	2	454.24	908.48			0.1					1		15	
	3-1	2	0.5	1	2907.21	5814.42	5888.68	11777.36	0.1	166335	7.08	11640	0.0140	1	0.005	15	0.1009
			0.5	2	2981.47	5962.94			0.1					1		15	
	3-2	2	0.5	1	2559.5	5119	5396.45	10792.9	0.1	166335	6.49	10656	0.0128	1	0.005	15	0.0923
			0.5	2	2836.95	5673.9			0.1					1		15	
	3-3	2	0.5	1	3172.78	6345.56	6319.53	12639.06	0.1	166335	7.60	12502	0.0150	1	0.005	15	0.1083
			0.5	2	3146.75	6293.5			0.1					1		15	
	4-1	2	0.5	1	6405.38	12810.76	12833.9	25667.8	0.1	166335	15.43	25631	0.0307	1	0.005	15	0.2212
			0.5	2	6428.52	12857.04			0.1					1		15	
	4-2	2	0.5	1	6889.75	13781.5	13789.44	27578.88	0.1	166335	16.58	27442	0.0330	1	0.005	15	0.2378
			0.5	2	6898.69	13797.38			0.1					1		15	
	4-3	2	0.5	1	6463.36	12926.72	12978.78	25957.56	0.1	166335	15.61	25820	0.0310	1	0.005	15	0.2237
			0.5	2	6515.42	13030.84			0.1					1		15	
	5-1	2	0.5	1	10218.22	20432.44	20457.88	40915.72	0.1	166335	24.60	40779	0.0490	1	0.005	15	0.3533
			0.5	2	10241.64	20483.28			0.1					1		15	
	5-2	2	0.5	1	9094.82	18189.64	18308.91	36617.82	0.1	166335	22.01	36481	0.0438	1	0.005	15	0.3161
			0.5	2	9214.09	18428.18			0.1					1		15	
	5-3	2	0.5	1	9179.88	18359.76	18486.38	36972.76	0.1	166335	22.23	36836	0.0443	1	0.005	15	0.3192
			0.5	2	9306.5	18613			0.1					1		15	
	6-1	2	0.5	1	11498.21	22996.42	23208.26	46416.52	0.1	166335	27.91	46279	0.0556	1	0.005	15	0.4010
			0.5	2	11710.05	23420.1			0.1					1		15	
	6-2	2	0.5	1	11699.53	23399.06	23557.41	47114.82	0.1	166335	28.33	46978	0.0565	1	0.005	15	0.4070
			0.5	2	11857.88	23715.76			0.1					1		15	
	6-3	2	0.5	1	11426.2	22852.4	22932.47	45864.94	0.1	166335	27.57	45728	0.0550	1	0.005	15	0.3962
			0.5	2	11506.27	23012.54			0.1					1		15	
	7-1	2	0.5	1	12695.24	25390.48	25391.18	50782.36	0.1	166335	30.53	50645	0.0609	1	0.005	15	0.4388
			0.5	2	12725.94	25451.88			0.1					1		15	
	7-2	2	0.5	1	12732.01	25464.02	25565.57	51131.14	0.1	166335	30.74	50994	0.0613	1	0.005	15	0.4418
			0.5	2	12833.56	25667.12			0.1					1		15	
	7-3	2	0.5	1	12080.52	24161.04	24420.07	48840.14	0.1	166335	29.36	48703	0.0585	1	0.005	15	0.4220
			0.5	2	12339.55	24679.1			0.1					1		15	
	8-1	2	0.5	1	12874.07	25748.14	26111.99	52223.98	0.1	166335	31.40	52087	0.0626	1	0.005	15	0.4513
			0.5	2	13237.92	26475.84			0.1					1		15	
	8-2	2	0.5	1	12320.68	24641.36	24078.46	48156.92	0.1	166335	28.95	48020	0.0577	1	0.005	15	0.4161
			0.5	2	11757.78	23515.56			0.1					1		15	
	8-3	2	0.5	1	12959.39	25918.78	25884.68	51769.36	0.1	166335	31.12	51632	0.0621	1	0.005	15	0.4474
			0.5	2	12925.29	25850.58			0.1					1		15	

Assay Date	8/10/2005	ID	RC3	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0 Technician ID	TNB	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.4334	0.0092
Full activity	End	0.3967	0.0191
Full activity	Overall	0.4150	0.0245
Background	Beginning	0.0001	1.92373E-05
Background	End	-0.0001	0.000214796
Background	Overall	0.0000	0.000140841
Positive	Beginning	0.2390	0.0058
Positive	End	0.2125	0.0143
Positive	Overall	0.2257	0.0177
Negative	Beginning	0.4332	0.0013
Negative	End	0.4121	0.0068
Negative	Overall	0.4227	0.0128

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC3	1	1	1.00E-05	-5.00	0.0021
RC3	1	2	1.00E-05	-5.00	0.0017
RC3	1	3	1.00E-05	-5.00	0.0014
RC3	2	1	1.00E-06	-6.00	0.0163
RC3	2	2	1.00E-06	-6.00	0.0146
RC3	2	3	1.00E-06	-6.00	0.0146
RC3	3	1	1.00E-07	-7.00	0.1009
RC3	3	2	1.00E-07	-7.00	0.0923
RC3	3	3	1.00E-07	-7.00	0.1083
RC3	4	1	3.00E-08	-7.52	0.2212
RC3	4	2	3.00E-08	-7.52	0.2378
RC3	4	3	3.00E-08	-7.52	0.2237
RC3	5	1	1.00E-08	-8.00	0.3533
RC3	5	2	1.00E-08	-8.00	0.3161
RC3	5	3	1.00E-08	-8.00	0.3192
RC3	6	1	3.00E-09	-8.52	0.4010
RC3	6	2	3.00E-09	-8.52	0.4070
RC3	6	3	3.00E-09	-8.52	0.3962
RC3	7	1	1.00E-09	-9.00	0.4388
RC3	7	2	1.00E-09	-9.00	0.4418
RC3	7	3	1.00E-09	-9.00	0.4220
RC3	8	1	1.00E-10	-10.00	0.4513
RC3	8	2	1.00E-10	-10.00	0.4161
RC3	8	3	1.00E-10	-10.00	0.4474

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-5.00	0.50	0.40	0.34
2	-6.00	3.93	3.51	3.52
3	-7.00	24.30	22.24	26.10
4	-7.52	53.30	57.29	53.90
5	-8.00	85.13	76.16	76.90
6	-8.52	96.61	98.07	95.46
7	-9.00	105.72	106.45	101.67
8	-10.00	108.73	100.24	107.79

Aromatase Assay Spreadsheet

Assay Date	9/20/2005	Test Chemical ID	RC4	# Concentrations tested	8
ID	JG	Replicate #	Rep 1	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	34210.41	1727798	
2	0.0200	35633.46	1781673	
3	0.0199	35415.33	1779665	
4	0.0198	34426.24	1738699	
5	0.0204	35141.13	1722604	
			Average DPM/g soln	1750088
			SD	28523
			CV	1.63
			µCi/g soln	0.788

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	8.2516 g
Mass of dilution B used in substrate prep	4.6427 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.585148 µg/g

Calculation of Substrate Solution Specific Activity

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

a. µCi/g soln 0.788
b. Specific activity of [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.

= 0.585148 + 0.00892
= 0.594072 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.327 µCi/µg ASDN

843711 dpm/nmol

Test

Assay Date 9/20/2005 Chemical ID RC4 tested 8

ID JG Replicate # Rep 1 Microsome type Recombinant Microsome ID 0

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	0.344	0.142	0.088	0.048	0.040	0.034	25	100	
	0.563	0.333	0.146	0.078	0.051	0.040	0.036			
	0.572	0.339	0.146	0.072	0.046	0.044	0.036			

Samples:	10	100	microsomes
	0.058	0.280	0.060
	0.058	0.277	0.054
			0.057

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	0.535	0.0485
0.125	100	200	0.00013	200	0.0250	0.339	0.303	0.0277
0.05	200	1000	0.00005	200	0.0100	0.145	0.109	0.0102
0.025	100	1000	0.00003	200	0.0050	0.079	0.044	0.0043
0.01	40	1000	0.00001	200	0.0020	0.048	0.013	0.0015
0.005	20	1000	0.00001	200	0.0010	0.041	0.006	0.0009

Blank 0.035

Variables	Regression results	
m, b	0.090	0.000
se_m, se_b	0.003	0.001
r , se_y	0.994	0.002
F, df	713	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

	r^2=	0.994							
	m=	0.090							
	b=	0.000							

	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	0.023	0.002	200	1	1	0.000	0.000	0.012
10	0.058	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.280	0.245	0.022	200	1	1	0.000	0.000	0.111
100	0.277	0.242	0.022	200	1	1	0.000		
100									
	0.060	0.025	0.003	200	114	69814	0.008	0.007	6.955
	0.054	0.019	0.002	200	114	69814	0.006		
	0.057	0.022	0.002	200	114	69814	0.007		

Test

Assay Date 9/20/2005 Chemical ID RC4 tested 8

ID JG Replicate # Rep 1 Microsome type Recombinant Microsome ID 0

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	0.344	0.142	0.088	0.048	0.040	0.034	25	100	
	0.563	0.333	0.146	0.078	0.051	0.040	0.036			
	0.572	0.339	0.146	0.072	0.046	0.044	0.036			

Samples:	10	100	microsomes
	0.058	0.280	0.060
	0.058	0.277	0.054
			0.057

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	0.535	0.0435	m, b	0.080	0.001
0.125	100	200	0.00013	200	0.0250	0.339	0.303	0.0252	se_m, se_b	0.002	0.000
0.05	200	1000	0.00005	200	0.0100	0.145	0.109	0.0097	r , se_y	0.998	0.000
0.025	100	1000	0.00003	200	0.0050	0.079	0.044	0.0045	F, df	1983	3
0.01	40	1000	0.00001	200	0.0020	0.048	0.013	0.0021	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.041	0.006	0.0015			

Blank 0.035

r^2= 0.998
m= 0.080
b= 0.001

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	0.023	0.003	200	1	1	0.000	0.000	0.014
10	0.058	0.023	0.003	200	1	1	0.000		
10					1	1			
100	0.280	0.245	0.020	200	1	1	0.000	0.000	0.102
100	0.277	0.242	0.020	200	1	1	0.000		
100									
	0.060	0.025	0.003	200	114	69814	0.009	0.008	8.449
	0.054	0.019	0.003	200	114	69814	0.008		
	0.057	0.022	0.003	200	114	69814	0.008		

Assay Date	9/20/2005	Test Chemical ID	RC4	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

								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	9/20/2005	Chemical ID	RC4	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.449
Protein Concentration (dilution added to assay, mg/mL):	0.013796

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	9/20/2005	Test Chemical ID	RC4	# Concentrations tested	8	Microsome type		Microsome ID		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	10950.39	21900.78	21771.87	43543.74	0.1	175009	24.86	43413	0.0515	1	0.007	15	0.2486
			0.5	2	10821.48	21642.96			0.1					1		15	
	2	2	0.5	1	10158.93	20317.86	20548.78	41097.56	0.1	175009	23.48	40967	0.0486	1	0.007	15	0.2346
			0.5	2	10389.85	20779.7			0.1					1		15	
	3	2	0.5	1	10190.87	20381.74	20511.34	41022.68	0.1	175009	23.44	40892	0.0485	1	0.007	15	0.2342
			0.5	2	10320.47	20640.94			0.1					1		15	
	4	2	0.5	1	10931.14	21862.28	22065.41	44130.82	0.1	175009	25.22	44000	0.0522	1	0.007	15	0.2520
			0.5	2	11134.27	22268.54			0.1					1		15	
Background control	1	2	0.5	1	36.59	73.18	68.5	137	0.1	175009	0.08	7	0.0000	1	0.007	15	0.0000
			0.5	2	31.91	63.82			0.1					1		15	
	2	2	0.5	1	30.59	61.18	70.54	141.08	0.1	175009	0.08	11	0.0000	1	0.007	15	0.0001
			0.5	2	39.95	79.9			0.1					1		15	
	3	2	0.5	1	31.22	62.44	68.98	137.96	0.1	175009	0.08	8	0.0000	1	0.007	15	0.0000
			0.5	2	37.76	75.52			0.1					1		15	
	4	2	0.5	1	26.26	56.52	52.65	105.3	0.1	175009	0.06	-25	0.0000	1	0.007	15	-0.0001
			0.5	2	24.39	48.78			0.1					1		15	
Positive control	1	2	0.5	1	4831.88	9663.76	9961.87	19923.74	0.1	175009	11.38	19793	0.0235	1	0.007	15	0.1134
			0.5	2	5129.98	10259.96			0.1					1		15	
	2	2	0.5	1	5465.36	10930.72	10896.82	21793.64	0.1	175009	12.45	21663	0.0257	1	0.007	15	0.1241
			0.5	2	5431.46	10862.92			0.1					1		15	
	3	2	0.5	1	4794.87	9589.74	9488.03	18976.06	0.1	175009	10.84	18846	0.0223	1	0.007	15	0.1079
			0.5	2	4693.16	9386.32			0.1					1		15	
	4	2	0.5	1	5251.21	10502.42	10522.2	21044.4	0.1	175009	12.02	20914	0.0248	1	0.007	15	0.1198
			0.5	2	5270.99	10541.98			0.1					1		15	
Negative Control	1	2	0.5	1	10636.07	21672.14	21757.37	43514.74	0.1	175009	24.86	43384	0.0514	1	0.007	15	0.2485
			0.5	2	10921.30	21842.6			0.1					1		15	
	2	2	0.5	1	10711.95	21423.9	21469.1	42938.2	0.1	175009	24.53	42808	0.0507	1	0.007	15	0.2452
			0.5	2	10757.15	21514.3			0.1					1		15	
	3	2	0.5	1	9819.56	19639.12	19817.97	39635.94	0.1	175009	22.65	39506	0.0468	1	0.007	15	0.2263
			0.5	2	9998.41	19996.82			0.1					1		15	
	4	2	0.5	1	9785.05	19570.1	19321.05	38642.1	0.1	175009	22.06	38512	0.0456	1	0.007	15	0.2206
			0.5	2	9536.00	19072			0.1					1		15	
RC4	1-1	2	0.5	1	54.79	109.58	110.5	221	0.1	175009	0.13	91	0.0001	1	0.007	15	0.0005
			0.5	2	55.71	111.42			0.1					1		15	
	1-2	2	0.5	1	45.01	90.02	100.48	200.96	0.1	175009	0.11	71	0.0001	1	0.007	15	0.0004
			0.5	2	55.47	110.94			0.1					1		15	
	1-3	2	0.5	1	43.62	87.24	98.39	196.78	0.1	175009	0.11	66	0.0001	1	0.007	15	0.0004
			0.5	2	54.77	109.54			0.1					1		15	
	2-1	2	0.5	1	192.00	384	384.35	768.7	0.1	175009	0.44	636	0.0008	1	0.007	15	0.0037
			0.5	2	192.35	384.7			0.1					1		15	
	2-2	2	0.5	1	190.24	380.48	381.74	763.48	0.1	175009	0.44	633	0.0008	1	0.007	15	0.0036
			0.5	2	191.50	383			0.1					1		15	
	2-3	2	0.5	1	211.36	422.72	417.18	834.36	0.1	175009	0.48	704	0.0008	1	0.007	15	0.0040
			0.5	2	205.82	411.64			0.1					1		15	
	3-1	2	0.5	1	7965.76	15931.52	15803.34	31606.68	0.1	175009	18.06	31476	0.0373	1	0.007	15	0.1803
			0.5	2	7837.58	15675.16			0.1					1		15	
	3-2	2	0.5	1	7427.44	14854.88	14796.76	29593.52	0.1	175009	16.91	29463	0.0349	1	0.007	15	0.1687
			0.5	2	7369.32	14738.64			0.1					1		15	
	3-3	2	0.5	1	4757.13	9514.26	9557.9	19115.8	0.1	175009	10.92	18985	0.0225	1	0.007	15	0.1087
			0.5	2	4800.77	9601.54			0.1					1		15	
	4-1	2	0.5	1	10571.89	21143.38	21086.23	42172.46	0.1	175009	24.10	42042	0.0498	1	0.007	15	0.2408
			0.5	2	10514.54	21029.08			0.1					1		15	
	4-2	2	0.5	1	10540.50	21081	21044.5	42089	0.1	175009	24.05	41959	0.0497	1	0.007	15	0.2403
			0.5	2	10504.00	21008			0.1					1		15	
	4-3	2	0.5	1	10153.61	20307.22	20179.89	40359.78	0.1	175009	23.08	40229	0.0477	1	0.007	15	0.2304
			0.5	2	10026.28	20052.56			0.1					1		15	
	5-1	2	0.5	1	9986.06	19972.12	19889.39	39778.78	0.1	175009	22.73	39648	0.0470	1	0.007	15	0.2271
			0.5	2	9903.33	19806.66			0.1					1		15	
	5-2	2	0.5	1	10660.41	21320.82	21454.98	42909.96	0.1	175009	24.52	42780	0.0507	1	0.007	15	0.2450
			0.5	2	10794.58	21589.16			0.1					1		15	
	5-3	2	0.5	1	10146.14	20292.28	20318.72	40637.44	0.1	175009	23.22	40507	0.0480	1	0.007	15	0.2320
			0.5	2	10172.58	20345.16			0.1					1		15	
	6-1	2	0.5	1	10521.81	21043.62	21114.15	42228.3	0.1	175009	24.13	42098	0.0499	1	0.007	15	0.2411
			0.5	2	10592.34	21184.68			0.1					1		15	
	6-2	2	0.5	1	10738.34	21476.68	21412.07	42824.14	0.1	175009	24.47	42694	0.0506	1	0.007	15	0.2445
			0.5	2	10673.73	21347.46			0.1					1		15	
	6-3	2	0.5	1	10675.61	21351.22	21299.35	42598.7	0.1	175009	24.34	42468	0.0503	1	0.007	15	0.2432
			0.5	2	10623.74	21247.48			0.1					1		15	
	7-1	2	0.5	1	10506.91	21013.82	21086.86	42173.72	0.1	175009	24.10	42043	0.0498	1	0.007	15	0.2408
			0.5	2	10579.95	21159.9			0.1					1		15	
	7-2	2	0.5	1	10394.73	20789.46	20823.8	41647.6	0.1	175009	23.80	41517	0.0492	1	0.007	15	0.2378
			0.5	2	10429.07	20858.14			0.1					1		15	
	7-3	2	0.5	1	10861.45	21722.9	21580.46	43160.92	0.1	175009	24.66	43031	0.0510	1	0.007	15	0.2464
			0.5	2	10719.01	21438.02			0.1					1		15	
	8-1	2	0.5	1	10425.86	20851.72	20874.41	41748.82	0.1	175009	23.86	41618	0.0493	1	0.007	15	0.2384
			0.5	2	10448.55	20897.1			0.1					1		15	
	8-2	2	0.5	1	10406.43	20812.86	20785.94	41571.88	0.1	175009	23.75	41442	0.0491	1	0.007	15	0.2373
			0.5	2	10379.51	20759.02			0.1					1		15	
	8-3	2	0.5	1	10157.59	20315.18	20517.72	41035.44	0.1	175009	23.45	40905	0.0485	1	0.007	15	0.2343
			0.5	2	10360.13	20720.26			0.1					1		15	

Assay Date	9/20/2005	ID	RC4	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.2416	0.0099
Full activity	End	0.2431	0.0126
Full activity	Overall	0.2424	0.0093
Background	Beginning	0.0000	1.65231E-05
Background	End	0.0000	0.000132266
Background	Overall	0.0000	9.61072E-05
Positive	Beginning	0.1187	0.0076
Positive	End	0.1139	0.0084
Positive	Overall	0.1163	0.0071
Negative	Beginning	0.2468	0.0023
Negative	End	0.2234	0.0040
Negative	Overall	0.2351	0.0138

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC4	1	1	1.00E-03	-3.00	0.0005
RC4	1	2	1.00E-03	-3.00	0.0004
RC4	1	3	1.00E-03	-3.00	0.0004
RC4	2	1	1.00E-04	-4.00	0.0037
RC4	2	2	1.00E-04	-4.00	0.0036
RC4	2	3	1.00E-04	-4.00	0.0040
RC4	3	1	1.00E-05	-5.00	0.1803
RC4	3	2	1.00E-05	-5.00	0.1687
RC4	3	3	1.00E-05	-5.00	0.1087
RC4	4	1	1.00E-06	-6.00	0.2408
RC4	4	2	1.00E-06	-6.00	0.2403
RC4	4	3	1.00E-06	-6.00	0.2304
RC4	5	1	1.00E-07	-7.00	0.2271
RC4	5	2	1.00E-07	-7.00	0.2450
RC4	5	3	1.00E-07	-7.00	0.2320
RC4	6	1	1.00E-08	-8.00	0.2411
RC4	6	2	1.00E-08	-8.00	0.2445
RC4	6	3	1.00E-08	-8.00	0.2432
RC4	7	1	1.00E-09	-9.00	0.2408
RC4	7	2	1.00E-09	-9.00	0.2378
RC4	7	3	1.00E-09	-9.00	0.2464
RC4	8	1	1.00E-10	-10.00	0.2384
RC4	8	2	1.00E-10	-10.00	0.2373
RC4	8	3	1.00E-10	-10.00	0.2343

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	0.21	0.17	0.16
2	-4.00	1.51	1.50	1.66
3	-5.00	74.38	69.62	44.86
4	-6.00	99.35	99.15	95.06
5	-7.00	93.69	101.09	95.72
6	-8.00	99.48	100.89	100.35
7	-9.00	99.35	98.11	101.68
8	-10.00	98.35	97.93	96.66

Aromatase Assay Spreadsheet

Assay Date	9/23/2005	Test Chemical ID	RC4	# Concentrations tested	8
ID	JG	Replicate #	Rep 2	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0199	33956.54	1706359		
2	0.0200	35131.29	1756565		
3	0.0199	36000.66	1809078		
4	0.0199	35292.26	1773480		
5	0.0200	35458.43	1772922		
			Average DPM/g soln		1763681
			SD		37346
			CV		2.12
			µCi/g soln		0.794

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	8.1914 g
Mass of dilution B used in substrate prep	4.5701 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.580231 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00899 µg/g soln.
µg/g soln.

a. µCi/g soln	0.794
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.

= 0.580231 + 0.00899
= 0.589224 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.348 µCi/µg ASDN

857260 dpm/nmol

Test

Assay Date 9/23/2005 Chemical ID RC4 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.570	0.354	0.160	0.093	0.054	0.044	0.032	25	100	
	0.579	0.349	0.162	0.091	0.052	0.043	0.030			
	0.576	0.347	0.164	0.097	0.055	0.044	0.033			

Samples:	10	100	microsomes
	0.058	0.289	0.053
	0.058	0.290	0.056
			0.053

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	0.575	0.543	0.0482	m, b	0.090	-0.001
0.125	100	200	0.00013	200	0.0250	0.350	0.318	0.0279	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.162	0.130	0.0109	r , se_y	0.992	0.002
0.025	100	1000	0.00003	200	0.0050	0.094	0.062	0.0047	F, df	521	4
0.01	40	1000	0.00001	200	0.0020	0.054	0.022	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.044	0.012	0.0002			

Blank 0.032

r^2= 0.992
m= 0.090
b= -0.001

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	0.026	0.002	200	1	1	0.000	0.000	0.008
10	0.058	0.026	0.002	200	1	1	0.000		
10					1	1			
100	0.289	0.257	0.022	200	1	1	0.000	0.000	0.112
100	0.290	0.258	0.022	200	1	1	0.000		
100									
	0.053	0.021	0.001	200	114	69814	0.003	0.003	3.498
	0.056	0.024	0.001	200	114	69814	0.004		
	0.053	0.021	0.001	200	114	69814	0.003		

Assay Date	9/23/2005	Test Chemical ID	RC4	tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.570	0.354	0.160	0.093	0.054	0.044	0.032	25	100	
	0.579	0.349	0.162	0.091	0.052	0.043	0.030			
	0.576	0.347	0.164	0.097	0.055	0.044	0.033			

Samples:	10	100	microsomes
	0.058	0.289	0.053
	0.058	0.290	0.056
			0.053

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	0.575	0.543	0.0424	m, b	0.078	0.000
0.125	100	200	0.00013	200	0.0250	0.350	0.318	0.0249	se_m, se_b	0.001	0.000
0.05	200	1000	0.00005	200	0.0100	0.162	0.130	0.0103	r , se_y	1.000	0.000
0.025	100	1000	0.00003	200	0.0050	0.094	0.062	0.0049	F, df	10118	3
0.01	40	1000	0.00001	200	0.0020	0.054	0.022	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.044	0.012	0.0010			

Regression results are calculated using the function LINEST

Blank 0.032

r^2= 1.000
m= 0.078
b= 0.000

	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	0.026	0.002	200	1	1	0.000	0.000	0.011
10	0.058	0.026	0.002	200	1	1	0.000		
10					1	1			
100	0.289	0.257	0.020	200	1	1	0.000	0.000	0.101
100	0.290	0.258	0.020	200	1	1	0.000		
100									
	0.053	0.021	0.002	200	114	69814	0.005	0.006	5.677
	0.056	0.024	0.002	200	114	69814	0.006		
	0.053	0.021	0.002	200	114	69814	0.005		

Assay Date	9/23/2005	Test Chemical ID	RC4		tested		8		
ID	JG	Replicate #	Rep 2		Microsome type	Recombinant	Microsome ID		0

								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

					Final vol. Diluted usomes			
A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	(µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	9/23/2005	Chemical ID	RC4	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	5.00E-05
4	1.00E-05
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-09

Protein Concentration (stock microsomes, mg/mL):	5.677
Protein Concentration (dilution added to assay, mg/mL):	0.00927

Assay Date	9/23/2005	Test Chemical ID	RC4	# Concentrations tested		8 Microsome type		Microsome ID		0 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	9476.95	18953.9	19001.02	38002.04	0.1	176368	21.55	37864	0.0442	1	0.005	15	0.3176
			0.5	2	9524.07	19048.14			0.1					1		15	
	2	2	0.5	1	9123.65	18247.3	18353.28	36706.56	0.1	176368	20.81	36589	0.0427	1	0.005	15	0.3068
			0.5	2	9229.63	18459.26			0.1					1		15	
	3	2	0.5	1	9361.27	18722.54	19066.9	38133.8	0.1	176368	21.62	37996	0.0443	1	0.005	15	0.3188
			0.5	2	9705.63	19411.26			0.1					1		15	
	4	2	0.5	1	9811.88	19623.76	19654.88	39309.76	0.1	176368	22.29	39172	0.0457	1	0.005	15	0.3286
			0.5	2	9843	19686			0.1					1		15	
Background control	1	2	0.5	1	33.16	66.32	59.79	119.58	0.1	176368	0.07	-18	0.0000	1	0.005	15	-0.0002
			0.5	2	26.63	53.26			0.1					1		15	
	2	2	0.5	1	32.18	64.36	69.7	139.4	0.1	176368	0.08	2	0.0000	1	0.005	15	0.0000
			0.5	2	37.52	75.04			0.1					1		15	
	3	2	0.5	1	40.8	81.6	62.13	124.26	0.1	176368	0.07	-14	0.0000	1	0.005	15	-0.0001
			0.5	2	21.33	42.66			0.1					1		15	
	4	2	0.5	1	41.75	83.5	84.01	168.02	0.1	176368	0.10	30	0.0000	1	0.005	15	0.0003
			0.5	2	42.26	84.52			0.1					1		15	
Positive control	1	2	0.5	1	5031.59	10063.18	10153.95	20307.9	0.1	176368	11.51	20170	0.0235	1	0.005	15	0.1692
			0.5	2	5122.36	10244.72			0.1					1		15	
	2	2	0.5	1	4956.38	9912.76	10005.4	20010.8	0.1	176368	11.35	19873	0.0232	1	0.005	15	0.1667
			0.5	2	5049.02	10098.04			0.1					1		15	
	3	2	0.5	1	4910.36	9820.72	9858.87	19717.74	0.1	176368	11.18	19580	0.0228	1	0.005	15	0.1643
			0.5	2	4948.51	9897.02			0.1					1		15	
	4	2	0.5	1	4905.79	9811.58	9890.54	19781.08	0.1	176368	11.22	19643	0.0229	1	0.005	15	0.1648
			0.5	2	4984.75	9969.5			0.1					1		15	
Negative Control	1	2	0.5	1	9554.67	19109.34	19364.02	38728.04	0.1	176368	21.98	38590	0.0450	1	0.005	15	0.3237
			0.5	2	9809.35	19618.7			0.1					1		15	
	2	2	0.5	1	9662.22	19324.44	19569.35	39138.7	0.1	176368	22.16	39001	0.0455	1	0.005	15	0.3272
			0.5	2	9907.13	19814.26			0.1					1		15	
	3	2	0.5	1	9712.48	19424.96	19223.14	38446.28	0.1	176368	21.80	38308	0.0447	1	0.005	15	0.3214
			0.5	2	9510.86	19021.32			0.1					1		15	
	4	2	0.5	1	9370.69	18741.38	18725.72	37451.44	0.1	176368	21.23	37314	0.0435	1	0.005	15	0.3130
			0.5	2	9355.03	18710.06			0.1					1		15	
RC4	1-1	2	0.5	1	43.72	87.44	93.41	186.82	0.1	176368	0.11	49	0.0001	1	0.005	15	0.0004
			0.5	2	49.69	99.38			0.1					1		15	
	1-2	2	0.5	1	40.34	80.68	85.02	170.04	0.1	176368	0.10	32	0.0000	1	0.005	15	0.0003
			0.5	2	44.68	89.36			0.1					1		15	
	1-3	2	0.5	1	38.47	76.94	82.98	165.96	0.1	176368	0.09	28	0.0000	1	0.005	15	0.0002
			0.5	2	44.51	89.02			0.1					1		15	
	2-1	2	0.5	1	199.03	398.06	406.55	813.1	0.1	176368	0.46	675	0.0008	1	0.005	15	0.0057
			0.5	2	207.52	415.04			0.1					1		15	
	2-2	2	0.5	1	199.77	399.54	407.01	814.02	0.1	176368	0.46	676	0.0008	1	0.005	15	0.0057
			0.5	2	207.24	414.48			0.1					1		15	
	2-3	2	0.5	1	224.85	449.7	440.47	880.94	0.1	176368	0.50	743	0.0009	1	0.005	15	0.0062
			0.5	2	215.62	431.24			0.1					1		15	
	3-1	2	0.5	1	1109.86	2219.72	2192	4384	0.1	176368	2.49	4246	0.0050	1	0.005	15	0.0356
			0.5	2	1082.14	2164.28			0.1					1		15	
	3-2	2	0.5	1	931.56	1863.12	1853.67	3707.34	0.1	176368	2.10	3570	0.0042	1	0.005	15	0.0299
			0.5	2	922.11	1844.22			0.1					1		15	
	3-3	2	0.5	1	1056.64	2113.28	2037.24	4074.48	0.1	176368	2.31	3937	0.0046	1	0.005	15	0.0330
			0.5	2	980.6	1961.2			0.1					1		15	
	4-1	2	0.5	1	6886.43	13776.86	13737.08	27474.16	0.1	176368	15.58	27336	0.0319	1	0.005	15	0.2293
			0.5	2	6848.65	13697.3			0.1					1		15	
	4-2	2	0.5	1	6997.07	13994.14	13968.73	27977.46	0.1	176368	15.86	27840	0.0325	1	0.005	15	0.2335
			0.5	2	6891.66	13983.32			0.1					1		15	
	4-3	2	0.5	1	6659.39	13316.78	13242.24	26484.48	0.1	176368	15.02	26347	0.0307	1	0.005	15	0.2210
			0.5	2	6583.85	13167.7			0.1					1		15	
	5-1	2	0.5	1	9644.65	19289.3	19160.58	38321.16	0.1	176368	21.73	38183	0.0445	1	0.005	15	0.3203
			0.5	2	9515.93	19031.86			0.1					1		15	
	5-2	2	0.5	1	9583.31	19166.62	19106.25	38216.5	0.1	176368	21.67	38079	0.0444	1	0.005	15	0.3194
			0.5	2	9524.94	19049.88			0.1					1		15	
	5-3	2	0.5	1	9674.12	19348.24	19279.94	38559.88	0.1	176368	21.86	38422	0.0448	1	0.005	15	0.3223
			0.5	2	9605.82	19211.64			0.1					1		15	
	6-1	2	0.5	1	10019.96	20039.92	20069.79	40139.58	0.1	176368	22.76	40002	0.0467	1	0.005	15	0.3356
			0.5	2	10049.83	20099.66			0.1					1		15	
	6-2	2	0.5	1	10146.7	20293.4	20405.51	40811.02	0.1	176368	23.14	40673	0.0474	1	0.005	15	0.3412
			0.5	2	10258.81	20517.62			0.1					1		15	
	6-3	2	0.5	1	8967.17	19934.34	20108.99	40217.98	0.1	176368	22.80	40080	0.0468	1	0.005	15	0.3362
			0.5	2	10141.82	20283.64			0.1					1		15	
	7-1	2	0.5	1	9986.72	19973.44	20029.94	40059.88	0.1	176368	22.71	39922	0.0466	1	0.005	15	0.3349
			0.5	2	10043.22	20086.44			0.1					1		15	
	7-2	2	0.5	1	9716.29	19432.58	19749.64	39499.28	0.1	176368	22.40	39381	0.0459	1	0.005	15	0.3302
			0.5	2	10033.35	20066.7			0.1					1		15	
	7-3	2	0.5	1	9558.04	19116.08	19245.96	38491.92	0.1	176368	21.82	38354	0.0447	1	0.005	15	0.3218
			0.5	2	9687.92	19375.84			0.1					1		15	
	8-1	2	0.5	1	9855.66	19711.32	19781.16	39562.32	0.1	176368	22.43	39425	0.0460	1	0.005	15	0.3307
			0.5	2	9925.5	19851			0.1					1		15	
	8-2	2	0.5	1	9836.27	19672.54	19782.91	39565.82	0.1	176368	22.43	39428	0.0460	1	0.005	15	0.3308
			0.5	2	9946.64	19893.28			0.1					1		15	
	8-3	2	0.5	1	8153.67	16307.34	16363.33	32726.66	0.1	176368	18.56	32588	0.0380	1	0.005	15	0.2734
			0.5	2	8209.66	16419.32			0.1					1		15	

Assay Date	9/23/2005	ID	RC4	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.3122	0.0077
Full activity	End	0.3237	0.0070
Full activity	Overall	0.3179	0.0089
Background	Beginning	-0.0001	0.000117572
Background	End	0.0001	0.000259584
Background	Overall	0.0000	0.000183228
Positive	Beginning	0.1680	0.0018
Positive	End	0.1645	0.0004
Positive	Overall	0.1662	0.0022
Negative	Beginning	0.3255	0.0024
Negative	End	0.3172	0.0059
Negative	Overall	0.3213	0.0060

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	0.0003
RC4	1	3	1.00E-03	-3.00	0.0002
RC4	2	1	1.00E-04	-4.00	0.0057
RC4	2	2	1.00E-04	-4.00	0.0057
RC4	2	3	1.00E-04	-4.00	0.0062
RC4	3	1	5.00E-05	-4.30	0.0356
RC4	3	2	5.00E-05	-4.30	0.0299
RC4	3	3	5.00E-05	-4.30	0.0330
RC4	4	1	1.00E-05	-5.00	0.2293
RC4	4	2	1.00E-05	-5.00	0.2335
RC4	4	3	1.00E-05	-5.00	0.2210
RC4	5	1	1.00E-06	-6.00	0.3203
RC4	5	2	1.00E-06	-6.00	0.3194
RC4	5	3	1.00E-06	-6.00	0.3223
RC4	6	1	1.00E-07	-7.00	0.3356
RC4	6	2	1.00E-07	-7.00	0.3412
RC4	6	3	1.00E-07	-7.00	0.3362
RC4	7	1	1.00E-08	-8.00	0.3349
RC4	7	2	1.00E-08	-8.00	0.3302
RC4	7	3	1.00E-08	-8.00	0.3218
RC4	8	1	1.00E-09	-9.00	0.3307
RC4	8	2	1.00E-09	-9.00	0.3308
RC4	8	3	1.00E-09	-9.00	0.2734

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	0.13	0.09	0.07
2	-4.00	1.78	1.78	1.96
3	-4.30	11.20	9.42	10.39
4	-5.00	72.13	73.46	69.52
5	-6.00	100.75	100.47	101.38
6	-7.00	105.54	107.32	105.75
7	-8.00	105.33	103.86	101.20
8	-9.00	104.02	104.03	85.99

Aromatase Assay Spreadsheet

Assay Date	9/27/2005	Test Chemical ID	RC4	# Concentrations tested	8
ID	JG	Replicate #	Rep 3	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	36510.32	1825516	
2	0.0200	35579.4	1778970	
3	0.0200	37945.33	1897267	
4	0.0200	37505.18	1875259	
5	0.0200	37887.21	1894361	
			Average DPM/g soln	1854274
			SD	50974
			CV	2.75
			µCi/g soln	0.835

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	8.1897 g
Mass of dilution B used in substrate prep	4.6025 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.584466 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00946 µg/g soln.
µg/g soln.

a. µCi/g soln	0.835
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.

= 0.584466 + 0.00946
= 0.593921 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.406 µCi/µg ASDN

894166 dpm/nmol

Assay Date 9/27/2005 Test Chemical ID RC4 tested 8

ID JG Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.573	0.356	0.161	0.092	0.050	0.041	0.034	25	100	
	0.589	0.353	0.165	0.094	0.057	0.040	0.034			
	0.577	0.354	0.157	0.091	0.054	0.037	0.033			

Samples:	10	100	microsomes
	0.057	0.281	0.055
	0.055	0.296	0.053
			0.054

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	0.580	0.546	0.0481	m, b	0.089	0.000
0.125	100	200	0.00013	200	0.0250	0.354	0.321	0.0280	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.161	0.127	0.0108	r , se_y	0.992	0.002
0.025	100	1000	0.00003	200	0.0050	0.092	0.059	0.0047	F, df	475	4
0.01	40	1000	0.00001	200	0.0020	0.054	0.020	0.0013	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.039	0.006	0.0000			

Regression results are calculated using the function LINEST

Blank 0.034

r^2= 0.992
m= 0.089
b= 0.000

	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	0.023	0.002	200	1	1	0.000	0.000	0.007
10	0.055	0.021	0.001	200	1	1	0.000		
10					1	1			
100	0.281	0.247	0.022	200	1	1	0.000	0.000	0.111
100	0.296	0.262	0.023	200	1	1	0.000		
100									
	0.055	0.021	0.001	200	114	69814	0.004	0.004	4.043
	0.053	0.019	0.001	200	114	69814	0.004		
	0.054	0.020	0.001	200	114	69814	0.004		

Assay Date	9/27/2005	Test Chemical ID	RC4	tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.573	0.356	0.161	0.092	0.050	0.041	0.034	25	100	
	0.589	0.353	0.165	0.094	0.057	0.040	0.034			
	0.577	0.354	0.157	0.091	0.054	0.037	0.033			
Samples:	10	100	microsomes							
	0.057	0.281	0.055							
	0.055	0.296	0.053							
			0.054							

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	0.580	0.546	0.0421	m, b	0.076	0.000
0.125	100	200	0.00013	200	0.0250	0.354	0.321	0.0249	se_m, se_b	0.001	0.000
0.05	200	1000	0.00005	200	0.0100	0.161	0.127	0.0102	r , se_y	1.000	0.000
0.025	100	1000	0.00003	200	0.0050	0.092	0.059	0.0050	F, df	22889	3
0.01	40	1000	0.00001	200	0.0020	0.054	0.020	0.0020	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.039	0.006	0.0009			

Regression results are calculated using the function LINEST

Blank 0.034

r^2= 1.000
m= 0.076
b= 0.000

	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	0.023	0.002	200	1	1	0.000	0.000	0.011
10	0.055	0.021	0.002	200	1	1	0.000		
10					1	1			
100	0.281	0.247	0.019	200	1	1	0.000	0.000	0.100
100	0.296	0.262	0.020	200	1	1	0.000		
100									
	0.055	0.021	0.002	200	114	69814	0.006	0.006	6.222
	0.053	0.019	0.002	200	114	69814	0.006		
	0.054	0.020	0.002	200	114	69814	0.006		

Assay Date	9/27/2005	Test Chemical ID	RC4		tested		8		
ID	JG	Replicate #	Rep 3		Microsome type	Recombinant	Microsome ID		0

								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	9/27/2005	Chemical ID	RC4	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 3

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	5.00E-05
4	1.00E-05
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-09

Protein Concentration (stock microsomes, mg/mL):	6.222
Protein Concentration (dilution added to assay, mg/mL):	0.01016

Assay Date	9/27/2005	Test Chemical ID	RC4	# Concentrations tested	8	Microsome type		Microsome ID		D 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	14485.23	28970.46	29058.12	58116.24	0.1	185427	31.34	57936	0.0648	1	0.005	15	0.4252
			0.5	2	14572.89	29145.78			0.1					1		15	
	2	2	0.5	1	13954.36	27908.72	28004.45	56008.9	0.1	185427	30.21	55829	0.0624	1	0.005	15	0.4097
			0.5	2	14050.09	28100.18			0.1					1		15	
	3	2	0.5	1	13472.21	26944.42	26929.26	53858.52	0.1	185427	29.05	53678	0.0600	1	0.005	15	0.3939
			0.5	2	13457.05	26914.1			0.1					1		15	
	4	2	0.5	1	13132.31	26264.62	26404.04	52808.08	0.1	185427	28.48	52628	0.0589	1	0.005	15	0.3862
			0.5	2	13271.73	26543.46			0.1					1		15	
Background control	1	2	0.5	1	50.41	100.82	98.29	196.58	0.1	185427	0.11	18	0.0000	1	0.005	15	0.0001
			0.5	2	48.88	97.76			0.1					1		15	
	2	2	0.5	1	49.94	99.88	96.3	192.6	0.1	185427	0.10	12	0.0000	1	0.005	15	0.0001
			0.5	2	46.36	92.72			0.1					1		15	
	3	2	0.5	1	45.44	90.88	90.05	180.1	0.1	185427	0.10	0	0.0000	1	0.005	15	0.0000
			0.5	2	44.61	89.22			0.1					1		15	
	4	2	0.5	1	33.97	67.94	74.63	149.26	0.1	185427	0.08	-31	0.0000	1	0.005	15	-0.0002
			0.5	2	40.66	81.32			0.1					1		15	
Positive control	1	2	0.5	1	7056.51	14113.02	14129.66	28259.32	0.1	185427	15.24	28079	0.0314	1	0.005	15	0.2061
			0.5	2	7073.15	14146.3			0.1					1		15	
	2	2	0.5	1	7003.53	14007.06	13907.43	27814.86	0.1	185427	15.00	27635	0.0309	1	0.005	15	0.2028
			0.5	2	6903.9	13807.8			0.1					1		15	
	3	2	0.5	1	6589.75	13179.5	13175.56	26351.12	0.1	185427	14.21	26171	0.0293	1	0.005	15	0.1921
			0.5	2	6585.81	13171.62			0.1					1		15	
	4	2	0.5	1	6864.61	13729.22	13737.77	27475.54	0.1	185427	14.82	27295	0.0305	1	0.005	15	0.2003
			0.5	2	6873.16	13746.32			0.1					1		15	
Negative Control	1	2	0.5	1	13576.34	27152.68	27247.66	54495.32	0.1	185427	29.39	54315	0.0607	1	0.005	15	0.3986
			0.5	2	13671.32	27342.64			0.1					1		15	
	2	2	0.5	1	13921.25	27842.5	27966.5	55933	0.1	185427	30.16	55753	0.0624	1	0.005	15	0.4091
			0.5	2	14045.25	28090.5			0.1					1		15	
	3	2	0.5	1	13509.48	27018.96	27286.41	54572.82	0.1	185427	29.43	54393	0.0608	1	0.005	15	0.3992
			0.5	2	13776.93	27553.86			0.1					1		15	
	4	2	0.5	1	13365.33	26730.66	26541.3	53082.6	0.1	185427	28.63	52902	0.0592	1	0.005	15	0.3882
			0.5	2	13175.97	26351.94			0.1					1		15	
RC4	1-1	2	0.5	1	74.94	148.88	135.48	270.96	0.1	185427	0.15	91	0.0001	1	0.005	15	0.0007
			0.5	2	60.54	121.08			0.1					1		15	
	1-2	2	0.5	1	47.98	95.96	109.68	219.36	0.1	185427	0.12	39	0.0000	1	0.005	15	0.0003
			0.5	2	61.7	123.4			0.1					1		15	
	1-3	2	0.5	1	65.23	130.46	129.31	258.62	0.1	185427	0.14	78	0.0001	1	0.005	15	0.0006
			0.5	2	64.08	128.16			0.1					1		15	
	2-1	2	0.5	1	325.03	650.06	638.62	1277.24	0.1	185427	0.69	1097	0.0012	1	0.005	15	0.0081
			0.5	2	313.59	627.18			0.1					1		15	
	2-2	2	0.5	1	314.55	629.1	630.62	1261.24	0.1	185427	0.68	1081	0.0012	1	0.005	15	0.0079
			0.5	2	316.07	632.14			0.1					1		15	
	2-3	2	0.5	1	316.35	632.7	628.45	1256.9	0.1	185427	0.68	1077	0.0012	1	0.005	15	0.0079
			0.5	2	312.1	624.2			0.1					1		15	
	3-1	2	0.5	1	1891.95	3783.9	3766.29	7532.58	0.1	185427	4.06	7352	0.0062	1	0.005	15	0.0540
			0.5	2	1874.34	3748.68			0.1					1		15	
	3-2	2	0.5	1	2100	4200	4179.14	8358.28	0.1	185427	4.51	8178	0.0091	1	0.005	15	0.0600
			0.5	2	2079.14	4158.28			0.1					1		15	
	3-3	2	0.5	1	1845.28	3690.56	3848.95	7697.9	0.1	185427	4.15	7518	0.0084	1	0.005	15	0.0552
			0.5	2	1903.67	3807.34			0.1					1		15	
	4-1	2	0.5	1	8952.42	17904.84	18026.67	36053.34	0.1	185427	19.44	35873	0.0401	1	0.005	15	0.2632
			0.5	2	9074.25	18148.5			0.1					1		15	
	4-2	2	0.5	1	9845.75	19691.5	19678.35	39356.7	0.1	185427	21.22	39177	0.0438	1	0.005	15	0.2875
			0.5	2	9832.6	19665.2			0.1					1		15	
	4-3	2	0.5	1	9680.86	19361.72	19346.27	38692.54	0.1	185427	20.87	38512	0.0431	1	0.005	15	0.2826
			0.5	2	9665.41	19330.82			0.1					1		15	
	5-1	2	0.5	1	13144.01	26288.02	26384.54	52769.08	0.1	185427	28.46	52589	0.0588	1	0.005	15	0.3859
			0.5	2	13240.53	26481.06			0.1					1		15	
	5-2	2	0.5	1	13204.11	26408.22	26256.03	52512.06	0.1	185427	28.32	52332	0.0585	1	0.005	15	0.3840
			0.5	2	13051.92	26103.84			0.1					1		15	
	5-3	2	0.5	1	13148.08	26296.16	26297.48	52594.96	0.1	185427	28.36	52415	0.0586	1	0.005	15	0.3846
			0.5	2	13149.4	26298.8			0.1					1		15	
	6-1	2	0.5	1	13394.46	26788.92	26693.42	53386.84	0.1	185427	28.79	53207	0.0595	1	0.005	15	0.3904
			0.5	2	13298.96	26597.92			0.1					1		15	
	6-2	2	0.5	1	13106.47	26212.94	26388.4	52776.8	0.1	185427	28.46	52597	0.0588	1	0.005	15	0.3860
			0.5	2	13281.93	26563.86			0.1					1		15	
	6-3	2	0.5	1	13162.12	26324.24	26412.03	52824.06	0.1	185427	28.49	52644	0.0589	1	0.005	15	0.3863
			0.5	2	13249.91	26499.82			0.1					1		15	
	7-1	2	0.5	1	12852.15	25704.3	25650.13	51300.26	0.1	185427	27.67	51120	0.0572	1	0.005	15	0.3751
			0.5	2	12797.98	25595.96			0.1					1		15	
	7-2	2	0.5	1	12599.75	25199.5	25136.73	50273.46	0.1	185427	27.11	50093	0.0560	1	0.005	15	0.3678
			0.5	2	12536.96	25073.96			0.1					1		15	
	7-3	2	0.5	1	13271.54	26543.08	26577.14	53154.28	0.1	185427	28.67	52974	0.0592	1	0.005	15	0.3887
			0.5	2	13305.6	26611.2			0.1					1		15	
	8-1	2	0.5	1	13129.65	26259.3	26271.96	52543.92	0.1	185427	28.34	52364	0.0586	1	0.005	15	0.3843
			0.5	2	13142.31	26284.62			0.1					1		15	
	8-2	2	0.5	1	13066.59	26133.18	26066.86	52133.72	0.1	185427	28.12	51954	0.0581	1	0.005	15	0.3813
			0.5	2	13000.27	26000.54			0.1					1		15	
	8-3	2	0.5	1	13022.8	26045.6	26067.2	52134.4	0.1	185427	28.12	51954	0.0581	1	0.005	15	0.3813
			0.5	2	13044.4	26088.8			0.1					1		15	

Assay Date	9/27/2005	ID	RC4	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0 Technician ID	JG	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.4174	0.0109
Full activity	End	0.3901	0.0055
Full activity	Overall	0.4037	0.0173
Background	Beginning	0.0001	3.10302E-05
Background	End	-0.0001	0.000160029
Background	Overall	0.0000	0.000161269
Positive	Beginning	0.2044	0.0023
Positive	End	0.1962	0.0058
Positive	Overall	0.2003	0.0060
Negative	Beginning	0.4039	0.0075
Negative	End	0.3937	0.0077
Negative	Overall	0.3988	0.0085

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC4	1	1	1.00E-03	-3.00	0.0007
RC4	1	2	1.00E-03	-3.00	0.0003
RC4	1	3	1.00E-03	-3.00	0.0006
RC4	2	1	1.00E-04	-4.00	0.0081
RC4	2	2	1.00E-04	-4.00	0.0079
RC4	2	3	1.00E-04	-4.00	0.0079
RC4	3	1	5.00E-05	-4.30	0.0540
RC4	3	2	5.00E-05	-4.30	0.0600
RC4	3	3	5.00E-05	-4.30	0.0552
RC4	4	1	1.00E-05	-5.00	0.2632
RC4	4	2	1.00E-05	-5.00	0.2875
RC4	4	3	1.00E-05	-5.00	0.2826
RC4	5	1	1.00E-06	-6.00	0.3859
RC4	5	2	1.00E-06	-6.00	0.3840
RC4	5	3	1.00E-06	-6.00	0.3846
RC4	6	1	1.00E-07	-7.00	0.3904
RC4	6	2	1.00E-07	-7.00	0.3860
RC4	6	3	1.00E-07	-7.00	0.3863
RC4	7	1	1.00E-08	-8.00	0.3751
RC4	7	2	1.00E-08	-8.00	0.3676
RC4	7	3	1.00E-08	-8.00	0.3887
RC4	8	1	1.00E-09	-9.00	0.3843
RC4	8	2	1.00E-09	-9.00	0.3813
RC4	8	3	1.00E-09	-9.00	0.3813

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	0.17	0.07	0.14
2	-4.00	1.99	1.97	1.96
3	-4.30	13.36	14.86	13.66
4	-5.00	65.20	71.21	70.00
5	-6.00	95.59	95.12	95.27
6	-7.00	96.71	95.60	95.69
7	-8.00	92.92	91.05	96.29
8	-9.00	95.18	94.43	94.43

Aromatase Assay Spreadsheet

Assay Date	9/29/2005	Test Chemical ID	RC5	# Concentrations tested	8
ID	TNB	Replicate #	Rep 1	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0204	40617.16	1991037	
2	0.0204	40016.84	1961610	
3	0.0198	39359.99	1987878	
4	0.0201	39381.37	1959272	
5	0.0202	39408.74	1950928	
			Average DPM/g soln	1970145
			SD	18106
			CV	0.92
			µCi/g soln	0.887

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	8.1365 g
Mass of dilution B used in substrate prep	4.6119 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.589489 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01005 µg/g soln.
µg/g soln.

a. µCi/g soln	0.887
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.

= 0.589489 + 0.01005
= 0.599535 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.480 µCi/µg ASDN

941145 dpm/nmol

Test
Assay Date 9/29/2005 Chemical ID RC5 tested 8

ID TNB Replicate # Rep 1 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.574	0.344	0.164	0.092	0.055	0.044	0.035	25	100	
	0.571	0.351	0.160	0.095	0.059	0.045	0.038			
	0.565	0.354	0.163	0.097	0.054	0.047	0.037			
Samples:	10	100	microsomes							
	0.057	0.287	0.061							
	0.056	0.294	0.058							
			0.056							

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	0.533	0.0481
0.125	100	200	0.00013	200	0.0250	0.350	0.313	0.0280
0.05	200	1000	0.00005	200	0.0100	0.162	0.126	0.0109
0.025	100	1000	0.00003	200	0.0050	0.095	0.058	0.0047
0.01	40	1000	0.00001	200	0.0020	0.056	0.019	0.0012
0.005	20	1000	0.00001	200	0.0010	0.045	0.009	0.0002

Blank 0.037

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	489	4
ss_{reg}, ss_{resid}	0.002	0.000

r^2= 0.992
m= 0.091
b= -0.001

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	0.020	0.001	200	1	1	0.000	0.000	0.006
10	0.056	0.019	0.001	200	1	1	0.000		
10					1	1			
100	0.287	0.250	0.022	200	1	1	0.000	0.000	0.113
100	0.294	0.257	0.023	200	1	1	0.000		
100									
	0.061	0.024	0.002	200	114	69814	0.005	0.004	4.189
	0.058	0.021	0.001	200	114	69814	0.004		
	0.056	0.019	0.001	200	114	69814	0.004		

Test

Assay Date 9/29/2005 Chemical ID RC5 tested 8

ID TNB Replicate # Rep 1 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.574	0.344	0.164	0.092	0.055	0.044	0.035	25	100
	0.571	0.351	0.160	0.095	0.059	0.045	0.038		
	0.565	0.354	0.163	0.097	0.054	0.047	0.037		

Protein stock ID

Samples:	10	100	microsomes
	0.057	0.287	0.061
	0.056	0.294	0.058
			0.056

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	0.533	0.0422
0.125	100	200	0.00013	200	0.0250	0.350	0.313	0.0249
0.05	200	1000	0.00005	200	0.0100	0.162	0.126	0.0102
0.025	100	1000	0.00003	200	0.0050	0.095	0.058	0.0049
0.01	40	1000	0.00001	200	0.0020	0.056	0.019	0.0019
0.005	20	1000	0.00001	200	0.0010	0.045	0.009	0.0010

Blank 0.037

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	14534	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	Final vol. Diluted usomes prep. (µL)	(µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.057	0.020	0.002	200	1	1	0.000	0.000	0.010
10	0.056	0.019	0.002	200	1	1	0.000		
10					1	1			
100	0.287	0.250	0.020	200	1	1	0.000	0.000	0.101
100	0.294	0.257	0.021	200	1	1	0.000		
100									
	0.061	0.024	0.002	200	114	69814	0.007	0.006	6.322
	0.058	0.021	0.002	200	114	69814	0.006		
	0.056	0.019	0.002	200	114	69814	0.006		

Assay Date	9/29/2005	Test Chemical ID	RC5	tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

								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	9/29/2005	Chemical ID	RC5	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 1

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.322
Protein Concentration (dilution added to assay, mg/mL):	0.010323

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	9/29/2005	Test Chemical ID	RC5	# Concentrations tested	8	Microsome type		Microsome ID		0	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	11568.56	23137.12	23278.83	46557.66	0.1	197015	23.63	46414	0.0493	1	0.005	15	0.3165
			0.5	2	11710.27	23420.54			0.1					1		15	
	2	2	0.5	1	12590.64	25181.28	25285.22	50570.44	0.1	197015	25.67	50427	0.0536	1	0.005	15	0.3460
			0.5	2	12694.58	25389.16			0.1					1		15	
	3	2	0.5	1	12315.18	24630.36	24467.68	48935.36	0.1	197015	24.84	48792	0.0518	1	0.005	15	0.3348
			0.5	2	12152.5	24305			0.1					1		15	
	4	2	0.5	1	12314.59	24629.18	24610.32	49220.64	0.1	197015	24.96	49077	0.0521	1	0.005	15	0.3368
			0.5	2	12295.73	24591.46			0.1					1		15	
Background control	1	2	0.5	1	42.95	85.9	74.78	149.56	0.1	197015	0.08	6	0.0000	1	0.005	15	0.0000
			0.5	2	31.83	63.66			0.1					1		15	
	2	2	0.5	1	27.44	54.88	63.54	127.08	0.1	197015	0.06	-16	0.0000	1	0.005	15	-0.0001
			0.5	2	36.1	72.2			0.1					1		15	
	3	2	0.5	1	33.43	66.86	66.79	133.58	0.1	197015	0.07	-10	0.0000	1	0.005	15	-0.0001
			0.5	2	33.36	66.72			0.1					1		15	
	4	2	0.5	1	42.68	85.36	82.01	164.02	0.1	197015	0.08	20	0.0000	1	0.005	15	0.0001
			0.5	2	39.33	78.66			0.1					1		15	
Positive control	1	2	0.5	1	6280.14	12560.28	12621.07	25242.14	0.1	197015	12.81	25099	0.0267	1	0.005	15	0.1722
			0.5	2	6340.93	12681.86			0.1					1		15	
	2	2	0.5	1	5509.73	11019.46	11005.95	22011.9	0.1	197015	11.17	21868	0.0232	1	0.005	15	0.1501
			0.5	2	5496.22	10992.44			0.1					1		15	
	3	2	0.5	1	6490.8	12981.6	12994.79	25989.58	0.1	197015	13.19	25846	0.0275	1	0.005	15	0.1773
			0.5	2	6503.99	13007.98			0.1					1		15	
	4	2	0.5	1	5515.27	11030.54	11130.76	22261.52	0.1	197015	11.30	22118	0.0235	1	0.005	15	0.1518
			0.5	2	5615.49	11230.98			0.1					1		15	
Negative Control	1	2	0.5	1	7768.37	15536.74	15642.47	31284.94	0.1	197015	15.88	31141	0.0331	1	0.005	15	0.2137
			0.5	2	7874.1	15748.2			0.1					1		15	
	2	2	0.5	1	12152.74	24305.48	24567.39	49134.78	0.1	197015	24.94	48991	0.0521	1	0.005	15	0.3362
			0.5	2	12414.65	24829.3			0.1					1		15	
	3	2	0.5	1	10021.88	20043.76	19778.23	39556.46	0.1	197015	20.08	39413	0.0419	1	0.005	15	0.2704
			0.5	2	9756.35	19512.7			0.1					1		15	
	4	2	0.5	1	12427.37	24854.74	25239.05	50478.1	0.1	197015	25.62	50335	0.0535	1	0.005	15	0.3454
			0.5	2	12811.68	25623.36			0.1					1		15	
RC5	1-1	2	0.5	1	12248.86	24497.72	24738.74	49477.48	0.1	197015	25.11	49334	0.0524	1	0.005	15	0.3385
			0.5	2	12489.88	24979.76			0.1					1		15	
	1-2	2	0.5	1	11287	22574	22684.88	45369.76	0.1	197015	23.03	45226	0.0481	1	0.005	15	0.3103
			0.5	2	11397.88	22795.76			0.1					1		15	
	1-3	2	0.5	1	10711.53	21423.06	21063.81	42127.62	0.1	197015	21.38	41984	0.0446	1	0.005	15	0.2881
			0.5	2	10352.28	20704.56			0.1					1		15	
	2-1	2	0.5	1	13482.95	26965.9	26824.95	53649.9	0.1	197015	27.23	53506	0.0569	1	0.005	15	0.3671
			0.5	2	13342	26684			0.1					1		15	
	2-2	2	0.5	1	12015.05	24030.1	24426.42	48852.84	0.1	197015	24.80	48709	0.0518	1	0.005	15	0.3342
			0.5	2	12411.37	24822.74			0.1					1		15	
	2-3	2	0.5	1	11767.35	23534.7	23683.72	47367.44	0.1	197015	24.04	47224	0.0502	1	0.005	15	0.3240
			0.5	2	11916.37	23832.74			0.1					1		15	
	3-1	2	0.5	1	12738.91	25477.82	25518.84	51037.68	0.1	197015	25.91	50894	0.0541	1	0.005	15	0.3492
			0.5	2	12779.93	25559.86			0.1					1		15	
	3-2	2	0.5	1	12515.26	25030.52	24895.47	49990.94	0.1	197015	25.37	49847	0.0530	1	0.005	15	0.3420
			0.5	2	12400.21	24860.42			0.1					1		15	
	3-3	2	0.5	1	12480.85	24961.9	25031.58	50063.16	0.1	197015	25.41	49920	0.0530	1	0.005	15	0.3425
			0.5	2	12550.63	25101.26			0.1					1		15	
	4-1	2	0.5	1	12804.79	25609.58	25516.63	51033.26	0.1	197015	25.90	50890	0.0541	1	0.005	15	0.3492
			0.5	2	12711.84	25423.68			0.1					1		15	
	4-2	2	0.5	1	12628.81	25257.62	25402.74	50805.48	0.1	197015	25.79	50662	0.0538	1	0.005	15	0.3476
			0.5	2	12773.93	25547.86			0.1					1		15	
	4-3	2	0.5	1	12073.54	24147.08	24344.83	48689.66	0.1	197015	24.71	48546	0.0516	1	0.005	15	0.3331
			0.5	2	12271.29	24542.58			0.1					1		15	
	5-1	2	0.5	1	12474.33	24948.66	24956.35	49912.7	0.1	197015	25.33	49769	0.0529	1	0.005	15	0.3415
			0.5	2	12482.02	24964.04			0.1					1		15	
	5-2	2	0.5	1	11037.24	22074.48	23342.96	46685.92	0.1	197015	23.70	46542	0.0495	1	0.005	15	0.3194
			0.5	2	12305.72	24611.44			0.1					1		15	
	5-3	2	0.5	1	7170.75	14341.5	14585.26	29170.52	0.1	197015	14.81	29027	0.0308	1	0.005	15	0.1992
			0.5	2	7414.51	14829.02			0.1					1		15	
	6-1	2	0.5	1	12546.55	25093.1	25170.73	50341.46	0.1	197015	25.55	50198	0.0533	1	0.005	15	0.3444
			0.5	2	12624.18	25248.36			0.1					1		15	
	6-2	2	0.5	1	12851.78	25703.56	25788.93	51577.86	0.1	197015	26.18	51434	0.0547	1	0.005	15	0.3529
			0.5	2	12937.15	25874.3			0.1					1		15	
	6-3	2	0.5	1	12500.45	25000.9	25191.68	50383.36	0.1	197015	25.57	50240	0.0534	1	0.005	15	0.3447
			0.5	2	12691.23	25382.46			0.1					1		15	
	7-1	2	0.5	1	12661.79	25323.58	25453.8	50907.6	0.1	197015	25.84	50764	0.0539	1	0.005	15	0.3483
			0.5	2	12792.01	25584.02			0.1					1		15	
	7-2	2	0.5	1	12800.16	25600.32	25731.7	51463.4	0.1	197015	26.12	51320	0.0545	1	0.005	15	0.3521
			0.5	2	12931.54	25863.08			0.1					1		15	
	7-3	2	0.5	1	12795.57	25591.14	25609.2	51218.4	0.1	197015	26.00	51075	0.0543	1	0.005	15	0.3505
			0.5	2	12813.63	25627.26			0.1					1		15	
	8-1	2	0.5	1	12344.71	24689.42	24747.25	49494.5	0.1	197015	25.12	49351	0.0524	1	0.005	15	0.3386
			0.5	2	12402.54	24805.08			0.1					1		15	
	8-2	2	0.5	1	12474.91	24949.82	24875.47	49750.94	0.1	197015	25.25	49607	0.0527	1	0.005	15	0.3404
			0.5	2	12400.56	24801.12			0.1					1		15	
	8-3	2	0.5	1	12813.56	25627.12	25443.92	50887.84	0.1	197015	25.83	50744	0.0539	1	0.005	15	0.3482
			0.5	2	12630.36	25260.72			0.1					1		15	

Assay Date	9/29/2005	ID	RC5	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0	Technician ID	TNB	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.3322	0.0195
Full activity	End	0.3358	0.0014
Full activity	Overall	0.3340	0.0115
Background	Beginning	0.0000	0.000109073
Background	End	0.0000	0.000147695
Background	Overall	0.0000	0.000113845
Positive	Beginning	0.1611	0.0157
Positive	End	0.1646	0.0181
Positive	Overall	0.1628	0.0140
Negative	Beginning	0.2749	0.0866
Negative	End	0.3079	0.0530
Negative	Overall	0.2914	0.0616

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC5	1	1	1.00E-04	-4.00	0.3385
RC5	1	2	1.00E-04	-4.00	0.3103
RC5	1	3	1.00E-04	-4.00	0.2881
RC5	2	1	1.00E-05	-5.00	0.3671
RC5	2	2	1.00E-05	-5.00	0.3342
RC5	2	3	1.00E-05	-5.00	0.3240
RC5	3	1	5.00E-06	-5.30	0.3492
RC5	3	2	5.00E-06	-5.30	0.3420
RC5	3	3	5.00E-06	-5.30	0.3425
RC5	4	1	1.00E-06	-6.00	0.3492
RC5	4	2	1.00E-06	-6.00	0.3476
RC5	4	3	1.00E-06	-6.00	0.3331
RC5	5	1	1.00E-07	-7.00	0.3415
RC5	5	2	1.00E-07	-7.00	0.3194
RC5	5	3	1.00E-07	-7.00	0.1992
RC5	6	1	1.00E-08	-8.00	0.3444
RC5	6	2	1.00E-08	-8.00	0.3529
RC5	6	3	1.00E-08	-8.00	0.3447
RC5	7	1	1.00E-09	-9.00	0.3483
RC5	7	2	1.00E-09	-9.00	0.3521
RC5	7	3	1.00E-09	-9.00	0.3505
RC5	8	1	1.00E-10	-10.00	0.3386
RC5	8	2	1.00E-10	-10.00	0.3404
RC5	8	3	1.00E-10	-10.00	0.3482

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-4.00	101.35	92.91	86.25
2	-5.00	109.92	100.07	97.01
3	-5.30	104.55	102.40	102.55
4	-6.00	104.54	104.08	99.73
5	-7.00	102.24	95.61	59.63
6	-8.00	103.12	105.66	103.21
7	-9.00	104.29	105.43	104.93
8	-10.00	101.38	101.91	104.25

Aromatase Assay Spreadsheet

Assay Date	10/3/2005	Test Chemical ID	RC5	# Concentrations tested	8
ID	TNB	Replicate #	Rep 2	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0202	38465.28	1904222	
2	0.0202	38148.55	1888542	
3	0.0200	37712.89	1885645	
4	0.0201	39277.98	1954128	
5	0.0203	39501.52	1945888	
			Average DPM/g soln	1915685
			SD	32252
			CV	1.68
			µCi/g soln	0.863

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	8.1822 g
Mass of dilution B used in substrate prep	4.619 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.570163 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00977 µg/g soln.
µg/g soln.

a. µCi/g soln	0.863
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.

= 0.570163 + 0.00977
= 0.579932 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.488 µCi/µg ASDN

946063 dpm/nmol

Assay Date	10/3/2005	Test Chemical ID	RC5	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.584	0.343	0.142	0.086	0.056	0.045	0.037	25	100	
	0.583	0.357	0.143	0.086	0.056	0.046	0.037			
	0.572	0.355	0.147	0.088	0.058	0.047	0.037			

Samples:	10	100	microsomes
	0.058	0.287	0.060
	0.059	0.290	0.059
			0.058

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	0.580	0.543	0.0484	m, b	0.089	0.000
0.125	100	200	0.00013	200	0.0250	0.352	0.315	0.0281	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.144	0.107	0.0095	r , se_y	0.993	0.002
0.025	100	1000	0.00003	200	0.0050	0.087	0.050	0.0044	F, df	572	4
0.01	40	1000	0.00001	200	0.0020	0.057	0.020	0.0018	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.046	0.009	0.0008			

Regression results are calculated using the function LINEST

Blank	0.037	r^2=	0.993	
		m=	0.089	
		b=	0.000	

	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	0.021	0.002	200	1	1	0.000	0.000	0.010
10	0.059	0.022	0.002	200	1	1	0.000		
10					1	1			
100	0.287	0.250	0.022	200	1	1	0.000	0.000	0.112
100	0.290	0.253	0.023	200	1	1	0.000		
100									
	0.060	0.023	0.002	200	114	69814	0.006	0.006	6.038
	0.059	0.022	0.002	200	114	69814	0.006		
	0.058	0.021	0.002	200	114	69814	0.006		

Assay Date	10/3/2005	Test Chemical ID	RC5	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.584	0.343	0.142	0.086	0.056	0.045	0.037	25	100	
	0.583	0.357	0.143	0.086	0.056	0.046	0.037			
	0.572	0.355	0.147	0.088	0.058	0.047	0.037			

Samples:	10	100	microsomes
	0.058	0.287	0.060
	0.059	0.290	0.059
			0.058

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	0.580	0.543	0.0430	m, b	0.078	0.001
0.125	100	200	0.00013	200	0.0250	0.352	0.315	0.0253	se_m, se_b	0.003	0.000
0.05	200	1000	0.00005	200	0.0100	0.144	0.107	0.0091	r , se_y	0.997	0.001
0.025	100	1000	0.00003	200	0.0050	0.087	0.050	0.0047	F, df	876	3
0.01	40	1000	0.00001	200	0.0020	0.057	0.020	0.0023	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.046	0.009	0.0015			

Blank 0.037

r^2= 0.997
m= 0.078
b= 0.001

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	0.021	0.002	200	1	1	0.000	0.000	0.012
10	0.059	0.022	0.003	200	1	1	0.000		
10					1	1			
100	0.287	0.250	0.020	200	1	1	0.000	0.000	0.102
100	0.290	0.253	0.021	200	1	1	0.000		
100									
	0.060	0.023	0.003	200	114	69814	0.008	0.008	7.748
	0.059	0.022	0.003	200	114	69814	0.008		
	0.058	0.021	0.002	200	114	69814	0.008		

Assay Date	10/3/2005	Test Chemical ID	RC5	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

								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	10/3/2005	Chemical ID	RC5	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 2

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.403509	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
	612.403509	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	7.748
Protein Concentration (dilution added to assay, mg/mL):	0.012652

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	10/3/2005	Test Chemical ID	RC5	# Concentrations tested		S Microsome type		Microsome ID		O 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 H_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	11285.39	22570.78	22634.76	45269.52	0.1	191568	23.63	45165	0.0477	1	0.006	15	0.2516
			0.5	2	11349.37	22698.74			0.1					1		15	
	2	2	0.5	1	11674.08	23348.16	23405.53	46811.06	0.1	191568	24.44	46706	0.0494	1	0.006	15	0.2601
			0.5	2	11731.45	23462.9			0.1					1		15	
	3	2	0.5	1	11471.68	22943.36	23143.79	46287.58	0.1	191568	24.16	46183	0.0488	1	0.006	15	0.2572
			0.5	2	11672.11	23344.22			0.1					1		15	
	4	2	0.5	1	11514.18	23028.36	22926.79	45853.58	0.1	191568	23.94	45749	0.0484	1	0.006	15	0.2548
			0.5	2	11412.61	22825.22			0.1					1		15	
Background control	1	2	0.5	1	30	60	51.88	103.76	0.1	191568	0.05	-1	0.0000	1	0.006	15	0.0000
			0.5	2	21.88	43.76			0.1					1		15	
	2	2	0.5	1	29.27	58.54	55.95	111.9	0.1	191568	0.06	7	0.0000	1	0.006	15	0.0000
			0.5	2	26.68	53.36			0.1					1		15	
	3	2	0.5	1	30.37	60.74	53.13	106.26	0.1	191568	0.06	1	0.0000	1	0.006	15	0.0000
			0.5	2	22.76	45.52			0.1					1		15	
	4	2	0.5	1	25.38	50.76	48.98	97.96	0.1	191568	0.05	-7	0.0000	1	0.006	15	0.0000
			0.5	2	23.6	47.2			0.1					1		15	
Positive control	1	2	0.5	1	5935.45	11870.9	11774.25	23548.5	0.1	191568	12.29	23444	0.0248	1	0.006	15	0.1306
			0.5	2	5838.8	11677.6			0.1					1		15	
	2	2	0.5	1	5781.81	11563.62	11578.49	23156.96	0.1	191568	12.09	23052	0.0244	1	0.006	15	0.1284
			0.5	2	5796.68	11573.36			0.1					1		15	
	3	2	0.5	1	5583.32	11166.64	11064.45	22128.9	0.1	191568	11.55	22024	0.0233	1	0.006	15	0.1227
			0.5	2	5481.13	10962.26			0.1					1		15	
	4	2	0.5	1	5444.81	10889.62	10814.82	21629.64	0.1	191568	11.29	21525	0.0228	1	0.006	15	0.1199
			0.5	2	5370.01	10740.02			0.1					1		15	
Negative Control	1	2	0.5	1	11698.72	23397.44	23466.62	46933.24	0.1	191568	24.50	46828	0.0495	1	0.006	15	0.2608
			0.5	2	11767.9	23535.8			0.1					1		15	
	2	2	0.5	1	11398.63	22793.26	22966.47	45932.94	0.1	191568	23.98	45828	0.0484	1	0.006	15	0.2553
			0.5	2	11569.84	23139.68			0.1					1		15	
	3	2	0.5	1	11576.94	23153.88	22965.17	45930.34	0.1	191568	23.98	45825	0.0484	1	0.006	15	0.2552
			0.5	2	11388.23	22776.46			0.1					1		15	
	4	2	0.5	1	10694.79	21389.58	21043.32	42086.64	0.1	191568	21.97	41982	0.0444	1	0.006	15	0.2338
			0.5	2	10348.53	20697.06			0.1					1		15	
RC5	1-1	2	0.5	1	10864.34	21728.68	21839.64	43679.28	0.1	191568	22.80	43574	0.0461	1	0.006	15	0.2427
			0.5	2	10975.3	21950.6			0.1					1		15	
	1-2	2	0.5	1	11313.63	22627.26	22662.49	45324.98	0.1	191568	23.66	45220	0.0478	1	0.006	15	0.2519
			0.5	2	11348.86	22697.72			0.1					1		15	
	1-3	2	0.5	1	10898.92	21797.84	21897.96	43795.92	0.1	191568	22.86	43691	0.0462	1	0.006	15	0.2433
			0.5	2	10999.04	21998.08			0.1					1		15	
	2-1	2	0.5	1	12043.86	24087.72	24073.15	48146.3	0.1	191568	25.13	48041	0.0508	1	0.006	15	0.2676
			0.5	2	12029.29	24058.58			0.1					1		15	
	2-2	2	0.5	1	12013.39	24026.78	24229.12	48458.24	0.1	191568	25.30	48353	0.0511	1	0.006	15	0.2693
			0.5	2	12215.73	24431.46			0.1					1		15	
	2-3	2	0.5	1	11737.6	23475.2	23842.35	47684.7	0.1	191568	24.89	47580	0.0503	1	0.006	15	0.2650
			0.5	2	12104.75	24209.5			0.1					1		15	
	3-1	2	0.5	1	11683.41	23366.82	23593.95	47187.9	0.1	191568	24.63	47083	0.0498	1	0.006	15	0.2622
			0.5	2	11910.54	23821.08			0.1					1		15	
	3-2	2	0.5	1	11763.41	23526.82	23487.15	46974.3	0.1	191568	24.52	46869	0.0495	1	0.006	15	0.2611
			0.5	2	11723.74	23447.48			0.1					1		15	
	3-3	2	0.5	1	12432.95	24865.9	24606.27	49212.54	0.1	191568	25.69	49108	0.0519	1	0.006	15	0.2735
			0.5	2	12173.32	24346.64			0.1					1		15	
	4-1	2	0.5	1	12133.91	24267.82	24222.52	48445.04	0.1	191568	25.29	48340	0.0511	1	0.006	15	0.2692
			0.5	2	12088.61	24177.22			0.1					1		15	
	4-2	2	0.5	1	12208.44	24416.88	24136.14	48272.28	0.1	191568	25.20	48167	0.0509	1	0.006	15	0.2683
			0.5	2	11927.7	23855.4			0.1					1		15	
	4-3	2	0.5	1	11802.95	23605.9	23468.79	46937.58	0.1	191568	24.50	46833	0.0495	1	0.006	15	0.2608
			0.5	2	11665.84	23331.68			0.1					1		15	
	5-1	2	0.5	1	11564.72	23129.44	22861.38	45722.76	0.1	191568	23.87	45618	0.0482	1	0.006	15	0.2541
			0.5	2	11296.67	22593.34			0.1					1		15	
	5-2	2	0.5	1	11697.09	23394.18	23249.79	46499.58	0.1	191568	24.27	46395	0.0490	1	0.006	15	0.2584
			0.5	2	11552.7	23105.4			0.1					1		15	
	5-3	2	0.5	1	12077.86	24155.72	24027.92	48055.64	0.1	191568	25.09	47951	0.0507	1	0.006	15	0.2671
			0.5	2	11950.06	23900.12			0.1					1		15	
	6-1	2	0.5	1	12263.44	24526.88	24466.19	48932.38	0.1	191568	25.54	48827	0.0516	1	0.006	15	0.2720
			0.5	2	12202.75	24405.5			0.1					1		15	
	6-2	2	0.5	1	11939.66	23879.32	24240.12	48480.24	0.1	191568	25.31	48375	0.0511	1	0.006	15	0.2694
			0.5	2	12300.46	24600.92			0.1					1		15	
	6-3	2	0.5	1	12604.43	25208.86	24465.46	48930.92	0.1	191568	25.54	48826	0.0516	1	0.006	15	0.2719
			0.5	2	11861.03	23722.06			0.1					1		15	
	7-1	2	0.5	1	12054.78	24109.56	23346.59	46693.18	0.1	191568	24.37	46588	0.0492	1	0.006	15	0.2595
			0.5	2	11291.61	22583.62			0.1					1		15	
	7-2	2	0.5	1	12119.51	24239.02	24228.97	48467.94	0.1	191568	25.30	48353	0.0511	1	0.006	15	0.2693
			0.5	2	12109.46	24218.92			0.1					1		15	
	7-3	2	0.5	1	11957.08	23914.16	23749.4	47498.8	0.1	191568	24.79	47394	0.0501	1	0.006	15	0.2640
			0.5	2	11792.32	23584.64			0.1					1		15	
	8-1	2	0.5	1	12499.92	24999.84	25065.69	50131.38	0.1	191568	26.17	50026	0.0529	1	0.006	15	0.2786
			0.5	2	12565.77	25131.54			0.1					1		15	
	8-2	2	0.5	1	11710.8	23421.6	23426.94	46853.88	0.1	191568	24.46	46749	0.0494	1	0.006	15	0.2604
			0.5	2	11716.14	23432.28			0.1					1		15	
	8-3	2	0.5	1	12282.59	24565.18	24832.59	49665.18	0.1	191568	25.93	49560	0.0524	1	0.006	15	0.2760
			0.5	2	12550	25100			0.1					1		15	

Assay Date	10/3/2005	ID	RC5	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	TNB	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.2558	0.0061
Full activity	End	0.2560	0.0017
Full activity	Overall	0.2559	0.0036
Background	Beginning	0.0000	3.20587E-05
Background	End	0.0000	3.26889E-05
Background	Overall	0.0000	3.22041E-05
Positive	Beginning	0.1295	0.0015
Positive	End	0.1213	0.0020
Positive	Overall	0.1254	0.0050
Negative	Beginning	0.2580	0.0039
Negative	End	0.2445	0.0151
Negative	Overall	0.2513	0.0119

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC5	1	1	1.00E-04	-4.00	0.2427
RC5	1	2	1.00E-04	-4.00	0.2519
RC5	1	3	1.00E-04	-4.00	0.2433
RC5	2	1	1.00E-05	-5.00	0.2676
RC5	2	2	1.00E-05	-5.00	0.2693
RC5	2	3	1.00E-05	-5.00	0.2650
RC5	3	1	5.00E-06	-5.30	0.2622
RC5	3	2	5.00E-06	-5.30	0.2611
RC5	3	3	5.00E-06	-5.30	0.2735
RC5	4	1	1.00E-06	-6.00	0.2692
RC5	4	2	1.00E-06	-6.00	0.2683
RC5	4	3	1.00E-06	-6.00	0.2608
RC5	5	1	1.00E-07	-7.00	0.2541
RC5	5	2	1.00E-07	-7.00	0.2584
RC5	5	3	1.00E-07	-7.00	0.2671
RC5	6	1	1.00E-08	-8.00	0.2720
RC5	6	2	1.00E-08	-8.00	0.2694
RC5	6	3	1.00E-08	-8.00	0.2719
RC5	7	1	1.00E-09	-9.00	0.2595
RC5	7	2	1.00E-09	-9.00	0.2693
RC5	7	3	1.00E-09	-9.00	0.2640
RC5	8	1	1.00E-10	-10.00	0.2786
RC5	8	2	1.00E-10	-10.00	0.2604
RC5	8	3	1.00E-10	-10.00	0.2760

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-4.00	94.83	98.41	95.08
2	-5.00	104.55	105.23	103.55
3	-5.30	102.46	102.00	106.87
4	-6.00	105.20	104.82	101.92
5	-7.00	99.28	100.97	104.35
6	-8.00	106.26	105.28	106.26
7	-9.00	101.39	105.23	103.14
8	-10.00	108.87	101.74	107.86

Aromatase Assay Spreadsheet

Assay Date	10/5/2005	Test Chemical ID	RC5	# Concentrations tested	8
ID	TNB	Replicate #	Rep 3	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0202	37801.65	1871369	
2	0.0201	38926.56	1936645	
3	0.0203	37946.41	1869281	
4	0.0204	38392.64	1881992	
5	0.0204	40045.7	1963025	
			Average DPM/g soln	1904462
			SD	42729
			CV	2.24
			µCi/g soln	0.858

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	8.1776 g
Mass of dilution B used in substrate prep	4.6127 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.586628 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00971 µg/g soln.
µg/g soln.

a. µCi/g soln	0.858
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.

= 0.586628 + 0.00971
= 0.596339 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.439 µCi/µg ASDN

914644 dpm/nmol

Test

Assay Date 10/5/2005 Chemical ID RC5 tested 8

ID TNB Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.463	0.294	0.119	0.071	0.044	0.037	0.031	25	100	
	0.450	0.313	0.130	0.070	0.048	0.043	0.033			
	0.447	0.320	0.127	0.076	0.046	0.039	0.032			

Samples:	10	100	microsomes
	0.052	0.243	0.056
	0.048	0.261	0.053
			0.058

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	0.453	0.421	0.0465	m, b	0.111	0.000
0.125	100	200	0.00013	200	0.0250	0.309	0.277	0.0305	se_m, se_b	0.009	0.002
0.05	200	1000	0.00005	200	0.0100	0.125	0.093	0.0101	r , se_y	0.976	0.003
0.025	100	1000	0.00003	200	0.0050	0.072	0.040	0.0042	F, df	162	4
0.01	40	1000	0.00001	200	0.0020	0.046	0.014	0.0012	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.040	0.008	0.0005			

Blank 0.032

r^2= 0.976
m= 0.111
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	0.052	0.020	0.002	200	1	1	0.000	0.000	0.008
10	0.048	0.016	0.001	200	1	1	0.000		
10					1	1			
100	0.243	0.211	0.023	200	1	1	0.000	0.000	0.121
100	0.261	0.229	0.025	200	1	1	0.000		
100									
	0.056	0.024	0.002	200	114	69814	0.007	0.007	7.110
	0.053	0.021	0.002	200	114	69814	0.006		
	0.058	0.026	0.003	200	114	69814	0.008		

Assay Date 10/5/2005 | Test Chemical ID RC5 | tested 8

ID | TNB | Replicate # Rep 3 | Microsome type Recombinant | Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.463	0.294	0.119	0.071	0.044	0.037	0.031	25	100
	0.450	0.313	0.130	0.070	0.048	0.043	0.033		
	0.447	0.320	0.127	0.076	0.046	0.039	0.032		

Protein stock ID

Samples:	10	100	microsomes
	0.052	0.243	0.056
	0.048	0.261	0.053
			0.058

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	0.453	0.421	0.0380
0.125	100	200	0.00013	200	0.0250	0.309	0.277	0.0253
0.05	200	1000	0.00005	200	0.0100	0.125	0.093	0.0092
0.025	100	1000	0.00003	200	0.0050	0.072	0.040	0.0046
0.01	40	1000	0.00001	200	0.0020	0.046	0.014	0.0022
0.005	20	1000	0.00001	200	0.0010	0.040	0.008	0.0017

Variables	Regression results	
m, b	0.088	0.001
se_m, se_b	0.003	0.000
r , se_y	0.996	0.001
F, df	785	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

Blank 0.032

r^2= 0.996
m= 0.088
b= 0.001

	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	0.052	0.020	0.003	200	1	1	0.000	0.000	0.013
10	0.048	0.016	0.002	200	1	1	0.000		
10					1	1			
100	0.243	0.211	0.020	200	1	1	0.000	0.000	0.102
100	0.261	0.229	0.021	200	1	1	0.000		
100									
	0.056	0.024	0.003	200	114	69814	0.010	0.009	9.477
	0.053	0.021	0.003	200	114	69814	0.009		
	0.058	0.026	0.003	200	114	69814	0.010		

Assay Date	10/5/2005	Test Chemical ID	RC5	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

								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

					Final vol. Diluted usomes			
A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	prep. (µL)	(µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	10/5/2005	Chemical ID	RC5	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 3

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.40351	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
	612.40351	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	9.477
Protein Concentration (dilution added to assay, mg/mL):	0.015475

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	10/5/2005	Test Chemical ID	RC5	# Concentrations tested	8	Microsome type		Microsome ID		0 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	12483.1	24966.2	25108.46	50216.92	0.1	190446	26.37	50028	0.0547	1	0.008	15	0.2356
			0.5	2	12625.36	25250.72			0.1					1		15	
	2	2	0.5	1	12741.55	25483.1	25443.51	50887.02	0.1	190446	26.72	50698	0.0554	1	0.008	15	0.2388
			0.5	2	12701.96	25403.92			0.1					1		15	
	3	2	0.5	1	10376.93	20753.86	20431.51	40863.02	0.1	190446	21.46	40674	0.0445	1	0.008	15	0.1916
			0.5	2	10054.58	20109.16			0.1					1		15	
	4	2	0.5	1	11828.97	23657.94	23602.69	47205.38	0.1	190446	24.79	47016	0.0514	1	0.008	15	0.2214
			0.5	2	11773.72	23547.44			0.1					1		15	
Background control	1	2	0.5	1	52.7	105.4	104.89	209.78	0.1	190446	0.11	21	0.0000	1	0.008	15	0.0001
			0.5	2	52.19	104.38			0.1					1		15	
	2	2	0.5	1	38.7	77.4	85.14	170.28	0.1	190446	0.09	-19	0.0000	1	0.008	15	-0.0001
			0.5	2	46.44	92.88			0.1					1		15	
	3	2	0.5	1	42.46	84.92	83.74	167.46	0.1	190446	0.09	-21	0.0000	1	0.008	15	-0.0001
			0.5	2	41.28	82.56			0.1					1		15	
	4	2	0.5	1	50.34	100.68	104.02	208.04	0.1	190446	0.11	19	0.0000	1	0.008	15	0.0001
			0.5	2	53.68	107.36			0.1					1		15	
Positive control	1	2	0.5	1	6207.82	12415.64	12460.57	24921.14	0.1	190446	13.09	24732	0.0270	1	0.008	15	0.1165
			0.5	2	6252.75	12505.5			0.1					1		15	
	2	2	0.5	1	6474.65	12949.3	12848.1	25696.2	0.1	190446	13.49	25507	0.0279	1	0.008	15	0.1201
			0.5	2	6373.45	12746.9			0.1					1		15	
	3	2	0.5	1	5905.24	11810.48	11792.61	23585.22	0.1	190446	12.38	23396	0.0256	1	0.008	15	0.1102
			0.5	2	5887.37	11774.74			0.1					1		15	
	4	2	0.5	1	6056.35	12112.7	12099.75	24199.5	0.1	190446	12.71	24011	0.0263	1	0.008	15	0.1131
			0.5	2	6043.4	12086.8			0.1					1		15	
Negative Control	1	2	0.5	1	12560.1	25120.2	25029.91	50059.82	0.1	190446	26.29	49871	0.0545	1	0.008	15	0.2349
			0.5	2	12469.81	24939.62			0.1					1		15	
	2	2	0.5	1	12276.43	24552.86	24555.75	49111.5	0.1	190446	25.79	48923	0.0535	1	0.008	15	0.2304
			0.5	2	12279.32	24558.64			0.1					1		15	
	3	2	0.5	1	11812.46	23624.92	23622.48	47244.98	0.1	190446	24.81	47056	0.0514	1	0.008	15	0.2216
			0.5	2	11810.03	23620.06			0.1					1		15	
	4	2	0.5	1	10967.75	21935.5	22501.85	45003.7	0.1	190446	23.63	44815	0.0490	1	0.008	15	0.2111
			0.5	2	11534.1	23068.2			0.1					1		15	
RC5	1-1	2	0.5	1	11398.39	22796.78	22625.56	45251.12	0.1	190446	23.76	45062	0.0493	1	0.008	15	0.2122
			0.5	2	11227.17	22454.34			0.1					1		15	
	1-2	2	0.5	1	11892.96	23785.92	23539.79	47079.58	0.1	190446	24.72	46881	0.0513	1	0.008	15	0.2209
			0.5	2	11646.83	23293.66			0.1					1		15	
	1-3	2	0.5	1	11728.57	23457.14	23099.9	46199.8	0.1	190446	24.26	46011	0.0503	1	0.008	15	0.2167
			0.5	2	11371.33	22742.66			0.1					1		15	
	2-1	2	0.5	1	12058.84	24117.68	24114.44	48228.88	0.1	190446	25.32	48040	0.0525	1	0.008	15	0.2263
			0.5	2	12055.6	24111.2			0.1					1		15	
	2-2	2	0.5	1	12440.44	24880.88	24664.27	49328.54	0.1	190446	25.90	49140	0.0537	1	0.008	15	0.2314
			0.5	2	12223.83	24447.66			0.1					1		15	
	2-3	2	0.5	1	12218.09	24436.18	24394.83	48769.66	0.1	190446	25.61	48581	0.0531	1	0.008	15	0.2288
			0.5	2	12166.74	24333.48			0.1					1		15	
	3-1	2	0.5	1	12403.27	24806.54	24986.92	49973.84	0.1	190446	26.24	49785	0.0544	1	0.008	15	0.2345
			0.5	2	12583.65	25167.3			0.1					1		15	
	3-2	2	0.5	1	12407.54	24815.08	24846.48	49692.96	0.1	190446	26.09	49504	0.0541	1	0.008	15	0.2332
			0.5	2	12438.94	24877.88			0.1					1		15	
	3-3	2	0.5	1	12776.43	25552.86	25278.57	50557.14	0.1	190446	26.55	50368	0.0551	1	0.008	15	0.2372
			0.5	2	12502.14	25004.28			0.1					1		15	
	4-1	2	0.5	1	12109.68	24219.76	24151.68	48303.36	0.1	190446	25.36	48114	0.0526	1	0.008	15	0.2266
			0.5	2	12041.6	24083.6			0.1					1		15	
	4-2	2	0.5	1	12747.12	25494.24	25566.31	51132.62	0.1	190446	26.85	50944	0.0557	1	0.008	15	0.2399
			0.5	2	12819.19	25638.38			0.1					1		15	
	4-3	2	0.5	1	12667.98	25335.96	25483.61	50967.22	0.1	190446	26.76	50778	0.0555	1	0.008	15	0.2392
			0.5	2	12815.63	25631.26			0.1					1		15	
	5-1	2	0.5	1	12159.42	24318.84	24442.67	48885.34	0.1	190446	25.67	48696	0.0532	1	0.008	15	0.2294
			0.5	2	12283.25	24566.5			0.1					1		15	
	5-2	2	0.5	1	10434.34	20868.68	22888.78	45777.56	0.1	190446	24.04	45589	0.0498	1	0.008	15	0.2147
			0.5	2	12454.44	24908.88			0.1					1		15	
	5-3	2	0.5	1	11053.89	22107.78	22060.7	44121.4	0.1	190446	23.17	43933	0.0480	1	0.008	15	0.2069
			0.5	2	11006.71	22013.42			0.1					1		15	
	6-1	2	0.5	1	12762.28	25524.56	25431.47	50862.94	0.1	190446	26.71	50674	0.0554	1	0.008	15	0.2387
			0.5	2	12669.19	25338.38			0.1					1		15	
	6-2	2	0.5	1	12148.8	24297.6	24388.03	48776.06	0.1	190446	25.61	48587	0.0531	1	0.008	15	0.2288
			0.5	2	12239.23	24478.46			0.1					1		15	
	6-3	2	0.5	1	13058.89	26117.78	25984.45	51968.9	0.1	190446	27.29	51780	0.0566	1	0.008	15	0.2439
			0.5	2	12925.56	25851.12			0.1					1		15	
	7-1	2	0.5	1	11773.71	23547.42	23689.15	47378.3	0.1	190446	24.88	47189	0.0516	1	0.008	15	0.2223
			0.5	2	11915.44	23830.88			0.1					1		15	
	7-2	2	0.5	1	12418.98	24837.96	24948.45	49896.9	0.1	190446	26.20	49708	0.0543	1	0.008	15	0.2341
			0.5	2	12529.46	25058.92			0.1					1		15	
	7-3	2	0.5	1	12475.12	24950.24	24885.34	49770.68	0.1	190446	26.13	49582	0.0542	1	0.008	15	0.2335
			0.5	2	12410.22	24820.44			0.1					1		15	
	8-1	2	0.5	1	12508.47	25016.94	25024.71	50049.42	0.1	190446	26.28	49861	0.0545	1	0.008	15	0.2348
			0.5	2	12516.24	25032.48			0.1					1		15	
	8-2	2	0.5	1	12723.63	25447.26	21598.79	43197.58	0.1	190446	22.68	43009	0.0470	1	0.008	15	0.2026
			0.5	2	8875.16	17750.32			0.1					1		15	
	8-3	2	0.5	1	12747.7	25495.4	25553.09	51106.18	0.1	190446	26.83	50917	0.0557	1	0.008	15	0.2398
			0.5	2	12805.39	25610.78			0.1					1		15	

Assay Date	10/5/2005	ID	RC5	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0 Technician ID	TNB	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.2372	0.0022
Full activity	End	0.2065	0.0211
Full activity	Overall	0.2219	0.0216
Background	Beginning	0.0000	0.000131554
Background	End	0.0000	0.000135085
Background	Overall	0.0000	0.000109039
Positive	Beginning	0.1183	0.0026
Positive	End	0.1116	0.0020
Positive	Overall	0.1150	0.0043
Negative	Beginning	0.2327	0.0032
Negative	End	0.2164	0.0075
Negative	Overall	0.2245	0.0105

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC5	1	1	1.00E-04	-4.00	0.2122
RC5	1	2	1.00E-04	-4.00	0.2209
RC5	1	3	1.00E-04	-4.00	0.2167
RC5	2	1	1.00E-05	-5.00	0.2263
RC5	2	2	1.00E-05	-5.00	0.2314
RC5	2	3	1.00E-05	-5.00	0.2288
RC5	3	1	5.00E-06	-5.30	0.2345
RC5	3	2	5.00E-06	-5.30	0.2332
RC5	3	3	5.00E-06	-5.30	0.2372
RC5	4	1	1.00E-06	-6.00	0.2266
RC5	4	2	1.00E-06	-6.00	0.2399
RC5	4	3	1.00E-06	-6.00	0.2392
RC5	5	1	1.00E-07	-7.00	0.2294
RC5	5	2	1.00E-07	-7.00	0.2147
RC5	5	3	1.00E-07	-7.00	0.2069
RC5	6	1	1.00E-08	-8.00	0.2387
RC5	6	2	1.00E-08	-8.00	0.2288
RC5	6	3	1.00E-08	-8.00	0.2439
RC5	7	1	1.00E-09	-9.00	0.2223
RC5	7	2	1.00E-09	-9.00	0.2341
RC5	7	3	1.00E-09	-9.00	0.2335
RC5	8	1	1.00E-10	-10.00	0.2348
RC5	8	2	1.00E-10	-10.00	0.2026
RC5	8	3	1.00E-10	-10.00	0.2398

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-4.00	95.67	99.55	97.68
2	-5.00	101.99	104.32	103.13
3	-5.30	105.69	105.09	106.93
4	-6.00	102.14	108.15	107.80
5	-7.00	103.38	96.78	93.27
6	-8.00	107.58	103.15	109.93
7	-9.00	100.18	105.53	105.26
8	-10.00	105.85	91.31	108.10

Aromatase Assay Spreadsheet

Assay Date	8/4/2005	Test Chemical ID	RC6	# Concentrations tested	8
ID	TNB	Replicate #	1	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0198	32446.93	1638734	
2	0.0202	32884.73	1627957	
3	0.0202	32927.29	1630064	
4	0.0202	32969.04	1632131	
5	0.0202	33300.85	1648557	
			Average DPM/g soln	1635488
			SD	8348
			CV	0.51
			µCi/g soln	0.737

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	8.1661 g
Mass of dilution B used in substrate prep	4.5943 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.562606 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00834 µg/g soln.
µg/g soln.

a. µCi/g soln 0.737
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.

= 0.562606 + 0.00834
= 0.570946 µg ASDN/g soln.

3) Calculate Solution Specific Activity

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

820400 dpm/nmol

Test

Assay Date 8/4/2005 Chemical ID RC6 tested 8

ID TNB Replicate # 1 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.564	0.336	0.159	0.094	0.049	0.036	0.027	25	100	
	0.567	0.342	0.163	0.096	0.051	0.036	0.027			
	0.562	0.341	0.160	0.101	0.050	0.039	0.025			

Samples:	10	100	microsomes
	0.048	0.277	0.044
	0.050	0.278	0.045
			0.043

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	0.564	0.538	0.0482
0.125	100	200	0.00013	200	0.0250	0.339	0.313	0.0276
0.05	200	1000	0.00005	200	0.0100	0.160	0.134	0.0111
0.025	100	1000	0.00003	200	0.0050	0.097	0.070	0.0053
0.01	40	1000	0.00001	200	0.0020	0.050	0.024	0.0010
0.005	20	1000	0.00001	200	0.0010	0.037	0.010	-0.0002

Blank 0.026

r^2= 0.992
m= 0.092
b= -0.001

Variables	Regression results	
m, b	0.092	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.992	0.002
F, df	527	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	0.048	0.021	0.001	200	1	1	0.000	0.000	0.004
10	0.050	0.023	0.001	200	1	1	0.000		
10					1	1			
100	0.277	0.251	0.022	200	1	1	0.000	0.000	0.109
100	0.278	0.251	0.022	200	1	1	0.000		
100									
	0.044	0.018	0.000	200	114	69814	0.001	0.001	1.336
	0.045	0.019	0.001	200	114	69814	0.002		
	0.043	0.016	0.000	200	114	69814	0.001		

Test
Assay Date 8/4/2005 Chemical ID RC6 tested 8

ID TNB Replicate # 1 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.564	0.336	0.159	0.094	0.049	0.036	0.027	25	100	
	0.567	0.342	0.163	0.096	0.051	0.036	0.027			
	0.562	0.341	0.160	0.101	0.050	0.039	0.025			

Samples:	10	100	microsomes
	0.048	0.277	0.044
	0.050	0.278	0.045
			0.043

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	0.564	0.538	0.0426
0.125	100	200	0.00013	200	0.0250	0.339	0.313	0.0247
0.05	200	1000	0.00005	200	0.0100	0.160	0.134	0.0105
0.025	100	1000	0.00003	200	0.0050	0.097	0.070	0.0054
0.01	40	1000	0.00001	200	0.0020	0.050	0.024	0.0017
0.005	20	1000	0.00001	200	0.0010	0.037	0.010	0.0007

Blank 0.026

r^2= 0.998
m= 0.080
b= 0.000

Variables	Regression results	
m, b	0.080	0.000
se_m, se_b	0.002	0.000
r , se_y	0.998	0.000
F, df	1622	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	0.048	0.021	0.002	200	1	1	0.000	0.000	0.008
10	0.050	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.277	0.251	0.020	200	1	1	0.000	0.000	0.099
100	0.278	0.251	0.020	200	1	1	0.000		
100									
	0.044	0.018	0.001	200	114	69814	0.004	0.004	3.764
	0.045	0.019	0.001	200	114	69814	0.004		
	0.043	0.016	0.001	200	114	69814	0.003		

Assay Date	8/4/2005	Test Chemical ID	RC6	tested	8		
ID	TNB	Replicate #	1	Microsome type	Recombinant	Microsome ID	0

								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	8/4/2005	Chemical ID	RC6	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	1

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	3.764
Protein Concentration (dilution added to assay, mg/mL):	0.006146

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	8/4/2005	Test Chemical ID	RC6	# Concentrations tested		# Microsome type		Microsome ID		Technician ID	TNB	Replicate #	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					0.1	163549		#VALUE!	#VALUE!	1	0.003	15	#VALUE!
			0.5	2					0.1					1		15	
	2	2	0.5	1	8857.65	17715.3	16164.3	36328.6	0.1	163549	22.21	36184	0.0441	1	0.003	15	0.4784
			0.5	2	9006.65	18013.3			0.1					1		15	
	3	2	0.5	1	8333.77	16667.54	16714.37	33428.74	0.1	163549	20.44	33284	0.0406	1	0.003	15	0.4401
			0.5	2	8380.6	16761.2			0.1					1		15	
	4	2	0.5	1	8053.29	16106.58	15970.46	31940.92	0.1	163549	19.53	31796	0.0388	1	0.003	15	0.4204
			0.5	2	7917.17	15834.34			0.1					1		15	
Background control	1	2	0.5	1	37.64	75.28	69.65	139.3	0.1	163549	0.08	-5	0.0000	1	0.003	15	-0.0001
			0.5	2	32.01	64.02			0.1					1		15	
	2	2	0.5	1	40.68	81.36	82.98	165.96	0.1	163549	0.10	21	0.0000	1	0.003	15	0.0000
			0.5	2	42.3	84.6			0.1					1		15	
	3	2	0.5	1	35.77	71.54	74.85	149.7	0.1	163549	0.09	5	0.0000	1	0.003	15	0.0001
			0.5	2	39.06	78.16			0.1					1		15	
	4	2	0.5	1	32.79	65.58	61.55	123.1	0.1	163549	0.08	-21	0.0000	1	0.003	15	-0.0003
			0.5	2	28.76	57.52			0.1					1		15	
Positive control	1	2	0.5	1	4627.73	9255.46	9425.16	18850.32	0.1	163549	11.53	18708	0.0228	1	0.003	15	0.2473
			0.5	2	4797.43	9594.86			0.1					1		15	
	2	2	0.5	1	4291.48	8582.96	8707.44	17414.88	0.1	163549	10.65	17270	0.0211	1	0.003	15	0.2283
			0.5	2	4415.95	8831.9			0.1					1		15	
	3	2	0.5	1	3968.76	7937.52	7954.66	15909.32	0.1	163549	9.73	15765	0.0192	1	0.003	15	0.2084
			0.5	2	3985.9	7971.8			0.1					1		15	
	4	2	0.5	1	4127.92	8255.84	8311.73	16623.46	0.1	163549	10.16	16479	0.0201	1	0.003	15	0.2179
			0.5	2	4183.81	8367.62			0.1					1		15	
Negative Control	1	2	0.5	1	9151.12	18302.24	18380.11	36760.22	0.1	163549	22.49	36636	0.0447	1	0.003	15	0.4844
			0.5	2	9238.99	18477.98			0.1					1		15	
	2	2	0.5	1	8617.53	17235.06	17495.45	34990.9	0.1	163549	21.39	34846	0.0425	1	0.003	15	0.4607
			0.5	2	8877.92	17755.84			0.1					1		15	
	3	2	0.5	1	8148.7	16297.4	16099.32	32198.64	0.1	163549	19.69	32054	0.0391	1	0.003	15	0.4238
			0.5	2	7950.62	15901.24			0.1					1		15	
	4	2	0.5	1	7751.94	15503.88	15654.46	31308.92	0.1	163549	19.14	31164	0.0380	1	0.003	15	0.4120
			0.5	2	7902.52	15805.04			0.1					1		15	
RC6	1-1	2	0.5	1	84.78	169.56	173.55	347.1	0.1	163549	0.21	203	0.0002	1	0.003	15	0.0027
			0.5	2	88.77	177.54			0.1					1		15	
	1-2	2	0.5	1	117.98	235.96	232.88	465.76	0.1	163549	0.28	321	0.0004	1	0.003	15	0.0042
			0.5	2	114.9	229.8			0.1					1		15	
	1-3	2	0.5	1	133.51	267.02	275.92	551.84	0.1	163549	0.34	407	0.0005	1	0.003	15	0.0054
			0.5	2	142.41	284.82			0.1					1		15	
	2-1	2	0.5	1	527.2	1054.4	965.94	1931.88	0.1	163549	1.18	1787	0.0022	1	0.003	15	0.0236
			0.5	2	438.74	877.48			0.1					1		15	
	2-2	2	0.5	1	544.58	1089.16	1083.46	2166.92	0.1	163549	1.32	2022	0.0025	1	0.003	15	0.0267
			0.5	2	538.88	1077.76			0.1					1		15	
	2-3	2	0.5	1	578.17	1156.34	1157.97	2315.94	0.1	163549	1.42	2171	0.0026	1	0.003	15	0.0287
			0.5	2	579.8	1159.6			0.1					1		15	
	3-1	2	0.5	1	3241.47	6482.94	6447.61	12895.22	0.1	163549	7.88	12751	0.0155	1	0.003	15	0.1666
			0.5	2	3206.14	6412.28			0.1					1		15	
	3-2	2	0.5	1	3413.57	6827.14	6799.36	13598.72	0.1	163549	8.31	13454	0.0164	1	0.003	15	0.1779
			0.5	2	3385.79	6771.58			0.1					1		15	
	3-3	2	0.5	1	3297.02	6594.04	6525.52	13051.04	0.1	163549	7.98	12907	0.0157	1	0.003	15	0.1706
			0.5	2	3228.5	6457			0.1					1		15	
	4-1	2	0.5	1	7350.44	14700.88	14677.77	29355.54	0.1	163549	17.95	29211	0.0356	1	0.003	15	0.3862
			0.5	2	7327.33	14654.66			0.1					1		15	
	4-2	2	0.5	1	7337.23	14674.46	14684.6	29369.2	0.1	163549	17.96	29225	0.0356	1	0.003	15	0.3864
			0.5	2	7347.37	14694.74			0.1					1		15	
	4-3	2	0.5	1	7491.73	14983.46	15766.05	31532.1	0.1	163549	19.28	31386	0.0383	1	0.003	15	0.4150
			0.5	2	8274.32	16548.64			0.1					1		15	
	5-1	2	0.5	1	8520.62	17041.24	17047.22	34094.44	0.1	163549	20.85	33950	0.0414	1	0.003	15	0.4489
			0.5	2	8526.6	17053.2			0.1					1		15	
	5-2	2	0.5	1	8370.38	16740.76	17059.12	34118.24	0.1	163549	20.86	33974	0.0414	1	0.003	15	0.4492
			0.5	2	8688.74	17377.48			0.1					1		15	
	5-3	2	0.5	1	8545	17096	17236.2	34472.4	0.1	163549	21.08	34328	0.0418	1	0.003	15	0.4539
			0.5	2	8687.2	17374.4			0.1					1		15	
	6-1	2	0.5	1	8494.99	16989.98	17356.52	34713.04	0.1	163549	21.22	34569	0.0421	1	0.003	15	0.4570
			0.5	2	8861.53	17723.06			0.1					1		15	
	6-2	2	0.5	1	8963.19	17926.38	18042.67	36085.34	0.1	163549	22.06	35941	0.0438	1	0.003	15	0.4752
			0.5	2	9079.48	18158.96			0.1					1		15	
	6-3	2	0.5	1	8700.57	17401.14	17365.45	34730.9	0.1	163549	21.24	34586	0.0422	1	0.003	15	0.4573
			0.5	2	8664.88	17329.76			0.1					1		15	
	7-1	2	0.5	1	8273.13	16546.26	16653.91	33307.82	0.1	163549	20.37	33163	0.0404	1	0.003	15	0.4385
			0.5	2	8360.78	16761.56			0.1					1		15	
	7-2	2	0.5	1	8794.06	17588.12	17703.95	35407.9	0.1	163549	21.65	35263	0.0430	1	0.003	15	0.4662
			0.5	2	8909.89	17819.78			0.1					1		15	
	7-3	2	0.5	1	8648.32	17696.64	17578.2	35156.4	0.1	163549	21.50	35012	0.0427	1	0.003	15	0.4629
			0.5	2	8729.86	17459.76			0.1					1		15	
	8-1	2	0.5	1	8274.77	16549.54	16631.29	33262.58	0.1	163549	20.34	33116	0.0404	1	0.003	15	0.4379
			0.5	2	8356.52	16713.04			0.1					1		15	
	8-2	2	0.5	1	8302.77	16605.54	16697.58	33395.16	0.1	163549	20.42	33251	0.0405	1	0.003	15	0.4396
			0.5	2	8384.81	16769.62			0.1					1		15	
	8-3	2	0.5	1	8474.48	16948.96	16988.3	33976.6	0.1	163549	20.77	33832	0.0412	1	0.003	15	0.4473
			0.5	2	8513.82	17027.64			0.1					1		15	

Assay Date	8/4/2005	ID	RC6	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	TNB	Replicate #	1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4784	#DIV/0!
Full activity	End	0.4302	0.0139
Full activity	Overall	0.4463	0.0295
Background	Beginning	0.0001	0.000249239
Background	End	-0.0001	0.000248678
Background	Overall	0.0000	0.000238052
Positive	Beginning	0.2378	0.0134
Positive	End	0.2132	0.0067
Positive	Overall	0.2255	0.0167
Negative	Beginning	0.4725	0.0167
Negative	End	0.4179	0.0083
Negative	Overall	0.4452	0.0333

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
RC6	1	1	1.00E-03	-3.00	0.0027
RC6	1	2	1.00E-03	-3.00	0.0042
RC6	1	3	1.00E-03	-3.00	0.0054
RC6	2	1	1.00E-04	-4.00	0.0236
RC6	2	2	1.00E-04	-4.00	0.0267
RC6	2	3	1.00E-04	-4.00	0.0287
RC6	3	1	1.00E-05	-5.00	0.1686
RC6	3	2	1.00E-05	-5.00	0.1779
RC6	3	3	1.00E-05	-5.00	0.1706
RC6	4	1	1.00E-06	-6.00	0.3862
RC6	4	2	1.00E-06	-6.00	0.3864
RC6	4	3	1.00E-06	-6.00	0.4150
RC6	5	1	1.00E-07	-7.00	0.4489
RC6	5	2	1.00E-07	-7.00	0.4492
RC6	5	3	1.00E-07	-7.00	0.4539
RC6	6	1	1.00E-08	-8.00	0.4570
RC6	6	2	1.00E-08	-8.00	0.4752
RC6	6	3	1.00E-08	-8.00	0.4573
RC6	7	1	1.00E-09	-9.00	0.4385
RC6	7	2	1.00E-09	-9.00	0.4662
RC6	7	3	1.00E-09	-9.00	0.4629
RC6	8	1	1.00E-10	-10.00	0.4379
RC6	8	2	1.00E-10	-10.00	0.4396
RC6	8	3	1.00E-10	-10.00	0.4473

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	0.60	0.95	1.21
2	-4.00	5.30	5.99	6.43
3	-5.00	37.77	39.86	38.24
4	-6.00	86.54	86.58	92.99
5	-7.00	100.58	100.65	101.70
6	-8.00	102.41	106.48	102.46
7	-9.00	98.25	104.47	103.72
8	-10.00	98.11	98.51	100.23

Aromatase Assay Spreadsheet

Assay Date	8/8/2005	Test Chemical ID	Chem 6	# Concentrations tested	8
ID	TNB	Replicate #	Rep 2	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0202	31831.28	1575806	
2	0.0205	33482.48	1633292	
3	0.0201	32909.91	1637309	
4	0.0201	31529.07	1568610	
5	0.0203	32792.16	1615377	
			Average DPM/g soln	1606079
			SD	32104
			CV	2.00
			µCi/g soln	0.723

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	8.0555 g
Mass of dilution B used in substrate prep	4.5155 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.57176 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00819 µg/g soln.
µg/g soln.

a. µCi/g soln 0.723
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.

= 0.571760 + 0.00819
= 0.579949 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.247 µCi/µg ASDN

793140 dpm/nmol

Assay Date	8/8/2005	Test Chemical ID	Chem 6	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.581	0.340	0.166	0.101	0.053	0.043	0.034	25	100	
	0.587	0.351	0.160	0.092	0.050	0.044	0.041			
	0.585	0.358	0.160	0.105	0.062	0.042	0.031			

Samples:	10	100	microsomes
	0.052	0.274	0.061
	0.051	0.274	0.044
			0.053

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	0.584	0.549	0.0484	m, b	0.089	-0.001
0.125	100	200	0.00013	200	0.0250	0.349	0.314	0.0275	se_m, se_b	0.003	0.001
0.05	200	1000	0.00005	200	0.0100	0.162	0.126	0.0107	r , se_y	0.994	0.002
0.025	100	1000	0.00003	200	0.0050	0.099	0.064	0.0051	F, df	681	4
0.01	40	1000	0.00001	200	0.0020	0.055	0.019	0.0011	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.043	0.008	0.0001			

Blank 0.036

r^2= 0.994
m= 0.089
b= -0.001

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	0.052	0.017	0.001	200	1	1	0.000	0.000	0.004
10	0.051	0.016	0.001	200	1	1	0.000		
10					1	1			
100	0.274	0.239	0.021	200	1	1	0.000	0.000	0.104
100	0.274	0.238	0.021	200	1	1	0.000		
100									
	0.061	0.025	0.002	200	114	69814	0.005	0.003	2.896
	0.044	0.009	0.000	200	114	69814	0.001		
	0.053	0.017	0.001	200	114	69814	0.003		

Test

Assay Date 8/8/2005 Chemical ID Chem 6 tested 8

ID TNB Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.581	0.340	0.166	0.101	0.053	0.043	0.034	25	100	
	0.587	0.351	0.160	0.092	0.050	0.044	0.041			
	0.585	0.358	0.160	0.105	0.062	0.042	0.031			

Samples:	10	100	microsomes
	0.052	0.274	0.061
	0.051	0.274	0.044
			0.053

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	0.584	0.549	0.0433	m, b	0.078	0.000
0.125	100	200	0.00013	200	0.0250	0.349	0.314	0.0249	se_m, se_b	0.001	0.000
0.05	200	1000	0.00005	200	0.0100	0.162	0.126	0.0102	r , se_y	1.000	0.000
0.025	100	1000	0.00003	200	0.0050	0.099	0.064	0.0053	F, df	6323	3
0.01	40	1000	0.00001	200	0.0020	0.055	0.019	0.0018	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.043	0.008	0.0009			

Blank 0.036

r^2= 1.000
m= 0.078
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	0.052	0.017	0.002	200	1	1	0.000	0.000	0.008
10	0.051	0.016	0.001	200	1	1	0.000		
10					1	1			
100	0.274	0.239	0.019	200	1	1	0.000	0.000	0.095
100	0.274	0.238	0.019	200	1	1	0.000		
100									
	0.061	0.025	0.002	200	114	69814	0.007	0.005	4.936
	0.044	0.009	0.001	200	114	69814	0.003		
	0.053	0.017	0.002	200	114	69814	0.005		

Assay Date	8/8/2005	Test Chemical ID	Chem 6		tested		8		
ID	TNB	Replicate #	Rep 2		Microsome type	Recombinant	Microsome ID	0	

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	µ µSOMES	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL

Assay Date	8/8/2005	Chemical ID	Chem 6	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	4.936
Protein Concentration (dilution added to assay, mg/mL):	0.00806

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	8/8/2005	Test Chemical ID	Chem 6	# Concentrations tested	8	Microsome type		Microsome ID		0 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 ^1H_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	465.91	931.82	945.64	1891.28	0.1	160608	1.18	1774	0.0022	1	0.004	15	0.0185
			0.5	2	479.73	959.46			0.1					1		15	
	2	2	0.5	1	440.55	881.1	880.51	1761.02	0.1	160608	1.10	1643	0.0021	1	0.004	15	0.0171
			0.5	2	439.96	879.92			0.1					1		15	
	3	2	0.5	1	429.05	858.1	862.67	1725.34	0.1	160608	1.07	1608	0.0020	1	0.004	15	0.0168
			0.5	2	433.62	867.24			0.1					1		15	
	4	2	0.5	1	426.12	852.24	874.58	1749.16	0.1	160608	1.09	1632	0.0021	1	0.004	15	0.0170
			0.5	2	448.46	896.92			0.1					1		15	
Background control	1	2	0.5	1	19.28	38.56	54.91	109.82	0.1	160608	0.07	-8	0.0000	1	0.004	15	-0.0001
			0.5	2	35.63	71.26			0.1					1		15	
	2	2	0.5	1	31.97	63.94	62.97	125.94	0.1	160608	0.06	-8	0.0000	1	0.004	15	0.0001
			0.5	2	31	62			0.1					1		15	
	3	2	0.5	1	25.15	50.3	51.66	103.32	0.1	160608	0.06	-14	0.0000	1	0.004	15	-0.0001
			0.5	2	26.51	53.02			0.1					1		15	
	4	2	0.5	1	36.33	72.66	65.54	131.08	0.1	160608	0.08	14	0.0000	1	0.004	15	0.0001
			0.5	2	29.21	58.42			0.1					1		15	
Positive control	1	2	0.5	1	448.33	896.66	910.7	1821.4	0.1	160608	1.13	1704	0.0021	1	0.004	15	0.0178
			0.5	2	462.37	924.74			0.1					1		15	
	2	2	0.5	1	460.24	920.48	898.59	1797.18	0.1	160608	1.12	1680	0.0021	1	0.004	15	0.0175
			0.5	2	436.35	872.7			0.1					1		15	
	3	2	0.5	1	447.12	894.24	890.22	1780.44	0.1	160608	1.11	1663	0.0021	1	0.004	15	0.0173
			0.5	2	443.1	886.2			0.1					1		15	
	4	2	0.5	1	422.6	845.2	839.64	1679.28	0.1	160608	1.05	1562	0.0020	1	0.004	15	0.0163
			0.5	2	417.04	834.08			0.1					1		15	
Negative Control	1	2	0.5	1	447.5	895	895.94	1791.88	0.1	160608	1.12	1674	0.0021	1	0.004	15	0.0175
			0.5	2	448.44	896.88			0.1					1		15	
	2	2	0.5	1	446.84	893.68	891.51	1783.02	0.1	160608	1.11	1665	0.0021	1	0.004	15	0.0174
			0.5	2	444.67	889.34			0.1					1		15	
	3	2	0.15	1	136.82	912.133333	911.8333333	1823.6667	0.1	160608	1.14	1706	0.0022	1	0.004	15	0.0178
			0.15	2	136.73	911.533333			0.1					1		15	
	4	2	0.5	1	435.76	871.52	871.23	1742.46	0.1	160608	1.08	1625	0.0020	1	0.004	15	0.0169
			0.5	2	435.47	870.94			0.1					1		15	
Chem 6	1-1	2	0.5	1	120.64	241.28	257.28	514.56	0.1	160608	0.32	397	0.0005	1	0.004	15	0.0041
			0.5	2	136.64	273.28			0.1					1		15	
	1-2	2	0.5	1	133.95	267.9	259.92	519.84	0.1	160608	0.32	402	0.0005	1	0.004	15	0.0042
			0.5	2	125.97	251.94			0.1					1		15	
	1-3	2	0.5	1	116.77	233.54	228.48	456.96	0.1	160608	0.28	339	0.0004	1	0.004	15	0.0035
			0.5	2	111.71	223.42			0.1					1		15	
	2-1	2	0.5	1	305.54	611.08	611.24	1222.48	0.1	160608	0.76	1105	0.0014	1	0.004	15	0.0115
			0.5	2	305.7	611.4			0.1					1		15	
	2-2	2	0.5	1	337.19	674.38	659.33	1318.66	0.1	160608	0.82	1201	0.0015	1	0.004	15	0.0125
			0.5	2	322.14	644.28			0.1					1		15	
	2-3	2	0.5	1	340.75	681.5	664.5	1329	0.1	160608	0.83	1211	0.0015	1	0.004	15	0.0126
			0.5	2	323.75	647.5			0.1					1		15	
	3-1	2	0.5	1	405.23	812.46	811.32	1622.64	0.1	160608	1.01	1505	0.0019	1	0.004	15	0.0157
			0.5	2	405.09	810.18			0.1					1		15	
	3-2	2	0.5	1	390.47	780.94	784.62	1569.24	0.1	160608	0.98	1452	0.0018	1	0.004	15	0.0151
			0.5	2	394.15	788.3			0.1					1		15	
	3-3	2	0.5	1	440.06	880.12	849.78	1699.56	0.1	160608	1.06	1582	0.0020	1	0.004	15	0.0165
			0.5	2	409.72	819.44			0.1					1		15	
	4-1	2	0.5	1	437.75	875.5	887.79	1775.58	0.1	160608	1.11	1658	0.0021	1	0.004	15	0.0173
			0.5	2	450.04	900.08			0.1					1		15	
	4-2	2	0.5	1	427.61	855.22	881.28	1762.56	0.1	160608	1.10	1645	0.0021	1	0.004	15	0.0172
			0.5	2	453.87	907.34			0.1					1		15	
	4-3	2	0.5	1	460.43	920.86	909.38	1818.76	0.1	160608	1.13	1701	0.0021	1	0.004	15	0.0177
			0.5	2	448.95	897.9			0.1					1		15	
	5-1	2	0.5	1	425.31	850.62	863.21	1726.42	0.1	160608	1.07	1609	0.0020	1	0.004	15	0.0168
			0.5	2	437.9	875.8			0.1					1		15	
	5-2	2	0.5	1	458.33	916.66	898.16	1796.32	0.1	160608	1.12	1679	0.0021	1	0.004	15	0.0175
			0.5	2	439.83	879.66			0.1					1		15	
	5-3	2	0.5	1	396.06	792.12	813.79	1627.58	0.1	160608	1.01	1510	0.0019	1	0.004	15	0.0157
			0.5	2	417.73	835.46			0.1					1		15	
	6-1	2	0.5	1	420.93	841.86	835.72	1671.44	0.1	160608	1.04	1554	0.0020	1	0.004	15	0.0162
			0.5	2	414.79	829.58			0.1					1		15	
	6-2	2	0.5	1	415.26	830.52	837.71	1675.42	0.1	160608	1.04	1558	0.0020	1	0.004	15	0.0162
			0.5	2	422.45	844.9			0.1					1		15	
	6-3	2	0.5	1	455.91	911.82	907.79	1815.58	0.1	160608	1.13	1698	0.0021	1	0.004	15	0.0177
			0.5	2	451.68	903.76			0.1					1		15	
	7-1	2	0.5	1	461.45	922.9	905.99	1811.98	0.1	160608	1.13	1694	0.0021	1	0.004	15	0.0177
			0.5	2	444.54	889.08			0.1					1		15	
	7-2	2	0.5	1	456.57	913.14	912.14	1824.28	0.1	160608	1.14	1707	0.0022	1	0.004	15	0.0178
			0.5	2	455.57	911.14			0.1					1		15	
	7-3	2	0.5	1	429.88	859.76	867.95	1735.9	0.1	160608	1.08	1618	0.0020	1	0.004	15	0.0169
			0.5	2	438.06	876.12			0.1					1		15	
	8-1	2	0.5	1	438.79	877.58	877.71	1755.42	0.1	160608	1.09	1638	0.0021	1	0.004	15	0.0171
			0.5	2	438.92	877.84			0.1					1		15	
	8-2	2	0.5	1	453.51	907.02	913.77	1827.54	0.1	160608	1.14	1710	0.0022	1	0.004	15	0.0175
			0.5	2	460.26	920.52			0.1					1		15	
	8-3	2	0.5	1	441.78	883.56	859.83	1719.66	0.1	160608	1.07	1602	0.0020	1	0.004	15	0.0167
			0.5	2	418.05	836.1			0.1					1		15	

Assay Date	8/8/2005	ID	Chem 6	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	TNB	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.0178	0.0010
Full activity	End	0.0169	0.0002
Full activity	Overall	0.0174	0.0008
Background	Beginning	0.0000	0.00011887
Background	End	0.0000	0.000204704
Background	Overall	0.0000	0.000136728
Positive	Beginning	0.0176	0.0002
Positive	End	0.0168	0.0007
Positive	Overall	0.0172	0.0007
Negative	Beginning	0.0174	0.0001
Negative	End	0.0174	0.0006
Negative	Overall	0.0174	0.0003

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
Chem 6	1	1	1.00E-03	-3.00	0.0041
Chem 6	1	2	1.00E-03	-3.00	0.0042
Chem 6	1	3	1.00E-03	-3.00	0.0035
Chem 6	2	1	1.00E-04	-4.00	0.0115
Chem 6	2	2	1.00E-04	-4.00	0.0125
Chem 6	2	3	1.00E-04	-4.00	0.0126
Chem 6	3	1	3.00E-05	-4.52	0.0157
Chem 6	3	2	3.00E-05	-4.52	0.0151
Chem 6	3	3	3.00E-05	-4.52	0.0165
Chem 6	4	1	1.00E-05	-5.00	0.0173
Chem 6	4	2	1.00E-05	-5.00	0.0172
Chem 6	4	3	1.00E-05	-5.00	0.0177
Chem 6	5	1	3.00E-06	-5.52	0.0168
Chem 6	5	2	3.00E-06	-5.52	0.0175
Chem 6	5	3	3.00E-06	-5.52	0.0157
Chem 6	6	1	1.00E-06	-6.00	0.0162
Chem 6	6	2	1.00E-06	-6.00	0.0162
Chem 6	6	3	1.00E-06	-6.00	0.0177
Chem 6	7	1	1.00E-07	-7.00	0.0177
Chem 6	7	2	1.00E-07	-7.00	0.0178
Chem 6	7	3	1.00E-07	-7.00	0.0169
Chem 6	8	1	1.00E-09	-9.00	0.0171
Chem 6	8	2	1.00E-09	-9.00	0.0178
Chem 6	8	3	1.00E-09	-9.00	0.0167

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	23.86	24.17	20.40
2	-4.00	66.40	72.18	72.80
3	-4.52	90.44	87.23	95.06
4	-5.00	99.63	98.85	102.23
5	-5.52	96.68	100.88	90.74
6	-6.00	93.37	93.61	102.04
7	-7.00	101.82	102.56	97.25
8	-9.00	98.42	102.75	96.27

Aromatase Assay Spreadsheet

Assay Date	8/10/2005	Test Chemical ID	RC6	# Concentrations tested	8
ID	TNB	Replicate #	Rep 3	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0203	33657.08	1657984	
2	0.0200	33179.37	1658969	
3	0.0200	33143.17	1657159	
4	0.0201	33186.38	1651064	
5	0.0201	34001.11	1691598	
			Average DPM/g soln	1663354
			SD	16087
			CV	0.97
			µCi/g soln	0.749

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	8.1165 g
Mass of dilution B used in substrate prep	4.5788 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.564135 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00848 µg/g soln.
µg/g soln.

a. µCi/g soln	0.749
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.

= 0.564135 + 0.00848
= 0.572617 µg ASDN/g soln.

3) Calculate Solution Specific Activity

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

831944 dpm/nmol

Assay Date	8/10/2005	Test Chemical ID	RC6	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Rcombinant	Microsome ID	0

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.576	0.371	0.165	0.097	0.055	0.041	0.038	25	100	
	0.573	0.354	0.163	0.102	0.056	0.050	0.033			
	0.570	0.366	0.163	0.101	0.059	0.046	0.031			
Samples:	10	100	microsomes							
	0.059	0.292	0.060							
	0.061	0.298	0.055							
			0.054							

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	0.573	0.539	0.0476
0.125	100	200	0.00013	200	0.0250	0.364	0.330	0.0287
0.05	200	1000	0.00005	200	0.0100	0.163	0.129	0.0107
0.025	100	1000	0.00003	200	0.0050	0.100	0.066	0.0049
0.01	40	1000	0.00001	200	0.0020	0.057	0.023	0.0010
0.005	20	1000	0.00001	200	0.0010	0.046	0.012	0.0000
		Blank		0.034				

Variables	Regression results	
m, b	0.090	-0.001
se_m, se_b	0.005	0.001
r , se_y	0.988	0.002
F, df	325	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

r^2= 0.988
m= 0.090
b= -0.001

	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	0.059	0.025	0.001	200	1	1	0.000	0.000	0.007
10	0.061	0.027	0.001	200	1	1	0.000		
10					1	1			
100	0.292	0.258	0.022	200	1	1	0.000	0.000	0.113
100	0.298	0.264	0.023	200	1	1	0.000		
100									
	0.060	0.026	0.001	200	114	69814	0.004	0.003	3.015
	0.055	0.021	0.001	200	114	69814	0.003		
	0.054	0.020	0.001	200	114	69814	0.002		

Assay Date	8/10/2005	Test Chemical ID	RC6	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Rcombinant	Microsome ID	0

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	100	
	0.576	0.371	0.165	0.097	0.055	0.041	0.038	25		
	0.573	0.354	0.163	0.102	0.056	0.050	0.033			
	0.570	0.366	0.163	0.101	0.059	0.046	0.031			
Samples:	10	100	microsomes							
	0.059	0.292	0.060							
	0.061	0.298	0.055							
			0.054							

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	0.573	0.539	0.0408
0.125	100	200	0.00013	200	0.0250	0.364	0.330	0.0250
0.05	200	1000	0.00005	200	0.0100	0.163	0.129	0.0099
0.025	100	1000	0.00003	200	0.0050	0.100	0.066	0.0051
0.01	40	1000	0.00001	200	0.0020	0.057	0.023	0.0019
0.005	20	1000	0.00001	200	0.0010	0.046	0.012	0.0011
			Blank	0.034				

Variables	Regression results	
m, b	0.075	0.000
se_m, se_b	0.000	0.000
r , se_y	1.000	0.000
F, df	23843	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

r^2= 1.000
m= 0.075
b= 0.000

	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	0.059	0.025	0.002	200	1	1	0.000	0.000	0.011
10	0.061	0.027	0.002	200	1	1	0.000		
10					1	1			
100	0.292	0.258	0.020	200	1	1	0.000	0.000	0.099
100	0.298	0.264	0.020	200	1	1	0.000		
100									
	0.060	0.026	0.002	200	114	69814	0.006	0.006	5.664
	0.055	0.021	0.002	200	114	69814	0.005		
	0.054	0.020	0.002	200	114	69814	0.005		

Assay Date	8/10/2005	Test Chemical ID	RC6	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

								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	8/10/2005	Chemical ID	RC6	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 3

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	5.664
Protein Concentration (dilution added to assay, mg/mL):	0.009249

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	8/10/2005	Test Chemical ID	RC6	# Concentrations tested	8	Microsome type	Rcombinant	Microsome ID	0	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	12255.02	24510.04	24703.84	49407.68	0.1	166335	29.70	49270	0.0592	1	0.005	15	0.4269
			0.5	2	12448.82	24897.64			0.1					1		15	
	2	2	0.5	1	12613.56	25227.12	25454.84	50909.68	0.1	166335	30.61	50772	0.0610	1	0.005	15	0.4399
			0.5	2	12841.28	25682.56			0.1					1		15	
	3	2	0.5	1	11055.52	22111.04	22179.98	44359.98	0.1	166335	26.67	44223	0.0532	1	0.005	15	0.3832
			0.5	2	11124.47	22248.94			0.1					1		15	
	4	2	0.5	1	11880.98	23761.92	23741.34	47482.68	0.1	166335	28.55	47345	0.0569	1	0.005	15	0.4102
			0.5	2	11860.38	23720.76			0.1					1		15	
Background control	1	2	0.5	1	32.15	64.3	71.1	142.2	0.1	166335	0.09	5	0.0000	1	0.005	15	0.0000
			0.5	2	38.95	77.9			0.1					1		15	
	2	2	0.5	1	34.33	68.66	72.67	145.34	0.1	166335	0.09	8	0.0000	1	0.005	15	0.0001
			0.5	2	38.34	76.68			0.1					1		15	
	3	2	0.5	1	28.71	57.42	56.54	113.08	0.1	166335	0.07	-24	0.0000	1	0.005	15	-0.0002
			0.5	2	27.83	55.66			0.1					1		15	
	4	2	0.5	1	33.72	67.44	74.07	148.14	0.1	166335	0.09	11	0.0000	1	0.005	15	0.0001
			0.5	2	40.35	80.7			0.1					1		15	
Positive control	1	2	0.5	1	7024.25	14048.5	14096.28	28192.56	0.1	166335	16.95	28055	0.0337	1	0.005	15	0.2431
			0.5	2	7072.03	14144.06			0.1					1		15	
	2	2	0.5	1	6791.42	13582.84	13622.28	27244.56	0.1	166335	16.38	27107	0.0326	1	0.005	15	0.2349
			0.5	2	6830.86	13661.72			0.1					1		15	
	3	2	0.5	1	5848.77	11697.54	11745.35	23490.7	0.1	166335	14.12	23354	0.0281	1	0.005	15	0.2023
			0.5	2	5896.58	11793.16			0.1					1		15	
	4	2	0.5	1	6401.56	12803.16	12913.51	25827.02	0.1	166335	15.53	25690	0.0309	1	0.005	15	0.2226
			0.5	2	6511.93	13023.86			0.1					1		15	
Negative Control	1	2	0.5	1	12482	24964	25124.71	50248.42	0.1	166335	30.21	50112	0.0602	1	0.005	15	0.4342
			0.5	2	12642.71	25285.42			0.1					1		15	
	2	2	0.5	1	12438.21	24876.42	25015.41	50030.82	0.1	166335	30.08	49894	0.0600	1	0.005	15	0.4323
			0.5	2	12577.2	25154.4			0.1					1		15	
	3	2	0.5	1	11949.37	23898.74	24127.8	48255.6	0.1	166335	29.01	48118	0.0578	1	0.005	15	0.4169
			0.5	2	12178.43	24356.86			0.1					1		15	
	4	2	0.5	1	11918.71	23837.42	23574.5	47149	0.1	166335	28.35	47012	0.0565	1	0.005	15	0.4073
			0.5	2	11655.79	23311.58			0.1					1		15	
RC6	1-1	2	0.5	1	50.19	100.38	104.47	208.94	0.1	166335	0.13	72	0.0001	1	0.005	15	0.0006
			0.5	2	54.28	108.56			0.1					1		15	
	1-2	2	0.5	1	151.54	303.08	312.9	625.8	0.1	166335	0.38	489	0.0006	1	0.005	15	0.0042
			0.5	2	161.36	322.72			0.1					1		15	
	1-3	2	0.5	1	154.62	309.24	319.32	638.64	0.1	166335	0.38	501	0.0006	1	0.005	15	0.0043
			0.5	2	164.7	329.4			0.1					1		15	
	2-1	2	0.5	1	733.69	1467.38	1462.79	2925.58	0.1	166335	1.76	2788	0.0034	1	0.005	15	0.0242
			0.5	2	729.11	1458.2			0.1					1		15	
	2-2	2	0.5	1	566	1132	1163.64	2327.28	0.1	166335	1.40	2190	0.0026	1	0.005	15	0.0190
			0.5	2	597.64	1195.28			0.1					1		15	
	2-3	2	0.5	1	885.07	1770.14	1762.48	3524.96	0.1	166335	2.12	3388	0.0041	1	0.005	15	0.0294
			0.5	2	877.41	1754.82			0.1					1		15	
	3-1	2	0.5	1	2519.59	5039.18	5868.98	11737.96	0.1	166335	7.06	11601	0.0139	1	0.005	15	0.1005
			0.5	2	3349.39	6698.78			0.1					1		15	
	3-2	2	0.5	1	2316.14	4632.28	4729.37	9458.74	0.1	166335	5.69	9322	0.0112	1	0.005	15	0.0808
			0.5	2	2413.23	4826.46			0.1					1		15	
	3-3	2	0.5	1	2146.22	4292.44	4329.06	8658.12	0.1	166335	5.21	8521	0.0102	1	0.005	15	0.0738
			0.5	2	2182.84	4365.68			0.1					1		15	
	4-1	2	0.5	1	4454.7	8909.4	8909.2	17818.4	0.1	166335	10.71	17681	0.0213	1	0.005	15	0.1532
			0.5	2	4454.5	8909			0.1					1		15	
	4-2	2	0.5	1	4883.49	9766.98	9874.2	19748.4	0.1	166335	11.87	19611	0.0236	1	0.005	15	0.1699
			0.5	2	4990.71	9981.42			0.1					1		15	
	4-3	2	0.5	1	4568.97	9137.94	9184.38	18368.76	0.1	166335	11.04	18232	0.0219	1	0.005	15	0.1580
			0.5	2	4615.41	9230.82			0.1					1		15	
	5-1	2	0.5	1	8147.62	16295.24	16331.39	32662.78	0.1	166335	19.64	32526	0.0391	1	0.005	15	0.2818
			0.5	2	8183.77	16367.54			0.1					1		15	
	5-2	2	0.5	1	7835.97	15671.94	15904.92	31809.84	0.1	166335	19.12	31673	0.0381	1	0.005	15	0.2744
			0.5	2	8068.95	16137.9			0.1					1		15	
	5-3	2	0.5	1	8077.61	16155.22	16087.83	32175.66	0.1	166335	19.34	32038	0.0385	1	0.005	15	0.2776
			0.5	2	8010.22	16020.44			0.1					1		15	
	6-1	2	0.5	1	10118.27	20236.54	20476.65	40953.3	0.1	166335	24.62	40816	0.0491	1	0.005	15	0.3536
			0.5	2	10358.38	20716.76			0.1					1		15	
	6-2	2	0.5	1	10236.02	20472.04	20493.17	40986.34	0.1	166335	24.64	40849	0.0491	1	0.005	15	0.3539
			0.5	2	10257.15	20514.3			0.1					1		15	
	6-3	2	0.5	1	10091.38	20182.76	20272.79	40545.58	0.1	166335	24.38	40408	0.0486	1	0.005	15	0.3501
			0.5	2	10181.41	20362.82			0.1					1		15	
	7-1	2	0.5	1	10993.68	21987.36	22224.79	44449.58	0.1	166335	26.72	44312	0.0533	1	0.005	15	0.3839
			0.5	2	11231.11	22462.22			0.1					1		15	
	7-2	2	0.5	1	11472.93	22945.86	23145.87	46291.64	0.1	166335	27.83	46154	0.0555	1	0.005	15	0.3999
			0.5	2	11672.88	23345.78			0.1					1		15	
	7-3	2	0.5	1	11404.95	22809.9	23003.62	46007.24	0.1	166335	27.66	45870	0.0551	1	0.005	15	0.3974
			0.5	2	11598.67	23197.34			0.1					1		15	
	8-1	2	0.5	1	9662.86	19325.72	19203.34	38406.68	0.1	166335	23.09	38269	0.0460	1	0.005	15	0.3316
			0.5	2	9540.36	19080.76			0.1					1		15	
	8-2	2	0.5	1	11043.16	22086.32	22068.65	44137.3	0.1	166335	26.54	44000	0.0528	1	0.005	15	0.3812
			0.5	2	11025.49	22050.98			0.1					1		15	
	8-3	2	0.5	1	11053.51	22107.02	22021.64	44043.28	0.1	166335	26.48	43906	0.0528	1	0.005	15	0.3804
			0.5	2	10968.13	21936.26			0.1					1		15	

Assay Date	8/10/2005	ID	RC6	# Concentrations tested	Microsome 8 type	Rcombinant	Microsome ID	0 Technician ID	TNB	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.4334	0.0092
Full activity	End	0.3967	0.0191
Full activity	Overall	0.4150	0.0245
Background	Beginning	0.0001	1.92373E-05
Background	End	-0.0001	0.000214796
Background	Overall	0.0000	0.000140841
Positive	Beginning	0.2390	0.0058
Positive	End	0.2125	0.0143
Positive	Overall	0.2257	0.0177
Negative	Beginning	0.4332	0.0013
Negative	End	0.4121	0.0068
Negative	Overall	0.4227	0.0128

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	0.0042
RC6	1	3	1.00E-03	-3.00	0.0043
RC6	2	1	1.00E-04	-4.00	0.0242
RC6	2	2	1.00E-04	-4.00	0.0190
RC6	2	3	1.00E-04	-4.00	0.0294
RC6	3	1	3.00E-05	-4.52	0.1005
RC6	3	2	3.00E-05	-4.52	0.0808
RC6	3	3	3.00E-05	-4.52	0.0738
RC6	4	1	1.00E-05	-5.00	0.1532
RC6	4	2	1.00E-05	-5.00	0.1699
RC6	4	3	1.00E-05	-5.00	0.1580
RC6	5	1	3.00E-06	-5.52	0.2818
RC6	5	2	3.00E-06	-5.52	0.2744
RC6	5	3	3.00E-06	-5.52	0.2776
RC6	6	1	1.00E-06	-6.00	0.3536
RC6	6	2	1.00E-06	-6.00	0.3539
RC6	6	3	1.00E-06	-6.00	0.3501
RC6	7	1	1.00E-07	-7.00	0.3839
RC6	7	2	1.00E-07	-7.00	0.3999
RC6	7	3	1.00E-07	-7.00	0.3974
RC6	8	1	1.00E-09	-9.00	0.3316
RC6	8	2	1.00E-09	-9.00	0.3812
RC6	8	3	1.00E-09	-9.00	0.3804

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	0.15	1.02	1.05
2	-4.00	5.82	4.57	7.07
3	-4.52	24.22	19.46	17.79
4	-5.00	36.91	40.94	38.06
5	-5.52	67.90	66.12	66.88
6	-6.00	85.21	85.28	84.35
7	-7.00	92.50	96.35	95.76
8	-9.00	79.89	91.85	91.66

Aromatase Assay Spreadsheet

Assay Date	8/18/2005	Test Chemical ID	RC7	# Concentrations tested	8
ID	JG	Replicate #	Rep 1	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	33036.82	1643623	
2	0.0201	33530.88	1668203	
3	0.0204	33680.85	1651022	
4	0.0200	33247.93	1662397	
5	0.0204	33054.31	1620309	
			Average DPM/g soln	1649111
			SD	18737
			CV	1.14
			µCi/g soln	0.743

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	8.2255 g
Mass of dilution B used in substrate prep	4.6184 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.572703 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00841 µg/g soln.
µg/g soln.

a. µCi/g soln 0.743
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.

= 0.572703 + 0.00841
= 0.581112 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.278 µCi/µg ASDN

812761 dpm/nmol

Test

Assay Date 8/18/2005 Chemical ID RC7 tested 8

ID JG Replicate # Rep 1 Microsome type Recombinant Microsome ID 0

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	0.350	0.144	0.090	0.050	0.037	0.030	25	100	
	0.597	0.372	0.144	0.089	0.049	0.039	0.030			
	0.576	0.360	0.149	0.090	0.053	0.037	0.032			
Samples:	10	100	microsomes							
	0.056	0.295	0.047							
	0.054	0.297	0.051							
			0.050							

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	0.583	0.552	0.0480
0.125	100	200	0.00013	200	0.0250	0.361	0.330	0.0286
0.05	200	1000	0.00005	200	0.0100	0.146	0.115	0.0098
0.025	100	1000	0.00003	200	0.0050	0.089	0.059	0.0049
0.01	40	1000	0.00001	200	0.0020	0.051	0.020	0.0015
0.005	20	1000	0.00001	200	0.0010	0.038	0.007	0.0003

Blank 0.031

r^2= 0.990
m= 0.087
b= 0.000

Variables	Regression results	
m, b	0.087	0.000
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	prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL
10	0.056	0.026	0.002	200	1	1	0.000	0.000	0.009
10	0.054	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.295	0.264	0.023	200	1	1	0.000	0.000	0.115
100	0.297	0.267	0.023	200	1	1	0.000		
100									
	0.047	0.016	0.001	200	114	69814	0.004	0.004	4.182
	0.051	0.020	0.002	200	114	69814	0.005		
	0.050	0.019	0.001	200	114	69814	0.004		

Test

Assay Date 8/18/2005 Chemical ID RC7 tested 8

ID	JG	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

									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	0.350	0.144	0.090	0.050	0.037	0.030	25	100	
	0.597	0.372	0.144	0.089	0.049	0.039	0.030			
	0.576	0.360	0.149	0.090	0.053	0.037	0.032			

Samples:	10	100	microsomes
	0.056	0.295	0.047
	0.054	0.297	0.051
			0.050

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	0.583	0.552	0.0417	m, b	0.074	0.001
0.125	100	200	0.00013	200	0.0250	0.361	0.330	0.0252	se_m, se_b	0.002	0.000
0.05	200	1000	0.00005	200	0.0100	0.146	0.115	0.0093	r , se_y	0.998	0.000
0.025	100	1000	0.00003	200	0.0050	0.089	0.059	0.0051	F, df	1606	3
0.01	40	1000	0.00001	200	0.0020	0.051	0.020	0.0022	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.038	0.007	0.0012			

Blank 0.031

r^2= 0.998
m= 0.074
b= 0.001

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	0.056	0.026	0.003	200	1	1	0.000	0.000	0.013
10	0.054	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.295	0.264	0.020	200	1	1	0.000	0.000	0.102
100	0.297	0.267	0.021	200	1	1	0.000		
100									
	0.047	0.016	0.002	200	114	69814	0.006	0.006	6.432
	0.051	0.020	0.002	200	114	69814	0.007		
	0.050	0.019	0.002	200	114	69814	0.007		

Assay Date	8/18/2005	Test Chemical ID	RC7	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

								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	8/18/2005	Chemical ID	RC7	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

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

Protein Concentration (stock microsomes, mg/mL):	6.432
Protein Concentration (dilution added to assay, mg/mL):	0.010503

Assay Date	8/18/2005	Test Chemical ID	RC7	# Concentrations tested	8	Microsome type		Microsome ID		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	12866.37	25732.74	25674.44	51348.88	0.1	164911	31.14	51217	0.0630	1	0.005	15	0.4000
			0.5	2	12808.07	25616.14			0.1					1		15	
	2	2	0.5	1	13029.68	26059.36	26022.14	52044.28	0.1	164911	31.56	51913	0.0639	1	0.005	15	0.4054
			0.5	2	12992.46	25984.92			0.1					1		15	
	3	2	0.5	1	12025.9	24051.8	24053.91	48107.82	0.1	164911	29.17	47976	0.0590	1	0.005	15	0.3747
			0.5	2	12028.01	24056.02			0.1					1		15	
	4	2	0.5	1	12927.74	25855.48	25111.22	50222.44	0.1	164911	30.45	50091	0.0616	1	0.005	15	0.3912
			0.5	2	12183.48	24366.96			0.1					1		15	
Background control	1	2	0.5	1	26.2	52.4	56.63	113.26	0.1	164911	0.07	-18	0.0000	1	0.005	15	-0.0001
			0.5	2	30.43	60.86			0.1					1		15	
	2	2	0.5	1	37.34	74.68	65.78	131.56	0.1	164911	0.08	0	0.0000	1	0.005	15	0.0000
			0.5	2	28.44	56.88			0.1					1		15	
	3	2	0.5	1	34.75	69.5	72.24	144.48	0.1	164911	0.09	13	0.0000	1	0.005	15	0.0001
			0.5	2	37.49	74.98			0.1					1		15	
	4	2	0.5	1	37.06	74.12	68.67	137.34	0.1	164911	0.08	6	0.0000	1	0.005	15	0.0000
			0.5	2	31.61	63.22			0.1					1		15	
Positive control	1	2	0.5	1	6144.74	12288.48	12341.91	24683.82	0.1	164911	14.97	24552	0.0302	1	0.005	15	0.1917
			0.5	2	6197.17	12394.34			0.1					1		15	
	2	2	0.5	1	6092.95	12185.9	12156.03	24312.06	0.1	164911	14.74	24180	0.0298	1	0.005	15	0.1888
			0.5	2	6063.08	12126.16			0.1					1		15	
	3	2	0.5	1	5646.74	11293.48	11364.56	22729.12	0.1	164911	13.78	22597	0.0278	1	0.005	15	0.1765
			0.5	2	5717.82	11435.64			0.1					1		15	
	4	2	0.5	1	5636.27	11272.54	11234.53	22469.06	0.1	164911	13.62	22337	0.0275	1	0.005	15	0.1744
			0.5	2	5598.26	11196.52			0.1					1		15	
Negative Control	1	2	0.5	1	11511.25	23022.5	23313.83	46627.66	0.1	164911	28.27	46496	0.0572	1	0.005	15	0.3631
			0.5	2	11802.58	23605.16			0.1					1		15	
	2	2	0.5	1	11742.13	23484.26	23906.77	47813.54	0.1	164911	28.99	47682	0.0587	1	0.005	15	0.3724
			0.5	2	12164.64	24329.28			0.1					1		15	
	3	2	0.5	1	11549.23	23098.46	22972.11	45944.22	0.1	164911	27.86	45813	0.0564	1	0.005	15	0.3578
			0.5	2	11422.88	22845.76			0.1					1		15	
	4	2	0.5	1	11138.58	22277.16	21921.75	43843.5	0.1	164911	26.59	43712	0.0538	1	0.005	15	0.3414
			0.5	2	10783.17	21566.34			0.1					1		15	
RC7	1-1	2	0.5	1	40.73	81.46	77.75	155.5	0.1	164911	0.09	24	0.0000	1	0.005	15	0.0002
			0.5	2	37.02	74.04			0.1					1		15	
	1-2	2	0.5	1	28.3	56.6	60.93	121.86	0.1	164911	0.07	-10	0.0000	1	0.005	15	-0.0001
			0.5	2	32.63	65.26			0.1					1		15	
	1-3	2	0.5	1	31.66	63.32	63.43	126.86	0.1	164911	0.08	-5	0.0000	1	0.005	15	0.0000
			0.5	2	31.77	63.54			0.1					1		15	
	2-1	2	0.5	1	44.72	89.44	96.85	193.7	0.1	164911	0.12	62	0.0001	1	0.005	15	0.0005
			0.5	2	52.13	104.26			0.1					1		15	
	2-2	2	0.5	1	22.05	44.1	42.98	85.96	0.1	164911	0.05	-46	-0.0001	1	0.005	15	-0.0004
			0.5	2	20.93	41.86			0.1					1		15	
	2-3	2	0.5	1	32.14	64.28	58.94	117.88	0.1	164911	0.07	-14	0.0000	1	0.005	15	-0.0001
			0.5	2	26.8	53.6			0.1					1		15	
	3-1	2	0.5	1	37.2	74.4	71.3	142.6	0.1	164911	0.09	11	0.0000	1	0.005	15	0.0001
			0.5	2	34.1	68.2			0.1					1		15	
	3-2	2	0.5	1	31.97	63.94	61.58	123.16	0.1	164911	0.07	-9	0.0000	1	0.005	15	-0.0001
			0.5	2	29.61	59.22			0.1					1		15	
	3-3	2	0.5	1	31.8	63.6	64.42	128.84	0.1	164911	0.08	-3	0.0000	1	0.005	15	0.0000
			0.5	2	32.62	65.24			0.1					1		15	
	4-1	2	0.5	1	55.53	111.06	116.1	232.2	0.1	164911	0.14	101	0.0001	1	0.005	15	0.0008
			0.5	2	60.57	121.14			0.1					1		15	
	4-2	2	0.5	1	46.64	93.28	102.75	205.5	0.1	164911	0.12	74	0.0001	1	0.005	15	0.0006
			0.5	2	56.11	112.22			0.1					1		15	
	4-3	2	0.5	1	59.64	119.28	120.4	240.8	0.1	164911	0.15	109	0.0001	1	0.005	15	0.0009
			0.5	2	60.76	121.52			0.1					1		15	
	5-1	2	0.5	1	252.03	504.06	488.51	977.02	0.1	164911	0.59	845	0.0010	1	0.005	15	0.0066
			0.5	2	236.48	472.96			0.1					1		15	
	5-2	2	0.5	1	252.87	505.74	512.57	1025.14	0.1	164911	0.62	893	0.0011	1	0.005	15	0.0070
			0.5	2	259.7	519.4			0.1					1		15	
	5-3	2	0.5	1	248.89	497.78	483.3	966.6	0.1	164911	0.59	835	0.0010	1	0.005	15	0.0065
			0.5	2	234.41	468.82			0.1					1		15	
	6-1	2	0.5	1	2084.02	4168.04	4158.91	8317.82	0.1	164911	5.04	8186	0.0101	1	0.005	15	0.0639
			0.5	2	2094.89	4189.78			0.1					1		15	
	6-2	2	0.5	1	1952.78	3905.56	3939.37	7878.74	0.1	164911	4.78	7747	0.0095	1	0.005	15	0.0605
			0.5	2	1986.59	3973.18			0.1					1		15	
	6-3	2	0.5	1	1970.06	3940.12	3876.27	7752.54	0.1	164911	4.70	7621	0.0094	1	0.005	15	0.0595
			0.5	2	1906.21	3812.42			0.1					1		15	
	7-1	2	0.5	1	9257.63	18515.26	18580.79	37161.58	0.1	164911	22.53	37030	0.0456	1	0.005	15	0.2892
			0.5	2	9323.16	18646.32			0.1					1		15	
	7-2	2	0.5	1	9084	18168	18114.76	36229.52	0.1	164911	21.97	36098	0.0444	1	0.005	15	0.2819
			0.5	2	9030.76	18061.52			0.1					1		15	
	7-3	2	0.5	1	9278.12	18556.24	18529.94	37059.88	0.1	164911	22.47	36928	0.0454	1	0.005	15	0.2884
			0.5	2	9251.82	18503.64			0.1					1		15	
	8-1	2	0.5	1	12375.64	24751.28	24736.18	49472.36	0.1	164911	30.00	49341	0.0607	1	0.005	15	0.3853
			0.5	2	12360.54	24721.08			0.1					1		15	
	8-2	2	0.5	1	12245.56	24491.12	24557.67	49115.34	0.1	164911	29.78	48984	0.0603	1	0.005	15	0.3826
			0.5	2	12312.11	24624.22			0.1					1		15	
	8-3	2	0.5	1	12531.98	25063.96	25291.47	50582.94	0.1	164911	30.67	50451	0.0621	1	0.005	15	0.3940
			0.5	2	12759.49	25518.98			0.1					1		15	

Assay Date	8/18/2005	ID	RC7	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4027	0.0038
Full activity	End	0.3829	0.0117
Full activity	Overall	0.3928	0.0134
Background	Beginning	-0.0001	0.000101059
Background	End	0.0001	3.94294E-05
Background	Overall	0.0000	0.000104311
Positive	Beginning	0.1903	0.0021
Positive	End	0.1755	0.0014
Positive	Overall	0.1829	0.0087
Negative	Beginning	0.3678	0.0065
Negative	End	0.3496	0.0116
Negative	Overall	0.3587	0.0130

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC7	1	1	1.00E-03	-3.00	0.0002
RC7	1	2	1.00E-03	-3.00	-0.0001
RC7	1	3	1.00E-03	-3.00	0.0000
RC7	2	1	1.00E-04	-4.00	0.0005
RC7	2	2	1.00E-04	-4.00	-0.0004
RC7	2	3	1.00E-04	-4.00	-0.0001
RC7	3	1	1.00E-05	-5.00	0.0001
RC7	3	2	1.00E-05	-5.00	-0.0001
RC7	3	3	1.00E-05	-5.00	0.0000
RC7	4	1	1.00E-06	-6.00	0.0008
RC7	4	2	1.00E-06	-6.00	0.0006
RC7	4	3	1.00E-06	-6.00	0.0009
RC7	5	1	1.00E-07	-7.00	0.0066
RC7	5	2	1.00E-07	-7.00	0.0070
RC7	5	3	1.00E-07	-7.00	0.0065
RC7	6	1	1.00E-08	-8.00	0.0639
RC7	6	2	1.00E-08	-8.00	0.0605
RC7	6	3	1.00E-08	-8.00	0.0595
RC7	7	1	1.00E-09	-9.00	0.2892
RC7	7	2	1.00E-09	-9.00	0.2819
RC7	7	3	1.00E-09	-9.00	0.2884
RC7	8	1	1.00E-10	-10.00	0.3853
RC7	8	2	1.00E-10	-10.00	0.3826
RC7	8	3	1.00E-10	-10.00	0.3940

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	0.05	-0.02	-0.01
2	-4.00	0.12	-0.09	-0.03
3	-5.00	0.02	-0.02	-0.01
4	-6.00	0.20	0.15	0.22
5	-7.00	1.68	1.78	1.66
6	-8.00	16.27	15.40	15.15
7	-9.00	73.62	71.77	73.42
8	-10.00	98.09	97.38	100.30

Aromatase Assay Spreadsheet

Assay Date	8/29/2005	Test Chemical ID	RC 7	# Concentrations tested	8
ID	JG	Replicate #	Rep 2	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	64716.17	3235809	
2	0.0202	67135.36	3323533	
3	0.0199	66261.05	3329701	
4	0.0199	66071.05	3320153	
5	0.0201	67801.89	3373228	
			Average DPM/g soln	3316485
			SD	49916
			CV	1.51
			µCi/g soln	1.494

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	8.2891 g
Mass of dilution B used in substrate prep	4.6697 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563354 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01691 µg/g soln.
µg/g soln.

a. µCi/g soln 1.494
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.

= 0.563354 + 0.01691
= 0.580266 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 2.575 µCi/µg ASDN

1636908 dpm/nmol

Test

Assay Date 8/29/2005 Chemical ID RC 7 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.600	0.344	0.142	0.083	0.045	0.036	0.028	25	100
	0.619	0.364	0.143	0.083	0.047	0.036	0.027		
	0.610	0.365	0.143	0.085	0.045	0.035	0.027		

Protein stock ID

Samples:	10	100	microsomes
	0.056	0.296	0.049
	0.050	0.297	0.058
			0.051

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	0.610	0.583	0.0486
0.125	100	200	0.00013	200	0.0250	0.358	0.331	0.0276
0.05	200	1000	0.00005	200	0.0100	0.143	0.115	0.0097
0.025	100	1000	0.00003	200	0.0050	0.084	0.056	0.0047
0.01	40	1000	0.00001	200	0.0020	0.046	0.019	0.0016
0.005	20	1000	0.00001	200	0.0010	0.036	0.009	0.0008

Blank 0.027

r^2= 0.995
m= 0.083
b= 0.000

Variables	Regression results	
m, b	0.083	0.000
se_m, se_b	0.003	0.001
r , se_y	0.995	0.002
F, df	789	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	0.056	0.029	0.002	200	1	1	0.000	0.000	0.011
10	0.050	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.296	0.269	0.022	200	1	1	0.000	0.000	0.113
100	0.297	0.270	0.023	200	1	1	0.000		
100									
	0.049	0.022	0.002	200	114	69814	0.006	0.007	6.607
	0.058	0.031	0.003	200	114	69814	0.008		
	0.051	0.023	0.002	200	114	69814	0.006		

Test

Assay Date 8/29/2005 Chemical ID RC 7 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.600	0.344	0.142	0.083	0.045	0.036	0.028	25	100	
	0.619	0.364	0.143	0.083	0.047	0.036	0.027			
	0.610	0.365	0.143	0.085	0.045	0.035	0.027			
Samples:	10	100	microsomes							
	0.056	0.296	0.049							
	0.050	0.297	0.058							
			0.051							

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	0.610	0.583	0.0439
0.125	100	200	0.00013	200	0.0250	0.358	0.331	0.0252
0.05	200	1000	0.00005	200	0.0100	0.143	0.115	0.0093
0.025	100	1000	0.00003	200	0.0050	0.084	0.056	0.0049
0.01	40	1000	0.00001	200	0.0020	0.046	0.019	0.0021
0.005	20	1000	0.00001	200	0.0010	0.036	0.009	0.0014

Blank 0.027

r^2= 0.998
m= 0.074
b= 0.001

Variables	Regression results	
m, b	0.074	0.001
se_m, se_b	0.002	0.000
r , se_y	0.998	0.000
F, df	1610	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	0.056	0.029	0.003	200	1	1	0.000	0.000	0.013
10	0.050	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.296	0.269	0.021	200	1	1	0.000	0.000	0.103
100	0.297	0.270	0.021	200	1	1	0.000		
100									
	0.049	0.022	0.002	200	114	69814	0.007	0.008	8.073
	0.058	0.031	0.003	200	114	69814	0.009		
	0.051	0.023	0.002	200	114	69814	0.008		

Assay Date 8/29/2005 Test Chemical ID RC 7 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

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	8/29/2005	Chemical ID	RC 7	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 2

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.073
Protein Concentration (dilution added to assay, mg/mL):	0.013182

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	8/29/2005	Test Chemical ID	RC 7	# Concentrations tested	8	Microsome type		Microsome ID		0 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	25802.62	51605.24	50035.92	100071.84	0.1	331648	30.17	99791	0.0610	1	0.007	15	0.3083
			0.5	2	24233.3	48466.6			0.1					1		15	
	2	2	0.5	1	24032.61	48065.22	47286.19	94572.38	0.1	331648	28.52	94292	0.0576	1	0.007	15	0.2913
			0.5	2	23253.58	46507.16			0.1					1		15	
	3	2	0.5	1	21674.65	43349.3	42781.01	85562.02	0.1	331648	25.80	85281	0.0521	1	0.007	15	0.2635
			0.5	2	21106.36	42212.72			0.1					1		15	
	4	2	0.5	1	21052.54	42105.08	42102.04	84204.08	0.1	331648	25.39	83923	0.0513	1	0.007	15	0.2593
			0.5	2	21049.5	42099			0.1					1		15	
Background control	1	2	0.5	1	69.74	139.48	127.68	255.36	0.1	331648	0.08	-25	0.0000	1	0.007	15	-0.0001
			0.5	2	57.94	115.88			0.1					1		15	
	2	2	0.5	1	69.48	138.96	147.56	295.12	0.1	331648	0.09	14	0.0000	1	0.007	15	0.0000
			0.5	2	78.08	156.16			0.1					1		15	
	3	2	0.5	1	67.81	135.62	142.27	284.54	0.1	331648	0.09	4	0.0000	1	0.007	15	0.0000
			0.5	2	74.46	148.92			0.1					1		15	
	4	2	0.5	1	73.76	147.52	143.74	287.48	0.1	331648	0.09	7	0.0000	1	0.007	15	0.0000
			0.5	2	69.98	139.96			0.1					1		15	
Positive control	1	2	0.5	1	12467.32	24934.64	25071.88	50143.76	0.1	331648	15.12	49863	0.0305	1	0.007	15	0.1541
			0.5	2	12604.56	25209.12			0.1					1		15	
	2	2	0.5	1	11628.74	23257.48	22813.1	45626.2	0.1	331648	13.76	45346	0.0277	1	0.007	15	0.1401
			0.5	2	11184.36	22368.72			0.1					1		15	
	3	2	0.5	1	10409.5	20819	20725.37	41450.74	0.1	331648	12.50	41170	0.0252	1	0.007	15	0.1272
			0.5	2	10315.87	20631.74			0.1					1		15	
	4	2	0.5	1	10149.44	20298.88	20039.02	40078.04	0.1	331648	12.08	39797	0.0243	1	0.007	15	0.1230
			0.5	2	9889.58	19779.16			0.1					1		15	
Negative Control	1	2	0.5	1	23007.28	46014.56	45416.55	90833.1	0.1	331648	27.39	90552	0.0553	1	0.007	15	0.2798
			0.5	2	22409.29	44818.58			0.1					1		15	
	2	2	0.5	1	23027.84	46055.68	45650.26	91300.52	0.1	331648	27.53	91020	0.0556	1	0.007	15	0.2812
			0.5	2	22622.42	45244.84			0.1					1		15	
	3	2	0.5	1	20619.28	41238.56	41199.41	82398.82	0.1	331648	24.85	82116	0.0502	1	0.007	15	0.2537
			0.5	2	20360.13	40720.26			0.1					1		15	
	4	2	0.5	1	19843.8	39687.6	39572.49	79144.98	0.1	331648	23.86	78864	0.0482	1	0.007	15	0.2437
			0.5	2	19828.69	39657.38			0.1					1		15	
RC 7	1-1	2	0.5	1	93.64	187.28	190.17	380.34	0.1	331648	0.11	100	0.0001	1	0.007	15	0.0003
			0.5	2	96.53	193.06			0.1					1		15	
	1-2	2	0.5	1	80.23	160.46	162.55	325.1	0.1	331648	0.10	44	0.0000	1	0.007	15	0.0001
			0.5	2	82.32	164.64			0.1					1		15	
	1-3	2	0.5	1	61.35	122.7	130.51	261.02	0.1	331648	0.08	-20	0.0000	1	0.007	15	-0.0001
			0.5	2	69.16	138.32			0.1					1		15	
	2-1	2	0.5	1	134.7	269.4	265.8	531.6	0.1	331648	0.16	251	0.0002	1	0.007	15	0.0008
			0.5	2	131.1	262.2			0.1					1		15	
	2-2	2	0.5	1	120.94	241.88	242.78	485.56	0.1	331648	0.15	205	0.0001	1	0.007	15	0.0006
			0.5	2	121.84	243.68			0.1					1		15	
	2-3	2	0.5	1	115.92	231.84	228.32	456.64	0.1	331648	0.14	176	0.0001	1	0.007	15	0.0005
			0.5	2	112.4	224.8			0.1					1		15	
	3-1	2	0.5	1	502.06	1004.12	1002.52	2005.04	0.1	331648	0.60	1724	0.0011	1	0.007	15	0.0053
			0.5	2	500.46	1000.92			0.1					1		15	
	3-2	2	0.5	1	509.1	1018.2	1004.05	2008.1	0.1	331648	0.61	1727	0.0011	1	0.007	15	0.0053
			0.5	2	494.95	989.9			0.1					1		15	
	3-3	2	0.5	1	493.52	987.04	969.4	1938.8	0.1	331648	0.58	1658	0.0010	1	0.007	15	0.0051
			0.5	2	475.88	951.76			0.1					1		15	
	4-1	2	0.5	1	3779.33	7559.86	7588.36	15176.72	0.1	331648	4.58	14896	0.0091	1	0.007	15	0.0460
			0.5	2	3808.43	7616.86			0.1					1		15	
	4-2	2	0.5	1	3601.18	7202.36	7193.11	14386.22	0.1	331648	4.34	14106	0.0086	1	0.007	15	0.0436
			0.5	2	3591.93	7183.86			0.1					1		15	
	4-3	2	0.5	1	3792.77	7585.54	7557.65	15115.3	0.1	331648	4.56	14835	0.0091	1	0.007	15	0.0458
			0.5	2	3764.88	7529.76			0.1					1		15	
	5-1	2	0.5	1	9795.24	19590.48	19439.41	38878.82	0.1	331648	11.72	38598	0.0236	1	0.007	15	0.1192
			0.5	2	9644.17	19288.34			0.1					1		15	
	5-2	2	0.5	1	8793.94	17587.88	17550.76	35101.52	0.1	331648	10.58	34821	0.0213	1	0.007	15	0.1076
			0.5	2	8756.82	17513.64			0.1					1		15	
	5-3	2	0.5	1	9917.07	19834.14	19803.16	39606.32	0.1	331648	12.00	39526	0.0241	1	0.007	15	0.1221
			0.5	2	9886.09	19772.18			0.1					1		15	
	6-1	2	0.5	1	16721.47	33442.94	33219.22	66438.44	0.1	331648	20.03	66158	0.0404	1	0.007	15	0.2044
			0.5	2	16497.75	32995.5			0.1					1		15	
	6-2	2	0.5	1	17035.14	34070.28	33768.1	67536.2	0.1	331648	20.36	67256	0.0411	1	0.007	15	0.2078
			0.5	2	16732.96	33465.92			0.1					1		15	
	6-3	2	0.5	1	16727.75	33455.5	33512.32	67024.64	0.1	331648	20.21	66744	0.0408	1	0.007	15	0.2062
			0.5	2	16784.57	33569.14			0.1					1		15	
	7-1	2	0.5	1	21322.94	42645.88	42575.84	85151.68	0.1	331648	25.68	84871	0.0518	1	0.007	15	0.2622
			0.5	2	21252.9	42505.8			0.1					1		15	
	7-2	2	0.5	1	20905.79	41811.58	42008.25	84016.5	0.1	331648	25.33	83736	0.0512	1	0.007	15	0.2587
			0.5	2	21102.46	42204.92			0.1					1		15	
	7-3	2	0.5	1	20746.08	41492.16	41319.04	82638.08	0.1	331648	24.92	82357	0.0503	1	0.007	15	0.2544
			0.5	2	20572.96	41145.92			0.1					1		15	
	8-1	2	0.5	1	22482.9	44965.8	44721.21	89442.42	0.1	331648	26.97	89162	0.0545	1	0.007	15	0.2755
			0.5	2	22238.31	44476.62			0.1					1		15	
	8-2	2	0.5	1	22020.82	44041.64	44061.1	88122.2	0.1	331648	26.57	87842	0.0537	1	0.007	15	0.2714
			0.5	2	22040.16	44080.36			0.1					1		15	
	8-3	2	0.5	1	21818.1	43636.2	43799.41	87598.82	0.1	331648	26.41	87318	0.0533	1	0.007	15	0.2698
			0.5	2	21981.31	43962.62			0.1					1		15	

Assay Date	8/29/2005	ID	RC 7	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.2998	0.0120
Full activity	End	0.2614	0.0030
Full activity	Overall	0.2806	0.0233
Background	Beginning	0.0000	8.68598E-05
Background	End	0.0000	6.42273E-06
Background	Overall	0.0000	5.383E-05
Positive	Beginning	0.1471	0.0099
Positive	End	0.1251	0.0030
Positive	Overall	0.1361	0.0140
Negative	Beginning	0.2805	0.0010
Negative	End	0.2487	0.0071
Negative	Overall	0.2646	0.0188

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC 7	1	1	1.00E-05	-5.00	0.0003
RC 7	1	2	1.00E-05	-5.00	0.0001
RC 7	1	3	1.00E-05	-5.00	-0.0001
RC 7	2	1	1.00E-06	-6.00	0.0008
RC 7	2	2	1.00E-06	-6.00	0.0006
RC 7	2	3	1.00E-06	-6.00	0.0005
RC 7	3	1	1.00E-07	-7.00	0.0053
RC 7	3	2	1.00E-07	-7.00	0.0053
RC 7	3	3	1.00E-07	-7.00	0.0051
RC 7	4	1	1.00E-08	-8.00	0.0460
RC 7	4	2	1.00E-08	-8.00	0.0436
RC 7	4	3	1.00E-08	-8.00	0.0458
RC 7	5	1	3.00E-09	-8.52	0.1192
RC 7	5	2	3.00E-09	-8.52	0.1076
RC 7	5	3	3.00E-09	-8.52	0.1221
RC 7	6	1	1.00E-09	-9.00	0.2044
RC 7	6	2	1.00E-09	-9.00	0.2078
RC 7	6	3	1.00E-09	-9.00	0.2062
RC 7	7	1	3.00E-10	-9.52	0.2622
RC 7	7	2	3.00E-10	-9.52	0.2587
RC 7	7	3	3.00E-10	-9.52	0.2544
RC 7	8	1	1.00E-10	-10.00	0.2755
RC 7	8	2	1.00E-10	-10.00	0.2714
RC 7	8	3	1.00E-10	-10.00	0.2698

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-5.00	0.11	0.05	-0.02
2	-6.00	0.28	0.23	0.19
3	-7.00	1.90	1.90	1.83
4	-8.00	16.40	15.53	16.33
5	-8.52	42.50	38.34	43.52
6	-9.00	72.84	74.05	73.49
7	-9.52	93.45	92.20	90.68
8	-10.00	98.17	96.72	96.14

Aromatase Assay Spreadsheet

Assay Date	8/31/2005	Test Chemical ID	RC7	# Concentrations tested	8
ID	JG	Replicate #	Rep 3	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	31822.08	1583188	
2	0.0201	32473.87	1615615	
3	0.0200	32677.33	1633867	
4	0.0203	32663.61	1609045	
5	0.0202	32799.48	1623737	
			Average DPM/g soln	1613090
			SD	19117
			CV	1.19
			µCi/g soln	0.727

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	8.2604 g
Mass of dilution B used in substrate prep	4.6325 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.583241 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00823 µg/g soln.
µg/g soln.

a. µCi/g soln 0.727
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.

= 0.583241 + 0.00823
= 0.591466 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.229 µCi/µg ASDN

781092 dpm/nmol

Assay Date	8/31/2005	Test Chemical ID	RC7	tested	8		
ID	JG	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.470	0.322	0.137	0.087	0.054	0.044	0.038	25	100	
	0.474	0.300	0.127	0.090	0.054	0.044	0.039			
	0.460	0.323	0.130	0.093	0.057	0.041	0.039			
Samples:	10	100	microsomes							
	0.061	0.230	0.056							
	0.066	0.224	0.061							
			0.054							

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	0.468	0.430	0.0469
0.125	100	200	0.00013	200	0.0250	0.315	0.277	0.0300
0.05	200	1000	0.00005	200	0.0100	0.131	0.093	0.0097
0.025	100	1000	0.00003	200	0.0050	0.090	0.052	0.0052
0.01	40	1000	0.00001	200	0.0020	0.055	0.017	0.0013
0.005	20	1000	0.00001	200	0.0010	0.043	0.005	0.0000

Variables	Regression results	
m, b	0.110	-0.001
se_m, se_b	0.008	0.002
r , se_y	0.980	0.003
F, df	195	4
ss_{reg}, ss_{resid}	0.002	0.000

Regression results are calculated using the function LINEST

Blank 0.038

r^2= 0.980
m= 0.110
b= -0.001

	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	0.061	0.022	0.002	200	1	1	0.000	0.000	0.011
10	0.066	0.028	0.003	200	1	1	0.000		
10					1	1			
100	0.230	0.191	0.021	200	1	1	0.000	0.000	0.101
100	0.224	0.185	0.020	200	1	1	0.000		
100									
	0.056	0.018	0.001	200	114	69814	0.004	0.005	4.535
	0.061	0.022	0.002	200	114	69814	0.006		
	0.054	0.015	0.001	200	114	69814	0.003		

Test

Assay Date 8/31/2005 Chemical ID RC7 tested 8

ID JG Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.470	0.322	0.137	0.087	0.054	0.044	0.038	25	100	
	0.474	0.300	0.127	0.090	0.054	0.044	0.039			
	0.460	0.323	0.130	0.093	0.057	0.041	0.039			

Samples:	10	100	microsomes
	0.061	0.230	0.056
	0.066	0.224	0.061
			0.054

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	0.468	0.430	0.0388	m, b	0.089	0.001
0.125	100	200	0.00013	200	0.0250	0.315	0.277	0.0253	se_m, se_b	0.003	0.000
0.05	200	1000	0.00005	200	0.0100	0.131	0.093	0.0090	r , se_y	0.997	0.001
0.025	100	1000	0.00003	200	0.0050	0.090	0.052	0.0053	F, df	865	3
0.01	40	1000	0.00001	200	0.0020	0.055	0.017	0.0022	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.043	0.005	0.0012			

Regression results are calculated using the function LINEST

Blank 0.038

r^2= 0.997
m= 0.089
b= 0.001

	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	0.061	0.022	0.003	200	1	1	0.000	0.000	0.015
10	0.066	0.028	0.003	200	1	1	0.000		
10					1	1			
100	0.230	0.191	0.018	200	1	1	0.000	0.000	0.087
100	0.224	0.185	0.017	200	1	1	0.000		
100									
	0.056	0.018	0.002	200	114	69814	0.007	0.007	7.306
	0.061	0.022	0.003	200	114	69814	0.008		
	0.054	0.015	0.002	200	114	69814	0.006		

Assay Date 8/31/2005 Test Chemical ID RC7 tested 8

ID JG Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

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	8/31/2005	Chemical ID	RC7	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 3

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	7.306
Protein Concentration (dilution added to assay, mg/mL):	0.01193

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	8/31/2005	Test Chemical ID	RC7	# Concentrations tested	8	Microsome type		Microsome ID		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	10634.68	21269.32	21282.12	42564.24	0.1	161309	26.39	42405	0.0543	1	0.006	15	0.3034
			0.5	2	10647.46	21294.92			0.1					1		15	
	2	2	0.5	1	10820.91	21641.82	21465.02	42930.04	0.1	161309	26.61	42771	0.0548	1	0.006	15	0.3060
			0.5	2	10644.11	21288.22			0.1					1		15	
	3	2	0.5	1	9492.78	18985.56	18963.33	37926.66	0.1	161309	23.51	37768	0.0484	1	0.006	15	0.2702
			0.5	2	9470.55	18941.1			0.1					1		15	
	4	2	0.5	1	9839.56	19679.12	19503.46	39006.92	0.1	161309	24.18	38848	0.0497	1	0.006	15	0.2779
			0.5	2	9663.9	19327.8			0.1					1		15	
Background control	1	2	0.5	1	44.8	89.6	77.13	154.26	0.1	161309	0.10	-5	0.0000	1	0.006	15	0.0000
			0.5	2	32.33	64.66			0.1					1		15	
	2	2	0.5	1	36.52	73.04	72.3	144.6	0.1	161309	0.09	-14	0.0000	1	0.006	15	-0.0001
			0.5	2	35.78	71.56			0.1					1		15	
	3	2	0.5	1	33.84	67.68	83.04	166.08	0.1	161309	0.10	7	0.0000	1	0.006	15	0.0001
			0.5	2	49.2	98.4			0.1					1		15	
	4	2	0.5	1	42.08	84.16	85.57	171.14	0.1	161309	0.11	12	0.0000	1	0.006	15	0.0001
			0.5	2	43.49	86.98			0.1					1		15	
Positive control	1	2	0.5	1	5463.82	10967.64	10884.04	21768.08	0.1	161309	13.49	21609	0.0277	1	0.006	15	0.1546
			0.5	2	5400.22	10800.44			0.1					1		15	
	2	2	0.5	1	5619.27	11238.54	11210.02	22420.04	0.1	161309	13.90	22261	0.0285	1	0.006	15	0.1593
			0.5	2	5590.75	11181.5			0.1					1		15	
	3	2	0.5	1	5136.01	10272.02	10203.01	20406.02	0.1	161309	12.65	20247	0.0259	1	0.006	15	0.1449
			0.5	2	5067	10134			0.1					1		15	
	4	2	0.5	1	4952.86	9905.72	9978.2	19956.4	0.1	161309	12.37	19797	0.0253	1	0.006	15	0.1416
			0.5	2	5025.34	10050.68			0.1					1		15	
Negative Control	1	2	0.5	1	8844.76	17689.52	17686.33	35372.66	0.1	161309	21.93	35214	0.0451	1	0.006	15	0.2519
			0.5	2	8841.57	17683.14			0.1					1		15	
	2	2	0.5	1	8725.89	17451.78	17446.4	34892.8	0.1	161309	21.63	34734	0.0445	1	0.006	15	0.2485
			0.5	2	8720.51	17441.02			0.1					1		15	
	3	2	0.5	1	7928.31	15856.62	15762.67	31525.34	0.1	161309	19.54	31366	0.0402	1	0.006	15	0.2244
			0.5	2	7834.36	15668.72			0.1					1		15	
	4	2	0.5	1	8353.06	16706.12	16617.93	33235.86	0.1	161309	20.60	33077	0.0423	1	0.006	15	0.2366
			0.5	2	8264.87	16529.74			0.1					1		15	
RC7	1-1	2	0.5	1	58.23	116.46	106.57	213.14	0.1	161309	0.13	54	0.0001	1	0.006	15	0.0004
			0.5	2	48.34	96.68			0.1					1		15	
	1-2	2	0.5	1	40.83	81.66	82.87	165.74	0.1	161309	0.10	7	0.0000	1	0.006	15	0.0000
			0.5	2	42.04	84.08			0.1					1		15	
	1-3	2	0.5	1	44.48	88.96	88.2	176.4	0.1	161309	0.11	17	0.0000	1	0.006	15	0.0001
			0.5	2	43.72	87.44			0.1					1		15	
	2-1	2	0.5	1	61.21	122.42	117.54	235.08	0.1	161309	0.15	76	0.0001	1	0.006	15	0.0005
			0.5	2	56.33	112.66			0.1					1		15	
	2-2	2	0.5	1	67.38	134.76	130.86	261.72	0.1	161309	0.16	103	0.0001	1	0.006	15	0.0007
			0.5	2	63.48	126.96			0.1					1		15	
	2-3	2	0.5	1	59.26	118.52	111.55	223.1	0.1	161309	0.14	64	0.0001	1	0.006	15	0.0005
			0.5	2	52.29	104.58			0.1					1		15	
	3-1	2	0.5	1	240.12	480.24	477.1	954.2	0.1	161309	0.59	795	0.0010	1	0.006	15	0.0057
			0.5	2	236.98	473.96			0.1					1		15	
	3-2	2	0.5	1	233.47	466.94	465.99	931.98	0.1	161309	0.58	773	0.0010	1	0.006	15	0.0055
			0.5	2	232.52	465.04			0.1					1		15	
	3-3	2	0.5	1	223.02	446.04	453.05	906.1	0.1	161309	0.56	747	0.0010	1	0.006	15	0.0053
			0.5	2	230.03	460.06			0.1					1		15	
	4-1	2	0.5	1	1669.78	3339.56	3303.91	6607.82	0.1	161309	4.10	6449	0.0085	1	0.006	15	0.0461
			0.5	2	1634.13	3268.26			0.1					1		15	
	4-2	2	0.5	1	1795.65	3591.3	3559.83	7119.66	0.1	161309	4.41	6961	0.0089	1	0.006	15	0.0498
			0.5	2	1764.18	3528.36			0.1					1		15	
	4-3	2	0.5	1	1768.5	3537	3569.19	7138.38	0.1	161309	4.43	6979	0.0089	1	0.006	15	0.0499
			0.5	2	1780.69	3561.38			0.1					1		15	
	5-1	2	0.5	1	4717.32	9434.64	9387.97	18775.94	0.1	161309	11.64	18617	0.0238	1	0.006	15	0.1332
			0.5	2	4670.65	9341.3			0.1					1		15	
	5-2	2	0.5	1	4602.72	9205.44	9278.68	18557.36	0.1	161309	11.50	18398	0.0236	1	0.006	15	0.1316
			0.5	2	4675.96	9351.92			0.1					1		15	
	5-3	2	0.5	1	4664.56	9329.12	9515.47	19030.94	0.1	161309	11.80	18872	0.0242	1	0.006	15	0.1350
			0.5	2	4850.91	9701.82			0.1					1		15	
	6-1	2	0.5	1	8200.37	16400.74	16336.91	32673.82	0.1	161309	20.26	32515	0.0416	1	0.006	15	0.2326
			0.5	2	8136.54	16273.08			0.1					1		15	
	6-2	2	0.5	1	7710.82	15421.64	15482.76	30965.52	0.1	161309	19.20	30807	0.0394	1	0.006	15	0.2204
			0.5	2	7771.94	15543.88			0.1					1		15	
	6-3	2	0.5	1	7900.3	15800.6	15699.83	31399.66	0.1	161309	19.47	31241	0.0400	1	0.006	15	0.2235
			0.5	2	7799.53	15599.06			0.1					1		15	
	7-1	2	0.5	1	9659.61	19319.02	19470	38940	0.1	161309	24.14	38781	0.0496	1	0.006	15	0.2774
			0.5	2	9810.19	19620.38			0.1					1		15	
	7-2	2	0.5	1	9717.5	19435	19521.23	39042.46	0.1	161309	24.20	38883	0.0496	1	0.006	15	0.2782
			0.5	2	9803.73	19607.46			0.1					1		15	
	7-3	2	0.5	1	9702.22	19404.44	19464.56	38929.12	0.1	161309	24.13	38770	0.0496	1	0.006	15	0.2774
			0.5	2	9762.34	19524.68			0.1					1		15	
	8-1	2	0.5	1	10362.67	20725.34	20744.97	41489.94	0.1	161309	25.72	41331	0.0529	1	0.006	15	0.2957
			0.5	2	10382.3	20764.6			0.1					1		15	
	8-2	2	0.5	1	9809.63	19619.26	19728.44	39462.88	0.1	161309	24.46	39294	0.0503	1	0.006	15	0.2811
			0.5	2	9916.81	19833.62			0.1					1		15	
	8-3	2	0.5	1	10383.85	20767.7	20706.46	41412.92	0.1	161309	25.67	41254	0.0528	1	0.006	15	0.2951
			0.5	2	10322.61	20645.22			0.1					1		15	

Assay Date	8/31/2005	ID	RC7	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3047	0.0019
Full activity	End	0.2741	0.0055
Full activity	Overall	0.2894	0.0180
Background	Beginning	-0.0001	4.88683E-05
Background	End	0.0001	2.55977E-05
Background	Overall	0.0000	8.5386E-05
Positive	Beginning	0.1569	0.0033
Positive	End	0.1432	0.0023
Positive	Overall	0.1501	0.0082
Negative	Beginning	0.2502	0.0024
Negative	End	0.2305	0.0087
Negative	Overall	0.2404	0.0125

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC7	1	1	1.00E-05	-5.00	0.0004
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	0.0005
RC7	2	2	1.00E-06	-6.00	0.0007
RC7	2	3	1.00E-06	-6.00	0.0005
RC7	3	1	1.00E-07	-7.00	0.0057
RC7	3	2	1.00E-07	-7.00	0.0055
RC7	3	3	1.00E-07	-7.00	0.0053
RC7	4	1	1.00E-08	-8.00	0.0461
RC7	4	2	1.00E-08	-8.00	0.0498
RC7	4	3	1.00E-08	-8.00	0.0499
RC7	5	1	3.00E-09	-8.52	0.1332
RC7	5	2	3.00E-09	-8.52	0.1316
RC7	5	3	3.00E-09	-8.52	0.1350
RC7	6	1	1.00E-09	-9.00	0.2326
RC7	6	2	1.00E-09	-9.00	0.2204
RC7	6	3	1.00E-09	-9.00	0.2235
RC7	7	1	3.00E-10	-9.52	0.2774
RC7	7	2	3.00E-10	-9.52	0.2782
RC7	7	3	3.00E-10	-9.52	0.2774
RC7	8	1	1.00E-10	-10.00	0.2957
RC7	8	2	1.00E-10	-10.00	0.2811
RC7	8	3	1.00E-10	-10.00	0.2951

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-5.00	0.13	0.02	0.04
2	-6.00	0.19	0.25	0.16
3	-7.00	1.97	1.91	1.85
4	-8.00	15.94	17.21	17.26
5	-8.52	46.03	45.49	46.66
6	-9.00	80.39	76.16	77.24
7	-9.52	95.88	96.13	95.85
8	-10.00	102.18	97.15	101.99

Aromatase Assay Spreadsheet

Assay Date	8/18/2005	Test Chemical ID	RC8	# Concentrations tested	8
ID	JG	Replicate #	Rep 1	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	33036.82	1643623	
2	0.0201	33530.88	1668203	
3	0.0204	33680.85	1651022	
4	0.0200	33247.93	1662397	
5	0.0204	33054.31	1620309	
			Average DPM/g soln	1649111
			SD	18737
			CV	1.14
			µCi/g soln	0.743

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	8.2255 g
Mass of dilution B used in substrate prep	4.6184 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.572703 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00841 µg/g soln.
µg/g soln.

a. µCi/g soln 0.743
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.

= 0.572703 + 0.00841
= 0.581112 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.278 µCi/µg ASDN

812761 dpm/nmol

Assay Date	8/18/2005	Test Chemical ID	RC8	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

									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	0.350	0.144	0.090	0.050	0.037	0.030	25	100	
	0.597	0.372	0.144	0.089	0.049	0.039	0.030			
	0.576	0.360	0.149	0.090	0.053	0.037	0.032			

Samples:	10	100	microsomes
	0.056	0.295	0.047
	0.054	0.297	0.051
			0.050

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	0.583	0.552	0.0480	m, b	0.087	0.000
0.125	100	200	0.00013	200	0.0250	0.361	0.330	0.0286	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.146	0.115	0.0098	r , se_y	0.990	0.002
0.025	100	1000	0.00003	200	0.0050	0.089	0.059	0.0049	F, df	408	4
0.01	40	1000	0.00001	200	0.0020	0.051	0.020	0.0015	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.038	0.007	0.0003			

Regression results are calculated using the function LINEST

	Blank	0.031			r^2=	0.990						
					m=	0.087						
					b=	0.000						

	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	0.056	0.026	0.002	200	1	1	0.000	0.000	0.009
10	0.054	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.295	0.264	0.023	200	1	1	0.000	0.000	0.115
100	0.297	0.267	0.023	200	1	1	0.000		
100									
	0.047	0.016	0.001	200	114	69814	0.004	0.004	4.182
	0.051	0.020	0.002	200	114	69814	0.005		
	0.050	0.019	0.001	200	114	69814	0.004		

Assay Date	8/18/2005	Test Chemical ID	RC8	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

									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	0.350	0.144	0.090	0.050	0.037	0.030	25		
	0.597	0.372	0.144	0.089	0.049	0.039	0.030			
	0.576	0.360	0.149	0.090	0.053	0.037	0.032			
Samples:	10	100	microsomes							
	0.056	0.295	0.047							
	0.054	0.297	0.051							
			0.050							

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	0.583	0.552	0.0417	m, b	0.074	0.001
0.125	100	200	0.00013	200	0.0250	0.361	0.330	0.0252	se_m, se_b	0.002	0.000
0.05	200	1000	0.00005	200	0.0100	0.146	0.115	0.0093	r , se_y	0.998	0.000
0.025	100	1000	0.00003	200	0.0050	0.089	0.059	0.0051	F, df	1606	3
0.01	40	1000	0.00001	200	0.0020	0.051	0.020	0.0022	ss_{reg}, ss_{resid}	0.000	0.000
0.005	20	1000	0.00001	200	0.0010	0.038	0.007	0.0012			

Blank 0.031

r^2= 0.998
m= 0.074
b= 0.001

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	0.056	0.026	0.003	200	1	1	0.000	0.000	0.013
10	0.054	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.295	0.264	0.020	200	1	1	0.000	0.000	0.102
100	0.297	0.267	0.021	200	1	1	0.000		
100									
	0.047	0.016	0.002	200	114	69814	0.006	0.006	6.432
	0.051	0.020	0.002	200	114	69814	0.007		
	0.050	0.019	0.002	200	114	69814	0.007		

Assay Date	8/18/2005	Test Chemical ID	RC8	tested	8		
ID	JG	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

								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	8/18/2005	Chemical ID	RC8	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	6.432
Protein Concentration (dilution added to assay, mg/mL):	0.010503

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	8/18/2005	Test Chemical ID	RC6	# Concentrations tested	8	Microsome type		Microsome ID		0 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	12866.37	25732.74	25674.44	51348.88	0.1	164911	31.14	51217	0.0630	1	0.005	15	0.4009
			0.5	2	12808.07	25616.14			0.1					1		15	
	2	2	0.5	1	13029.68	26059.36	26022.14	52044.28	0.1	164911	31.56	51913	0.0639	1	0.005	15	0.4054
			0.5	2	12992.46	25984.92			0.1					1		15	
	3	2	0.5	1	12025.9	24051.8	24053.91	48107.82	0.1	164911	29.17	47976	0.0590	1	0.005	15	0.3747
			0.5	2	12028.01	24056.02			0.1					1		15	
	4	2	0.5	1	12927.74	25855.48	25111.22	50222.44	0.1	164911	30.45	50091	0.0616	1	0.005	15	0.3912
			0.5	2	12183.48	24366.96			0.1					1		15	
Background control	1	2	0.5	1	26.2	52.4	56.63	113.26	0.1	164911	0.07	-18	0.0000	1	0.005	15	-0.0001
			0.5	2	30.43	60.86			0.1					1		15	
	2	2	0.5	1	37.34	74.68	65.78	131.56	0.1	164911	0.08	0	0.0000	1	0.005	15	0.0000
			0.5	2	28.44	56.88			0.1					1		15	
	3	2	0.5	1	34.75	69.5	72.24	144.48	0.1	164911	0.09	13	0.0000	1	0.005	15	0.0001
			0.5	2	37.49	74.98			0.1					1		15	
	4	2	0.5	1	37.06	74.12	68.67	137.34	0.1	164911	0.08	6	0.0000	1	0.005	15	0.0000
			0.5	2	31.61	63.22			0.1					1		15	
Positive control	1	2	0.5	1	6144.74	12289.48	12341.91	24683.82	0.1	164911	14.97	24552	0.0302	1	0.005	15	0.1917
			0.5	2	6197.17	12394.34			0.1					1		15	
	2	2	0.5	1	6092.95	12185.9	12156.03	24312.06	0.1	164911	14.74	24180	0.0298	1	0.005	15	0.1888
			0.5	2	6063.08	12126.16			0.1					1		15	
	3	2	0.5	1	5646.74	11293.48	11364.56	22728.12	0.1	164911	13.78	22597	0.0278	1	0.005	15	0.1765
			0.5	2	5717.82	11435.64			0.1					1		15	
	4	2	0.5	1	5636.27	11272.54	11234.53	22469.06	0.1	164911	13.62	22337	0.0275	1	0.005	15	0.1744
			0.5	2	5598.26	11196.52			0.1					1		15	
Negative Control	1	2	0.5	1	11511.25	23022.5	23313.83	46627.66	0.1	164911	28.27	46496	0.0572	1	0.005	15	0.3631
			0.5	2	11802.58	23605.16			0.1					1		15	
	2	2	0.5	1	11742.13	23484.26	23906.77	47813.54	0.1	164911	28.99	47682	0.0587	1	0.005	15	0.3724
			0.5	2	12164.64	24329.28			0.1					1		15	
	3	2	0.5	1	11549.23	23098.46	22972.11	45944.22	0.1	164911	27.86	45813	0.0564	1	0.005	15	0.3578
			0.5	2	11422.88	22845.76			0.1					1		15	
	4	2	0.5	1	11138.58	22277.16	21921.75	43843.5	0.1	164911	26.59	43712	0.0538	1	0.005	15	0.3414
			0.5	2	10783.17	21566.34			0.1					1		15	
RC6	1-1	2	0.5	1	2110.76	4221.52	4235.56	8471.12	0.1	164911	5.14	8339	0.0103	1	0.005	15	0.0651
			0.5	2	2124.8	4249.6			0.1					1		15	
	1-2	2	0.5	1	1876.96	3753.92	3750.92	7501.84	0.1	164911	4.55	7370	0.0091	1	0.005	15	0.0576
			0.5	2	1873.96	3747.92			0.1					1		15	
	1-3	2	0.5	1	1512.5	3025	3049.4	6098.8	0.1	164911	3.70	5967	0.0073	1	0.005	15	0.0466
			0.5	2	1536.9	3073.8			0.1					1		15	
	2-1	2	0.5	1	3719.73	7439.46	7411.68	14823.36	0.1	164911	8.99	14692	0.0181	1	0.005	15	0.1147
			0.5	2	3691.95	7383.9			0.1					1		15	
	2-2	2	0.5	1	3707.92	7415.84	7368.5	14737	0.1	164911	8.94	14605	0.0180	1	0.005	15	0.1141
			0.5	2	3660.58	7321.16			0.1					1		15	
	2-3	2	0.5	1	3648.75	7297.56	7387.13	14774.26	0.1	164911	8.96	14643	0.0180	1	0.005	15	0.1144
			0.5	2	3738.35	7476.7			0.1					1		15	
	3-1	2	0.5	1	5777.12	11554.24	11542.34	23084.68	0.1	164911	14.00	22953	0.0282	1	0.005	15	0.1793
			0.5	2	5765.22	11530.44			0.1					1		15	
	3-2	2	0.5	1	5655	11310	11418.19	22836.38	0.1	164911	13.85	22705	0.0279	1	0.005	15	0.1773
			0.5	2	5763.19	11526.38			0.1					1		15	
	3-3	2	0.5	1	5500.97	11001.94	10981.17	21962.34	0.1	164911	13.32	21831	0.0269	1	0.005	15	0.1705
			0.5	2	5480.2	10960.4			0.1					1		15	
	4-1	2	0.5	1	9782.16	19564.32	19598.4	39196.8	0.1	164911	23.77	39065	0.0481	1	0.005	15	0.3051
			0.5	2	9816.24	19632.48			0.1					1		15	
	4-2	2	0.5	1	9928.46	19856.92	19858.79	39717.58	0.1	164911	24.06	39586	0.0487	1	0.005	15	0.3092
			0.5	2	9930.33	19860.66			0.1					1		15	
	4-3	2	0.5	1	10007.38	20014.76	19911.43	39822.86	0.1	164911	24.15	39691	0.0488	1	0.005	15	0.3100
			0.5	2	9904.05	19808.1			0.1					1		15	
	5-1	2	0.5	1	11983.62	23967.24	24074.11	48148.22	0.1	164911	29.20	48017	0.0591	1	0.005	15	0.3750
			0.5	2	12090.49	24180.98			0.1					1		15	
	5-2	2	0.5	1	12051.74	24103.48	24032.29	48064.58	0.1	164911	29.15	47933	0.0590	1	0.005	15	0.3743
			0.5	2	11980.55	23961.1			0.1					1		15	
	5-3	2	0.5	1	12319.03	24638.06	24634.76	49269.52	0.1	164911	29.88	49138	0.0605	1	0.005	15	0.3836
			0.5	2	12315.73	24631.46			0.1					1		15	
	6-1	2	0.5	1	12366.47	24732.94	24721.51	49443.02	0.1	164911	29.98	49311	0.0607	1	0.005	15	0.3851
			0.5	2	12355.04	24710.08			0.1					1		15	
	6-2	2	0.5	1	12413.33	24826.66	23713.9	47427.8	0.1	164911	28.76	47296	0.0582	1	0.005	15	0.3694
			0.5	2	11000.57	22001.14			0.1					1		15	
	6-3	2	0.5	1	12335.19	24670.38	24832.73	49665.46	0.1	164911	30.12	49534	0.0609	1	0.005	15	0.3868
			0.5	2	12497.54	24995.08			0.1					1		15	
	7-1	2	0.5	1	12625.75	25251.5	25104.36	50206.72	0.1	164911	30.45	50077	0.0616	1	0.005	15	0.3911
			0.5	2	12478.61	24957.22			0.1					1		15	
	7-2	2	0.5	1	12536.37	25072.74	25127.25	50254.5	0.1	164911	30.47	50123	0.0617	1	0.005	15	0.3914
			0.5	2	12590.88	25181.76			0.1					1		15	
	7-3	2	0.5	1	12807.86	25615.72	25685.2	51370.4	0.1	164911	31.15	51239	0.0630	1	0.005	15	0.4002
			0.5	2	12877.34	25754.68			0.1					1		15	
	8-1	2	0.5	1	12261.61	24523.22	24394.52	48789.04	0.1	164911	29.59	48657	0.0599	1	0.005	15	0.3800
			0.5	2	12132.91	24265.82			0.1					1		15	
	8-2	2	0.5	1	12319.89	24639.78	24736.78	49473.56	0.1	164911	30.00	49342	0.0607	1	0.005	15	0.3853
			0.5	2	12416.89	24833.78			0.1					1		15	
	8-3	2	0.5	1	12388.52	24777.04	24747.66	49495.32	0.1	164911	30.01	49364	0.0607	1	0.005	15	0.3855
			0.5	2	12359.14	24718.28			0.1					1		15	

Assay Date	8/18/2005	ID	RC8	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0 Technician ID	JG	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.4027	0.0038
Full activity	End	0.3829	0.0117
Full activity	Overall	0.3928	0.0134
Background	Beginning	-0.0001	0.000101059
Background	End	0.0001	3.94294E-05
Background	Overall	0.0000	0.000104311
Positive	Beginning	0.1903	0.0021
Positive	End	0.1755	0.0014
Positive	Overall	0.1829	0.0087
Negative	Beginning	0.3678	0.0065
Negative	End	0.3496	0.0116
Negative	Overall	0.3587	0.0130

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC8	1	1	1.00E-04	-4.00	0.0651
RC8	1	2	1.00E-04	-4.00	0.0576
RC8	1	3	1.00E-04	-4.00	0.0466
RC8	2	1	1.00E-05	-5.00	0.1147
RC8	2	2	1.00E-05	-5.00	0.1141
RC8	2	3	1.00E-05	-5.00	0.1144
RC8	3	1	5.00E-06	-5.30	0.1793
RC8	3	2	5.00E-06	-5.30	0.1773
RC8	3	3	5.00E-06	-5.30	0.1705
RC8	4	1	1.00E-06	-6.00	0.3051
RC8	4	2	1.00E-06	-6.00	0.3092
RC8	4	3	1.00E-06	-6.00	0.3100
RC8	5	1	1.00E-07	-7.00	0.3750
RC8	5	2	1.00E-07	-7.00	0.3743
RC8	5	3	1.00E-07	-7.00	0.3838
RC8	6	1	1.00E-08	-8.00	0.3851
RC8	6	2	1.00E-08	-8.00	0.3694
RC8	6	3	1.00E-08	-8.00	0.3868
RC8	7	1	1.00E-09	-9.00	0.3911
RC8	7	2	1.00E-09	-9.00	0.3914
RC8	7	3	1.00E-09	-9.00	0.4002
RC8	8	1	1.00E-10	-10.00	0.3800
RC8	8	2	1.00E-10	-10.00	0.3853
RC8	8	3	1.00E-10	-10.00	0.3855

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-4.00	16.58	14.65	11.86
2	-5.00	29.21	29.04	29.11
3	-5.30	45.63	45.14	43.40
4	-6.00	77.67	78.70	78.91
5	-7.00	95.46	95.30	97.69
6	-8.00	98.04	94.03	98.48
7	-9.00	99.56	99.65	101.87
8	-10.00	96.74	98.10	98.14

Aromatase Assay Spreadsheet

Assay Date	8/29/2005	Test Chemical ID	RC 8	# Concentrations tested	8
ID	JG	Replicate #	Rep 2	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0200	64716.17	3235809	
2	0.0202	67135.36	3323533	
3	0.0199	66261.05	3329701	
4	0.0199	66071.05	3320153	
5	0.0201	67801.89	3373228	
			Average DPM/g soln	3316485
			SD	49916
			CV	1.51
			µCi/g soln	1.494

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	8.2891 g
Mass of dilution B used in substrate prep	4.6697 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.563354 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.01691 µg/g soln.
µg/g soln.

a. µCi/g soln 1.494
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.

= 0.563354 + 0.01691
= 0.580266 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 2.575 µCi/µg ASDN

1636908 dpm/nmol

Assay Date	8/29/2005	Test Chemical ID	RC 8	tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.600	0.344	0.142	0.083	0.045	0.036	0.028	25	100	
	0.619	0.364	0.143	0.083	0.047	0.036	0.027			
	0.610	0.365	0.143	0.085	0.045	0.035	0.027			

Samples:	10	100	microsomes
	0.056	0.296	0.049
	0.050	0.297	0.058
			0.051

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	0.610	0.583	0.0486	m, b	0.083	0.000
0.125	100	200	0.00013	200	0.0250	0.358	0.331	0.0276	se_m, se_b	0.003	0.001
0.05	200	1000	0.00005	200	0.0100	0.143	0.115	0.0097	r , se_y	0.995	0.002
0.025	100	1000	0.00003	200	0.0050	0.084	0.056	0.0047	F, df	789	4
0.01	40	1000	0.00001	200	0.0020	0.046	0.019	0.0016	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.036	0.009	0.0008			

Regression results are calculated using the function LINEST

Blank	0.027	r^2=	0.995	
		m=	0.083	
		b=	0.000	

	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	0.056	0.029	0.002	200	1	1	0.000	0.000	0.011
10	0.050	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.296	0.269	0.022	200	1	1	0.000	0.000	0.113
100	0.297	0.270	0.023	200	1	1	0.000		
100									
	0.049	0.022	0.002	200	114	69814	0.006	0.007	6.607
	0.058	0.031	0.003	200	114	69814	0.008		
	0.051	0.023	0.002	200	114	69814	0.006		

Assay Date 8/29/2005 Test Chemical ID RC 8 tested 8

ID JG Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.600	0.344	0.142	0.083	0.045	0.036	0.028	25	100	
	0.619	0.364	0.143	0.083	0.047	0.036	0.027			
	0.610	0.365	0.143	0.085	0.045	0.035	0.027			

Samples:	10	100	microsomes
	0.056	0.296	0.049
	0.050	0.297	0.058
			0.051

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	0.610	0.583	0.0439
0.125	100	200	0.00013	200	0.0250	0.358	0.331	0.0252
0.05	200	1000	0.00005	200	0.0100	0.143	0.115	0.0093
0.025	100	1000	0.00003	200	0.0050	0.084	0.056	0.0049
0.01	40	1000	0.00001	200	0.0020	0.046	0.019	0.0021
0.005	20	1000	0.00001	200	0.0010	0.036	0.009	0.0014
			Blank	0.027				

Variables	Regression results	
m, b	0.074	0.001
se_m, se_b	0.002	0.000
r , se_y	0.998	0.000
F, df	1610	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

r^2= 0.998
m= 0.074
b= 0.001

	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	0.056	0.029	0.003	200	1	1	0.000	0.000	0.013
10	0.050	0.023	0.002	200	1	1	0.000		
10					1	1			
100	0.296	0.269	0.021	200	1	1	0.000	0.000	0.103
100	0.297	0.270	0.021	200	1	1	0.000		
100									
	0.049	0.022	0.002	200	114	69814	0.007	0.008	8.073
	0.058	0.031	0.003	200	114	69814	0.009		
	0.051	0.023	0.002	200	114	69814	0.008		

Assay Date	8/29/2005	Test Chemical ID	RC 8	tested	8		
ID	JG	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

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	8/29/2005	Chemical ID	RC 8	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	2.50E-06
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-10

Protein Concentration (stock microsomes, mg/mL):	8.073
Protein Concentration (dilution added to assay, mg/mL):	0.013182

Assay Date	8/29/2005	Test Chemical ID	RC 8	# Concentrations tested	8	Microsome type		Microsome ID	0	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	25802.62	51605.24	50035.92	100071.84	0.1	331648	30.17	99791	0.0610	1	0.007	15	0.3063
			0.5	2	24233.3	48466.6			0.1					1		15	
	2	2	0.5	1	24032.81	48065.22	47286.19	94572.38	0.1	331648	28.52	94292	0.0576	1	0.007	15	0.2913
			0.5	2	23253.58	46507.16			0.1					1		15	
	3	2	0.5	1	21674.65	43349.3	42781.01	85562.02	0.1	331648	25.80	85281	0.0521	1	0.007	15	0.2635
			0.5	2	21106.36	42212.72			0.1					1		15	
	4	2	0.5	1	21052.54	42105.08	42102.04	84204.08	0.1	331648	25.39	83923	0.0513	1	0.007	15	0.2593
			0.5	2	21049.5	42099			0.1					1		15	
Background control	1	2	0.5	1	69.74	139.48	127.68	255.36	0.1	331648	0.08	-25	0.0000	1	0.007	15	-0.0001
			0.5	2	57.94	115.88			0.1					1		15	
	2	2	0.5	1	69.48	138.96	147.56	295.12	0.1	331648	0.09	14	0.0000	1	0.007	15	0.0000
			0.5	2	78.08	156.16			0.1					1		15	
	3	2	0.5	1	67.81	135.62	142.27	284.54	0.1	331648	0.09	4	0.0000	1	0.007	15	0.0000
			0.5	2	74.46	148.92			0.1					1		15	
	4	2	0.5	1	73.76	147.52	143.74	287.48	0.1	331648	0.09	7	0.0000	1	0.007	15	0.0000
			0.5	2	69.98	139.96			0.1					1		15	
Positive control	1	2	0.5	1	12467.32	24934.64	25071.68	50143.76	0.1	331648	15.12	49863	0.0305	1	0.007	15	0.1541
			0.5	2	12604.36	25209.12			0.1					1		15	
	2	2	0.5	1	11628.74	23257.48	22813.1	45626.2	0.1	331648	13.76	45346	0.0277	1	0.007	15	0.1401
			0.5	2	11184.36	22368.72			0.1					1		15	
	3	2	0.5	1	10409.5	20819	20725.37	41450.74	0.1	331648	12.50	41170	0.0252	1	0.007	15	0.1272
			0.5	2	10315.87	20631.74			0.1					1		15	
	4	2	0.5	1	10149.44	20298.88	20039.02	40078.04	0.1	331648	12.08	39797	0.0243	1	0.007	15	0.1230
			0.5	2	9889.58	19779.16			0.1					1		15	
Negative Control	1	2	0.5	1	23007.26	46014.52	45416.55	90833.1	0.1	331648	27.39	90552	0.0553	1	0.007	15	0.2798
			0.5	2	22409.29	44818.58			0.1					1		15	
	2	2	0.5	1	23027.84	46055.68	45650.26	91300.52	0.1	331648	27.53	91020	0.0556	1	0.007	15	0.2812
			0.5	2	22622.42	45244.84			0.1					1		15	
	3	2	0.5	1	20619.28	41238.56	41199.41	82398.82	0.1	331648	24.85	82118	0.0502	1	0.007	15	0.2537
			0.5	2	20380.13	40760.26			0.1					1		15	
	4	2	0.5	1	19643.8	39287.6	39572.49	79144.98	0.1	331648	23.86	78864	0.0482	1	0.007	15	0.2437
			0.5	2	19928.69	39857.38			0.1					1		15	
RC 8	1-1	2	0.5	1	3958.7	7917.4	7950.97	15901.94	0.1	331648	4.79	15621	0.0095	1	0.007	15	0.0483
			0.5	2	3982.27	7964.54			0.1					1		15	
	1-2	2	0.5	1	2340.82	4681.64	4658.49	9316.98	0.1	331648	2.81	9036	0.0055	1	0.007	15	0.0279
			0.5	2	2317.67	4635.34			0.1					1		15	
	1-3	2	0.5	1	3679	7358	7424.85	14849.7	0.1	331648	4.48	14569	0.0089	1	0.007	15	0.0450
			0.5	2	3745.85	7491.7			0.1					1		15	
	2-1	2	0.5	1	7218.04	14436.08	14549.84	29099.68	0.1	331648	8.77	28819	0.0176	1	0.007	15	0.0890
			0.5	2	7331.9	14663.8			0.1					1		15	
	2-2	2	0.5	1	7231.86	14463.72	14448.44	28896.88	0.1	331648	8.71	28616	0.0175	1	0.007	15	0.0884
			0.5	2	7216.58	14433.16			0.1					1		15	
	2-3	2	0.5	1	7063.69	14127.38	13963.44	27926.88	0.1	331648	8.42	27646	0.0169	1	0.007	15	0.0854
			0.5	2	6899.75	13799.5			0.1					1		15	
	3-1	2	0.5	1	10113.58	20227.16	20106.23	40212.46	0.1	331648	12.13	39932	0.0244	1	0.007	15	0.1234
			0.5	2	9992.65	19985.3			0.1					1		15	
	3-2	2	0.5	1	10056.78	20113.56	20208.96	40417.92	0.1	331648	12.19	40137	0.0245	1	0.007	15	0.1240
			0.5	2	10152.18	20304.36			0.1					1		15	
	3-3	2	0.5	1	9633.38	19266.76	19326.99	38653.98	0.1	331648	11.66	38373	0.0234	1	0.007	15	0.1186
			0.5	2	9633.6	19387.2			0.1					1		15	
	4-1	2	0.5	1	13763.76	27527.52	27660.89	55321.78	0.1	331648	16.68	55041	0.0336	1	0.007	15	0.1700
			0.5	2	13897.13	27794.25			0.1					1		15	
	4-2	2	0.5	1	13687.06	27374.12	27332.65	54665.3	0.1	331648	16.48	54385	0.0332	1	0.007	15	0.1680
			0.5	2	13645.59	27291.18			0.1					1		15	
	4-3	2	0.5	1	13418.64	26837.28	26777.47	53554.94	0.1	331648	16.15	53274	0.0325	1	0.007	15	0.1646
			0.5	2	13358.63	26717.66			0.1					1		15	
	5-1	2	0.5	1	17098.97	34197.94	34205.12	68410.24	0.1	331648	20.63	68130	0.0416	1	0.007	15	0.2105
			0.5	2	17106.15	34212.3			0.1					1		15	
	5-2	2	0.5	1	17283.26	34566.52	34419.05	68838.1	0.1	331648	20.76	68557	0.0419	1	0.007	15	0.2118
			0.5	2	17135.79	34271.58			0.1					1		15	
	5-3	2	0.5	1	17313.4	34626.8	34390.09	68780.18	0.1	331648	20.74	68500	0.0418	1	0.007	15	0.2116
			0.5	2	17076.69	34153.38			0.1					1		15	
	6-1	2	0.5	1	21068.12	42136.24	42282.99	84565.98	0.1	331648	25.50	84285	0.0515	1	0.007	15	0.2604
			0.5	2	21214.87	42429.74			0.1					1		15	
	6-2	2	0.5	1	21051.73	42103.46	42081.22	84162.44	0.1	331648	25.38	83882	0.0512	1	0.007	15	0.2592
			0.5	2	21029.49	42058.98			0.1					1		15	
	6-3	2	0.5	1	21163.89	42327.78	42189.88	84379.76	0.1	331648	25.44	84098	0.0514	1	0.007	15	0.2598
			0.5	2	21025.99	42051.98			0.1					1		15	
	7-1	2	0.5	1	20781.89	41563.78	40724.82	81449.64	0.1	331648	24.56	81169	0.0496	1	0.007	15	0.2508
			0.5	2	19942.93	39885.86			0.1					1		15	
	7-2	2	0.5	1	21143.21	42286.42	42220.25	84440.5	0.1	331648	25.46	84160	0.0514	1	0.007	15	0.2600
			0.5	2	21077.04	42154.08			0.1					1		15	
	7-3	2	0.5	1	21114.63	42229.26	42333.94	84667.88	0.1	331648	25.53	84387	0.0516	1	0.007	15	0.2607
			0.5	2	21219.31	42438.62			0.1					1		15	
	8-1	2	0.5	1	19780.26	39560.52	39794.38	79588.76	0.1	331648	24.00	79308	0.0484	1	0.007	15	0.2450
			0.5	2	20014.12	40028.24			0.1					1		15	
	8-2	2	0.5	1	20104.51	40209.02	40211.73	80423.46	0.1	331648	24.25	80143	0.0490	1	0.007	15	0.2476
			0.5	2	20107.22	40214.44			0.1					1		15	
	8-3	2	0.5	1	20280.87	40521.74	40642.44	81284.88	0.1	331648	24.51	81004	0.0495	1	0.007	15	0.2503
			0.5	2	20381.57	40763.14			0.1					1		15	

Assay Date	8/29/2005	ID	RC 8	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.2998	0.0120
Full activity	End	0.2614	0.0030
Full activity	Overall	0.2806	0.0233
Background	Beginning	0.0000	8.68598E-05
Background	End	0.0000	6.42273E-06
Background	Overall	0.0000	5.383E-05
Positive	Beginning	0.1471	0.0099
Positive	End	0.1251	0.0030
Positive	Overall	0.1361	0.0140
Negative	Beginning	0.2805	0.0010
Negative	End	0.2487	0.0071
Negative	Overall	0.2646	0.0188

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC 8	1	1	1.00E-04	-4.00	0.0483
RC 8	1	2	1.00E-04	-4.00	0.0279
RC 8	1	3	1.00E-04	-4.00	0.0450
RC 8	2	1	1.00E-05	-5.00	0.0890
RC 8	2	2	1.00E-05	-5.00	0.0884
RC 8	2	3	1.00E-05	-5.00	0.0854
RC 8	3	1	5.00E-06	-5.30	0.1234
RC 8	3	2	5.00E-06	-5.30	0.1240
RC 8	3	3	5.00E-06	-5.30	0.1186
RC 8	4	1	2.50E-06	-5.60	0.1700
RC 8	4	2	2.50E-06	-5.60	0.1680
RC 8	4	3	2.50E-06	-5.60	0.1646
RC 8	5	1	1.00E-06	-6.00	0.2105
RC 8	5	2	1.00E-06	-6.00	0.2118
RC 8	5	3	1.00E-06	-6.00	0.2116
RC 8	6	1	1.00E-07	-7.00	0.2604
RC 8	6	2	1.00E-07	-7.00	0.2592
RC 8	6	3	1.00E-07	-7.00	0.2598
RC 8	7	1	1.00E-08	-8.00	0.2508
RC 8	7	2	1.00E-08	-8.00	0.2600
RC 8	7	3	1.00E-08	-8.00	0.2607
RC 8	8	1	1.00E-10	-10.00	0.2450
RC 8	8	2	1.00E-10	-10.00	0.2476
RC 8	8	3	1.00E-10	-10.00	0.2503

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-4.00	17.20	9.95	16.04
2	-5.00	31.73	31.51	30.44
3	-5.30	43.97	44.19	42.25
4	-5.60	60.60	59.88	58.66
5	-6.00	75.01	75.49	75.42
6	-7.00	92.80	92.36	92.60
7	-8.00	89.37	92.66	92.92
8	-10.00	87.32	88.24	89.19

Aromatase Assay Spreadsheet

Assay Date	8/31/2005	Test Chemical ID	RC8	# Concentrations tested	8
ID	JG	Replicate #	Rep3	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	31822.08	1583188	
2	0.0201	32473.87	1615615	
3	0.0200	32677.33	1633867	
4	0.0203	32663.61	1609045	
5	0.0202	32799.48	1623737	
			Average DPM/g soln	1613090
			SD	19117
			CV	1.19
			µCi/g soln	0.727

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	8.2604 g
Mass of dilution B used in substrate prep	4.6325 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.583241 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00823 µg/g soln.
µg/g soln.

a. µCi/g soln 0.727
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.

= 0.583241 + 0.00823
= 0.591466 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.229 µCi/µg ASDN

781092 dpm/nmol

Assay Date	8/31/2005	Test Chemical ID	RC8	tested	8		
ID	JG	Replicate #	Rep3	Microsome type	Recombinant	Microsome ID	0

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.470	0.322	0.137	0.087	0.054	0.044	0.038	25	100	
	0.474	0.300	0.127	0.090	0.054	0.044	0.039			
	0.460	0.323	0.130	0.093	0.057	0.041	0.039			

Samples:	10	100	microsomes
	0.061	0.230	0.056
	0.066	0.224	0.051
			0.054

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	0.468	0.430	0.0469
0.125	100	200	0.00013	200	0.0250	0.315	0.277	0.0300
0.05	200	1000	0.00005	200	0.0100	0.131	0.093	0.0097
0.025	100	1000	0.00003	200	0.0050	0.090	0.052	0.0052
0.01	40	1000	0.00001	200	0.0020	0.055	0.017	0.0013
0.005	20	1000	0.00001	200	0.0010	0.043	0.005	0.0000

Blank	0.038	r^2=	0.980	
		m=	0.110	
		b=	-0.001	

Variables	Regression results	
m, b	0.110	-0.001
se_m, se_b	0.008	0.002
r , se_y	0.980	0.003
F, df	195	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	0.061	0.022	0.002	200	1	1	0.000	0.000	0.011
10	0.066	0.028	0.003	200	1	1	0.000		
10					1	1			
100	0.230	0.191	0.021	200	1	1	0.000	0.000	0.101
100	0.224	0.185	0.020	200	1	1	0.000		
100									
	0.056	0.018	0.001	200	114	69814	0.004	0.005	4.535
	0.061	0.022	0.002	200	114	69814	0.006		
	0.054	0.015	0.001	200	114	69814	0.003		

Test

Assay Date 8/31/2005 Chemical ID RC8 tested 8

ID JG Replicate # Rep3 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.470	0.322	0.137	0.087	0.054	0.044	0.038	25	100	
	0.474	0.300	0.127	0.090	0.054	0.044	0.039			
	0.460	0.323	0.130	0.093	0.057	0.041	0.039			

Samples:	10	100	microsomes
	0.061	0.230	0.056
	0.066	0.224	0.061
			0.054

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	0.468	0.430	0.0388
0.125	100	200	0.00013	200	0.0250	0.315	0.277	0.0253
0.05	200	1000	0.00005	200	0.0100	0.131	0.093	0.0090
0.025	100	1000	0.00003	200	0.0050	0.090	0.052	0.0053
0.01	40	1000	0.00001	200	0.0020	0.055	0.017	0.0022
0.005	20	1000	0.00001	200	0.0010	0.043	0.005	0.0012

Blank 0.038

r^2= 0.997
m= 0.089
b= 0.001

Variables	Regression results	
m, b	0.089	0.001
se_m, se_b	0.003	0.000
r , se_y	0.997	0.001
F, df	865	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	0.061	0.022	0.003	200	1	1	0.000	0.000	0.015
10	0.066	0.028	0.003	200	1	1	0.000		
10					1	1			
100	0.230	0.191	0.018	200	1	1	0.000	0.000	0.087
100	0.224	0.185	0.017	200	1	1	0.000		
100									
	0.056	0.018	0.002	200	114	69814	0.007	0.007	7.306
	0.061	0.022	0.003	200	114	69814	0.008		
	0.054	0.015	0.002	200	114	69814	0.006		

Assay Date	8/31/2005	Test Chemical ID	RC8	tested		8		
ID	JG	Replicate #	Rep3	Microsome type	Recombinant	Microsome ID		0

							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	8/31/2005	Chemical ID	RC8	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	JG	Replicate #	Rep3

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	5.00E-06
4	2.50E-06
5	1.00E-06
6	1.00E-07
7	1.00E-08
8	1.00E-10

Protein Concentration (stock microsomes, mg/mL):	7.306
Protein Concentration (dilution added to assay, mg/mL):	0.01193

Assay Date	8/31/2005	Test Chemical ID	RC8	# Concentrations tested	8	Microsome type		Microsome ID		0	Technician ID	JG	Replicate #	Rep3

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	10634.66	21269.32	21282.12	42564.24	0.1	161309	26.39	42405	0.0543	1	0.006	15	0.3034
			0.5	2	10647.46	21294.92			0.1					1		15	
	2	2	0.5	1	10820.91	21641.82	21465.02	42930.04	0.1	161309	26.61	42771	0.0548	1	0.006	15	0.3060
			0.5	2	10644.11	21288.22			0.1					1		15	
	3	2	0.5	1	9492.78	18985.56	18963.33	37926.66	0.1	161309	23.51	37768	0.0484	1	0.006	15	0.2702
			0.5	2	9470.55	18941.1			0.1					1		15	
	4	2	0.5	1	9839.56	19679.12	19503.46	39006.92	0.1	161309	24.18	38848	0.0497	1	0.006	15	0.2779
			0.5	2	9663.9	19327.8			0.1					1		15	
Background control	1	2	0.5	1	44.8	89.6	77.13	154.26	0.1	161309	0.10	-5	0.0000	1	0.006	15	0.0000
			0.5	2	32.33	64.66			0.1					1		15	
	2	2	0.5	1	36.52	73.04	72.3	144.6	0.1	161309	0.09	-14	0.0000	1	0.006	15	-0.0001
			0.5	2	35.76	71.56			0.1					1		15	
	3	2	0.5	1	33.84	67.68	83.04	166.08	0.1	161309	0.10	7	0.0000	1	0.006	15	0.0001
			0.5	2	49.2	98.4			0.1					1		15	
	4	2	0.5	1	42.08	84.16	85.57	171.14	0.1	161309	0.11	12	0.0000	1	0.006	15	0.0001
			0.5	2	43.49	86.98			0.1					1		15	
Positive control	1	2	0.5	1	5483.82	10967.64	10884.04	21768.08	0.1	161309	13.49	21609	0.0277	1	0.006	15	0.1546
			0.5	2	5400.22	10800.44			0.1					1		15	
	2	2	0.5	1	5619.27	11238.54	11210.02	22420.04	0.1	161309	13.90	22261	0.0285	1	0.006	15	0.1593
			0.5	2	5590.75	11181.5			0.1					1		15	
	3	2	0.5	1	5136.01	10272.02	10203.01	20406.02	0.1	161309	12.65	20247	0.0259	1	0.006	15	0.1449
			0.5	2	5067	10134			0.1					1		15	
	4	2	0.5	1	4952.86	9905.72	9878.2	19956.4	0.1	161309	12.37	19797	0.0253	1	0.006	15	0.1416
			0.5	2	5025.34	10050.68			0.1					1		15	
Negative Control	1	2	0.5	1	8844.76	17689.52	17686.33	35372.66	0.1	161309	21.93	35214	0.0451	1	0.006	15	0.2519
			0.5	2	8841.57	17683.14			0.1					1		15	
	2	2	0.5	1	8725.89	17451.78	17446.4	34892.8	0.1	161309	21.63	34734	0.0445	1	0.006	15	0.2485
			0.5	2	8720.51	17441.02			0.1					1		15	
	3	2	0.5	1	7938.31	15876.62	15762.67	31525.34	0.1	161309	19.54	31366	0.0402	1	0.006	15	0.2244
			0.5	2	7834.36	15668.72			0.1					1		15	
	4	2	0.5	1	8353.06	16706.12	16617.93	33235.86	0.1	161309	20.60	33077	0.0423	1	0.006	15	0.2366
			0.5	2	8264.87	16529.74			0.1					1		15	
RC8	1-1	2	0.5	1	2249.09	4498.18	4494.05	8988.1	0.1	161309	5.57	8829	0.0113	1	0.006	15	0.0632
			0.5	2	2244.96	4489.92			0.1					1		15	
	1-2	2	0.5	1	1405.89	2813.98	2785.12	5570.24	0.1	161309	3.45	5411	0.0069	1	0.006	15	0.0387
			0.5	2	1378.13	2756.26			0.1					1		15	
	1-3	2	0.5	1	1774.24	3548.48	3514.66	7029.32	0.1	161309	4.36	6870	0.0088	1	0.006	15	0.0492
			0.5	2	1740.42	3480.84			0.1					1		15	
	2-1	2	0.5	1	2937.44	5874.88	5988.96	11977.92	0.1	161309	7.43	11819	0.0151	1	0.006	15	0.0846
			0.5	2	3051.52	6103.04			0.1					1		15	
	2-2	2	0.5	1	3110.27	6220.54	6199.52	12399.04	0.1	161309	7.69	12240	0.0157	1	0.006	15	0.0876
			0.5	2	3089.25	6178.5			0.1					1		15	
	2-3	2	0.5	1	3249.55	6499.1	6539.21	13078.42	0.1	161309	8.11	12919	0.0165	1	0.006	15	0.0924
			0.5	2	3289.86	6579.32			0.1					1		15	
	3-1	2	0.5	1	4874.92	9749.84	9779.45	19558.9	0.1	161309	12.13	19400	0.0248	1	0.006	15	0.1388
			0.5	2	4904.53	9809.06			0.1					1		15	
	3-2	2	0.5	1	5064.14	10128.28	10079.53	20159.06	0.1	161309	12.50	20000	0.0256	1	0.006	15	0.1431
			0.5	2	5015.39	10030.78			0.1					1		15	
	3-3	2	0.5	1	4857.67	9715.34	9708.28	19416.56	0.1	161309	12.04	19256	0.0247	1	0.006	15	0.1378
			0.5	2	4850.62	9701.24			0.1					1		15	
	4-1	2	0.5	1	6358.64	12717.28	12661.43	25322.86	0.1	161309	15.70	25164	0.0322	1	0.006	15	0.1800
			0.5	2	6302.79	12605.58			0.1					1		15	
	4-2	2	0.5	1	6679.14	13358.28	13340.88	26681.76	0.1	161309	16.54	26523	0.0340	1	0.006	15	0.1898
			0.5	2	6661.74	13323.48			0.1					1		15	
	4-3	2	0.5	1	6556.46	13112.92	13144.63	26289.26	0.1	161309	16.30	26130	0.0335	1	0.006	15	0.1869
			0.5	2	6588.17	13176.34			0.1					1		15	
	5-1	2	0.5	1	8155.09	16310.18	16267.57	32535.14	0.1	161309	20.17	32376	0.0414	1	0.006	15	0.2316
			0.5	2	8112.48	16224.96			0.1					1		15	
	5-2	2	0.5	1	8429.97	16859.94	16942.25	33884.5	0.1	161309	21.01	33725	0.0432	1	0.006	15	0.2413
			0.5	2	8512.28	17024.56			0.1					1		15	
	5-3	2	0.5	1	8424.06	16848.12	16875.29	33750.58	0.1	161309	20.92	33592	0.0430	1	0.006	15	0.2403
			0.5	2	8451.23	16902.46			0.1					1		15	
	6-1	2	0.5	1	10123.56	20247.12	20006.66	40013.32	0.1	161309	24.81	39854	0.0510	1	0.006	15	0.2851
			0.5	2	9883.1	19766.2			0.1					1		15	
	6-2	2	0.5	1	10296.32	20592.64	20631.28	41262.56	0.1	161309	25.59	41104	0.0526	1	0.006	15	0.2941
			0.5	2	10334.96	20669.92			0.1					1		15	
	6-3	2	0.5	1	10144.8	20289.6	20001.68	40003.36	0.1	161309	24.80	39844	0.0510	1	0.006	15	0.2851
			0.5	2	9856.76	19713.56			0.1					1		15	
	7-1	2	0.5	1	10089.82	20179.64	20090.46	40180.92	0.1	161309	24.91	40022	0.0512	1	0.006	15	0.2863
			0.5	2	10000.64	20001.28			0.1					1		15	
	7-2	2	0.5	1	10047.34	20094.68	20197.3	40394.6	0.1	161309	25.04	40236	0.0515	1	0.006	15	0.2879
			0.5	2	10149.96	20299.92			0.1					1		15	
	7-3	2	0.5	1	9900.27	19800.54	19739.64	39479.28	0.1	161309	24.47	39320	0.0503	1	0.006	15	0.2813
			0.5	2	9839.37	19678.74			0.1					1		15	
	8-1	2	0.5	1	8086.47	16172.94	16250.55	32501.1	0.1	161309	20.15	32342	0.0414	1	0.006	15	0.2314
			0.5	2	8164.08	16328.16			0.1					1		15	
	8-2	2	0.5	1	8351.37	16702.74	16719.52	33439.04	0.1	161309	20.73	33280	0.0426	1	0.006	15	0.2381
			0.5	2	8368.15	16736.3			0.1					1		15	
	8-3	2	0.5	1	8145.36	16290.72	16225.98	32451.96	0.1	161309	20.12	32293	0.0413	1	0.005	15	0.2310
			0.5	2	8080.62	16161.24			0.1					1		15	

Assay Date	8/31/2005	ID	RC8	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	JG	Replicate #	Rep3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3047	0.0019
Full activity	End	0.2741	0.0055
Full activity	Overall	0.2894	0.0180
Background	Beginning	-0.0001	4.88683E-05
Background	End	0.0001	2.55977E-05
Background	Overall	0.0000	8.5386E-05
Positive	Beginning	0.1569	0.0033
Positive	End	0.1432	0.0023
Positive	Overall	0.1501	0.0082
Negative	Beginning	0.2502	0.0024
Negative	End	0.2305	0.0087
Negative	Overall	0.2404	0.0125

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC8	1	1	1.00E-04	-4.00	0.0632
RC8	1	2	1.00E-04	-4.00	0.0387
RC8	1	3	1.00E-04	-4.00	0.0492
RC8	2	1	1.00E-05	-5.00	0.0846
RC8	2	2	1.00E-05	-5.00	0.0876
RC8	2	3	1.00E-05	-5.00	0.0924
RC8	3	1	5.00E-06	-5.30	0.1388
RC8	3	2	5.00E-06	-5.30	0.1431
RC8	3	3	5.00E-06	-5.30	0.1378
RC8	4	1	2.50E-06	-5.60	0.1800
RC8	4	2	2.50E-06	-5.60	0.1898
RC8	4	3	2.50E-06	-5.60	0.1869
RC8	5	1	1.00E-06	-6.00	0.2316
RC8	5	2	1.00E-06	-6.00	0.2413
RC8	5	3	1.00E-06	-6.00	0.2403
RC8	6	1	1.00E-07	-7.00	0.2851
RC8	6	2	1.00E-07	-7.00	0.2941
RC8	6	3	1.00E-07	-7.00	0.2851
RC8	7	1	1.00E-08	-8.00	0.2863
RC8	7	2	1.00E-08	-8.00	0.2879
RC8	7	3	1.00E-08	-8.00	0.2813
RC8	8	1	1.00E-10	-10.00	0.2314
RC8	8	2	1.00E-10	-10.00	0.2381
RC8	8	3	1.00E-10	-10.00	0.2310

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-4.00	21.83	13.38	16.99
2	-5.00	29.22	30.26	31.94
3	-5.30	47.96	49.45	47.61
4	-5.60	62.21	65.57	64.60
5	-6.00	80.04	83.38	83.05
6	-7.00	98.53	101.62	98.51
7	-8.00	98.95	99.47	97.21
8	-10.00	79.96	82.28	79.84

Aromatase Assay Spreadsheet

Assay Date	9/2/2005	Test Chemical ID	RC9	# Concentrations tested	8
ID	TNB	Replicate #	Rep 1	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.		
1	0.0201	37503.44	1865843		
2	0.0202	36666.75	1815186		
3	0.0201	36967.36	1839172		
4	0.0200	36785.67	1839284		
5	0.0201	36360.55	1808983		
			Average DPM/g soln	1833693	
			SD	22627	
			CV	1.23	
			µCi/g soln	0.826	

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	8.1344 g
Mass of dilution B used in substrate prep	4.5926 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56459 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00935 µg/g soln.
µg/g soln.

a. µCi/g soln 0.826
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.

= 0.564590 + 0.00935
= 0.573940 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.439 µCi/µg ASDN

915025 dpm/nmol

Test

Assay Date 9/2/2005 Chemical ID RC9 tested 8

ID	TNB	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.608	0.364	0.131	0.080	0.052	0.040	0.043	25	100	
	0.610	0.369	0.132	0.080	0.056	0.046	0.037			
	0.602	0.377	0.135	0.083	0.056	0.042	0.038			

Samples:	10	100	microsomes
	0.067	0.289	0.060
	0.061	0.290	0.064
			0.057

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	0.607	0.568	0.0482
0.125	100	200	0.00013	200	0.0250	0.370	0.331	0.0285
0.05	200	1000	0.00005	200	0.0100	0.133	0.094	0.0087
0.025	100	1000	0.00003	200	0.0050	0.081	0.042	0.0043
0.01	40	1000	0.00001	200	0.0020	0.055	0.016	0.0021
0.005	20	1000	0.00001	200	0.0010	0.043	0.004	0.0012

Blank 0.039

r^2= 0.990
m= 0.084
b= 0.001

Variables	Regression results	
m, b	0.084	0.001
se_m, se_b	0.004	0.001
r , se_y	0.990	0.002
F, df	412	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	0.067	0.028	0.003	200	1	1	0.000	0.000	0.015
10	0.061	0.022	0.003	200	1	1	0.000		
10					1	1			
100	0.289	0.250	0.022	200	1	1	0.000	0.000	0.109
100	0.290	0.251	0.022	200	1	1	0.000		
100									
	0.060	0.021	0.003	200	114	69814	0.008	0.008	7.970
	0.064	0.025	0.003	200	114	69814	0.009		
	0.057	0.018	0.002	200	114	69814	0.007		

Assay Date 9/2/2005 Test Chemical ID RC9 tested 8

ID TNB Replicate # Rep 1 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.608	0.364	0.131	0.080	0.052	0.040	0.043	25	100
	0.610	0.369	0.132	0.080	0.056	0.046	0.037		
	0.602	0.377	0.135	0.083	0.056	0.042	0.038		

Protein stock ID

Samples:	10	100	microsomes
	0.067	0.289	0.060
	0.061	0.290	0.064
			0.057

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	0.607	0.568	0.0426
0.125	100	200	0.00013	200	0.0250	0.370	0.331	0.0255
0.05	200	1000	0.00005	200	0.0100	0.133	0.094	0.0083
0.025	100	1000	0.00003	200	0.0050	0.081	0.042	0.0046
0.01	40	1000	0.00001	200	0.0020	0.055	0.016	0.0027
0.005	20	1000	0.00001	200	0.0010	0.043	0.004	0.0019

Blank 0.039

r^2= 0.989
m= 0.072
b= 0.002

Variables	Regression results	
m, b	0.072	0.002
se_m, se_b	0.004	0.001
r , se_y	0.989	0.001
F, df	262	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	0.067	0.028	0.004	200	1	1	0.000	0.000	0.017
10	0.061	0.022	0.003	200	1	1	0.000		
10					1	1			
100	0.289	0.250	0.020	200	1	1	0.000	0.000	0.098
100	0.290	0.251	0.020	200	1	1	0.000		
100									
	0.060	0.021	0.003	200	114	69814	0.009	0.010	9.528
	0.064	0.025	0.003	200	114	69814	0.010		
	0.057	0.018	0.003	200	114	69814	0.009		

Assay Date	9/2/2005	Test Chemical ID	RC9	tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

								Protein stock	
Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	(mg/10 mL)	Protein stock ID

Samples:

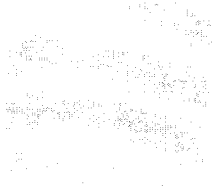
mg Protein per µL	µL Standard Used	mg Protein Measured	A_{raw}	A_{adj}	Curve Output	Variables	Regression results
0.00000	25	0.0000				m, b	
0.00000	25	0.0000				se_m, se_b	
0.00000	25	0.0000				r , se_y	
0.00000	25	0.0000				F, df	
0.00000	25	0.0000				ss_{reg}, ss_{resid}	
0.00000	25	0.0000					

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	9/2/2005	Chemical ID	RC9	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 1

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

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

Protein Concentration (stock microsomes, mg/mL):	9.535
Protein Concentration (dilution added to assay, mg/mL):	0.01557

Assay Date	9/2/2005	Test Chemical ID	RC9	# Concentrations tested	8	Microsome type		Microsome ID		0 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	12744.54	25489.08	25510.79	51021.58	0.1	183369	27.82	50671	0.0554	1	0.008	15	0.2371
			0.5	2	12766.25	25532.5			0.1					1		15	
	2	2	0.5	1	13037.76	26075.52	25877.4	51754.8	0.1	183369	28.22	51404	0.0562	1	0.008	15	0.2405
			0.5	2	12839.64	25679.28			0.1					1		15	
	3	2	0.5	1	12773.17	25546.34	25557.39	51114.78	0.1	183369	27.88	50764	0.0555	1	0.008	15	0.2375
			0.5	2	12784.22	25568.44			0.1					1		15	
	4	2	0.5	1	12743.18	25486.36	25496.46	50992.96	0.1	183369	27.81	50642	0.0553	1	0.008	15	0.2370
			0.5	2	12753.3	25506.6			0.1					1		15	
Background control	1	2	0.5	1	39.44	78.88	71.99	143.98	0.1	183369	0.08	-207	-0.0002	1	0.008	15	-0.0010
			0.5	2	32.55	65.1			0.1					1		15	
	2	2	0.5	1	45.34	90.68	90.18	180.36	0.1	183369	0.10	-170	-0.0002	1	0.008	15	-0.0008
			0.5	2	44.84	89.68			0.1					1		15	
	3	2	0.5	1	215.16	430.32	441.3	882.6	0.1	183369	0.48	532	0.0006	1	0.008	15	0.0025
			0.5	2	226.14	452.28			0.1					1		15	
	4	2	0.5	1	52.79	105.58	97.59	195.18	0.1	183369	0.11	-155	-0.0002	1	0.008	15	-0.0007
			0.5	2	44.8	89.6			0.1					1		15	
Positive control	1	2	0.5	1	6754.58	13509.16	13425.7	26851.4	0.1	183369	14.64	26501	0.0290	1	0.008	15	0.1240
			0.5	2	6671.12	13342.24			0.1					1		15	
	2	2	0.5	1	6691.97	13383.94	13332.41	26664.82	0.1	183369	14.54	26314	0.0288	1	0.008	15	0.1231
			0.5	2	6640.44	13280.88			0.1					1		15	
	3	2	0.5	1	6173.07	12346.14	12490.65	24981.3	0.1	183369	13.62	24631	0.0269	1	0.008	15	0.1153
			0.5	2	6317.58	12635.16			0.1					1		15	
	4	2	0.5	1	5941.29	11882.58	11952.92	23905.84	0.1	183369	13.04	23555	0.0257	1	0.008	15	0.1102
			0.5	2	6011.63	12023.26			0.1					1		15	
Negative Control	1	2	0.5	1	13114.71	26229.42	26332.1	52664.2	0.1	183369	26.72	52314	0.0572	1	0.008	15	0.2448
			0.5	2	13217.39	26434.78			0.1					1		15	
	2	2	0.5	1	13279.7	26559.4	26722.98	53445.96	0.1	183369	29.15	53095	0.0580	1	0.008	15	0.2485
			0.5	2	13443.28	26886.56			0.1					1		15	
	3	2	0.5	1	12399.3	24798.6	24927.89	49855.78	0.1	183369	27.19	49505	0.0541	1	0.008	15	0.2317
			0.5	2	12528.59	25057.18			0.1					1		15	
	4	2	0.5	1	11510.16	23020.32	23043.16	46086.32	0.1	183369	25.13	45736	0.0500	1	0.008	15	0.2140
			0.5	2	11533	23066			0.1					1		15	
RC9	1-1	2	0.5	1	377.25	754.5	751.49	1502.98	0.1	183369	0.82	1152	0.0013	1	0.008	15	0.0054
			0.5	2	374.24	748.48			0.1					1		15	
	1-2	2	0.5	1	383.01	766.02	731.86	1463.72	0.1	183369	0.80	1113	0.0012	1	0.008	15	0.0052
			0.5	2	348.85	697.7			0.1					1		15	
	1-3	2	0.5	1	496.82	993.64	1007.93	2015.86	0.1	183369	1.10	1665	0.0018	1	0.008	15	0.0078
			0.5	2	511.11	1022.22			0.1					1		15	
	2-1	2	0.5	1	3002.85	6005.7	6200.75	12401.5	0.1	183369	6.76	12051	0.0132	1	0.008	15	0.0564
			0.5	2	3197.9	6395.8			0.1					1		15	
	2-2	2	0.5	1	3233.1	6466.2	6513.12	13026.24	0.1	183369	7.10	12676	0.0139	1	0.008	15	0.0593
			0.5	2	3280.02	6560.04			0.1					1		15	
	2-3	2	0.5	1	3297.17	6594.34	6646.18	13292.36	0.1	183369	7.25	12942	0.0141	1	0.008	15	0.0606
			0.5	2	3349.01	6698.02			0.1					1		15	
	3-1	2	0.5	1	7916.06	15832.12	15853.77	31707.54	0.1	183369	17.29	31357	0.0343	1	0.008	15	0.1467
			0.5	2	7937.71	15875.42			0.1					1		15	
	3-2	2	0.5	1	9073.28	18146.56	18238.46	36476.92	0.1	183369	19.89	36126	0.0395	1	0.008	15	0.1691
			0.5	2	9165.18	18330.36			0.1					1		15	
	3-3	2	0.5	1	8904.56	17809.12	17871.77	35743.54	0.1	183369	19.49	35393	0.0387	1	0.008	15	0.1656
			0.5	2	8967.21	17934.42			0.1					1		15	
	4-1	2	0.5	1	12244.92	24489.84	24494.46	48988.92	0.1	183369	26.71	48618	0.0531	1	0.008	15	0.2275
			0.5	2	12239.54	24479.08			0.1					1		15	
	4-2	2	0.5	1	11980.04	23960.08	23915.33	47830.66	0.1	183369	26.08	47480	0.0519	1	0.008	15	0.2222
			0.5	2	11935.29	23870.58			0.1					1		15	
	4-3	2	0.5	1	11696.09	23392.18	23750.21	47500.42	0.1	183369	25.90	47150	0.0515	1	0.008	15	0.2206
			0.5	2	11854.12	23708.24			0.1					1		15	
	5-1	2	0.5	1	12178.79	24357.58	24587.43	49174.86	0.1	183369	26.82	48824	0.0534	1	0.008	15	0.2285
			0.5	2	12408.64	24817.28			0.1					1		15	
	5-2	2	0.5	1	12581.16	25162.32	25129.92	50259.84	0.1	183369	27.41	49909	0.0545	1	0.008	15	0.2335
			0.5	2	12548.76	25097.52			0.1					1		15	
	5-3	2	0.5	1	12220.67	24441.34	24559.83	49119.66	0.1	183369	26.79	48769	0.0533	1	0.008	15	0.2282
			0.5	2	12339.16	24678.32			0.1					1		15	
	6-1	2	0.5	1	13194.13	26388.26	26649.02	53298.04	0.1	183369	29.07	52848	0.0579	1	0.008	15	0.2478
			0.5	2	13454.89	26909.78			0.1					1		15	
	6-2	2	0.5	1	12683.81	25367.62	25494.05	50988.1	0.1	183369	27.81	50638	0.0553	1	0.008	15	0.2370
			0.5	2	12810.24	25620.48			0.1					1		15	
	6-3	2	0.5	1	13220.81	26441.62	26326.92	52653.84	0.1	183369	28.71	52303	0.0572	1	0.008	15	0.2447
			0.5	2	13106.11	26212.22			0.1					1		15	
	7-1	2	0.5	1	13329.63	26659.26	26445.67	52891.34	0.1	183369	28.84	52541	0.0574	1	0.008	15	0.2459
			0.5	2	13116.04	26232.08			0.1					1		15	
	7-2	2	0.5	1	13220.08	26440.16	26474.52	52949.04	0.1	183369	28.88	52599	0.0575	1	0.008	15	0.2461
			0.5	2	13254.43	26508.86			0.1					1		15	
	7-3	2	0.5	1	13057.79	26115.58	26284.15	52568.3	0.1	183369	28.66	52238	0.0571	1	0.008	15	0.2444
			0.5	2	13236.36	26472.72			0.1					1		15	
	8-1	2	0.5	1	13229.13	26458.26	26618.57	53237.14	0.1	183369	29.03	52887	0.0576	1	0.008	15	0.2475
			0.5	2	13389.44	26778.88			0.1					1		15	
	8-2	2	0.5	1	13098.45	26196.9	26340.87	52681.74	0.1	183369	28.73	52331	0.0572	1	0.008	15	0.2449
			0.5	2	13242.42	26484.84			0.1					1		15	
	8-3	2	0.5	1	12910.81	25821.62	25500.96	51001.92	0.1	183369	27.81	50651	0.0554	1	0.008	15	0.2370
			0.5	2	12590.15	25180.3			0.1					1		15	

Assay Date	9/2/2005	ID	RC9	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	TNB	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.2388	0.0024
Full activity	End	0.2373	0.0004
Full activity	Overall	0.2380	0.0017
Background	Beginning	-0.0009	0.000120376
Background	End	0.0009	0.002274573
Background	Overall	0.0000	0.001662904
Positive	Beginning	0.1236	0.0006
Positive	End	0.1127	0.0036
Positive	Overall	0.1182	0.0066
Negative	Beginning	0.2466	0.0026
Negative	End	0.2228	0.0125
Negative	Overall	0.2347	0.0156

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC9	1	1	1.00E-03	-3.00	0.0054
RC9	1	2	1.00E-03	-3.00	0.0052
RC9	1	3	1.00E-03	-3.00	0.0078
RC9	2	1	1.00E-04	-4.00	0.0564
RC9	2	2	1.00E-04	-4.00	0.0593
RC9	2	3	1.00E-04	-4.00	0.0606
RC9	3	1	1.00E-05	-5.00	0.1467
RC9	3	2	1.00E-05	-5.00	0.1691
RC9	3	3	1.00E-05	-5.00	0.1656
RC9	4	1	1.00E-06	-6.00	0.2275
RC9	4	2	1.00E-06	-6.00	0.2222
RC9	4	3	1.00E-06	-6.00	0.2206
RC9	5	1	1.00E-07	-7.00	0.2285
RC9	5	2	1.00E-07	-7.00	0.2335
RC9	5	3	1.00E-07	-7.00	0.2282
RC9	6	1	1.00E-08	-8.00	0.2478
RC9	6	2	1.00E-08	-8.00	0.2370
RC9	6	3	1.00E-08	-8.00	0.2447
RC9	7	1	1.00E-09	-9.00	0.2459
RC9	7	2	1.00E-09	-9.00	0.2461
RC9	7	3	1.00E-09	-9.00	0.2444
RC9	8	1	1.00E-10	-10.00	0.2475
RC9	8	2	1.00E-10	-10.00	0.2449
RC9	8	3	1.00E-10	-10.00	0.2370

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	2.27	2.19	3.27
2	-4.00	23.69	24.92	25.44
3	-5.00	61.64	71.02	69.57
4	-6.00	95.57	93.34	92.69
5	-7.00	95.98	98.11	95.87
6	-8.00	104.08	99.54	102.82
7	-9.00	103.28	103.40	102.69
8	-10.00	103.96	102.87	99.57

Aromatase Assay Spreadsheet

Assay Date	9/7/2005	Test Chemical ID	RC9	# Concentrations tested	8
ID	TNB	Replicate #	Rep 2	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	27485.55	1381183	
2	0.0203	28586.07	1408181	
3	0.0201	28589.92	1422384	
4	0.0202	28892.83	1430338	
5	0.0202	28994.39	1435366	
			Average DPM/g soln	1415490
			SD	21757
			CV	1.54
			µCi/g soln	0.638

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	8.0776 g
Mass of dilution B used in substrate prep	4.571 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.565886 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00722 µg/g soln.
µg/g soln.

a. µCi/g soln 0.638
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.

= 0.565886 + 0.00722
= 0.573104 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.113 µCi/µg ASDN

707370 dpm/nmol

Test

Assay Date 9/7/2005 Chemical ID RC9 tested 8

ID	TNB	Replicate #	Rep 2	Microsome type		Recombinant	Microsome ID	0		
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.571	0.346	0.125	0.075	0.046	0.037	0.030	25	100	
	0.580	0.355	0.128	0.076	0.044	0.037	0.030			
	0.573	0.357	0.127	0.073	0.046	0.037	0.031			
Samples:	10	100	microsomes							
	0.051	0.283	0.050							
	0.053	0.287	0.051							
			0.051							

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	0.0481
0.125	100	200	0.00013	200	0.0250	0.353	0.322	0.0287
0.05	200	1000	0.00005	200	0.0100	0.127	0.096	0.0089
0.025	100	1000	0.00003	200	0.0050	0.075	0.044	0.0044
0.01	40	1000	0.00001	200	0.0020	0.045	0.015	0.0018
0.005	20	1000	0.00001	200	0.0010	0.037	0.007	0.0011

Blank 0.030

r^2= 0.990
m= 0.087
b= 0.001

Variables	Regression results	
m, b	0.087	0.001
se_m, se_b	0.004	0.001
r , se_y	0.990	0.002
F, df	382	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	0.051	0.021	0.002	200	1	1	0.000	0.000	0.012
10	0.053	0.023	0.003	200	1	1	0.000		
10					1	1			
100	0.283	0.253	0.023	200	1	1	0.000	0.000	0.114
100	0.287	0.257	0.023	200	1	1	0.000		
100									
	0.050	0.020	0.002	200	114	69814	0.007	0.007	7.031
	0.051	0.021	0.002	200	114	69814	0.007		
	0.051	0.021	0.002	200	114	69814	0.007		

Assay Date	9/7/2005	Test Chemical ID	RC9	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.571	0.346	0.125	0.075	0.046	0.037	0.030	25	100	
	0.580	0.355	0.128	0.076	0.044	0.037	0.030			
	0.573	0.357	0.127	0.073	0.046	0.037	0.031			

Samples:	10	100	microsomes
	0.051	0.283	0.050
	0.053	0.287	0.051
			0.051

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	0.0420
0.125	100	200	0.00013	200	0.0250	0.353	0.322	0.0254
0.05	200	1000	0.00005	200	0.0100	0.127	0.096	0.0086
0.025	100	1000	0.00003	200	0.0050	0.075	0.044	0.0047
0.01	40	1000	0.00001	200	0.0020	0.045	0.015	0.0025
0.005	20	1000	0.00001	200	0.0010	0.037	0.007	0.0019

Variables	Regression results	
m, b	0.075	0.001
se_m, se_b	0.004	0.001
r , se_y	0.991	0.001
F, df	342	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

Blank 0.030

r^2= 0.991
m= 0.075
b= 0.001

	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	0.051	0.021	0.003	200	1	1	0.000	0.000	0.015
10	0.053	0.023	0.003	200	1	1	0.000		
10					1	1			
100	0.283	0.253	0.020	200	1	1	0.000	0.000	0.102
100	0.287	0.257	0.021	200	1	1	0.000		
100									
	0.050	0.020	0.003	200	114	69814	0.009	0.009	8.813
	0.051	0.021	0.003	200	114	69814	0.009		
	0.051	0.021	0.003	200	114	69814	0.009		

Assay Date	9/7/2005	Test Chemical ID	RC9	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

								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	9/7/2005	Chemical ID	RC9	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 2

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.813
Protein Concentration (dilution added to assay, mg/mL):	0.014391

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	9/7/2005	Test Chemical ID	RC9	# Concentrations tested	8	Microsome type		Microsome ID		0 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	7590.54	15181.08	15080.97	30161.94	0.1	141549	21.31	29997	0.0424	1	0.007	15	0.1964
			0.5	2	7490.43	14980.86			0.1					1		15	
	2	2	0.5	1	7517.7	15035.4	14928.95	29857.9	0.1	141549	21.09	29693	0.0420	1	0.007	15	0.1945
			0.5	2	7411.25	14822.5			0.1					1		15	
	3	2	0.5	1	6906.01	13812.02	14068.55	28137.1	0.1	141549	19.88	27972	0.0395	1	0.007	15	0.1832
			0.5	2	7162.54	14325.08			0.1					1		15	
	4	2	0.5	1	7170.17	14340.34	14392.02	28784.04	0.1	141549	20.34	28618	0.0405	1	0.007	15	0.1874
			0.5	2	7221.85	14443.7			0.1					1		15	
Background control	1	2	0.5	1	35.78	71.56	58.38	116.76	0.1	141549	0.08	-49	-0.0001	1	0.007	15	-0.0003
			0.5	2	22.6	45.2			0.1					1		15	
	2	2	0.5	1	50.25	100.5	106.62	213.24	0.1	141549	0.15	48	0.0001	1	0.007	15	0.0003
			0.5	2	56.37	112.74			0.1					1		15	
	3	2	0.5	1	40.97	81.94	74.27	148.54	0.1	141549	0.10	-17	0.0000	1	0.007	15	-0.0001
			0.5	2	33.3	66.6			0.1					1		15	
	4	2	0.5	1	40.31	80.62	91.44	182.88	0.1	141549	0.13	18	0.0000	1	0.007	15	0.0001
			0.5	2	51.13	102.26			0.1					1		15	
Positive control	1	2	0.5	1	4152.24	8304.48	8322.38	16644.76	0.1	141549	11.76	16479	0.0233	1	0.007	15	0.1079
			0.5	2	4170.14	8340.28			0.1					1		15	
	2	2	0.5	1	4225.45	8450.92	8299.03	16598.06	0.1	141549	11.73	16433	0.0232	1	0.007	15	0.1076
			0.5	2	4073.57	8147.14			0.1					1		15	
	3	2	0.5	1	3954.93	7909.86	7911.57	15823.14	0.1	141549	11.18	15658	0.0221	1	0.007	15	0.1025
			0.5	2	3956.64	7913.28			0.1					1		15	
	4	2	0.5	1	3977.78	7955.56	7885.53	15771.06	0.1	141549	11.14	15606	0.0221	1	0.007	15	0.1022
			0.5	2	3907.75	7815.5			0.1					1		15	
Negative Control	1	2	0.5	1	7155.47	14310.94	14352.28	28704.56	0.1	141549	20.26	28539	0.0403	1	0.007	15	0.1869
			0.5	2	7196.81	14393.62			0.1					1		15	
	2	2	0.5	1	7232.2	14464.4	14379.97	28759.94	0.1	141549	20.32	28595	0.0404	1	0.007	15	0.1873
			0.5	2	7147.77	14295.54			0.1					1		15	
	3	2	0.5	1	6807.02	13614.04	13601	27202	0.1	141549	19.22	27037	0.0382	1	0.007	15	0.1771
			0.5	2	6793.98	13587.96			0.1					1		15	
	4	2	0.5	1	6876.03	13752.06	13491.23	26982.46	0.1	141549	19.06	26817	0.0379	1	0.007	15	0.1756
			0.5	2	6615.2	13230.4			0.1					1		15	
RC9	1-1	2	0.5	1	330.97	661.94	682.21	1364.42	0.1	141549	0.96	1199	0.0017	1	0.007	15	0.0079
			0.5	2	351.24	702.48			0.1					1		15	
	1-2	2	0.5	1	270.41	540.82	548.59	1097.18	0.1	141548	0.76	932	0.0013	1	0.007	15	0.0061
			0.5	2	278.18	556.36			0.1					1		15	
	1-3	2	0.5	1	268.81	537.62	527.73	1055.46	0.1	141549	0.75	890	0.0013	1	0.007	15	0.0058
			0.5	2	258.92	517.84			0.1					1		15	
	2-1	2	0.5	1	937.28	1874.56	1845.51	3691.02	0.1	141549	2.61	3526	0.0050	1	0.007	15	0.0231
			0.5	2	908.23	1816.46			0.1					1		15	
	2-2	2	0.5	1	1006.82	2013.24	1964.56	3929.12	0.1	141549	2.78	3764	0.0053	1	0.007	15	0.0246
			0.5	2	957.94	1915.88			0.1					1		15	
	2-3	2	0.5	1	925.92	1851.84	1826.77	3653.54	0.1	141549	2.58	3488	0.0049	1	0.007	15	0.0228
			0.5	2	900.85	1801.7			0.1					1		15	
	3-1	2	0.5	1	2003.02	4006.04	4008.43	8016.86	0.1	141549	5.66	7852	0.0111	1	0.007	15	0.0514
			0.5	2	2005.41	4010.82			0.1					1		15	
	3-2	2	0.5	1	2298.2	4596.4	4556.02	9112.04	0.1	141549	6.44	8947	0.0126	1	0.007	15	0.0586
			0.5	2	2257.82	4515.64			0.1					1		15	
	3-3	2	0.5	1	2017.18	4034.36	4087.3	8174.6	0.1	141548	5.78	8009	0.0113	1	0.007	15	0.0525
			0.5	2	2070.12	4140.24			0.1					1		15	
	4-1	2	0.5	1	3703.44	7406.88	7370.26	14740.52	0.1	141549	10.41	14575	0.0206	1	0.007	15	0.0955
			0.5	2	3666.82	7333.64			0.1					1		15	
	4-2	2	0.5	1	4445.08	8890.16	8722.53	17445.06	0.1	141549	12.32	17260	0.0244	1	0.007	15	0.1132
			0.5	2	4277.45	8554.9			0.1					1		15	
	4-3	2	0.5	1	4554.89	9109.78	9097.4	18194.8	0.1	141548	12.85	18029	0.0255	1	0.007	15	0.1181
			0.5	2	4542.51	9085.02			0.1					1		15	
	5-1	2	0.5	1	4281.82	8563.64	8616.46	17232.92	0.1	141549	12.17	17068	0.0241	1	0.007	15	0.1118
			0.5	2	4334.64	8668.28			0.1					1		15	
	5-2	2	0.5	1	5139.47	10278.94	10242.4	20484.8	0.1	141549	14.47	20319	0.0287	1	0.007	15	0.1331
			0.5	2	5102.93	10205.86			0.1					1		15	
	5-3	2	0.5	1	5658.64	11317.28	11347.16	22694.32	0.1	141549	16.03	22529	0.0318	1	0.007	15	0.1475
			0.5	2	5688.52	11377.04			0.1					1		15	
	6-1	2	0.5	1	6041.85	12083.7	12183.01	24366.02	0.1	141549	17.21	24201	0.0342	1	0.007	15	0.1585
			0.5	2	6141.16	12282.32			0.1					1		15	
	6-2	2	0.5	1	6276.8	12557.6	12524.99	25049.98	0.1	141549	17.70	24885	0.0352	1	0.007	15	0.1630
			0.5	2	6246.19	12492.38			0.1					1		15	
	6-3	2	0.5	1	6464.37	12928.74	12751.06	25502.12	0.1	141549	18.02	25337	0.0358	1	0.007	15	0.1659
			0.5	2	6286.69	12573.38			0.1					1		15	
	7-1	2	0.5	1	6988.13	13976.26	14131.12	28262.24	0.1	141549	19.97	28097	0.0397	1	0.007	15	0.1840
			0.5	2	7142.98	14285.96			0.1					1		15	
	7-2	2	0.5	1	6654.65	13309.3	13411.85	26823.7	0.1	141549	18.95	26658	0.0377	1	0.007	15	0.1746
			0.5	2	6757.2	13514.4			0.1					1		15	
	7-3	2	0.5	1	6676.54	13353.08	13382.87	26765.74	0.1	141549	18.91	26600	0.0376	1	0.007	15	0.1742
			0.5	2	6706.33	13412.66			0.1					1		15	
	8-1	2	0.5	1	7193.5	14387	14415.67	28831.34	0.1	141549	20.37	28666	0.0405	1	0.007	15	0.1877
			0.5	2	7222.17	14444.34			0.1					1		15	
	8-2	2	0.5	1	7164.85	14329.7	14314.75	28629.5	0.1	141549	20.23	28464	0.0402	1	0.007	15	0.1864
			0.5	2	7149.9	14299.8			0.1					1		15	
	8-3	2	0.5	1	7226.67	14453.34	14565.58	29131.16	0.1	141549	20.58	28966	0.0409	1	0.007	15	0.1897
			0.5	2	7338.91	14677.82			0.1					1		15	

Assay Date	9/7/2005	ID	RC9	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0 Technician ID	TNB	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.1955	0.0014
Full activity	End	0.1853	0.0030
Full activity	Overall	0.1904	0.0062
Background	Beginning	0.0000	0.000446785
Background	End	0.0000	0.000159023
Background	Overall	0.0000	0.000273817
Positive	Beginning	0.1078	0.0002
Positive	End	0.1024	0.0002
Positive	Overall	0.1051	0.0031
Negative	Beginning	0.1871	0.0003
Negative	End	0.1763	0.0010
Negative	Overall	0.1817	0.0062

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC9	1	1	1.00E-03	-3.00	0.0079
RC9	1	2	1.00E-03	-3.00	0.0061
RC9	1	3	1.00E-03	-3.00	0.0058
RC9	2	1	3.00E-04	-3.52	0.0231
RC9	2	2	3.00E-04	-3.52	0.0246
RC9	2	3	3.00E-04	-3.52	0.0228
RC9	3	1	1.00E-04	-4.00	0.0514
RC9	3	2	1.00E-04	-4.00	0.0586
RC9	3	3	1.00E-04	-4.00	0.0525
RC9	4	1	3.00E-05	-4.52	0.0955
RC9	4	2	3.00E-05	-4.52	0.1132
RC9	4	3	3.00E-05	-4.52	0.1181
RC9	5	1	1.00E-05	-5.00	0.1118
RC9	5	2	1.00E-05	-5.00	0.1331
RC9	5	3	1.00E-05	-5.00	0.1475
RC9	6	1	3.00E-06	-5.52	0.1585
RC9	6	2	3.00E-06	-5.52	0.1630
RC9	6	3	3.00E-06	-5.52	0.1659
RC9	7	1	1.00E-06	-6.00	0.1840
RC9	7	2	1.00E-06	-6.00	0.1746
RC9	7	3	1.00E-06	-6.00	0.1742
RC9	8	1	1.00E-08	-8.00	0.1877
RC9	8	2	1.00E-08	-8.00	0.1864
RC9	8	3	1.00E-08	-8.00	0.1897

Percent of control values				
		Replicate		
Level	Log[test substance]	1	2	3
1	-3.00	4.12	3.21	3.06
2	-3.52	12.13	12.95	12.00
3	-4.00	27.01	30.78	27.55
4	-4.52	50.14	59.44	62.02
5	-5.00	58.71	69.90	77.50
6	-5.52	83.25	85.60	87.16
7	-6.00	96.65	91.70	91.50
8	-8.00	98.61	97.92	99.64

Aromatase Assay Spreadsheet

Assay Date	9/9/2005	Test Chemical ID	RC9	# Concentrations tested	8
ID	TNB	Replicate #	Rep 3	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0202	36051.51	1784728	
2	0.0205	37130.69	1811253	
3	0.0207	36549.05	1765655	
4	0.0206	36695.27	1781324	
5	0.0204	35839.02	1756815	
			Average DPM/g soln	1779955
			SD	20882
			CV	1.17
			µCi/g soln	0.802

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	8.1122 g
Mass of dilution B used in substrate prep	4.5911 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56595 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00908 µg/g soln.
µg/g soln.

a. µCi/g soln 0.802
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.

= 0.565950 + 0.00908
= 0.575026 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.394 µCi/µg ASDN

886532 dpm/nmol

Assay Date	9/9/2005	Test Chemical ID	RC9	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

									Total volume of stock (mL)	Protein stock ID
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)		
	0.540	0.329	0.154	0.094	0.051	0.038	0.032	25	100	
	0.547	0.335	0.160	0.091	0.050	0.038	0.032			
	0.552	0.338	0.159	0.094	0.053	0.038	0.032			

Samples:	10	100	microsomes
	0.053	0.280	0.058
	0.054	0.284	0.060
			0.057

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	0.546	0.514	0.0481
0.125	100	200	0.00013	200	0.0250	0.334	0.302	0.0279
0.05	200	1000	0.00005	200	0.0100	0.158	0.126	0.0112
0.025	100	1000	0.00003	200	0.0050	0.093	0.061	0.0050
0.01	40	1000	0.00001	200	0.0020	0.051	0.019	0.0011
0.005	20	1000	0.00001	200	0.0010	0.038	0.006	-0.0002

Blank	0.032	r^2=	0.991	
		m=	0.095	
		b=	-0.001	

Variables	Regression results	
m, b	0.095	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.991	0.002
F, df	454	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	0.053	0.021	0.001	200	1	1	0.000	0.000	0.006
10	0.054	0.022	0.001	200	1	1	0.000		
10					1	1			
100	0.280	0.248	0.023	200	1	1	0.000	0.000	0.115
100	0.284	0.252	0.023	200	1	1	0.000		
100									
	0.058	0.026	0.002	200	114	69814	0.005	0.005	5.291
	0.060	0.028	0.002	200	114	69814	0.006		
	0.057	0.025	0.002	200	114	69814	0.005		

Test

Assay Date 9/9/2005 Chemical ID RC9 tested 8

ID	TNB	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.540	0.329	0.154	0.094	0.051	0.038	0.032	25	100	
	0.547	0.335	0.160	0.091	0.050	0.038	0.032			
	0.552	0.338	0.159	0.094	0.053	0.038	0.032			

Samples:	10	100	microsomes
	0.053	0.280	0.058
	0.054	0.284	0.060
			0.057

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	0.546	0.514	0.0420
0.125	100	200	0.00013	200	0.0250	0.334	0.302	0.0248
0.05	200	1000	0.00005	200	0.0100	0.158	0.126	0.0105
0.025	100	1000	0.00003	200	0.0050	0.093	0.061	0.0052
0.01	40	1000	0.00001	200	0.0020	0.051	0.019	0.0018
0.005	20	1000	0.00001	200	0.0010	0.038	0.006	0.0007

Blank 0.032

r^2= 0.999
m= 0.081
b= 0.000

Variables	Regression results	
m, b	0.081	0.000
se_m, se_b	0.002	0.000
r , se_y	0.999	0.000
F, df	2881	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	0.053	0.021	0.002	200	1	1	0.000	0.000	0.010
10	0.054	0.022	0.002	200	1	1	0.000		
10					1	1			
100	0.280	0.248	0.020	200	1	1	0.000	0.000	0.103
100	0.284	0.252	0.021	200	1	1	0.000		
100									
	0.058	0.026	0.002	200	114	69814	0.007	0.007	7.309
	0.060	0.028	0.003	200	114	69814	0.008		
	0.057	0.025	0.002	200	114	69814	0.007		

Assay Date	9/9/2005	Test Chemical ID	RC9	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

								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	9/9/2005	Chemical ID	RC9	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 3

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	7.309
Protein Concentration (dilution added to assay, mg/mL):	0.011935

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	9/9/2005	Test Chemical ID	RC9	# Concentrations tested	8	Microsome type		Microsome ID		0	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	14084.03	28168.06	28146.15	56292.3	0.1	177995	31.63	56143	0.0633	1	0.006	15	0.3537
			0.5	2	14062.12	28124.24			0.1					1		15	
	2	2	0.5	1	13067.12	26134.24	26035.39	52070.78	0.1	177995	29.25	51922	0.0586	1	0.006	15	0.3271
			0.5	2	12968.27	25936.54			0.1					1		15	
	3	2	0.5	1	12804.25	25608.5	25934.24	51868.48	0.1	177995	29.14	51720	0.0583	1	0.006	15	0.3259
			0.5	2	13129.99	26259.98			0.1					1		15	
	4	2	0.5	1	13291.82	26583.64	26559.3	53118.6	0.1	177995	29.84	52970	0.0597	1	0.006	15	0.3338
			0.5	2	13267.48	26534.96			0.1					1		15	
Background control	1	2	0.5	1	34.92	69.84	61.39	122.78	0.1	177995	0.07	-26	0.0000	1	0.006	15	-0.0002
			0.5	2	26.47	52.94			0.1					1		15	
	2	2	0.5	1	39.41	78.82	77.24	154.48	0.1	177995	0.09	6	0.0000	1	0.006	15	0.0000
			0.5	2	37.83	75.66			0.1					1		15	
	3	2	0.5	1	42.7	85.4	84.2	168.4	0.1	177995	0.09	20	0.0000	1	0.006	15	0.0001
			0.5	2	41.5	83			0.1					1		15	
	4	2	0.5	1	36.26	72.52	74.88	149.76	0.1	177995	0.08	1	0.0000	1	0.006	15	0.0000
			0.5	2	38.62	77.24			0.1					1		15	
Positive control	1	2	0.5	1	6827.38	13654.76	13906.15	27812.3	0.1	177995	15.63	27663	0.0312	1	0.006	15	0.1743
			0.5	2	7078.76	14157.52			0.1					1		15	
	2	2	0.5	1	6842.03	13684.06	13769.54	27538.08	0.1	177995	15.47	27390	0.0309	1	0.006	15	0.1726
			0.5	2	6927.51	13855.02			0.1					1		15	
	3	2	0.5	1	6556.28	13112.56	13200.76	26401.52	0.1	177995	14.83	26253	0.0296	1	0.006	15	0.1654
			0.5	2	6644.48	13288.96			0.1					1		15	
	4	2	0.5	1	6628.44	13256.88	13338.54	26677.08	0.1	177995	14.99	26528	0.0299	1	0.006	15	0.1671
			0.5	2	6710.1	13420.2			0.1					1		15	
Negative Control	1	2	0.5	1	13301.11	26602.22	26723.37	53446.74	0.1	177995	30.03	53298	0.0601	1	0.006	15	0.3358
			0.5	2	13422.26	26844.52			0.1					1		15	
	2	2	0.5	1	13471.36	26942.72	27134.39	54268.78	0.1	177995	30.49	54120	0.0610	1	0.006	15	0.3410
			0.5	2	13663.03	27326.06			0.1					1		15	
	3	2	0.5	1	12586.38	25172.76	25039.8	50079.6	0.1	177995	28.14	49931	0.0563	1	0.006	15	0.3146
			0.5	2	12453.42	24906.84			0.1					1		15	
	4	2	0.5	1	11990.69	23981.38	24136.7	48273.4	0.1	177995	27.12	48125	0.0543	1	0.006	15	0.3032
			0.5	2	12146.01	24292.02			0.1					1		15	
RC9	1-1	2	0.5	1	614.03	1228.06	1221.12	2442.24	0.1	177995	1.37	2293	0.0026	1	0.006	15	0.0145
			0.5	2	607.09	1214.18			0.1					1		15	
	1-2	2	0.5	1	553.22	1106.44	1108.32	2216.64	0.1	177995	1.25	2068	0.0023	1	0.006	15	0.0130
			0.5	2	555.1	1110.2			0.1					1		15	
	1-3	2	0.5	1	467.53	935.06	940.73	1881.46	0.1	177995	1.06	1733	0.0020	1	0.006	15	0.0109
			0.5	2	473.2	946.4			0.1					1		15	
	2-1	2	0.5	1	1603.95	3207.9	3181.48	6362.96	0.1	177995	3.57	6214	0.0070	1	0.006	15	0.0392
			0.5	2	1577.53	3155.06			0.1					1		15	
	2-2	2	0.5	1	1552.33	3104.66	3066.17	6132.34	0.1	177995	3.45	5983	0.0067	1	0.006	15	0.0377
			0.5	2	1513.84	3027.68			0.1					1		15	
	2-3	2	0.5	1	1692	3384	3427.62	6855.24	0.1	177995	3.85	6706	0.0076	1	0.006	15	0.0423
			0.5	2	1735.62	3471.24			0.1					1		15	
	3-1	2	0.5	1	4793.29	9586.58	9475.44	18950.88	0.1	177995	10.65	18802	0.0212	1	0.006	15	0.1185
			0.5	2	4682.15	9364.3			0.1					1		15	
	3-2	2	0.5	1	3704.62	7409.24	7467.31	14934.62	0.1	177995	8.39	14786	0.0167	1	0.006	15	0.0932
			0.5	2	3762.69	7525.38			0.1					1		15	
	3-3	2	0.5	1	3812.64	7625.28	7717.52	15435.04	0.1	177995	8.67	15286	0.0172	1	0.006	15	0.0963
			0.5	2	3904.88	7809.76			0.1					1		15	
	4-1	2	0.5	1	7727.29	15454.58	15360	30720	0.1	177995	17.26	30571	0.0345	1	0.006	15	0.1926
			0.5	2	7632.71	15265.42			0.1					1		15	
	4-2	2	0.5	1	8420.11	16840.22	16944.21	33888.42	0.1	177995	19.04	33740	0.0381	1	0.006	15	0.2126
			0.5	2	8524.1	17048.2			0.1					1		15	
	4-3	2	0.5	1	8663.77	17367.54	17231.86	34463.72	0.1	177995	19.36	34315	0.0387	1	0.006	15	0.2162
			0.5	2	8548.09	17096.18			0.1					1		15	
	5-1	2	0.5	1	9338.16	18676.32	18961.96	37923.92	0.1	177995	21.31	37775	0.0426	1	0.006	15	0.2380
			0.5	2	9623.8	19247.6			0.1					1		15	
	5-2	2	0.5	1	9423.52	18847.04	18842.96	37685.92	0.1	177995	21.17	37537	0.0423	1	0.006	15	0.2365
			0.5	2	9419.44	18838.88			0.1					1		15	
	5-3	2	0.5	1	10040.15	20080.3	20195.81	40391.62	0.1	177995	22.69	40243	0.0454	1	0.006	15	0.2536
			0.5	2	10155.66	20311.32			0.1					1		15	
	6-1	2	0.5	1	11957.87	23915.74	23999.59	47999.18	0.1	177995	26.97	47850	0.0540	1	0.006	15	0.3015
			0.5	2	12041.72	24083.44			0.1					1		15	
	6-2	2	0.5	1	11338.54	22677.08	22925.41	45850.82	0.1	177995	25.76	45702	0.0516	1	0.006	15	0.2880
			0.5	2	11586.87	23173.74			0.1					1		15	
	6-3	2	0.5	1	11484.05	22968.1	22906.01	45892.02	0.1	177995	25.84	45843	0.0517	1	0.006	15	0.2888
			0.5	2	11511.96	23023.92			0.1					1		15	
	7-1	2	0.5	1	13236.7	26473.4	26255.63	52511.26	0.1	177995	29.50	52362	0.0591	1	0.006	15	0.3299
			0.5	2	13018.93	26037.86			0.1					1		15	
	7-2	2	0.5	1	13146	26292	26259.72	52519.44	0.1	177995	29.51	52371	0.0591	1	0.006	15	0.3300
			0.5	2	13313.72	26627.44			0.1					1		15	
	7-3	2	0.5	1	12753.75	25507.5	25101.66	50203.32	0.1	177995	28.20	50054	0.0565	1	0.006	15	0.3154
			0.5	2	12347.91	24695.82			0.1					1		15	
	8-1	2	0.5	1	14116.58	28233.16	28226.3	56452.6	0.1	177995	31.72	56304	0.0635	1	0.006	15	0.3548
			0.5	2	14109.72	28219.44			0.1					1		15	
	8-2	2	0.5	1	13640.46	27280.92	27014.63	54029.26	0.1	177995	30.35	53880	0.0608	1	0.006	15	0.3395
			0.5	2	13374.17	26748.34			0.1					1		15	
	8-3	2	0.5	1	13627.15	27254.3	27376.95	54753.9	0.1	177995	30.76	54605	0.0616	1	0.006	15	0.3441
			0.5	2	13749.8	27499.6			0.1					1		15	

Assay Date	9/9/2005	ID	RC9	# Concentrations tested	8	Microsome type	Recombinant	Microsome ID	0	Technician ID	TNB	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3404	0.0188
Full activity	End	0.3298	0.0056
Full activity	Overall	0.3351	0.0129
Background	Beginning	-0.0001	0.000141234
Background	End	0.0001	8.30472E-05
Background	Overall	0.0000	0.000120342
Positive	Beginning	0.1734	0.0012
Positive	End	0.1663	0.0012
Positive	Overall	0.1699	0.0043
Negative	Beginning	0.3384	0.0037
Negative	End	0.3089	0.0080
Negative	Overall	0.3237	0.0178

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC9	1	1	1.00E-03	-3.00	0.0145
RC9	1	2	1.00E-03	-3.00	0.0130
RC9	1	3	1.00E-03	-3.00	0.0109
RC9	2	1	3.00E-04	-3.52	0.0392
RC9	2	2	3.00E-04	-3.52	0.0377
RC9	2	3	3.00E-04	-3.52	0.0423
RC9	3	1	1.00E-04	-4.00	0.1185
RC9	3	2	1.00E-04	-4.00	0.0932
RC9	3	3	1.00E-04	-4.00	0.0963
RC9	4	1	3.00E-05	-4.52	0.1926
RC9	4	2	3.00E-05	-4.52	0.2126
RC9	4	3	3.00E-05	-4.52	0.2162
RC9	5	1	1.00E-05	-5.00	0.2380
RC9	5	2	1.00E-05	-5.00	0.2365
RC9	5	3	1.00E-05	-5.00	0.2536
RC9	6	1	3.00E-06	-5.52	0.3015
RC9	6	2	3.00E-06	-5.52	0.2880
RC9	6	3	3.00E-06	-5.52	0.2888
RC9	7	1	1.00E-06	-6.00	0.3299
RC9	7	2	1.00E-06	-6.00	0.3300
RC9	7	3	1.00E-06	-6.00	0.3154
RC9	8	1	1.00E-08	-8.00	0.3548
RC9	8	2	1.00E-08	-8.00	0.3395
RC9	8	3	1.00E-08	-8.00	0.3441

Percent of control values				
	Log[test	Replicate		
evel	ostance]	1	2	3
1	-3.00	4.31	3.89	3.26
2	-3.52	11.68	11.25	12.61
3	-4.00	35.35	27.80	28.74
4	-4.52	57.48	63.43	64.52
5	-5.00	71.02	70.57	75.66
6	-5.52	89.96	85.92	86.19
7	-6.00	98.45	98.46	94.11
8	-8.00	105.86	101.30	102.66

Aromatase Assay Spreadsheet

Assay Date	9/2/2005	Test Chemical ID	RC10	# Concentrations tested	8
ID	TNB	Replicate #	Rep 1	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0201	37503.44	1865843	
2	0.0202	36666.75	1815186	
3	0.0201	36967.36	1839172	
4	0.0200	36785.67	1839284	
5	0.0201	36360.55	1808983	
			Average DPM/g soln	1833693
			SD	22627
			CV	1.23
			µCi/g soln	0.826

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	8.1344 g
Mass of dilution B used in substrate prep	4.5926 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56459 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00935 µg/g soln.
µg/g soln.

a. µCi/g soln	0.826
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.

= 0.564590 + 0.00935
= 0.573940 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.439 µCi/µg ASDN

915025 dpm/nmol

Test
Assay Date 9/2/2005 Chemical ID RC10 tested 8

ID	TNB	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.608	0.364	0.131	0.080	0.052	0.040	0.043	25	100	
	0.610	0.369	0.132	0.080	0.056	0.046	0.037			
	0.602	0.377	0.135	0.083	0.056	0.042	0.038			

Samples:	10	100	microsomes
	0.067	0.289	0.060
	0.061	0.290	0.064
			0.057

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	0.607	0.568	0.0482	m, b	0.084	0.001
0.125	100	200	0.00013	200	0.0250	0.370	0.331	0.0285	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.133	0.094	0.0087	r , se_y	0.990	0.002
0.025	100	1000	0.00003	200	0.0050	0.081	0.042	0.0043	F, df	412	4
0.01	40	1000	0.00001	200	0.0020	0.055	0.016	0.0021	SS_{reg}, SS_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.043	0.004	0.0012			

Regression results are calculated using the function LINEST

Blank 0.039

r^2= 0.990
m= 0.084
b= 0.001

	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	0.067	0.028	0.003	200	1	1	0.000	0.000	0.015
10	0.061	0.022	0.003	200	1	1	0.000		
10					1	1			
100	0.289	0.250	0.022	200	1	1	0.000	0.000	0.109
100	0.290	0.251	0.022	200	1	1	0.000		
100									
	0.060	0.021	0.003	200	114	69814	0.008	0.008	7.970
	0.064	0.025	0.003	200	114	69814	0.009		
	0.057	0.018	0.002	200	114	69814	0.007		

Test

Assay Date 9/2/2005 Chemical ID RC10 tested 8

ID TNB Replicate # Rep 1 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.608	0.364	0.131	0.080	0.052	0.040	0.043	25	100	
	0.610		0.132	0.080	0.056	0.046	0.037			
	0.602	0.377	0.135	0.083	0.056	0.042	0.038			

Samples:	10	100	microsomes
	0.067	0.289	0.060
	0.061	0.290	0.064
			0.057

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	0.607	0.568	0.0425
0.125	100	200	0.00013	200	0.0250	0.370	0.331	0.0255
0.05	200	1000	0.00005	200	0.0100	0.133	0.094	0.0083
0.025	100	1000	0.00003	200	0.0050	0.081	0.042	0.0046
0.01	40	1000	0.00001	200	0.0020	0.055	0.016	0.0027
0.005	20	1000	0.00001	200	0.0010	0.043	0.004	0.0019

Variables	Regression results	
m, b	0.072	0.002
se_m, se_b	0.004	0.001
r , se_y	0.989	0.001
F, df	260	3
ss_{reg}, ss_{resid}	0.000	0.000

Regression results are calculated using the function LINEST

Blank 0.039

r^2= 0.989
m= 0.072
b= 0.002

	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	0.067	0.028	0.004	200	1	1	0.000	0.000	0.017
10	0.061	0.022	0.003	200	1	1	0.000		
10					1	1			
100	0.289	0.250	0.020	200	1	1	0.000	0.000	0.098
100	0.290	0.251	0.020	200	1	1	0.000		
100									
	0.060	0.021	0.003	200	114	69814	0.010	0.010	9.532
	0.064	0.025	0.003	200	114	69814	0.010		
	0.057	0.018	0.003	200	114	69814	0.009		

Assay Date	9/2/2005	Test Chemical ID	RC10	tested	8		
ID	TNB	Replicate #	Rep 1	Microsome type	Recombinant	Microsome ID	0

								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	9/2/2005	Chemical ID	RC10	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 1

Microsome Dilution Details

Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	9.535
Protein Concentration (dilution added to assay, mg/mL):	0.01557

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	9/2/2005	Test Chemical ID	RC10	# Concentrations tested	8	Microsome type		Microsome ID		0 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	12744.54	25489.08	25510.79	51021.58	0.1	183369	27.82	50671	0.0554	1	0.008	15	0.2371
			0.5	2	12766.25	25532.5			0.1					1		15	
	2	2	0.5	1	13037.76	26075.52	25877.4	51754.8	0.1	183369	28.22	51404	0.0562	1	0.008	15	0.2405
			0.5	2	12839.64	25679.28			0.1					1		15	
	3	2	0.5	1	12773.17	25546.34	25557.39	51114.78	0.1	183369	27.88	50764	0.0555	1	0.008	15	0.2375
			0.5	2	12784.22	25568.44			0.1					1		15	
	4	2	0.5	1	12743.18	25486.36	25496.48	50992.96	0.1	183369	27.81	50642	0.0553	1	0.008	15	0.2370
			0.5	2	12753.3	25506.6			0.1					1		15	
Background control	1	2	0.5	1	39.44	78.88	71.99	143.98	0.1	183369	0.08	-207	-0.0002	1	0.008	15	-0.0010
			0.5	2	32.55	65.1			0.1					1		15	
	2	2	0.5	1	45.34	90.68	90.18	180.36	0.1	183369	0.10	-170	-0.0002	1	0.008	15	-0.0008
			0.5	2	44.84	89.68			0.1					1		15	
	3	2	0.5	1	215.16	430.32	441.3	882.6	0.1	183369	0.48	532	0.0006	1	0.008	15	0.0025
			0.5	2	226.14	452.28			0.1					1		15	
	4	2	0.5	1	52.79	105.58	97.59	195.18	0.1	183369	0.11	-155	-0.0002	1	0.008	15	-0.0007
			0.5	2	44.8	89.6			0.1					1		15	
Positive control	1	2	0.5	1	6754.58	13509.16	13425.7	26851.4	0.1	183369	14.64	26501	0.0290	1	0.008	15	0.1240
			0.5	2	6667.12	13342.24			0.1					1		15	
	2	2	0.5	1	6691.97	13383.94	13332.41	26664.82	0.1	183369	14.54	26314	0.0288	1	0.008	15	0.1231
			0.5	2	6640.44	13280.88			0.1					1		15	
	3	2	0.5	1	6173.07	12346.14	12490.65	24981.3	0.1	183369	13.82	24631	0.0269	1	0.008	15	0.1153
			0.5	2	6317.58	12635.16			0.1					1		15	
	4	2	0.5	1	5941.29	11882.58	11952.92	23905.84	0.1	183369	13.04	23555	0.0257	1	0.008	15	0.1102
			0.5	2	6011.63	12023.26			0.1					1		15	
Negative Control	1	2	0.5	1	13114.71	26229.42	26332.1	52664.2	0.1	183369	28.72	52314	0.0572	1	0.008	15	0.2448
			0.5	2	13217.39	26434.78			0.1					1		15	
	2	2	0.5	1	13279.7	26559.4	26722.98	53445.96	0.1	183369	29.15	53095	0.0580	1	0.008	15	0.2485
			0.5	2	13443.28	26886.56			0.1					1		15	
	3	2	0.5	1	12399.3	24798.6	24927.89	49855.78	0.1	183369	27.19	49505	0.0541	1	0.008	15	0.2317
			0.5	2	12528.59	25057.18			0.1					1		15	
	4	2	0.5	1	11510.16	23020.32	23043.16	46086.32	0.1	183369	25.13	45736	0.0500	1	0.008	15	0.2140
			0.5	2	11533	23066			0.1					1		15	
RC10	1-1	2	0.5	1	10218.27	20436.54	20404.36	40808.72	0.1	183369	22.25	40458	0.0442	1	0.008	15	0.1893
			0.5	2	10186.09	20372.18			0.1					1		15	
	1-2	2	0.5	1	10708.58	21417.18	21299.43	42598.86	0.1	183369	23.23	42248	0.0462	1	0.008	15	0.1977
			0.5	2	10590.84	21181.68			0.1					1		15	
	1-3	2	0.5	1	11165.86	22331.72	22233.87	44467.74	0.1	183369	24.25	44117	0.0482	1	0.008	15	0.2064
			0.5	2	11068.01	22136.02			0.1					1		15	
	2-1	2	0.5	1	13161.16	26322.32	26348.89	52699.78	0.1	183369	28.74	52349	0.0572	1	0.008	15	0.2450
			0.5	2	13188.73	26377.46			0.1					1		15	
	2-2	2	0.5	1	12305.07	24610.14	24463.04	48926.08	0.1	183369	26.68	48576	0.0531	1	0.008	15	0.2273
			0.5	2	12157.97	24315.94			0.1					1		15	
	2-3	2	0.5	1	12724.94	25449.88	25344.56	50689.12	0.1	183369	27.64	50339	0.0550	1	0.008	15	0.2356
			0.5	2	12619.62	25239.24			0.1					1		15	
	3-1	2	0.5	1	13316.96	26633.92	26387.58	52775.16	0.1	183369	28.76	52425	0.0573	1	0.008	15	0.2453
			0.5	2	13070.62	26141.24			0.1					1		15	
	3-2	2	0.5	1	12978.23	25956.46	26049.89	52099.78	0.1	183369	28.41	51749	0.0566	1	0.008	15	0.2422
			0.5	2	13071.66	26143.32			0.1					1		15	
	3-3	2	0.5	1	12661.66	25323.32	25423.7	50847.4	0.1	183369	27.73	50497	0.0552	1	0.008	15	0.2363
			0.5	2	12762.04	25524.08			0.1					1		15	
	4-1	2	0.5	1	13137.54	26275.08	26389.16	52778.32	0.1	183369	28.76	52428	0.0573	1	0.008	15	0.2453
			0.5	2	13251.62	26503.24			0.1					1		15	
	4-2	2	0.5	1	12649.77	25299.54	25647.33	51294.66	0.1	183369	27.97	50944	0.0557	1	0.008	15	0.2384
			0.5	2	12997.56	25995.12			0.1					1		15	
	4-3	2	0.5	1	12706.69	25417.38	25621.31	51242.62	0.1	183369	27.95	50892	0.0556	1	0.008	15	0.2381
			0.5	2	12912.62	25825.24			0.1					1		15	
	5-1	2	0.5	1	12935.42	25870.84	25589.42	51178.84	0.1	183369	27.91	50828	0.0555	1	0.008	15	0.2378
			0.5	2	12654	25308			0.1					1		15	
	5-2	2	0.5	1	12684.31	25368.62	25601.11	51202.22	0.1	183369	27.92	50852	0.0556	1	0.008	15	0.2380
			0.5	2	12916.8	25833.6			0.1					1		15	
	5-3	2	0.5	1	13107.65	26215.7	26149.18	52296.36	0.1	183369	28.52	51948	0.0568	1	0.008	15	0.2431
			0.5	2	13041.33	26082.66			0.1					1		15	
	6-1	2	0.5	1	13337.45	26674.9	26672.93	53345.86	0.1	183369	29.09	52995	0.0579	1	0.008	15	0.2480
			0.5	2	13335.48	26670.96			0.1					1		15	
	6-2	2	0.5	1	13065.04	26130.08	26234.06	52468.12	0.1	183369	28.61	52118	0.0570	1	0.008	15	0.2439
			0.5	2	13169.02	26338.04			0.1					1		15	
	6-3	2	0.5	1	12936.73	25873.46	26172.14	52344.28	0.1	183369	28.55	51994	0.0568	1	0.008	15	0.2433
			0.5	2	13235.41	26470.82			0.1					1		15	
	7-1	2	0.5	1	12782.34	25564.68	25172.62	50345.24	0.1	183369	27.46	49995	0.0546	1	0.008	15	0.2339
			0.5	2	12390.28	24780.56			0.1					1		15	
	7-2	2	0.5	1	13074.53	26149.06	26167.82	52335.64	0.1	183369	28.54	51985	0.0568	1	0.008	15	0.2433
			0.5	2	13093.29	26186.58			0.1					1		15	
	7-3	2	0.5	1	12966.86	25933.72	26239.28	52478.56	0.1	183369	28.62	52128	0.0570	1	0.008	15	0.2439
			0.5	2	13272.42	26544.84			0.1					1		15	
	8-1	2	0.5	1	12788.24	25576.48	25300.56	50601.12	0.1	183369	27.60	50251	0.0549	1	0.008	15	0.2351
			0.5	2	12512.32	25024.64			0.1					1		15	
	8-2	2	0.5	1	12713.14	25426.28	25440.25	50880.5	0.1	183369	27.75	50530	0.0552	1	0.008	15	0.2365
			0.5	2	12727.11	25454.22			0.1					1		15	
	8-3	2	0.5	1	12430.72	24861.44	24567.64	49175.28	0.1	183369	26.82	48825	0.0534	1	0.008	15	0.2285
			0.5	2	12156.92	24313.84			0.1					1		15	

Assay Date	9/2/2005	ID	RC10	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0 Technician ID	TNB	Replicate #	Rep 1

Control Type	Portion	Average	SD
Full activity	Beginning	0.2388	0.0024
Full activity	End	0.2373	0.0004
Full activity	Overall	0.2380	0.0017
Background	Beginning	-0.0009	0.000120376
Background	End	0.0009	0.002274573
Background	Overall	0.0000	0.001662904
Positive	Beginning	0.1236	0.0006
Positive	End	0.1127	0.0036
Positive	Overall	0.1182	0.0066
Negative	Beginning	0.2466	0.0026
Negative	End	0.2228	0.0125
Negative	Overall	0.2347	0.0156

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC10	1	1	1.00E-03	-3.00	0.1893
RC10	1	2	1.00E-03	-3.00	0.1977
RC10	1	3	1.00E-03	-3.00	0.2064
RC10	2	1	1.00E-04	-4.00	0.2450
RC10	2	2	1.00E-04	-4.00	0.2273
RC10	2	3	1.00E-04	-4.00	0.2356
RC10	3	1	1.00E-05	-5.00	0.2453
RC10	3	2	1.00E-05	-5.00	0.2422
RC10	3	3	1.00E-05	-5.00	0.2363
RC10	4	1	1.00E-06	-6.00	0.2453
RC10	4	2	1.00E-06	-6.00	0.2384
RC10	4	3	1.00E-06	-6.00	0.2381
RC10	5	1	1.00E-07	-7.00	0.2378
RC10	5	2	1.00E-07	-7.00	0.2380
RC10	5	3	1.00E-07	-7.00	0.2431
RC10	6	1	1.00E-08	-8.00	0.2480
RC10	6	2	1.00E-08	-8.00	0.2439
RC10	6	3	1.00E-08	-8.00	0.2433
RC10	7	1	1.00E-09	-9.00	0.2339
RC10	7	2	1.00E-09	-9.00	0.2433
RC10	7	3	1.00E-09	-9.00	0.2439
RC10	8	1	1.00E-10	-10.00	0.2351
RC10	8	2	1.00E-10	-10.00	0.2365
RC10	8	3	1.00E-10	-10.00	0.2285

	Percent of control values			
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	79.53	83.05	86.72
2	-4.00	102.91	95.49	98.95
3	-5.00	103.06	101.73	99.27
4	-6.00	103.06	100.14	100.04
5	-7.00	99.92	99.96	102.12
6	-8.00	104.18	102.45	102.21
7	-9.00	98.28	102.19	102.47
8	-10.00	98.78	99.33	95.98

Aromatase Assay Spreadsheet

Assay Date	9/7/2005	Test Chemical ID	RC10	# Concentrations tested	8
ID	TNB	Replicate #	Rep 2	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0199	27485.55	1381183	
2	0.0203	28586.07	1408181	
3	0.0201	28589.92	1422384	
4	0.0202	28892.83	1430338	
5	0.0202	28994.39	1435366	
			Average DPM/g soln	1415490
			SD	21757
			CV	1.54
			µCi/g soln	0.638

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	8.0776 g
Mass of dilution B used in substrate prep	4.571 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.565886 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00722 µg/g soln.
µg/g soln.

a. µCi/g soln	0.638
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.

= 0.565886 + 0.00722
= 0.573104 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.113 µCi/µg ASDN

707370 dpm/nmol

Assay Date	9/7/2005	Test Chemical ID	RC10	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

									Total volume of	
Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	stock (mL)	Protein stock ID
	0.571	0.346	0.125	0.075	0.046	0.037	0.030	25	100	
	0.580	0.355	0.128	0.076	0.044	0.037	0.030			
	0.573	0.357	0.127	0.073	0.046	0.037	0.031			

Samples:	10	100	microsomes
	0.051	0.283	0.050
	0.053	0.287	0.051
			0.051

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	0.575	0.544	0.0481	m, b	0.087	0.001
0.125	100	200	0.00013	200	0.0250	0.353	0.322	0.0287	se_m, se_b	0.004	0.001
0.05	200	1000	0.00005	200	0.0100	0.127	0.096	0.0089	r , se_y	0.990	0.002
0.025	100	1000	0.00003	200	0.0050	0.075	0.044	0.0044	F, df	382	4
0.01	40	1000	0.00001	200	0.0020	0.045	0.015	0.0018	ss_{reg}, ss_{resid}	0.002	0.000
0.005	20	1000	0.00001	200	0.0010	0.037	0.007	0.0011			

Regression results are calculated using the function LINEST

Blank 0.030

r^2= 0.990
m= 0.087
b= 0.001

	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	0.051	0.021	0.002	200	1	1	0.000	0.000	0.012
10	0.053	0.023	0.003	200	1	1	0.000		
10					1	1			
100	0.283	0.253	0.023	200	1	1	0.000	0.000	0.114
100	0.287	0.257	0.023	200	1	1	0.000		
100									
	0.050	0.020	0.002	200	114	69814	0.007	0.007	7.031
	0.051	0.021	0.002	200	114	69814	0.007		
	0.051	0.021	0.002	200	114	69814	0.007		

Test

Assay Date 9/7/2005 Chemical ID RC10 tested 8

ID TNB Replicate # Rep 2 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.571	0.346	0.125	0.075	0.046	0.037	0.030	25	100	
	0.580	0.355	0.128	0.076	0.044	0.037	0.030			
	0.573	0.357	0.127	0.073	0.046	0.037	0.031			

Samples:	10	100	microsomes
	0.051	0.283	0.050
	0.053	0.287	0.051
			0.051

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	0.0420
0.125	100	200	0.00013	200	0.0250	0.353	0.322	0.0254
0.05	200	1000	0.00005	200	0.0100	0.127	0.096	0.0086
0.025	100	1000	0.00003	200	0.0050	0.075	0.044	0.0047
0.01	40	1000	0.00001	200	0.0020	0.045	0.015	0.0025
0.005	20	1000	0.00001	200	0.0010	0.037	0.007	0.0019

Blank	0.030	r^2=	0.991	
		m=	0.075	
		b=	0.001	

Variables	Regression results	
m, b	0.075	0.001
se_m, se_b	0.004	0.001
r , se_y	0.991	0.001
F, df	342	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	0.051	0.021	0.003	200	1	1	0.000	0.000	0.015
10	0.053	0.023	0.003	200	1	1	0.000		
10					1	1			
100	0.283	0.253	0.020	200	1	1	0.000	0.000	0.102
100	0.287	0.257	0.021	200	1	1	0.000		
100									
	0.050	0.020	0.003	200	114	69814	0.009	0.009	8.813
	0.051	0.021	0.003	200	114	69814	0.009		
	0.051	0.021	0.003	200	114	69814	0.009		

Assay Date	9/7/2005	Test Chemical ID	RC10	tested	8		
ID	TNB	Replicate #	Rep 2	Microsome type	Recombinant	Microsome ID	0

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	9/7/2005	Chemical ID	RC10	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 2

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

Protein Concentration (stock microsomes, mg/mL):	8.813
Protein Concentration (dilution added to assay, mg/mL):	0.014391

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	9/7/2005	Test Chemical ID	RC10	# Concentrations tested		β Microsome type		Microsome ID		α 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	7590.54	15181.08	15080.97	30161.94	0.1	141549	21.31	29997	0.0424	1	0.007	15	0.1984
			0.5	2	7490.43	14980.86			0.1					1		15	
	2	2	0.5	1	7517.7	15035.4	14928.95	29857.9	0.1	141549	21.09	29693	0.0420	1	0.007	15	0.1945
			0.5	2	7411.25	14822.5			0.1					1		15	
	3	2	0.5	1	6906.01	13812.02	14068.55	28137.1	0.1	141549	19.88	27972	0.0395	1	0.007	15	0.1832
			0.5	2	7162.54	14325.08			0.1					1		15	
	4	2	0.5	1	7170.17	14340.34	14392.02	28784.04	0.1	141549	20.34	28619	0.0405	1	0.007	15	0.1874
			0.5	2	7221.85	14443.7			0.1					1		15	
Background control	1	2	0.5	1	35.78	71.56	58.38	116.76	0.1	141549	0.08	-48	-0.0001	1	0.007	15	-0.0003
			0.5	2	22.6	45.2			0.1					1		15	
	2	2	0.5	1	50.25	100.5	106.62	213.24	0.1	141549	0.15	48	0.0001	1	0.007	15	0.0003
			0.5	2	56.37	112.74			0.1					1		15	
	3	2	0.5	1	40.97	81.94	74.27	148.54	0.1	141549	0.10	-17	0.0000	1	0.007	15	-0.0001
			0.5	2	33.3	66.6			0.1					1		15	
	4	2	0.5	1	40.31	80.62	91.44	182.88	0.1	141549	0.13	18	0.0000	1	0.007	15	0.0001
			0.5	2	51.13	102.26			0.1					1		15	
Positive control	1	2	0.5	1	4152.24	8304.48	8322.38	16644.76	0.1	141549	11.76	16479	0.0233	1	0.007	15	0.1079
			0.5	2	4170.14	8340.28			0.1					1		15	
	2	2	0.5	1	4225.46	8450.92	8299.03	16598.06	0.1	141549	11.73	16433	0.0232	1	0.007	15	0.1076
			0.5	2	4073.57	8147.14			0.1					1		15	
	3	2	0.5	1	3954.93	7909.86	7911.57	15823.14	0.1	141549	11.18	15658	0.0221	1	0.007	15	0.1025
			0.5	2	3956.64	7913.28			0.1					1		15	
	4	2	0.5	1	3977.78	7955.56	7885.53	15771.06	0.1	141549	11.14	15606	0.0221	1	0.007	15	0.1022
			0.5	2	3907.75	7815.5			0.1					1		15	
Negative Control	1	2	0.5	1	7155.47	14310.94	14352.28	28704.56	0.1	141549	20.28	28539	0.0403	1	0.007	15	0.1869
			0.5	2	7196.81	14393.62			0.1					1		15	
	2	2	0.5	1	7232.2	14464.4	14379.97	28759.54	0.1	141549	20.32	28595	0.0404	1	0.007	15	0.1873
			0.5	2	7147.77	14295.54			0.1					1		15	
	3	2	0.5	1	6807.02	13614.04	13601	27202	0.1	141549	19.22	27037	0.0382	1	0.007	15	0.1771
			0.5	2	6793.98	13587.96			0.1					1		15	
	4	2	0.5	1	6876.03	13752.06	13491.23	26982.46	0.1	141549	19.06	26817	0.0379	1	0.007	15	0.1756
			0.5	2	6615.2	13230.4			0.1					1		15	
RC10	1-1	2	0.5	1	6554.23	13108.46	13086.38	26172.76	0.1	141549	18.49	26007	0.0368	1	0.007	15	0.1703
			0.5	2	6532.15	13064.3			0.1					1		15	
	1-2	2	0.5	1	6563.19	13126.38	13167.57	26335.14	0.1	141549	18.60	26170	0.0370	1	0.007	15	0.1714
			0.5	2	6604.38	13208.76			0.1					1		15	
	1-3	2	0.5	1	6432.16	12864.32	12723.81	25447.62	0.1	141549	17.98	25282	0.0357	1	0.007	15	0.1656
			0.5	2	6291.65	12583.3			0.1					1		15	
	2-1	2	0.5	1	6836.64	13673.28	13904.03	27808.06	0.1	141549	19.65	27643	0.0391	1	0.007	15	0.1810
			0.5	2	7067.39	14134.78			0.1					1		15	
	2-2	2	0.5	1	6651.42	13302.84	13498.04	26996.08	0.1	141549	19.07	26831	0.0379	1	0.007	15	0.1757
			0.5	2	6846.62	13693.24			0.1					1		15	
	2-3	2	0.5	1	7151.53	14303.06	14359.49	28718.98	0.1	141549	20.29	28554	0.0404	1	0.007	15	0.1870
			0.5	2	7207.96	14415.92			0.1					1		15	
	3-1	2	0.5	1	7219.25	14438.5	14522.03	29044.06	0.1	141549	20.52	28879	0.0408	1	0.007	15	0.1891
			0.5	2	7302.78	14605.56			0.1					1		15	
	3-2	2	0.5	1	7026.76	14053.52	14216.89	28433.78	0.1	141549	20.09	28268	0.0400	1	0.007	15	0.1851
			0.5	2	7180.13	14360.26			0.1					1		15	
	3-3	2	0.5	1	7259.24	14518.48	14482.92	28965.84	0.1	141549	20.46	28800	0.0407	1	0.007	15	0.1886
			0.5	2	7223.68	14447.36			0.1					1		15	
	4-1	2	0.5	1	7250.86	14501.72	14403.3	28806.6	0.1	141549	20.35	28641	0.0405	1	0.007	15	0.1876
			0.5	2	7152.44	14304.88			0.1					1		15	
	4-2	2	0.5	1	6950.08	13900.16	13953.41	27906.82	0.1	141549	19.76	27801	0.0393	1	0.007	15	0.1821
			0.5	2	7033.33	14066.66			0.1					1		15	
	4-3	2	0.5	1	7167.38	14334.76	14305.7	28611.4	0.1	141549	20.21	28446	0.0402	1	0.007	15	0.1863
			0.5	2	7138.32	14276.64			0.1					1		15	
	5-1	2	0.5	1	7276.11	14552.22	14496.13	28992.26	0.1	141549	20.48	28827	0.0408	1	0.007	15	0.1888
			0.5	2	7220.02	14440.04			0.1					1		15	
	5-2	2	0.5	1	7285.4	14570.8	14409.33	28818.66	0.1	141549	20.36	28653	0.0405	1	0.007	15	0.1877
			0.5	2	7123.93	14247.86			0.1					1		15	
	5-3	2	0.5	1	7639.42	15278.84	14949.18	29898.36	0.1	141549	21.12	29733	0.0420	1	0.007	15	0.1947
			0.5	2	7309.76	14619.52			0.1					1		15	
	6-1	2	0.5	1	7154.33	14308.66	14212.5	28425	0.1	141549	20.08	28260	0.0400	1	0.007	15	0.1851
			0.5	2	7058.17	14116.34			0.1					1		15	
	6-2	2	0.5	1	7409.48	14818.96	14848.45	29696.9	0.1	141549	20.98	29532	0.0417	1	0.007	15	0.1934
			0.5	2	7438.97	14877.94			0.1					1		15	
	6-3	2	0.5	1	6842.29	13684.58	13917.34	27834.68	0.1	141549	19.66	27669	0.0391	1	0.007	15	0.1812
			0.5	2	7075.05	14150.1			0.1					1		15	
	7-1	2	0.5	1	7262.48	14524.96	14638.4	29276.8	0.1	141549	20.68	29111	0.0412	1	0.007	15	0.1907
			0.5	2	7375.92	14751.84			0.1					1		15	
	7-2	2	0.5	1	7136.72	14273.44	14368.02	28776.04	0.1	141549	20.33	28611	0.0404	1	0.007	15	0.1874
			0.5	2	7251.3	14502.6			0.1					1		15	
	7-3	2	0.5	1	7270.62	14541.24	14401.33	28802.66	0.1	141549	20.35	28637	0.0405	1	0.007	15	0.1875
			0.5	2	7130.71	14261.42			0.1					1		15	
	8-1	2	0.5	1	7216.85	14437.7	14329.67	28659.34	0.1	141549	20.25	28494	0.0403	1	0.007	15	0.1866
			0.5	2	7110.82	14221.64			0.1					1		15	
	8-2	2	0.5	1	7113.13	14226.26	14328.48	28656.96	0.1	141549	20.25	28492	0.0403	1	0.007	15	0.1866
			0.5	2	7215.35	14430.7			0.1					1		15	
	8-3	2	0.5	1	7211.63	14423.26	14522.5	29045	0.1	141549	20.52	28880	0.0408	1	0.007	15	0.1891
			0.5	2	7310.87	14621.74			0.1					1		15	

Assay Date	9/7/2005	ID	RC10	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0 Technician ID	TNB	Replicate #	Rep 2

Control Type	Portion	Average	SD
Full activity	Beginning	0.1955	0.0014
Full activity	End	0.1853	0.0030
Full activity	Overall	0.1904	0.0062
Background	Beginning	0.0000	0.000446785
Background	End	0.0000	0.000159023
Background	Overall	0.0000	0.000273817
Positive	Beginning	0.1078	0.0002
Positive	End	0.1024	0.0002
Positive	Overall	0.1051	0.0031
Negative	Beginning	0.1871	0.0003
Negative	End	0.1763	0.0010
Negative	Overall	0.1817	0.0062

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC10	1	1	1.00E-03	-3.00	0.1703
RC10	1	2	1.00E-03	-3.00	0.1714
RC10	1	3	1.00E-03	-3.00	0.1656
RC10	2	1	1.00E-04	-4.00	0.1810
RC10	2	2	1.00E-04	-4.00	0.1757
RC10	2	3	1.00E-04	-4.00	0.1870
RC10	3	1	1.00E-05	-5.00	0.1891
RC10	3	2	1.00E-05	-5.00	0.1851
RC10	3	3	1.00E-05	-5.00	0.1886
RC10	4	1	1.00E-06	-6.00	0.1876
RC10	4	2	1.00E-06	-6.00	0.1821
RC10	4	3	1.00E-06	-6.00	0.1863
RC10	5	1	1.00E-07	-7.00	0.1888
RC10	5	2	1.00E-07	-7.00	0.1877
RC10	5	3	1.00E-07	-7.00	0.1947
RC10	6	1	1.00E-08	-8.00	0.1851
RC10	6	2	1.00E-08	-8.00	0.1934
RC10	6	3	1.00E-08	-8.00	0.1812
RC10	7	1	1.00E-09	-9.00	0.1907
RC10	7	2	1.00E-09	-9.00	0.1874
RC10	7	3	1.00E-09	-9.00	0.1875
RC10	8	1	1.00E-10	-10.00	0.1866
RC10	8	2	1.00E-10	-10.00	0.1866
RC10	8	3	1.00E-10	-10.00	0.1891

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	89.47	90.02	86.97
2	-4.00	95.09	92.30	98.22
3	-5.00	99.34	97.24	99.07
4	-6.00	98.53	95.64	97.85
5	-7.00	99.16	98.57	102.28
6	-8.00	97.21	101.59	95.18
7	-9.00	100.14	98.42	98.51
8	-10.00	98.02	98.01	99.35

Aromatase Assay Spreadsheet

Assay Date	9/9/2005	Test Chemical ID	RC10	# Concentrations tested	8
ID	TNB	Replicate #	Rep 3	Microsome type	Microsome ID

Aliquot #	Weight of aliquot (g)	DPM/Aliq.	DPM/g soln.	
1	0.0202	36051.51	1784728	
2	0.0205	37130.69	1811253	
3	0.0207	36549.05	1765655	
4	0.0206	36695.27	1781324	
5	0.0204	35839.02	1756815	
			Average DPM/g soln	1779955
			SD	20882
			CV	1.17
			µCi/g soln	0.802

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	8.1122 g
Mass of dilution B used in substrate prep	4.5911 g
Concentration of nonradiolabeled ASDN in substrate soln.	0.56595 µg/g

Calculation of Substrate Solution Specific Activity

1) Calculate µg [^{3}H]ASDN/g soln. = 0.00908 µg/g soln.
µg/g soln.

a. µCi/g soln	0.802
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.

= 0.565950 + 0.00908
= 0.575026 µg ASDN/g soln.

3) Calculate Solution Specific Activity

= (µCi/g soln.)/(µg ASDN/g soln.)
= 1.394 µCi/µg ASDN

886532 dpm/nmol

Test

Assay Date 9/9/2005 Chemical ID RC10 tested 8

ID TNB Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)	Protein stock ID
	0.540	0.329	0.154	0.094	0.051	0.038	0.032	25	100	
	0.547	0.335	0.160	0.091	0.050	0.038	0.032			
	0.552	0.338	0.159	0.094	0.053	0.038	0.032			

Samples:	10	100	microsomes
	0.053	0.280	0.058
	0.054	0.284	0.060
			0.057

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	0.546	0.514	0.0481
0.125	100	200	0.00013	200	0.0250	0.334	0.302	0.0279
0.05	200	1000	0.00005	200	0.0100	0.158	0.126	0.0112
0.025	100	1000	0.00003	200	0.0050	0.093	0.061	0.0050
0.01	40	1000	0.00001	200	0.0020	0.051	0.019	0.0011
0.005	20	1000	0.00001	200	0.0010	0.038	0.006	-0.0002

Blank 0.032

r^2= 0.991
m= 0.095
b= -0.001

Variables	Regression results	
m, b	0.095	-0.001
se_m, se_b	0.004	0.001
r , se_y	0.991	0.002
F, df	454	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	0.053	0.021	0.001	200	1	1	0.000	0.000	0.006
10	0.054	0.022	0.001	200	1	1	0.000		
10					1	1			
100	0.280	0.248	0.023	200	1	1	0.000	0.000	0.115
100	0.284	0.252	0.023	200	1	1	0.000		
100									
	0.058	0.026	0.002	200	114	69814	0.005	0.005	5.291
	0.060	0.028	0.002	200	114	69814	0.006		
	0.057	0.025	0.002	200	114	69814	0.005		

Test

Assay Date 9/9/2005 Chemical ID RC10 tested 8

ID TNB Replicate # Rep 3 Microsome type Recombinant Microsome ID 0

Standards:	0.25	0.125	0.05	0.025	0.01	0.005	0	BSA)	Total volume of stock (mL)
	0.540	0.329	0.154	0.094	0.051	0.038	0.032	25	100
	0.547	0.335	0.160	0.091	0.050	0.038	0.032		
	0.552	0.338	0.159	0.094	0.053	0.038	0.032		

Protein stock ID

Samples:	10	100	microsomes								
	0.053	0.280	0.058								
	0.054	0.284	0.060								
			0.057								

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	0.546	0.514	0.0420
0.125	100	200	0.00013	200	0.0250	0.334	0.302	0.0248
0.05	200	1000	0.00005	200	0.0100	0.158	0.126	0.0105
0.025	100	1000	0.00003	200	0.0050	0.093	0.061	0.0052
0.01	40	1000	0.00001	200	0.0020	0.051	0.019	0.0018
0.005	20	1000	0.00001	200	0.0010	0.038	0.006	0.0007

Blank 0.032

r^2= 0.999
m= 0.081
b= 0.000

Variables	Regression results	
m, b	0.081	0.000
se_m, se_b	0.002	0.000
r , se_y	0.999	0.000
F, df	2881	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	0.053	0.021	0.002	200	1	1	0.000	0.000	0.010
10	0.054	0.022	0.002	200	1	1	0.000		
10					1	1			
100	0.280	0.248	0.020	200	1	1	0.000	0.000	0.103
100	0.284	0.252	0.021	200	1	1	0.000		
100									
	0.058	0.026	0.002	200	114	69814	0.007	0.007	7.309
	0.060	0.028	0.003	200	114	69814	0.008		
	0.057	0.025	0.002	200	114	69814	0.007		

Assay Date	9/9/2005	Test Chemical ID	RC10	tested	8		
ID	TNB	Replicate #	Rep 3	Microsome type	Recombinant	Microsome ID	0

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	9/9/2005	Chemical ID	RC10	# Concentrations tested	8	Microsome type		Microsome ID	0	Technician ID	TNB	Replicate #	Rep 3

Microsome Dilution Details		
Dilution A	0.114	mL microsome Stock used
	69.814	mL total volume
	612.4035	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
	612.4035	total dilution factor

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

Protein Concentration (stock microsomes, mg/mL):	7.309
Protein Concentration (dilution added to assay, mg/mL):	0.011935

Assay Date	9/8/2005	Test Chemical ID	RC10	# Concentrations tested	8	Microsome type		Microsome ID	0	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	14084.03	28168.06	28146.15	56292.3	0.1	177995	31.63	56143	0.0633	1	0.006	15	0.3537
			0.5	2	14062.12	28124.24			0.1					1		15	
	2	2	0.5	1	13967.12	26134.24	26035.39	52070.78	0.1	177995	29.25	51922	0.0586	1	0.006	15	0.3271
			0.5	2	12968.27	25936.54			0.1					1		15	
	3	2	0.5	1	12804.25	25608.5	25934.24	51868.48	0.1	177995	29.14	51720	0.0583	1	0.006	15	0.3259
			0.5	2	13129.99	26259.98			0.1					1		15	
	4	2	0.5	1	13291.82	26583.64	26559.3	53118.6	0.1	177995	29.84	52970	0.0597	1	0.006	15	0.3336
			0.5	2	13267.48	26534.96			0.1					1		15	
Background control	1	2	0.5	1	34.92	69.84	61.39	122.78	0.1	177995	0.07	-26	0.0000	1	0.006	15	-0.0002
			0.5	2	26.47	52.94			0.1					1		15	
	2	2	0.5	1	39.41	78.82	77.24	154.48	0.1	177995	0.09	6	0.0000	1	0.006	15	0.0000
			0.5	2	37.83	75.66			0.1					1		15	
	3	2	0.5	1	42.7	85.4	84.2	168.4	0.1	177995	0.09	20	0.0000	1	0.006	15	0.0001
			0.5	2	41.5	83			0.1					1		15	
	4	2	0.5	1	36.26	72.52	74.88	149.76	0.1	177995	0.08	1	0.0000	1	0.006	15	0.0000
			0.5	2	38.62	77.24			0.1					1		15	
Positive control	1	2	0.5	1	6827.39	13654.78	13906.15	27812.3	0.1	177995	15.63	27663	0.0312	1	0.006	15	0.1743
			0.5	2	7078.76	14157.52			0.1					1		15	
	2	2	0.5	1	6842.03	13684.06	13769.54	27539.08	0.1	177995	15.47	27390	0.0309	1	0.006	15	0.1728
			0.5	2	6927.51	13855.02			0.1					1		15	
	3	2	0.5	1	6556.28	13112.56	13200.76	26401.52	0.1	177995	14.83	26253	0.0296	1	0.006	15	0.1654
			0.5	2	6644.48	13288.96			0.1					1		15	
	4	2	0.5	1	6628.44	13256.88	13338.54	26677.08	0.1	177995	14.99	26528	0.0299	1	0.006	15	0.1671
			0.5	2	6710.1	13420.2			0.1					1		15	
Negative Control	1	2	0.5	1	13301.11	26602.22	26723.37	53446.74	0.1	177995	30.03	53298	0.0601	1	0.006	15	0.3358
			0.5	2	13422.26	26844.52			0.1					1		15	
	2	2	0.5	1	13471.36	26942.72	27134.39	54268.78	0.1	177995	30.49	54120	0.0610	1	0.006	15	0.3410
			0.5	2	13663.03	27326.06			0.1					1		15	
	3	2	0.5	1	12586.38	25172.76	25039.8	50079.6	0.1	177995	28.14	49931	0.0563	1	0.006	15	0.3146
			0.5	2	12453.42	24906.84			0.1					1		15	
	4	2	0.5	1	11990.69	23981.38	24136.7	48273.4	0.1	177995	27.12	48125	0.0543	1	0.006	15	0.3032
			0.5	2	12146.01	24292.02			0.1					1		15	
RC10	1-1	2	0.5	1	11433.17	22866.34	23224.67	46449.74	0.1	177995	26.10	46301	0.0522	1	0.006	15	0.2917
			0.5	2	11791.7	23583.4			0.1					1		15	
	1-2	2	0.5	1	11632.52	23265.04	23438.77	46877.54	0.1	177995	26.34	46729	0.0527	1	0.006	15	0.2944
			0.5	2	11606.25	23212.5			0.1					1		15	
	1-3	2	0.5	1	11561.39	23162.78	23047.53	46095.06	0.1	177995	25.90	45946	0.0518	1	0.006	15	0.2895
			0.5	2	11466.14	22932.28			0.1					1		15	
	2-1	2	0.5	1	13173.81	26347.62	26188.89	52377.78	0.1	177995	29.43	52229	0.0589	1	0.006	15	0.3291
			0.5	2	13015.08	26030.16			0.1					1		15	
	2-2	2	0.5	1	13232.92	26465.84	26356.82	52713.64	0.1	177995	29.62	52565	0.0593	1	0.006	15	0.3312
			0.5	2	13123.9	26247.8			0.1					1		15	
	2-3	2	0.5	1	12741.43	25482.86	25388.59	50777.18	0.1	177995	28.53	50628	0.0571	1	0.006	15	0.3190
			0.5	2	12647.16	25294.32			0.1					1		15	
	3-1	2	0.5	1	14044.14	28088.28	27896.03	55792.06	0.1	177995	31.34	55643	0.0628	1	0.006	15	0.3506
			0.5	2	13851.89	27703.78			0.1					1		15	
	3-2	2	0.5	1	13824.55	27649.1	28030.02	56180.04	0.1	177995	31.56	56031	0.0632	1	0.006	15	0.3530
			0.5	2	14265.47	28530.94			0.1					1		15	
	3-3	2	0.5	1	13585.06	27170.12	27360.95	54721.9	0.1	177995	30.74	54573	0.0616	1	0.006	15	0.3438
			0.5	2	13775.89	27551.78			0.1					1		15	
	4-1	2	0.5	1	14448.45	28896.9	28746.64	57493.28	0.1	177995	32.30	57344	0.0647	1	0.006	15	0.3613
			0.5	2	14298.19	28596.38			0.1					1		15	
	4-2	2	0.5	1	13762.05	27564.1	27625.73	55251.48	0.1	177995	31.04	55103	0.0622	1	0.006	15	0.3472
			0.5	2	13843.68	27687.36			0.1					1		15	
	4-3	2	0.5	1	13106.35	26212.7	26510.3	53020.6	0.1	177995	29.79	52872	0.0596	1	0.006	15	0.3331
			0.5	2	13403.95	26807.9			0.1					1		15	
	5-1	2	0.5	1	13621.64	27243.28	27345.78	54691.56	0.1	177995	30.73	54543	0.0615	1	0.006	15	0.3437
			0.5	2	13724.14	27448.28			0.1					1		15	
	5-2	2	0.5	1	13381.69	26763.78	26253.68	52507.36	0.1	177995	29.50	52359	0.0591	1	0.006	15	0.3299
			0.5	2	12871.79	25743.58			0.1					1		15	
	5-3	2	0.5	1	13839.63	27679.26	28027.91	56055.82	0.1	177995	31.49	55907	0.0631	1	0.006	15	0.3523
			0.5	2	14088.28	28176.56			0.1					1		15	
	6-1	2	0.5	1	14216.29	28432.58	28451.52	56903.04	0.1	177995	31.97	56754	0.0640	1	0.006	15	0.3576
			0.5	2	14235.23	28470.46			0.1					1		15	
	6-2	2	0.5	1	13342.08	26684.16	26713.88	53427.76	0.1	177995	30.02	53279	0.0601	1	0.006	15	0.3357
			0.5	2	13371.8	26743.6			0.1					1		15	
	6-3	2	0.5	1	13547.05	27094.1	26935.33	53870.66	0.1	177995	30.27	53722	0.0606	1	0.006	15	0.3385
			0.5	2	13388.28	26776.56			0.1					1		15	
	7-1	2	0.5	1	13470.28	26940.52	26884.55	53769.1	0.1	177995	30.21	53620	0.0605	1	0.006	15	0.3378
			0.5	2	13414.29	26828.56			0.1					1		15	
	7-2	2	0.5	1	13794.48	27588.96	27527.24	55054.48	0.1	177995	30.93	54906	0.0619	1	0.006	15	0.3459
			0.5	2	13732.76	27465.52			0.1					1		15	
	7-3	2	0.5	1	13805.71	27611.42	27573	55146	0.1	177995	30.98	54997	0.0620	1	0.006	15	0.3465
			0.5	2	13767.29	27534.58			0.1					1		15	
	8-1	2	0.5	1	13592.15	27184.3	27299.87	54599.74	0.1	177995	30.67	54451	0.0614	1	0.006	15	0.3431
			0.5	2	13707.72	27415.44			0.1					1		15	
	8-2	2	0.5	1	13424.66	26848.32	26760.37	53520.74	0.1	177995	30.07	53372	0.0602	1	0.006	15	0.3363
			0.5	2	13335.71	26671.42			0.1					1		15	
	8-3	2	0.5	1	13540.54	27081.08	26846.63	53693.26	0.1	177995	30.17	53544	0.0604	1	0.006	15	0.3374
			0.5	2	13306.09	26612.18			0.1					1		15	

Assay Date	9/9/2005	ID	RC10	# Concentrations tested	Microsome 8 type	Recombinant	Microsome ID	0	Technician ID	TNB	Replicate #	Rep 3

Control Type	Portion	Average	SD
Full activity	Beginning	0.3404	0.0188
Full activity	End	0.3298	0.0056
Full activity	Overall	0.3351	0.0129
Background	Beginning	-0.0001	0.000141234
Background	End	0.0001	8.30472E-05
Background	Overall	0.0000	0.000120342
Positive	Beginning	0.1734	0.0012
Positive	End	0.1663	0.0012
Positive	Overall	0.1699	0.0043
Negative	Beginning	0.3384	0.0037
Negative	End	0.3089	0.0080
Negative	Overall	0.3237	0.0178

Test Substance	Level	Replicate	[test substance] M	Log[test substance]	Activity
RC10	1	1	1.00E-03	-3.00	0.2917
RC10	1	2	1.00E-03	-3.00	0.2944
RC10	1	3	1.00E-03	-3.00	0.2895
RC10	2	1	1.00E-04	-4.00	0.3291
RC10	2	2	1.00E-04	-4.00	0.3312
RC10	2	3	1.00E-04	-4.00	0.3190
RC10	3	1	1.00E-05	-5.00	0.3506
RC10	3	2	1.00E-05	-5.00	0.3530
RC10	3	3	1.00E-05	-5.00	0.3439
RC10	4	1	1.00E-06	-6.00	0.3613
RC10	4	2	1.00E-06	-6.00	0.3472
RC10	4	3	1.00E-06	-6.00	0.3331
RC10	5	1	1.00E-07	-7.00	0.3437
RC10	5	2	1.00E-07	-7.00	0.3299
RC10	5	3	1.00E-07	-7.00	0.3523
RC10	6	1	1.00E-08	-8.00	0.3576
RC10	6	2	1.00E-08	-8.00	0.3357
RC10	6	3	1.00E-08	-8.00	0.3385
RC10	7	1	1.00E-09	-9.00	0.3378
RC10	7	2	1.00E-09	-9.00	0.3459
RC10	7	3	1.00E-09	-9.00	0.3465
RC10	8	1	1.00E-10	-10.00	0.3431
RC10	8	2	1.00E-10	-10.00	0.3363
RC10	8	3	1.00E-10	-10.00	0.3374

Percent of control values				
	Log[test	Replicate		
.evel	ostance]	1	2	3
1	-3.00	87.05	87.85	86.38
2	-4.00	98.20	98.83	95.19
3	-5.00	104.61	105.34	102.60
4	-6.00	107.81	103.60	99.40
5	-7.00	102.55	98.44	105.11
6	-8.00	106.70	100.17	101.00
7	-9.00	100.81	103.23	103.40
8	-10.00	102.37	100.34	100.67

Appendix E

Statistical Analysis Summary (BioSTAT Consultants, Inc.)

Statistical Analysis Summary
WIL Research Laboratories, LLC
Study Protocol – 431011
Version 1.1

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 one-way 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	Replicate 2	Replicate 3	Overall
1	Bottom	Prism Best Fit Value (S.E.)	-3.606 (2.239)	-1.775 (1.563)	-2.801 (2.267)	--
		Prism 95% CI (lower, upper)	(-8.276, 1.064)	(-5.035, 1.485)	(-7.529, 1.928)	--
		LSM (LSM s.e.)	--	--	--	-2.478 (1.116)
		95% CI (lower, upper)	--	--	--	(-7.278, 2.322)
	Slope (ß)	Prism Best Fit Value (S.E.)	-0.899 (0.048)	-0.917 (0.043)	-0.932 (0.050)	--
		Prism 95% CI (lower, upper)	(-0.998, -0.800)	(-1.007, -0.828)	(-1.037, -0.826)	--
		LSM (LSM s.e.)	--	--	--	-0.916 (0.027)
		95% CI (lower, upper)	--	--	--	(-1.031, -0.800)
	Top	Prism Best Fit Value (S.E.)	103.600 (0.510)	101.000 (0.670)	103.000 (0.640)	--
		Prism 95% CI (lower, upper)	(102.500, 104.700)	(99.590, 102.400)	(101.600, 104.300)	--
		LSM (LSM s.e.)	--	--	--	102.574 (0.779)
		95% CI (lower, upper)	--	--	--	(99.222, 105.926)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-4.138 (0.032)	-4.328 (0.022)	-4.085 (0.028)	--
		Prism 95% CI (lower, upper)	(-4.204, -4.072)	(-4.373, -4.282)	(-4.143, -4.027)	--
		LSM (LSM s.e.)	--	--	--	-4.185 (0.074)
		95% CI (lower, upper)	--	--	--	(-4.505, -3.864)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
2	Bottom	Prism Best Fit Value (S.E.)	-0.376 (1.064)	0.439 (2.203)	-0.068 (0.866)	--
		Prism 95% CI (lower, upper)	(-2.596, 1.843)	(-4.157, 5.035)	(-1.875, 1.740)	--
		LSM (LSM s.e.)	--	--	--	-0.137 (0.642)
		95% CI (lower, upper)	--	--	--	(-2.901, 2.627)
	Slope (ß)	Prism Best Fit Value (S.E.)	-0.935 (0.042)	-1.043 (0.115)	-0.991 (0.038)	--
		Prism 95% CI (lower, upper)	(-1.023, -0.847)	(-1.282, -0.803)	(-1.069, -0.912)	--
		LSM (LSM s.e.)	--	--	--	-0.970 (0.027)
		95% CI (lower, upper)	--	--	--	(-1.088, -0.853)
	Top	Prism Best Fit Value (S.E.)	99.710 (0.780)	97.950 (1.850)	98.900 (0.600)	--
		Prism 95% CI (lower, upper)	(98.070, 101.350)	(94.100, 101.800)	(97.650, 100.150)	--
		LSM (LSM s.e.)	--	--	--	99.124 (0.461)
		95% CI (lower, upper)	--	--	--	(97.142, 101.105)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-6.387 (0.027)	-6.550 (0.062)	-6.241 (0.020)	--
		Prism 95% CI (lower, upper)	(-6.443, -6.330)	(-6.679, -6.420)	(-6.283, -6.199)	--
		LSM (LSM s.e.)	--	--	--	-6.385 (0.087)
		95% CI (lower, upper)	--	--	--	(-6.760, -6.010)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
3	Bottom	Prism Best Fit Value (S.E.)	0.169 (0.724)	6.997 (4.124)	0.189 (1.268)	--
		Prism 95% CI (lower, upper)	(-1.341, 1.680)	(-1.607, 15.600)	(-2.457, 2.835)	--
		LSM (LSM s.e.)	--	--	--	0.329 (0.622)
		95% CI (lower, upper)	--	--	--	(-2.345, 3.003)
	Slope (ß)	Prism Best Fit Value (S.E.)	-0.974 (0.044)	-1.273 (0.248)	-1.002 (0.054)	--
		Prism 95% CI (lower, upper)	(-1.067, -0.882)	(-1.789, -0.757)	(-1.115, -0.889)	--
		LSM (LSM s.e.)	--	--	--	-0.991 (0.034)
		95% CI (lower, upper)	--	--	--	(-1.137, -0.844)
	Top	Prism Best Fit Value (S.E.)	109.740 (1.150)	102.390 (1.170)	106.600 (1.270)	--
		Prism 95% CI (lower, upper)	(107.330, 112.140)	(99.960, 104.810)	(104.000, 109.270)	--
		LSM (LSM s.e.)	--	--	--	106.245 (2.142)
		95% CI (lower, upper)	--	--	--	(97.027, 115.463)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-7.579 (0.026)	-6.023 (0.047)	-7.516 (0.025)	--
		Prism 95% CI (lower, upper)	(-7.634, -7.525)	(-6.121, -5.924)	(-7.568, -7.465)	--
		LSM (LSM s.e.)	--	--	--	-7.040 (0.508)
		95% CI (lower, upper)	--	--	--	(-9.226, -4.854)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
4	Bottom	Prism Best Fit Value (S.E.)	0.091 (3.042)	-0.459 (2.149)	-0.871 (0.975)	--
		Prism 95% CI (lower, upper)	(-6.256, 6.437)	(-4.943, 4.024)	(-2.906, 1.163)	--
		LSM (LSM s.e.)	--	--	--	-0.731 (0.852)
		95% CI (lower, upper)	--	--	--	(-4.398, 2.937)
	Slope (ß)	Prism Best Fit Value (S.E.)	-2.044 (1.158)	-1.881 (0.214)	-1.722 (0.766)	--
		Prism 95% CI (lower, upper)	(-4.460, 0.371)	(-2.327, -1.434)	(-1.882, -1.562)	--
		LSM (LSM s.e.)	--	--	--	-1.874 (0.203)
		95% CI (lower, upper)	--	--	--	(-2.747, -1.002)
	Top	Prism Best Fit Value (S.E.)	98.560 (1.420)	102.280 (1.220)	94.990 (0.540)	--
		Prism 95% CI (lower, upper)	(95.600, 101.520)	(99.740, 104.830)	(93.880, 96.110)	--
		LSM (LSM s.e.)	--	--	--	98.502 (2.159)
		95% CI (lower, upper)	--	--	--	(89.213, 107.791)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-4.879 (0.079)	-4.802 (0.033)	-4.750 (0.016)	--
		Prism 95% CI (lower, upper)	(-5.044, -4.714)	(-4.870, -4.733)	(-4.783, -4.717)	--
		LSM (LSM s.e.)	--	--	--	-4.782 (0.028)
		95% CI (lower, upper)	--	--	--	(-4.904, -4.660)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
6	Bottom	Prism Best Fit Value (S.E.)	0.598 (1.185)	18.284 (4.850)	0.909 (1.961)	--
		Prism 95% CI (lower, upper)	(-1.875, 3.070)	(8.166, 28.402)	(-3.183, 5.000)	--
		LSM (LSM s.e.)	--	--	--	5.732 (5.365)
		95% CI (lower, upper)	--	--	--	(-17.351, 28.815)
	Slope (ß)	Prism Best Fit Value (S.E.)	-1.052 (0.060)	-1.487 (0.340)	-1.077 (0.088)	--
		Prism 95% CI (lower, upper)	(-1.177, -0.928)	(-2.197, -0.777)	(-1.260, -0.894)	--
		LSM (LSM s.e.)	--	--	--	-1.069 (0.049)
		95% CI (lower, upper)	--	--	--	(-1.279, -0.859)
	Top	Prism Best Fit Value (S.E.)	101.800 (0.700)	98.640 (1.080)	92.160 (1.540)	--
		Prism 95% CI (lower, upper)	(100.330, 103.260)	(96.390, 100.900)	(88.940, 95.380)	--
		LSM (LSM s.e.)	--	--	--	97.664 (2.802)
		95% CI (lower, upper)	--	--	--	(85.607, 109.722)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-5.209 (0.026)	-3.822 (0.080)	-5.113 (0.039)	--
		Prism 95% CI (lower, upper)	(-5.263, -5.156)	(-3.990, -3.655)	(-5.193, -5.032)	--
		LSM (LSM s.e.)	--	--	--	-4.717 (0.446)
		95% CI (lower, upper)	--	--	--	(-6.636, -2.799)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
7	Bottom	Prism Best Fit Value (S.E.)	0.091 (0.168)	0.422 (0.421)	0.328 (0.422)	--
		Prism 95% CI (lower, upper)	(-0.259, 0.442)	(-0.455, 1.300)	(-0.553, 1.208)	--
		LSM (LSM s.e.)	--	--	--	0.160 (0.146)
		95% CI (lower, upper)	--	--	--	(-0.470, 0.789)
	Slope (ß)	Prism Best Fit Value (S.E.)	-1.149 (0.017)	-1.188 (0.034)	-1.217 (0.033)	--
		Prism 95% CI (lower, upper)	(-1.184, -1.114)	(-1.258, -1.117)	(-1.287, -1.148)	--
		LSM (LSM s.e.)	--	--	--	-1.177 (0.022)
		95% CI (lower, upper)	--	--	--	(-1.270, -1.083)
	Top	Prism Best Fit Value (S.E.)	101.330 (0.470)	99.820 (0.800)	102.690 (0.760)	--
		Prism 95% CI (lower, upper)	(100.360, 102.300)	(98.140, 101.500)	(101.110, 104.280)	--
		LSM (LSM s.e.)	--	--	--	101.298 (0.766)
		95% CI (lower, upper)	--	--	--	(98.003, 104.594)
	log10IC50 (µ)	Prism Best Fit Value (S.E.)	-8.644 (0.008)	-8.636 (0.011)	-8.593 (0.011)	--
		Prism 95% CI (lower, upper)	(-8.661, -8.628)	(-8.659, -8.612)	(-8.616, -8.571)	--
		LSM (LSM s.e.)	--	--	--	-8.625 (0.016)
		95% CI (lower, upper)	--	--	--	(-8.693, -8.557)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
8	Bottom	Prism Best Fit Value (S.E.)	12.265 (1.241)	12.989 (1.525)	16.572 (3.851)	--
		Prism 95% CI (lower, upper)	(9.676, 14.854)	(9.806, 16.171)	(8.539, 24.605)	--
		LSM (LSM s.e.)	--	--	--	12.790 (0.934)
		95% CI (lower, upper)	--	--	--	(8.772, 16.808)
	Slope (ß)	Prism Best Fit Value (S.E.)	-1.107 (0.050)	-1.155 (0.070)	-1.432 (0.235)	--
		Prism 95% CI (lower, upper)	(-1.212, -1.003)	(-1.302, -1.009)	(-1.922, -0.941)	--
		LSM (LSM s.e.)	--	--	--	-1.132 (0.040)
		95% CI (lower, upper)	--	--	--	(-1.305, -0.960)
	Top	Prism Best Fit Value (S.E.)	98.250 (0.550)	91.110 (0.780)	92.850 (2.100)	--
		Prism 95% CI (lower, upper)	(97.110, 99.400)	(89.490, 92.740)	(88.470, 97.220)	--
		LSM (LSM s.e.)	--	--	--	94.189 (2.299)
		95% CI (lower, upper)	--	--	--	(84.298, 104.081)
	log10IC50 (μ)	Prism Best Fit Value (S.E.)	-5.519 (0.021)	-5.459 (0.024)	-5.430 (0.056)	--
		Prism 95% CI (lower, upper)	(-5.563, -5.474)	(-5.510, -5.409)	(-5.548, -5.313)	--
		LSM (LSM s.e.)	--	--	--	-5.481 (0.026)
		95% CI (lower, upper)	--	--	--	(-5.593, -5.368)

Reference Chemical	Parameter	Statistic	Replicate 1	Replicate 2	Replicate 3	Overall
9	Bottom	Prism Best Fit Value (S.E.)	-4.646 (2.757)	-7.329 (5.595)	-9.718 (5.239)	--
		Prism 95% CI (lower, upper)	(-10.397, 1.105)	(-19.000, 4.341)	(-20.647, 1.211)	--
		LSM (LSM s.e.)	--	--	--	-5.999 (2.236)
		95% CI (lower, upper)	--	--	--	(-15.621, 3.624)
	Slope (ß)	Prism Best Fit Value (S.E.)	-0.733 (0.048)	-0.730 (0.085)	-0.715 (0.070)	--
		Prism 95% CI (lower, upper)	(-0.833, -0.633)	(-0.908, -0.553)	(-0.860, -0.569)	--
		LSM (LSM s.e.)	--	--	--	-0.728 (0.036)
		95% CI (lower, upper)	--	--	--	(-0.882, -0.573)
	Top	Prism Best Fit Value (S.E.)	101.750 (0.770)	98.830 (2.390)	102.720 (2.060)	--
		Prism 95% CI (lower, upper)	(100.140, 103.370)	(93.840, 103.820)	(98.430, 107.010)	--
		LSM (LSM s.e.)	--	--	--	101.615 (0.691)
		95% CI (lower, upper)	--	--	--	(98.644, 104.586)
	log10IC50 (µ)	Prism Best Fit Value (S.E.)	-4.565 (0.050)	-4.360 (0.084)	-4.310 (0.074)	--
		Prism 95% CI (lower, upper)	(-4.670, -4.460)	(-4.536, -4.184)	(-4.464, -4.155)	--
		LSM (LSM s.e.)	--	--	--	-4.421 (0.083)
		95% CI (lower, upper)	--	--	--	(-4.777, -4.064)

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	-0.000	0.019	0.009	-0.001	-0.016	-0.130	-0.020
		LSM s.e.	0.093	0.093	0.093	0.093	0.093	0.120	0.040
		95% CI - lower	-0.202	-0.183	-0.193	-0.203	-0.218	-0.392	-0.107
		95% CI - upper	0.202	0.222	0.211	0.201	0.186	0.132	0.067
	End	LSM	0.000	-0.019	-0.009	0.001	0.016	0.130	0.020
		LSM s.e.	0.093	0.093	0.093	0.093	0.093	0.120	0.040
		95% CI - lower	-0.202	-0.221	-0.211	-0.201	-0.186	-0.132	-0.067
		95% CI - upper	0.202	0.183	0.193	0.203	0.218	0.392	0.107
	Overall	LSM	-0.000	0.000	0.000	-0.000	-0.000	0.000	
		LSM s.e.	0.066	0.066	0.066	0.066	0.066	0.085	
		95% CI - lower	-0.143	-0.143	-0.143	-0.143	-0.143	-0.185	
		95% CI - upper	0.143	0.143	0.143	0.143	0.143	0.185	
	P-Values	Chemical = 1.0000							
		Portion = 0.4951							
		Chemical*Portion = 0.7946							
		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 = .							
		Portion*Replicate(Chemical) 4 = .							
		Portion*Replicate(Chemical) 5 = .							
		Portion*Replicate(Chemical) 7 & 8 = .							
		Portion*Replicate(Chemical) 9 & 10 = 0.2856							

Activity	Portion	Statistic	Chemical 1 & 2	3 & 6	4	5	7 & 8	9 & 10	Overall
Full Activity	Beginning	LSM	102.853	104.277	100.428	102.119	104.885	101.527	102.681
		LSM s.e.	1.216	1.332	1.543	2.404	1.267	1.216	0.635
		95% CI - lower	100.203	101.374	97.066	96.882	102.125	98.877	101.299
		95% CI - upper	105.502	107.180	103.789	107.356	107.645	104.177	104.064
	End	LSM	97.147	96.436	99.572	97.881	95.115	98.473	97.437
		LSM s.e.	1.216	1.216	1.543	2.404	1.267	1.216	0.628
		95% CI - lower	94.498	93.786	96.211	92.644	92.355	95.823	96.069
		95% CI - upper	99.797	99.086	102.934	103.118	97.875	101.123	98.806
	Overall	LSM	100.000	100.357	100.000	100.000	100.000	100.000	
		LSM s.e.	0.860	0.902	1.091	1.700	0.896	0.860	
		95% CI - lower	98.126	98.391	97.623	96.297	98.048	98.126	
		95% CI - upper	101.874	102.322	102.377	103.703	101.952	101.874	
	P-Values	Chemical = 0.9996							
		Portion = 0.0001							
		Chemical*Portion = 0.0662							
		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 = .							
		Portion*Replicate(Chemical) 4 = 0.2991							
		Portion*Replicate(Chemical) 5 = 0.1470							
		Portion*Replicate(Chemical) 7 & 8 = 0.4575							
		Portion*Replicate(Chemical) 9 & 10 = .							

Activity	Portion	Statistic	Chemical 1 & 2	3 & 6	4	5	7 & 8	9 & 10	Overall
Negative	Beginning	LSM	100.718	103.538	101.410	96.000	93.348	100.951	99.327
		LSM s.e.	2.511	2.073	2.073	5.032	3.664	2.073	1.269
		95% CI - lower	95.246	99.021	96.893	85.036	85.365	96.434	96.563
		95% CI - upper	106.190	108.055	105.927	106.963	101.331	105.468	102.092
	End	LSM	89.357	97.674	96.484	95.083	85.760	92.805	92.861
		LSM s.e.	2.511	2.073	2.073	5.032	3.664	2.073	1.269
		95% CI - lower	83.885	93.157	91.968	84.120	77.777	88.289	90.096
		95% CI - upper	94.829	102.190	101.001	106.047	93.742	97.322	95.625
	Overall	LSM	95.038	100.606	98.947	95.541	89.554	96.878	
		LSM s.e.	2.039	1.466	1.466	4.237	3.358	1.466	
		95% CI - lower	90.594	97.412	95.753	86.310	82.238	93.684	
		95% CI - upper	99.481	103.799	102.141	104.773	96.870	100.072	
	P-Values	Chemical = 0.0843							
		Portion = 0.0007							
		Chemical*Portion = 0.5365							
		Replicate(Chemical) 1 & 2 = 0.3153							
		Replicate(Chemical) 3 & 6 = .							
		Replicate(Chemical) 4 = .							
		Replicate(Chemical) 5 = 0.2927							
		Replicate(Chemical) 7 & 8 = 0.2093							
		Replicate(Chemical) 9 & 10 = .							
		Portion*Replicate(Chemical) 1 & 2 = .							
		Portion*Replicate(Chemical) 3 & 6 = .							
		Portion*Replicate(Chemical) 4 = .							
		Portion*Replicate(Chemical) 5 = 0.2397							
		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
Positive	Beginning	LSM	48.818	70.842	50.814	50.721	51.696	53.424	54.386
		LSM s.e.	4.260	15.656	1.378	1.203	1.668	1.796	2.752
		95% CI - lower	39.536	36.732	47.812	48.100	48.061	49.511	48.390
		95% CI - upper	58.099	104.952	53.816	53.343	55.331	57.338	60.381
	End	LSM	41.161	65.279	49.104	48.991	46.248	50.250	50.172
		LSM s.e.	4.260	15.656	1.378	1.203	1.668	1.796	2.752
		95% CI - lower	31.880	31.169	46.102	46.370	42.613	46.337	44.177
		95% CI - upper	50.442	99.389	52.107	51.613	49.883	54.164	56.168
	Overall	LSM	44.989	68.061	49.959	49.856	48.972	51.837	
		LSM s.e.	4.180	15.645	1.255	0.986	1.551	1.704	
		95% CI - lower	35.882	33.973	47.224	47.707	45.592	48.125	
		95% CI - upper	54.097	102.148	52.694	52.006	52.352	55.550	
	P-Values	Chemical = 0.5254							
		Portion = 0.0000							
		Chemical*Portion = 0.0498							
		Replicate(Chemical) 1 & 2 = 0.1683							
		Replicate(Chemical) 3 & 6 = 0.1590							
		Replicate(Chemical) 4 = 0.2135							
		Replicate(Chemical) 5 = 0.3227							
		Replicate(Chemical) 7 & 8 = 0.2023							
		Replicate(Chemical) 9 & 10 = 0.1871							
		Portion*Replicate(Chemical) 1 & 2 = 0.3026							
		Portion*Replicate(Chemical) 3 & 6 = .							
		Portion*Replicate(Chemical) 4 = .							
		Portion*Replicate(Chemical) 5 = 0.3759							
		Portion*Replicate(Chemical) 7 & 8 = 0.4444							
		Portion*Replicate(Chemical) 9 & 10 = .							

APPENDIX E

BATTELLE INTERLABORATORY STATISTICAL ANALYSIS REPORT

DRAFT REPORT

INTERLABORATORY STATISTICAL ANALYSIS

on

RECOMBINANT AROMATASE VALIDATION STUDY

CONDUCT MULTIPLE CHEMICAL STUDIES WITH HUMAN RECOMBINANT MICROSOMES

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-17, TASK 4

June 22, 2006

Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
ENDOCRINE DISRUPTOR SCREENING PROGRAM
WASHINGTON, D.C.

Prepared by

BATTELLE
505 King Avenue
Columbus, Ohio 43201

Recombinant Aromatase Validation Study

Conduct Multiple Chemical Studies with Human Recombinant Microsomes

Interlaboratory Statistical Analysis

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 4-17, TASK 4

Zhenxu J. Ma, Author _____ Date _____

Paul I. Feder, Reviewer _____ Date _____

Quality Assurance Statement

Study Number: WA 4-17 Task 4

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	6/19/2006	6/19/2006
Audit interlaboratory statistics draft report	6/19/2006	6/ 19/2006

Quality Assurance Unit **Date**

_____ _____

Terri L. Pollock

TABLE OF CONTENTS

List of Tables

List of Figures

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 studies with the recombinant aromatase assay under Work Assignment 4-17, Task 4, Conduct Multiple Chemical Studies with Human Recombinant Microsomes.

Summary and Conclusions

Human recombinant microsomes were distributed to the four participating laboratories. They were utilized to conduct recombinant 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). Recombinant 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 recombinant aromatase assays.

The intralaboratory analyses included concentration response relation 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 threshold parameters of the concentration response relation fits determined in the intralaboratory analyses for each chemical. The interlaboratory analysis also compared across the four laboratories the background activity, the full enzyme activity, the positive and 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.

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

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

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

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

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.

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

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

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

   c. For the negative control, the results for In Vitro differed from those of the other laboratories. The end portion was (nearly significantly) higher than the beginning

portion for In Vitro, and statistically significantly lower than the beginning for the other laboratories. As a result of this inconsistency, the estimated variance among the laboratories was about 4.6 times the average within laboratory variance. The end and the beginning portions did not differ significantly on average across laboratories.

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

In Vitro's results differed slightly from the other laboratories with respect to $\log_{10}IC_{50}$ and slope for ketoconazole, econazole and dicofol, for slope for 4-nonylphenol, for bottom for dicofol, for top for aminoglutethimide, and for differences between end and beginning portions for the negative and positive controls. It is not understood why the activity for the negative and positive controls at In Vitro increased between the beginning and end portions of replicates while those at the other laboratories decreased.

Introduction and Background

Task 4 of Work Assignment 4-17, the Recombinant Aromatase Validation Study, involved four laboratories (RTI, Battelle, WIL and In Vitro) independently carrying out the human recombinant aromatase assay on the aromatase activity of human recombinant microsomes with ten reference chemicals, 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. The intralaboratory analyses included concentration response relation fits for individual chemicals and analysis of variance comparisons of the background activity, full enzyme activity, positive and negative control responses averaged across the chemicals. The interlaboratory analysis compared across the four laboratories the $\log_{10}IC_{50}$, the slope, the top threshold, and the bottom threshold parameters of the concentration response relation fits determined in the intralaboratory analyses for each chemical. The interlaboratory analysis also compared across the four laboratories the background activity, full enzyme activity, positive and negative control responses between 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.

Test Organization

Human recombinant microsomes were distributed to the four participating laboratories. Recombinant assay aromatase activity levels were determined for various graded concentrations of each of the ten reference chemicals: aminoglutethimide, ketoconazole, prochloraz, 4-nonylphenol, dibenz[a,h]anthracene, fenarimol, econazole, chrysin, dicofol, and atrazine. Three replicates of the inhibitor assay were carried out at each laboratory for each chemical. Within each replicate three repetitions were run at each graded concentration of each of the ten reference chemicals. In addition, for each chemical two repeat tubes of the background activity, full enzyme activity, positive and negative activity controls were run prior to the inhibition chemical runs and two repeat tubes of the background activity, full enzyme activity, positive and negative controls were run following the inhibition chemical runs.

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 test results, 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 relation (four parameter) fit within each replicate to describe the trend in the percent of control activity across varying concentrations of each of the test chemicals

2. Estimates of the $\log_{10}IC_{50}$ concentration, the slope, the top and the bottom, and associated standard errors within each replicate for each chemical.

3. Average values across replicates for the $\log_{10}IC_{50}$ concentration, the slope, the top threshold, and the bottom threshold, and associated standard errors for each chemical.

4. Results of random effects 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 assay results were reported differently by the various laboratories (Appendix B). RTI and WIL reported least squares means results corresponding to the end and to the beginning portions, for each chemical (RTI) or for subsets of chemicals

(WIL). Battelle and In Vitro reported least squares means results corresponding to the difference between the end and the beginning portions (end minus beginning), averaged across chemicals.

It should be noted that within each of the laboratories the intralaboratory analyses of the control responses combined across chemicals were based on random effects analysis of variance models that assumed random replicate effects and independent responses within replicates. None of the models fully accounted 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 affected the standard errors that are presented in the tables in Appendix B. As an example, for RTI (Table B-1a) aminoglutethimide background activity control the replicate-to-replicate variability is zero and the standard error of the end minus beginning portions is presented as $0.036 \equiv 0.026\sqrt{2}$. The standard errors given in Appendix B for each of the laboratories do not account for the constraints on the sums of the background activity control values (0) and the full enzyme activity control values (100) within replicates.

The interlaboratory statistical analysis combined summary values developed in each of the four 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 among laboratories the average values for the $\log_{10}IC_{50}$, the slope, the top threshold, and the bottom threshold parameters of the concentration response relation fits determined by each of the participating laboratories, as reported in the intralaboratory analyses. It also compared among laboratories the background activity, the full enzyme activity, the negative and the positive control results obtained at the end of each replicate with those obtained at the beginning.

The objectives of the interlaboratory statistical analysis are 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 IC_{50}, the slope, and the top parameters.
- Estimate the ratio of the among laboratory variation to the average within laboratory variation for the parameters mentioned above.

Statistical Analysis Methods

Statistical analyses were carried out for each of the eight concentration response relation and control endpoints discussed above in the Test Organization section: $\log_{10}IC_{50}$, slope, top, bottom, and portion effects (end minus beginning) for the background activity, full enzyme activity, negative and positive controls. The analyses for the $\log_{10}IC_{50}$, the slope, the top and the bottom were performed separately for each chemical. The analyses for the controls were performed combining all chemicals.

For each endpoint, a random effects analysis of variance model with heterogeneous variances among the participating laboratories was fitted to the summary responses within

laboratories. Laboratory was treated as a random effect. For each endpoint, the within laboratory variances were based on the squares of the standard errors associated with the endpoint estimates obtained in each of the intralaboratory analyses. The analysis of variance provided an estimated weighted average across all the four laboratories and its associated standard error as well as an estimate of the laboratory-to-laboratory component of variation. The weights included in the weighted averages incorporated both laboratory-to-laboratory variation and within laboratory variation. The degrees of freedom associated with the overall weighted average was calculated as

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

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

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

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

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

1. For RTI, the differences (Diff) between the end and the beginning were first calculated for each chemical. The associated standard errors for these differences were calculated as $|Diff|/(F\text{-value})^{1/2}$, where the F-values were associated with test results for the portion effects for chemicals, as provided in the intralaboratory analysis reports. The differences between the beginning and the end portions were averaged across all chemicals resulting in the overall average differences between the end and the beginning portions. The associated standard errors were calculated as $(1/10)(\sum s_i^2)^{1/2}$, where s_i is the standard error associated with the difference for the i^{th} chemical (Tables B-1a, B-1b).

2. For WIL Laboratories, the ten chemicals were combined into subsets of size one or two. The subsets of chemicals were tested simultaneously, with common controls. This resulted in a total of six sets of independent controls. For each subset of chemical combinations, the mean values and the associated standard errors at the beginning and at

the end portions, as well as the averages across the two portions were reported. The differences between the end and beginning were first calculated for each chemical. The associated standard errors for these differences were calculated as $(2(S_e^2+S_b^2)-4S_s^2)^{1/2}$, where S_e, S_b, and S_s were the standard errors associated with the end, the beginning, and the averages across the end and the beginning respectively, as provided in the intralaboratory analysis report. The differences between the beginning and the end portions (end minus beginning) were averaged across the chemical combinations. The associated standard errors were calculated as $(1/6)(\sum s_i^2)^{1/2}$, where s_i is the standard error associated with the difference for the i^{th} chemical combination (Tables B-2a, B-2b).

3. Results for Battelle and In Vitro were as reported in the intralaboratory report, averaged across chemicals with associated standard errors (Tables B-3, B-4).

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

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

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

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

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

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

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

$$CV=|S/M|$$

where M is the mean and S is the associated standard deviation. S is approximated by $\sqrt{4}$ (se) where se is the standard error of the pooled mean estimate. The endpoints of the 95 percent confidence interval for the CV are the solutions to the two nonlinear equations:

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

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

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

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

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

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

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

where 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 eight endpoint parameters.

Round Off

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

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. The plotting symbols are circles. Some of the circles are filled while others are empty. This is due to an issue in the graphing program. For purposes of interpretation, it should be disregarded whether the circle is filled or is empty.

Statistical Analysis Results

As discussed in the methods section, the interlaboratory analyses for the $\log_{10}IC_{50}$, the slope, the top and the bottom parameters were performed separately for each chemical. The interlaboratory analyses for the background activity, the full enzyme activity, the negative and the positive activity control data were performed combined across chemicals.

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

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

Table 1 displays the estimated $\log_{10}IC_{50}$ and the slope within each laboratory and the associated 95 percent confidence intervals for each chemical[1]. This table also displays the overall mean values across laboratories and their associated 95 percent confidence intervals, incorporating among laboratory variation based on the random effects analysis of variance. These means and confidence intervals are displayed in Figures 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.

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 laboratory-to-laboratory random variations, the p-values associated with the test that the among laboratory variation is zero, and the squares of the standard errors of the overall mean values, as well as their associated degrees of freedom. The ratios of the among laboratory

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

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

variances to the unweighted average within laboratory variances are also displayed, with their associated 95 percent confidence intervals.

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

The estimates for $\log_{10}IC_{50}$ (Table 1) were in general consistent among the four laboratories for most chemicals. The In Vitro estimates were slightly lower than those for the other three laboratories for ketoconazole, econazole, and dicofol. The ratios of the among laboratory variance to the pooled within laboratory variance were relatively large for dicofol (9.5), ketoconazole (6.4), and econazole (4.9) and were less than 2.7 for the other chemicals (Table 2). The CVs for the IC_{50} were 39.5 percent for ketoconazole, 70.6 percent for dicofol, 26.9 percent for chrysin, 29.2 for prochloraz, and between 11.4 and 18.3 percent for the other chemicals (Table 1). All the CVs exceeded 10 percent.

The slope estimates (Table 1) were similar among the four laboratories for most chemicals. The In Vitro slope estimates were more negative than those for the other three laboratories for ketoconazole (Figure 4) and dicofol (Figure 16) and were less negative than those for the other three laboratories for 4-nonylphenol (Figure 8) and econazole (Figure 12). The slope CVs were 28.1 percent for dicofol, 14.5 percent for 4-nonylphenol, 7.4 percent for econazole, and less than 6.1 percent for the other five chemicals (Table 1). The ratios of the among laboratory variance to the pooled within laboratory variance were 2.5 or less for all of the chemicals (Table 2).

The estimates for the top thresholds varied between 85 and 111 percent. They were in general consistent among the four laboratories for most chemicals except for aminoglutethimide. The estimated top threshold for In Vitro was higher than the other three laboratories for aminoglutethimide (110.3 compared to 99 percent or less). The top threshold CVs were 6.0 percent for aminoglutethimide and less than 5.4 percent for the other seven chemicals (Table A-1). The ratios of the among laboratory variances to the pooled within laboratory variance ratios were 7.1 for aminoglutethimide and 3.9 or less for the other seven chemicals (Table A-2).

The estimated bottom thresholds were between approximately -2.5 and 11 for all chemicals except for chrysin and dicofol (Table A-1). The bottom thresholds were between 12.8 and 22.6 percent for chrysin, and consistent among laboratories. In Vitro had a bottom threshold of 41.1 percent for dicofol (Table A-1), which was inconsistent with those of the other three laboratories (-6.0 to 2.1 percent). The 95 percent confidence interval for In Vitro did not overlap with those for the other three laboratories (Figure A-16). The ratios of the among laboratory variances to the pooled within laboratory variance ratios were 21.4 for dicofol and less than 1.4 for any of the other seven chemicals (Table A-2).

Control Activity: Differences Between the Beginning and the End Portions

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 portions[3]. It also displays the overall mean differences across laboratories and their associated 95 percent confidence intervals, incorporating among laboratory variation based on the random effects analysis of variance. The mean differences (end minus beginning) and confidence intervals are displayed in Figures 17 to 20, one for each control endpoint. Each figure includes a reference line corresponding to the overall average.

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 variation and the p-values associated with their significance, and the squares of the standard errors of the overall mean values, as well as their associated degrees of freedom. The ratios of the among laboratory variances to the unweighted average within laboratory variances are also displayed, with their associated 95 percent confidence intervals.

The following results were observed:

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

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

For the negative controls, the result for In Vitro was different from the other three laboratories. The end portion was (nearly significantly) higher than the beginning portion for In Vitro, but statistically significantly lower than the beginning for the other three laboratories. As a result of this disagreement, the estimated variance among the laboratories was about 4.6 times of the average within laboratory variance. The two portions did not differ significantly on average across laboratories.

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

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

References

Hartung, J. and Makambi, K.H. *Simple non-iterative t-distribution based tests for meta-analysis*. South African Statistical Journal, 2001, Vol. 35, p. 1-17.

Lehmann, E. *Testing Statistical Hypotheses*. John Wiley & Sons, Inc., 1986, p. 352-3.

Table 1. Parameter Estimates and 95 Percent Confidence Intervals for the $Log_{10}IC_{50}$ and Slope Parameters of the Concentration Response Relations for the Recombinant Aromatase Assay. Estimated by Chemical.

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

1. The estimates and 95 percent confidence intervals are based on the intralaboratory analyses for the four participating laboratories. The intralaboratory analyses were performed by individual chemical.
2. The overall estimates and confidence intervals were estimated using a random effects analysis of variance, with laboratory as a random effect and with heterogeneous variances among the four laboratories. The variance for each laboratory was specified as the square of the within laboratory standard error. The degrees of freedom are given in Table 2.
3. CV was calculated for the IC_{50} and the slope parameters based on average results. CVs shown as associated with $log_{10}IC_{50}$ are actually CVs of the IC_{50}s.
4. Concentration response relations were not fitted for dibenz[a,h]anthracene and for atrazine, since they resulted in very little aromatase inhibition (i.e. they were noninhibitors).

Table 2. Variance Components and Ratio of Between and Within Laboratories Variances for the $Log_{10}IC_{50}$ and Slope Parameter of the Concentration Response Relations for the Recombinant Aromatase Assay. Estimated by Chemical.

Chemical[7]	Parameter	Within Laboratory Variance[1]					Among Laboratory Variance[3] and (p-value) (df=3)	Mean Variance[4,5]	Ratio and 95 percent CI[6]
		RTI	Battelle	WIL	In Vitro	Pooled Results[2]			
AG	Log_IC50	0.001/df=16.0	0.002/df=2.0	0.008/df=2.0	0.001/df=44.3	0.003/df=4.4	0.003 (0.17921)	0.001/df=5.1	0.896 (0.100, 13.458)
AG	Slope	0.004/df=16.0	0.001/df=56.1	0.001/df=2.0	0.002/df=53.2	0.002/df=37.7	0.000 (1.000)	0.000/df=37.7	0.000 (0.000, 0.000)
KCZ	Log_IC50	0.001/df=16.0	0.001/df=20.0	0.005/df=2.0	0.007/df=2.1	0.004/df=5.3	0.024 (0.12436)	0.007/df=3.5	6.431 (0.874, 95.409)
KCZ	Slope	0.002/df=16.0	0.003/df=20.6	0.001/df=2.0	0.016/df=3.1	0.005/df=6.0	0.000 (1.000)	0.000/df=6.0	0.000 (0.000, 0.000)
PCZ	Log_IC50	0.000/df=16.0	0.005/df=2.2	0.258/df=2.0	0.000/df=20.0	0.066/df=2.1	0.010 (0.12884)	0.004/df=2.7	0.155 (0.004, 2.471)
PCZ	Slope	0.001/df=16.0	0.002/df=8.6	0.001/df=2.0	0.003/df=20.0	0.002/df=31.9	0.000 (1.000)	0.000/df=31.9	0.000 (0.000, 0.000)
NYP	Log_IC50	0.000/df=16.0	0.000/df=5.1	0.001/df=2.0	0.001/df=19.8	0.001/df=17.3	0.002 (0.14043)	0.001/df=4.3	2.694 (0.675, 38.267)
NYP	Slope	0.028/df=16.0	0.017/df=44.2	0.041/df=2.0	0.006/df=26.8	0.023/df=9.4	0.058 (0.12870)	0.020/df=4.8	2.505 (0.504, 36.191)
FRM	Log_IC50	0.001/df=16.0	0.000/df=44.9	0.199/df=2.0	0.019/df=1.8	0.055/df=2.4	0.001 (0.24440)	0.001/df=2.5	0.019 (0.001, 0.295)
FRM	Slope	0.001/df=16.0	0.001/df=46.2	0.002/df=2.0	0.001/df=20.6	0.001/df=10.6	0.001 (0.34020)	0.000/df=3.5	0.401 (0.085, 5.776)
ECZ	Log_IC50	0.000/df=16.0	0.004/df=2.0	0.000/df=2.0	0.000/df=47.2	0.001/df=3.0	0.005 (0.09468)	0.002/df=4.8	4.873 (0.317, 75.225)
ECZ	Slope	0.002/df=16.0	0.006/df=2.0	0.000/df=2.0	0.001/df=43.5	0.002/df=4.7	0.005 (0.12045)	0.002/df=5.0	2.389 (0.287, 35.709)
CYN	Log_IC50	0.001/df=16.0	0.015/df=2.2	0.001/df=2.0	0.005/df=20.0	0.006/df=4.8	0.009 (0.16991)	0.003/df=4.1	1.670 (0.206, 24.922)
CYN	Slope	0.007/df=16.0	0.022/df=2.3	0.002/df=2.0	0.154/df=20.0	0.046/df=24.3	0.000 (1.000)	0.001/df=24.3	0.000 (0.000, 0.000)
DCF	Log_IC50	0.001/df=16.0	0.003/df=2.5	0.007/df=2.0	0.018/df=3.0	0.007/df=6.2	0.070 (0.11404)	0.019/df=3.5	9.519 (1.473, 140.080)
DCF	Slope	0.007/df=16.0	0.024/df=3.6	0.001/df=2.0	0.190/df=37.0	0.056/df=43.4	0.058 (0.27558)	0.021/df=2.7	1.038 (0.302, 14.554)

1. The within laboratory variance for each laboratory is the square of the standard error associated with the parameter estimate, which was reported in the intralaboratory analyses for each of the four participating laboratories.
2. Pooled average for the within laboratory variances is the unweighted average of the within laboratory variances among the four laboratories. Associated degrees of freedom were based on Satterthwaite's approximation
3. Variance among laboratories is based on a random effects analysis of variance model with heterogeneous variances among the individual laboratories equal to the squares of the within laboratory standard errors. P-values are associated with the test that the among laboratory variation is zero.
4. Mean variance is the square of the standard error of the pooled weighted mean value. It includes both within and among laboratory variation.
5. Degrees of freedom for the (mean) overall effect variance were estimated as $2*((1/K)*\sum(S_L^2 + S_i^2))^2/(var(S_L^2)+(2/K^2)*\sum(S_i^4/df_i))$, where S_L^2 is the among laboratory variance, S_i^2 and df_i are the reported variance and degrees of freedom for laboratory i, $var(S_L^2)$ is the variance of S_L^2, and K is the number of laboratories (Hartung and Makambi, 2001).
6. Ratio of the among-laboratory variance to the pooled average within laboratory variance.
7. Concentration response relations were not fitted for dibenz[a,h]anthracene and for atrazine, since they resulted in very little aromatase inhibition (i.e. they were noninhibitors).

Table 3. Parameter Estimate and the 95 Percent Confidence Intervals for Differences Between the Beginning and the End Portions for the Percent of Control Responses for the Recombinant Aromatase Assay.

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

1. The estimates and 95 percent confidence intervals are based on the intralaboratory analyses for the four participating laboratories.
2. The results from the four laboratories are given in Appendix B.
3. The overall (mean) effects and confidence intervals were estimated using a one-way random effects analysis of variance, with laboratory as a random effect, and with heterogeneous variances among the individual laboratories. The variances for each laboratory were specified as the squares of the within laboratory standard errors.

Table 4. Variance Components and Ratio of Among and Within Laboratories Variances for Differences Between the Beginning and the End Portions for the Percent of Control Responses for the Recombinant Aromatase Assay.

Parameter	Within Laboratory Variance[1]					Among Laboratory Variance[3] and (p-value) (df=3)	Mean Variance[4,5]	Ratio and 95 percent CI[6]
	RTI	Battelle	WIL	In Vitro	Pooled Results[2]			
Background Activity Control	0.000/df=20.0	0.013/df=16.0	0.003/df=12.0	0.004/df=14.0	0.005/df=33.2	0.000 (1.00)	0.000/df=33.2	0.000 (0.000, 0.000)
Full Enzyme Activity Control	0.730/df=20.0	1.523/df=35.9	0.797/df=12.0	3.698/df=23.2	1.687/df=62.0	13.112 (0.09369)	3.677/df=4.4	7.772 (2.331, 108.729)
Negative Control	1.648/df=20.0	1.100/df=20.4	2.012/df=12.0	12.982/df=16.2	4.435/df=28.7	20.380 (0.17558)	6.003/df=2.6	4.595 (1.272, 64.732)
Positive Control	0.288/df=20.0	0.448/df=12.5	0.280/df=12.0	2.307/df=16.9	0.831/df=32.4	1.314 (0.25770)	0.477/df=2.2	1.581 (0.445, 22.244)

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

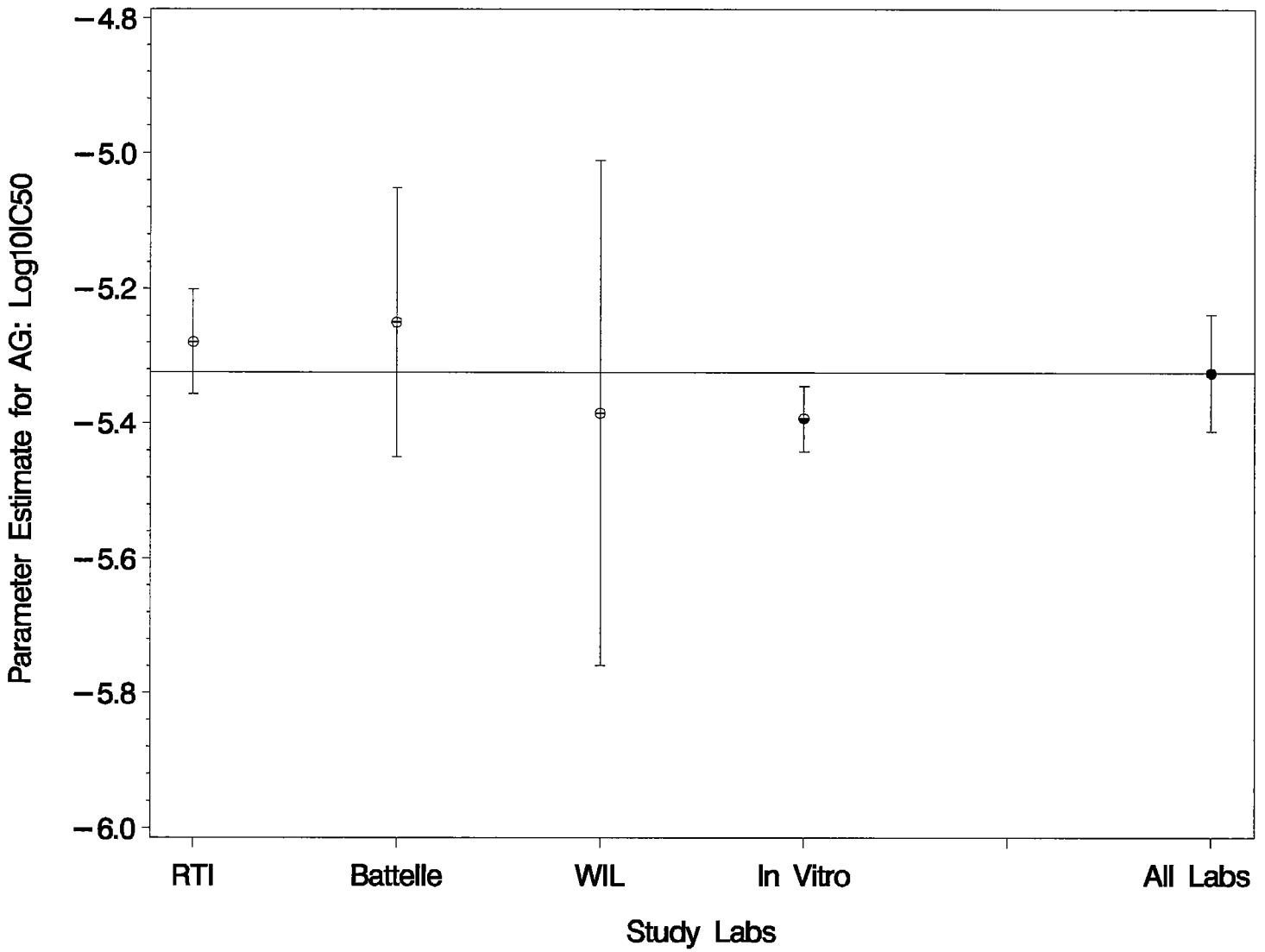


Figure 1. **Aminoglutethimide: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

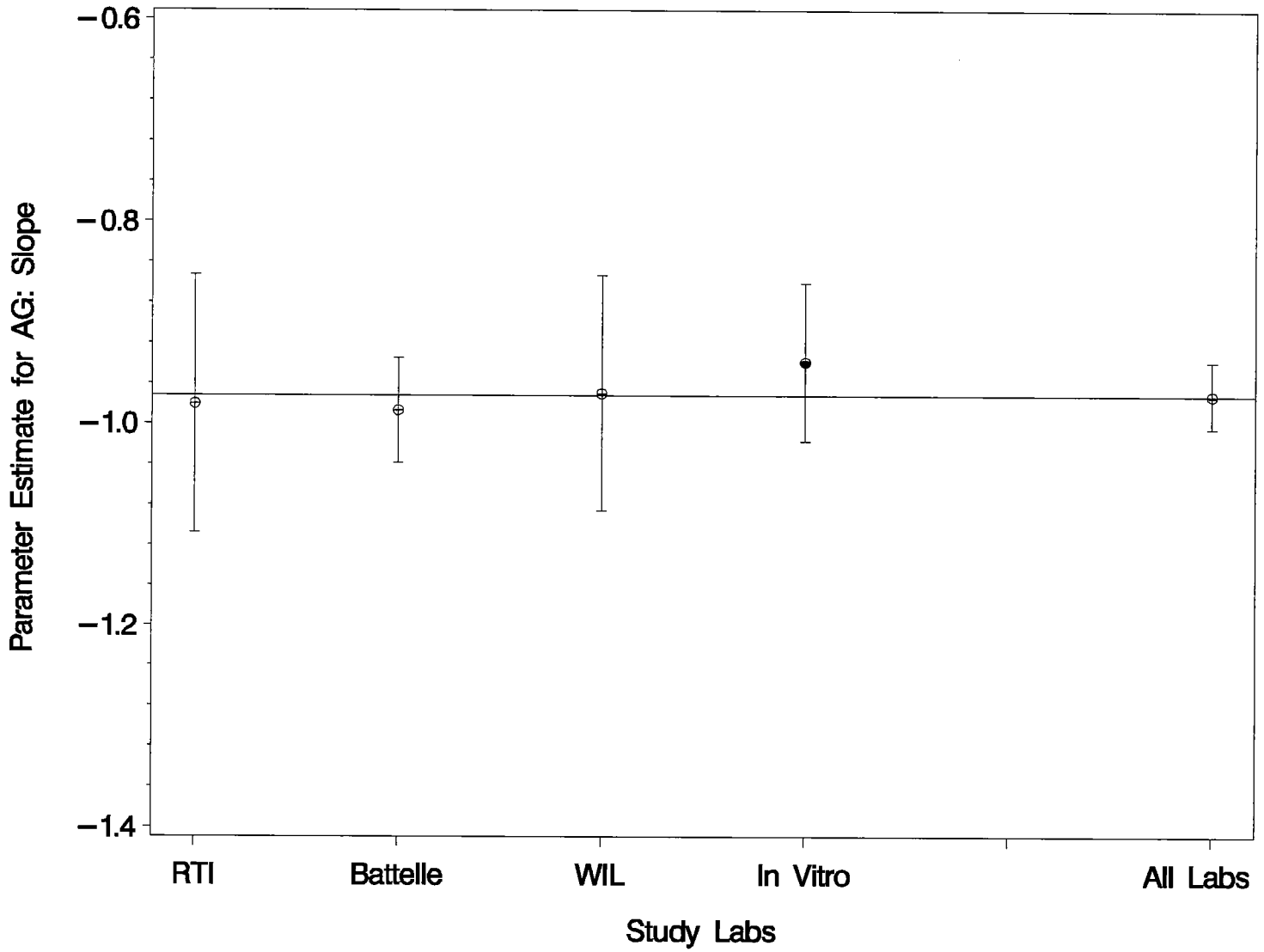


Figure 2. **Aminoglutethimide: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

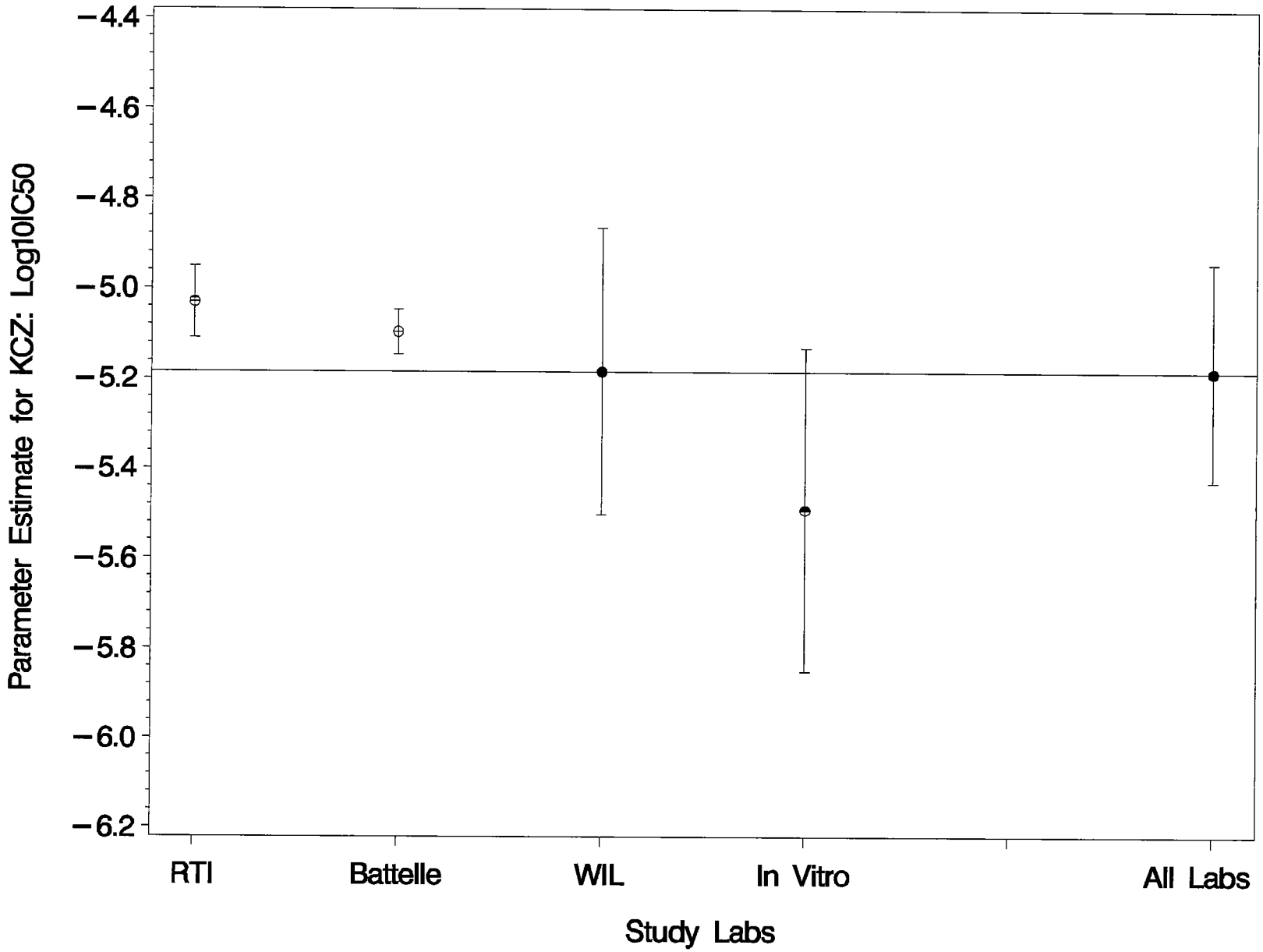


Figure 3. **Ketoconazole: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

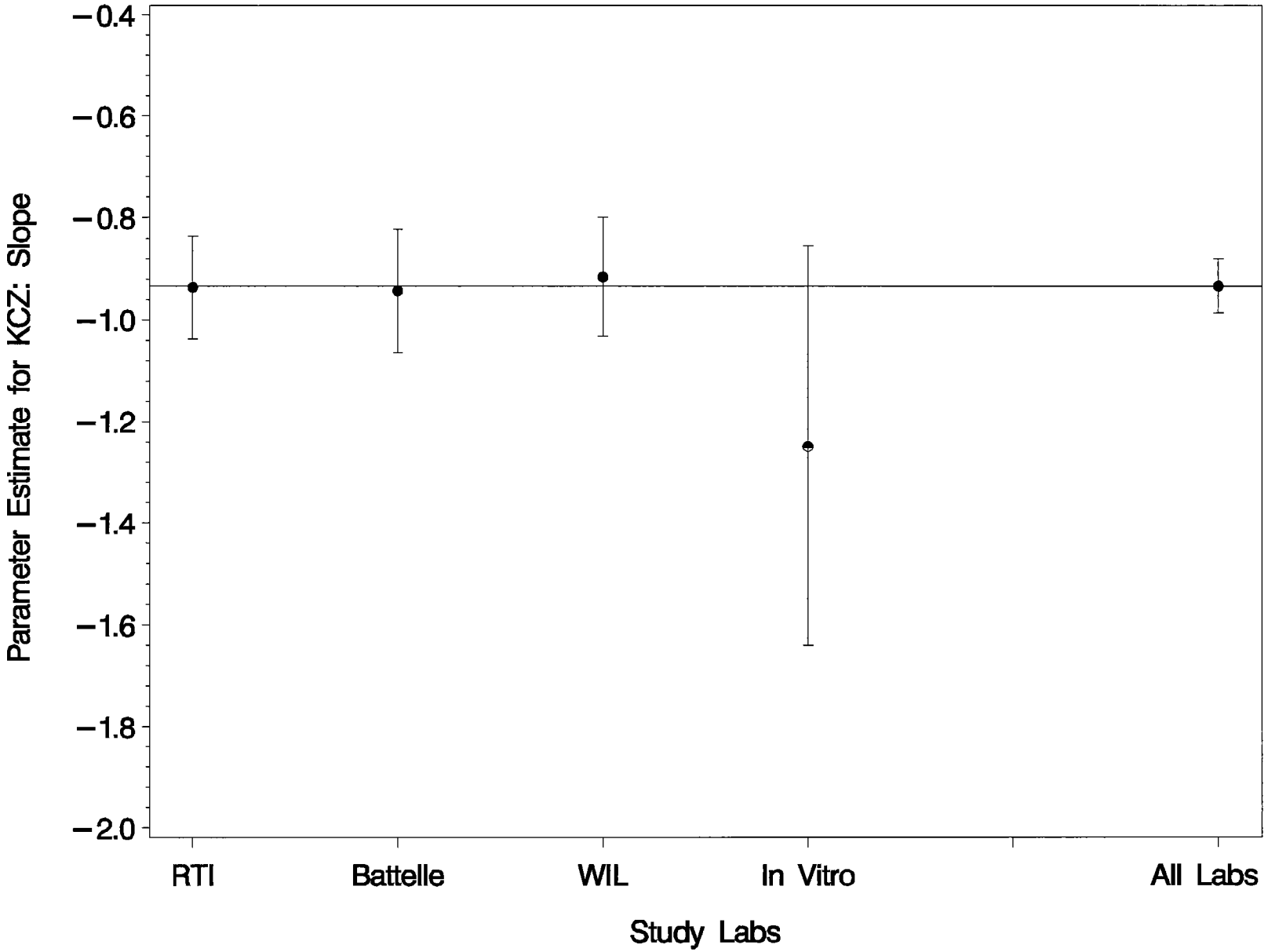


Figure 4. **Ketoconazole: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

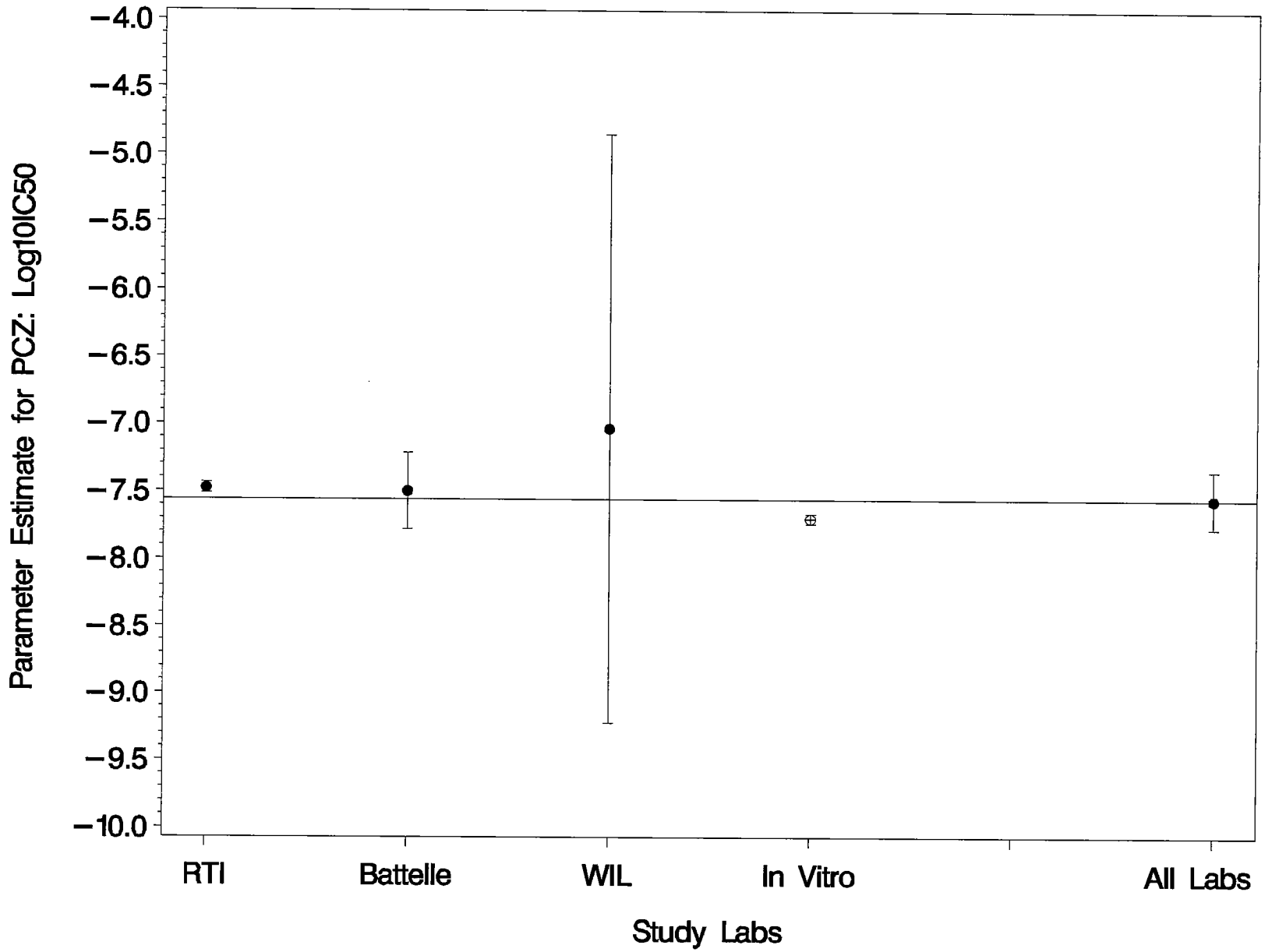


Figure 5. **Prochloraz: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

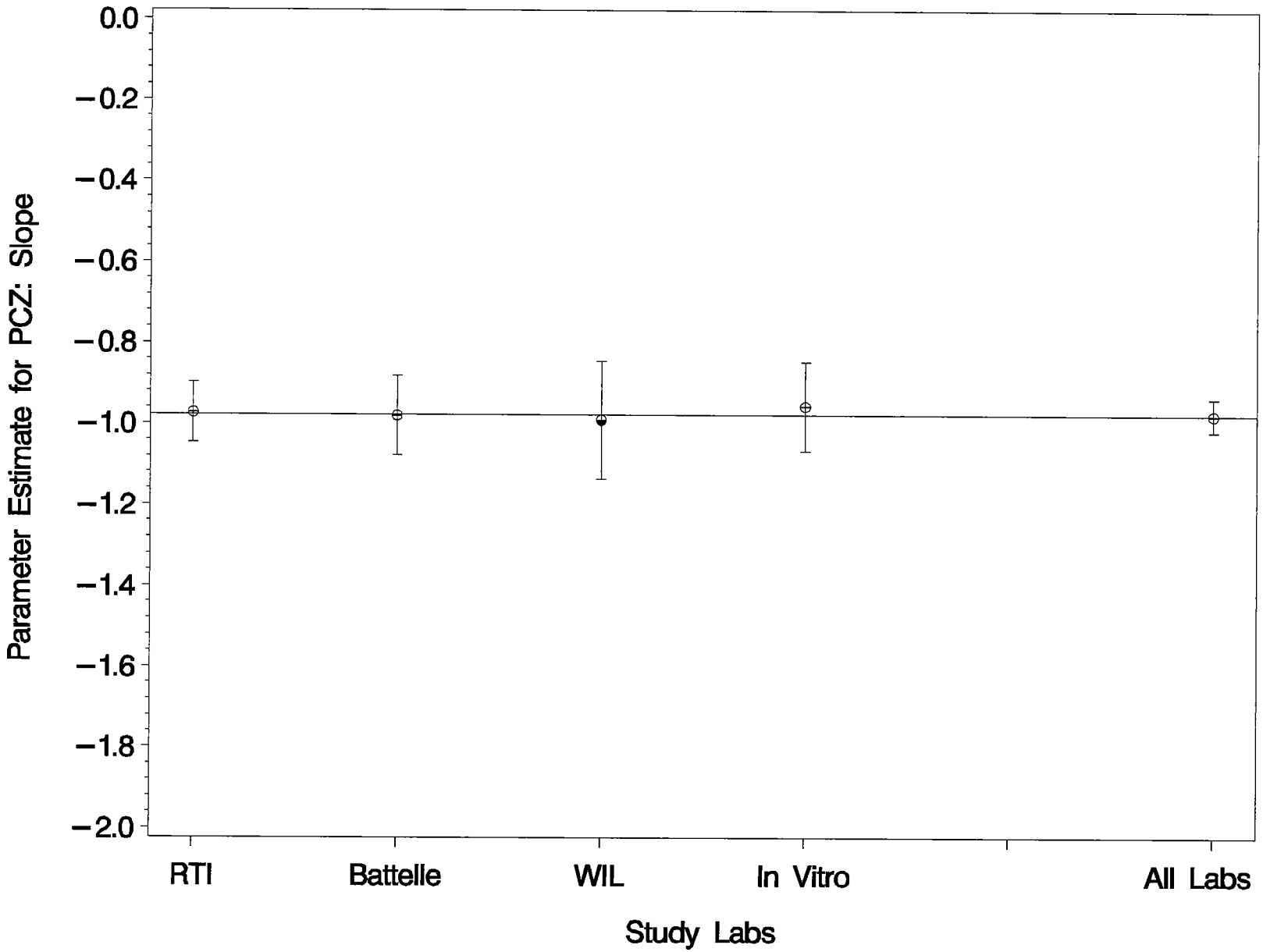


Figure 6. **<u>Prochloraz:</u> Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

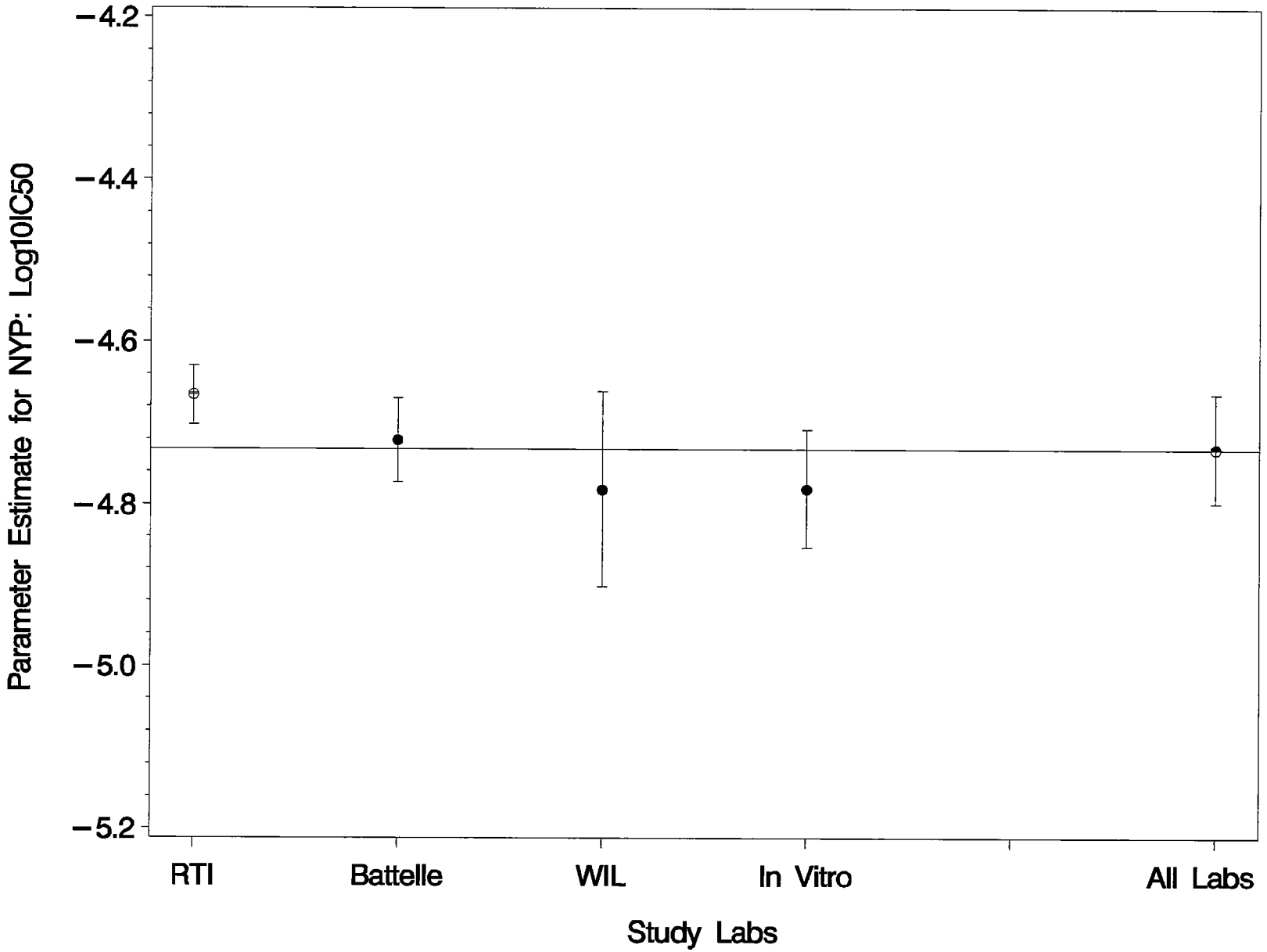


Figure 7. **4-Nonylphenol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

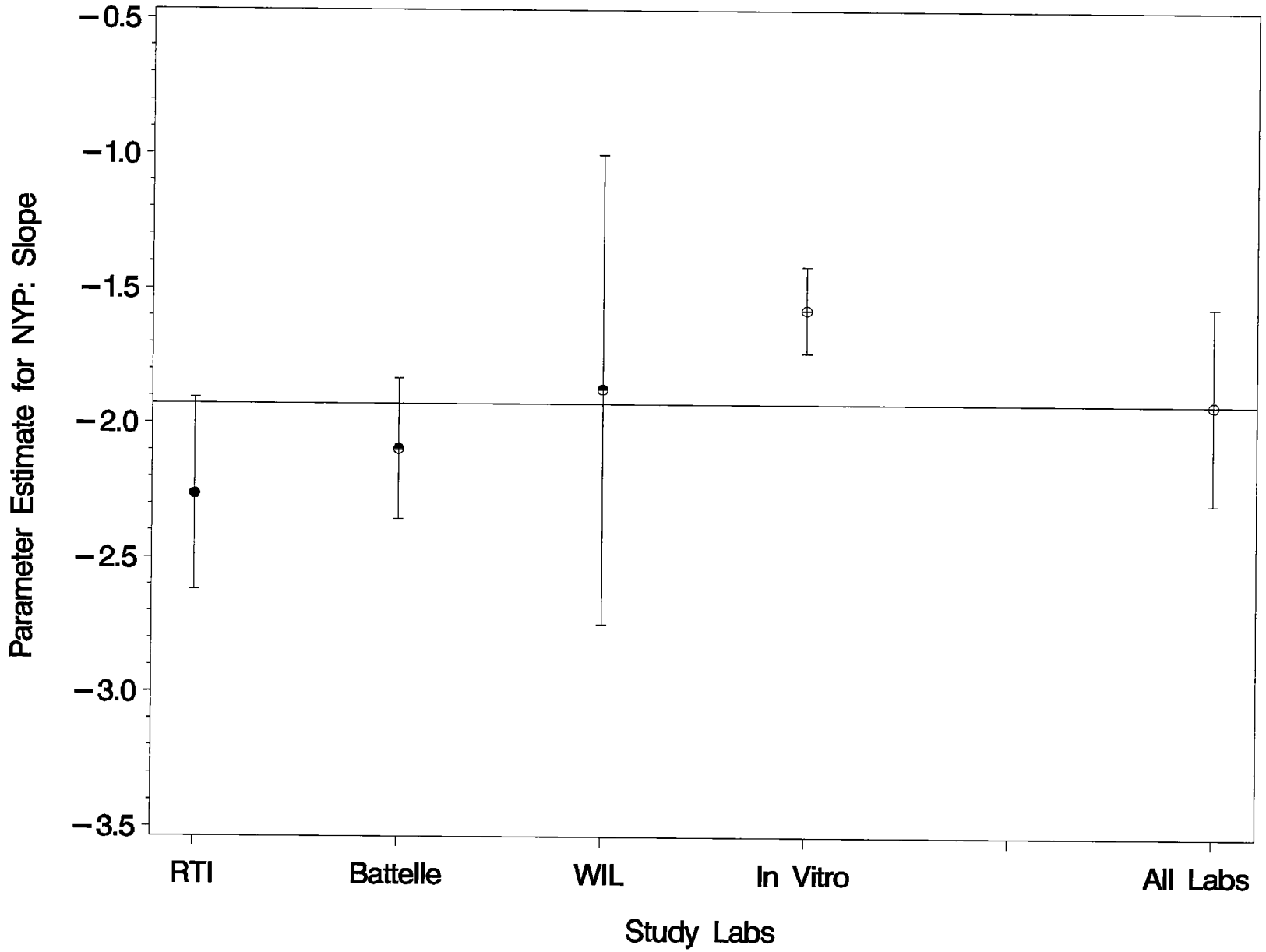


Figure 8. **4-Nonylphenol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

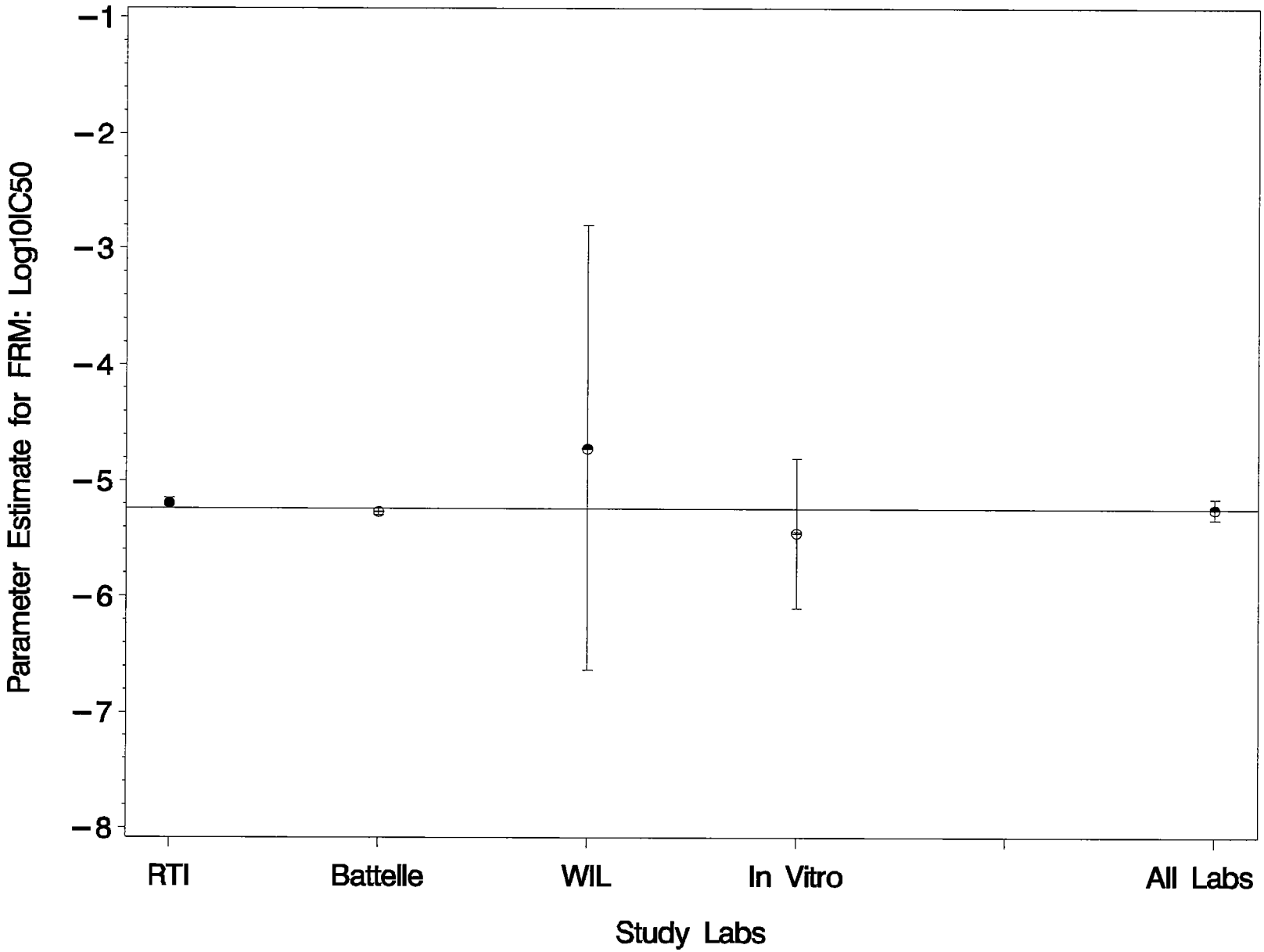


Figure 9. **Fenarimol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

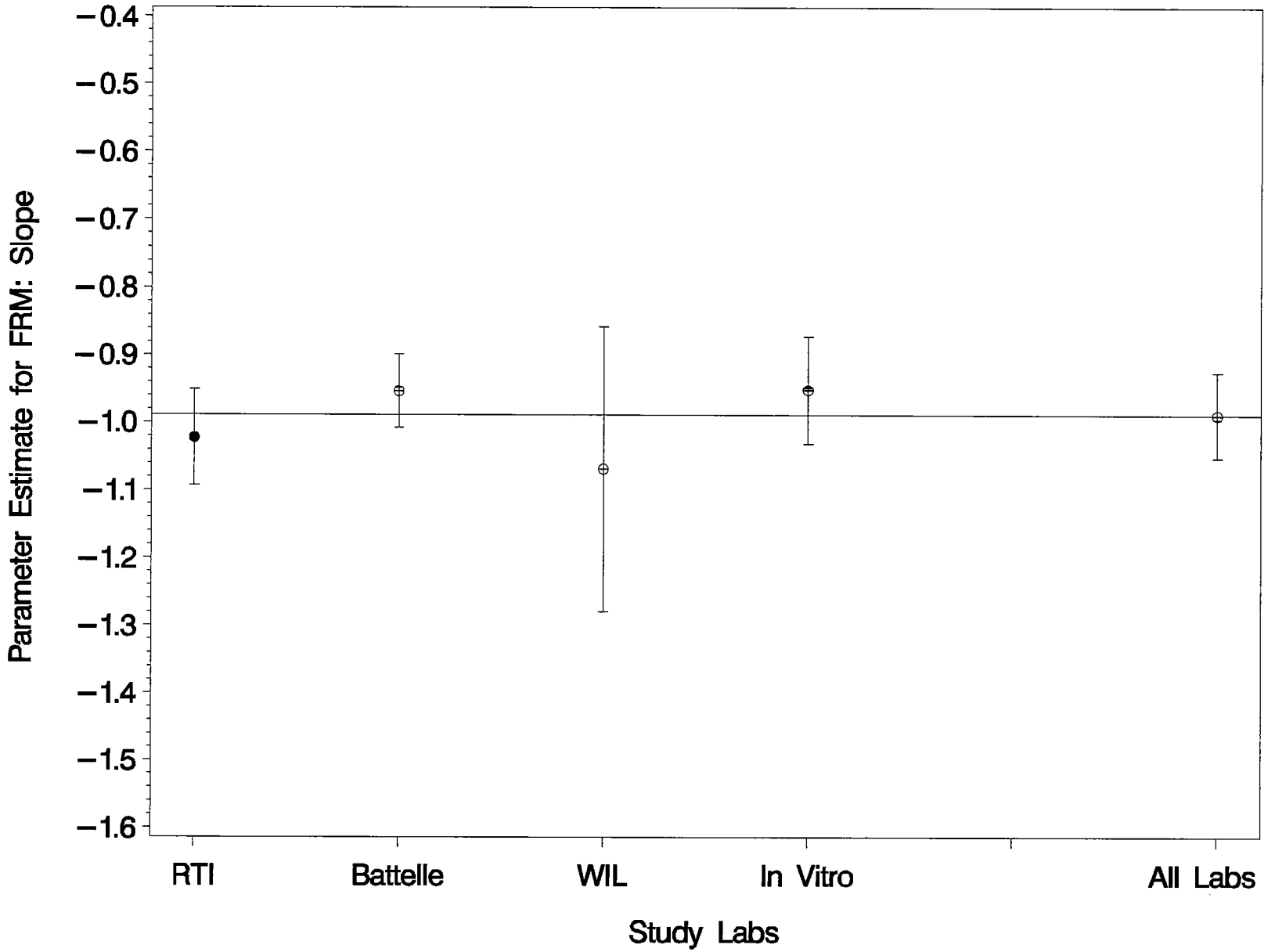


Figure 10. **Fenarimol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

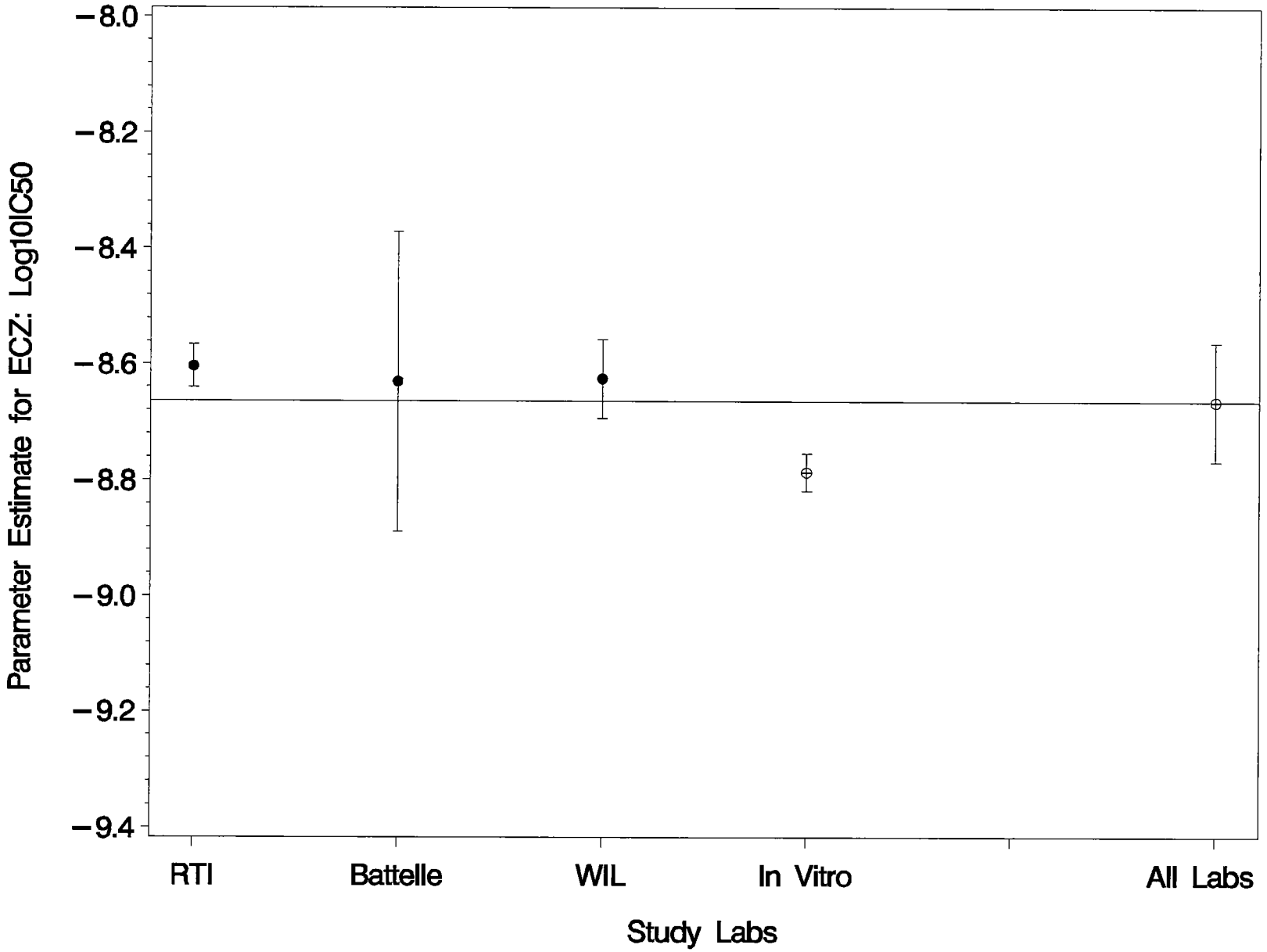


Figure 11. **<u>Econazole:</u> Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

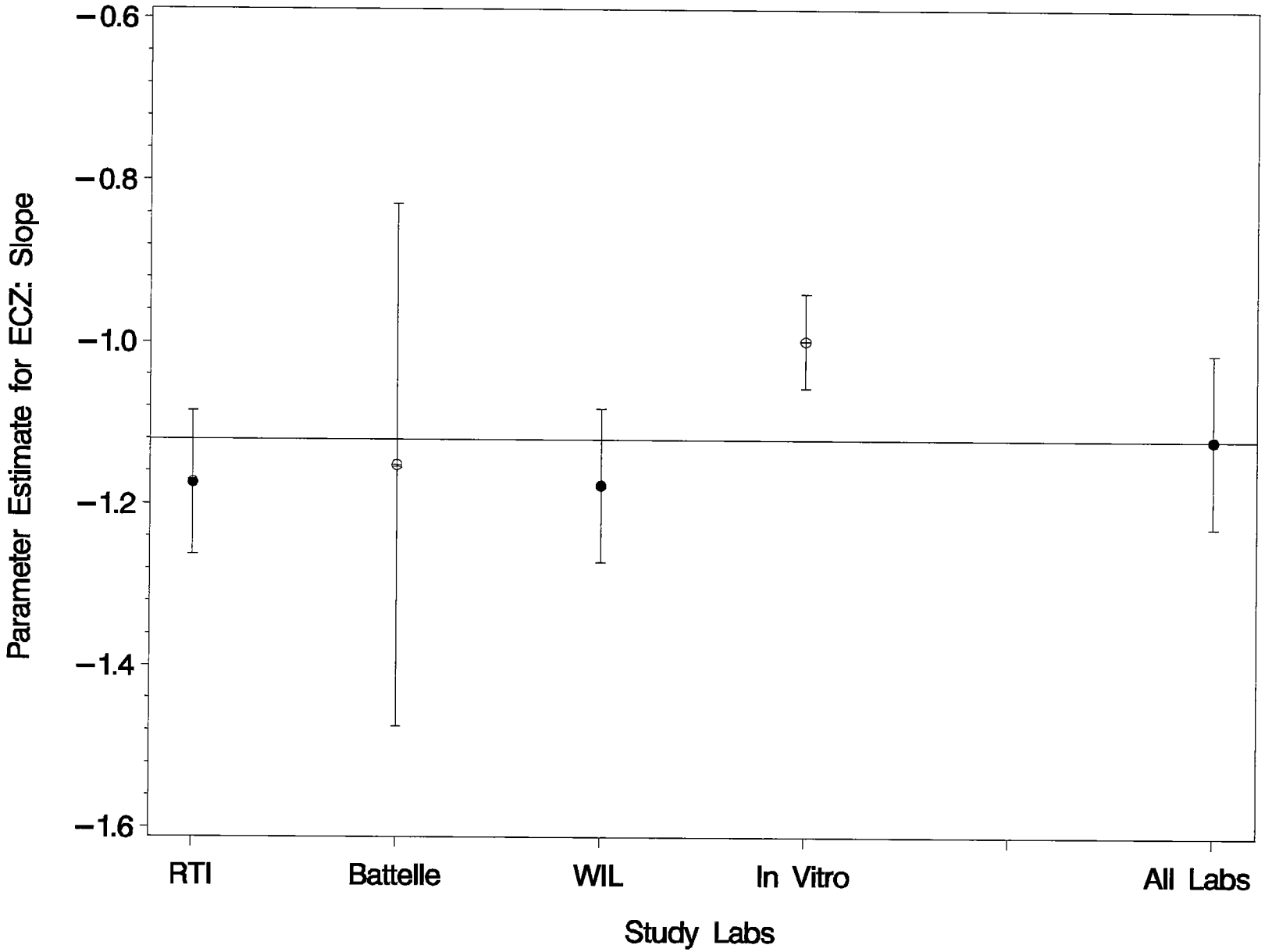


Figure 12. **Econazole: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

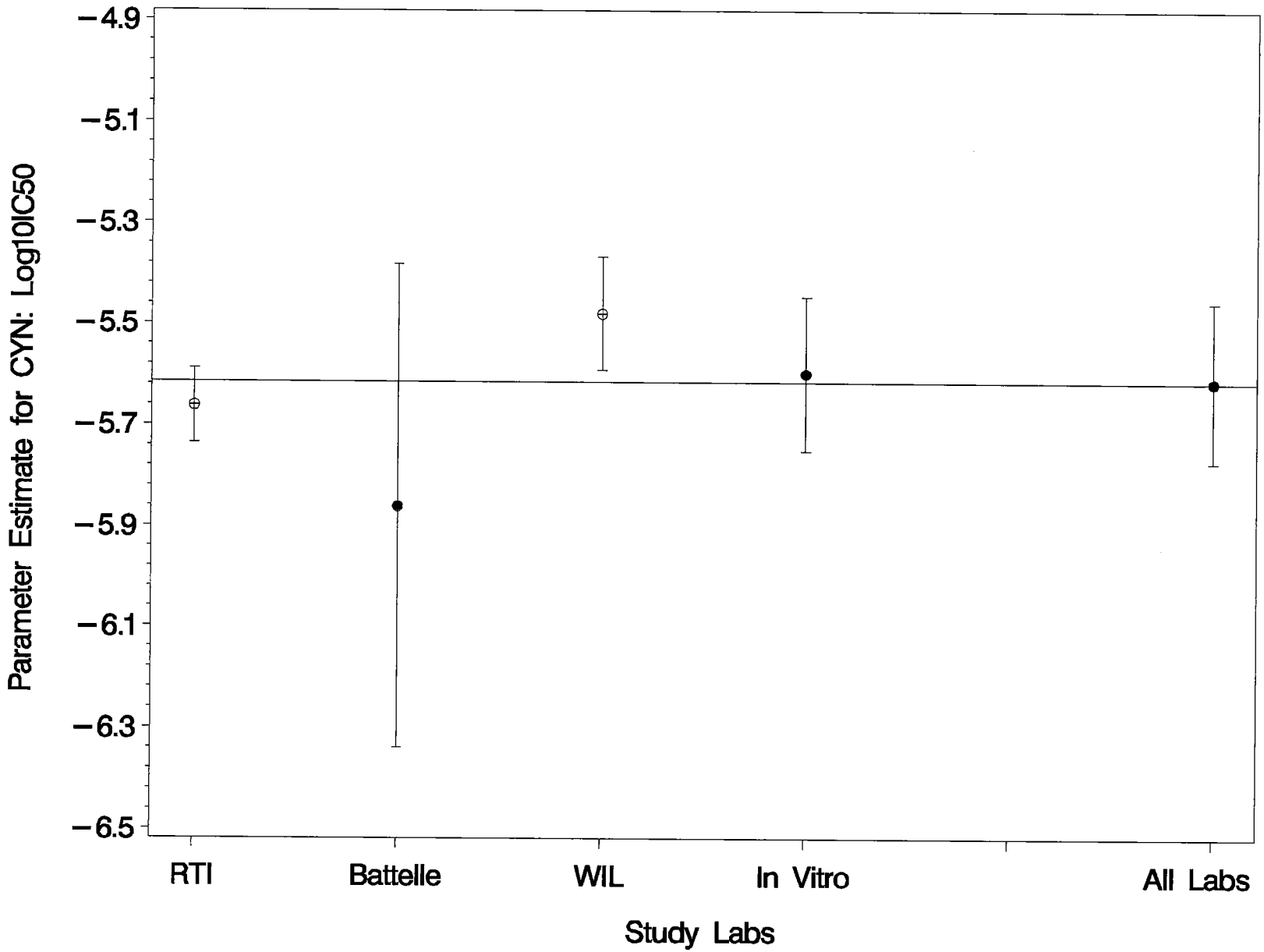


Figure 13. **Chrysin: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

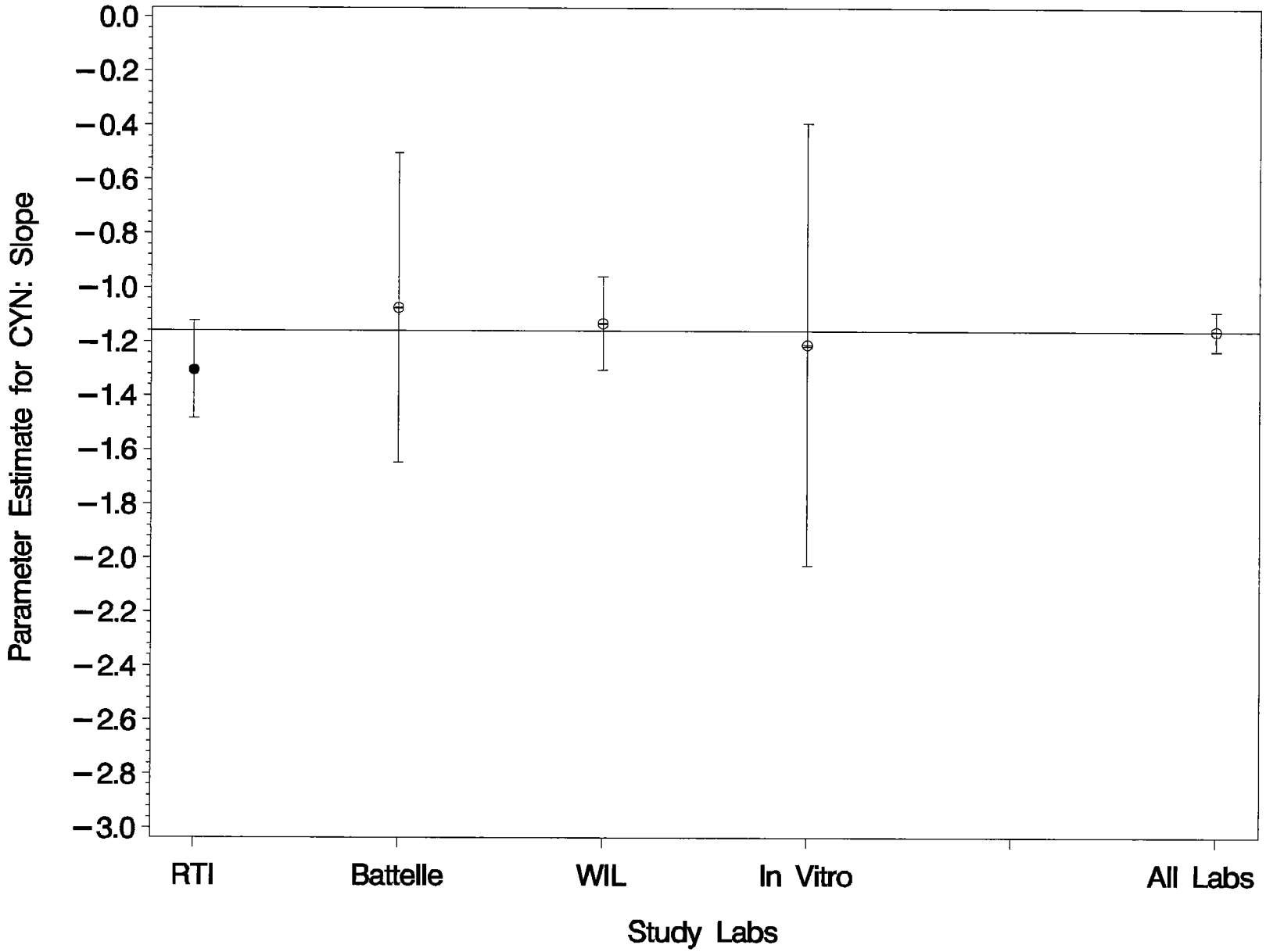


Figure 14. **<u>Chrysin:</u> Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

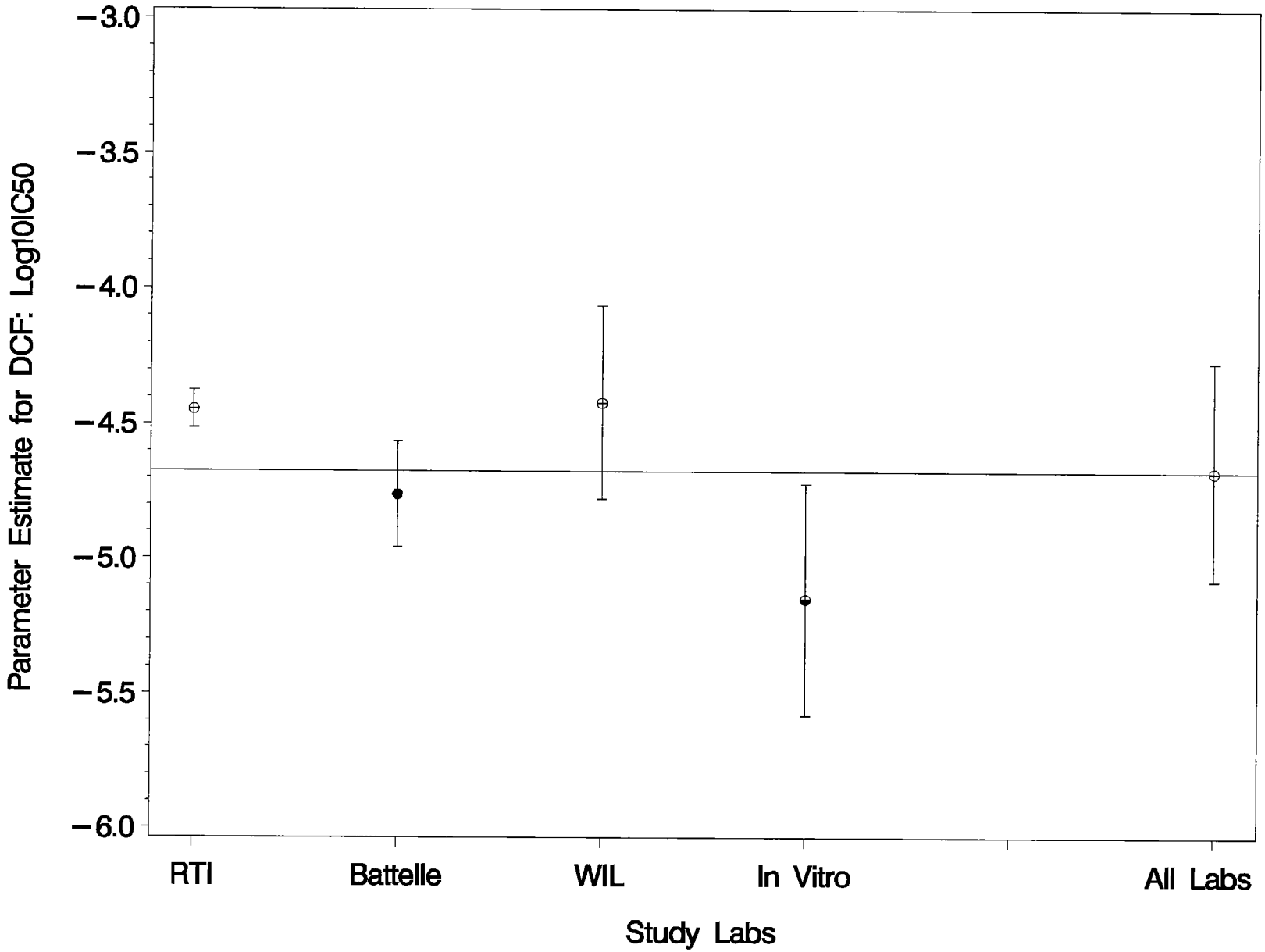


Figure 15. **Dicofol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for $Log_{10}IC_{50}$ in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

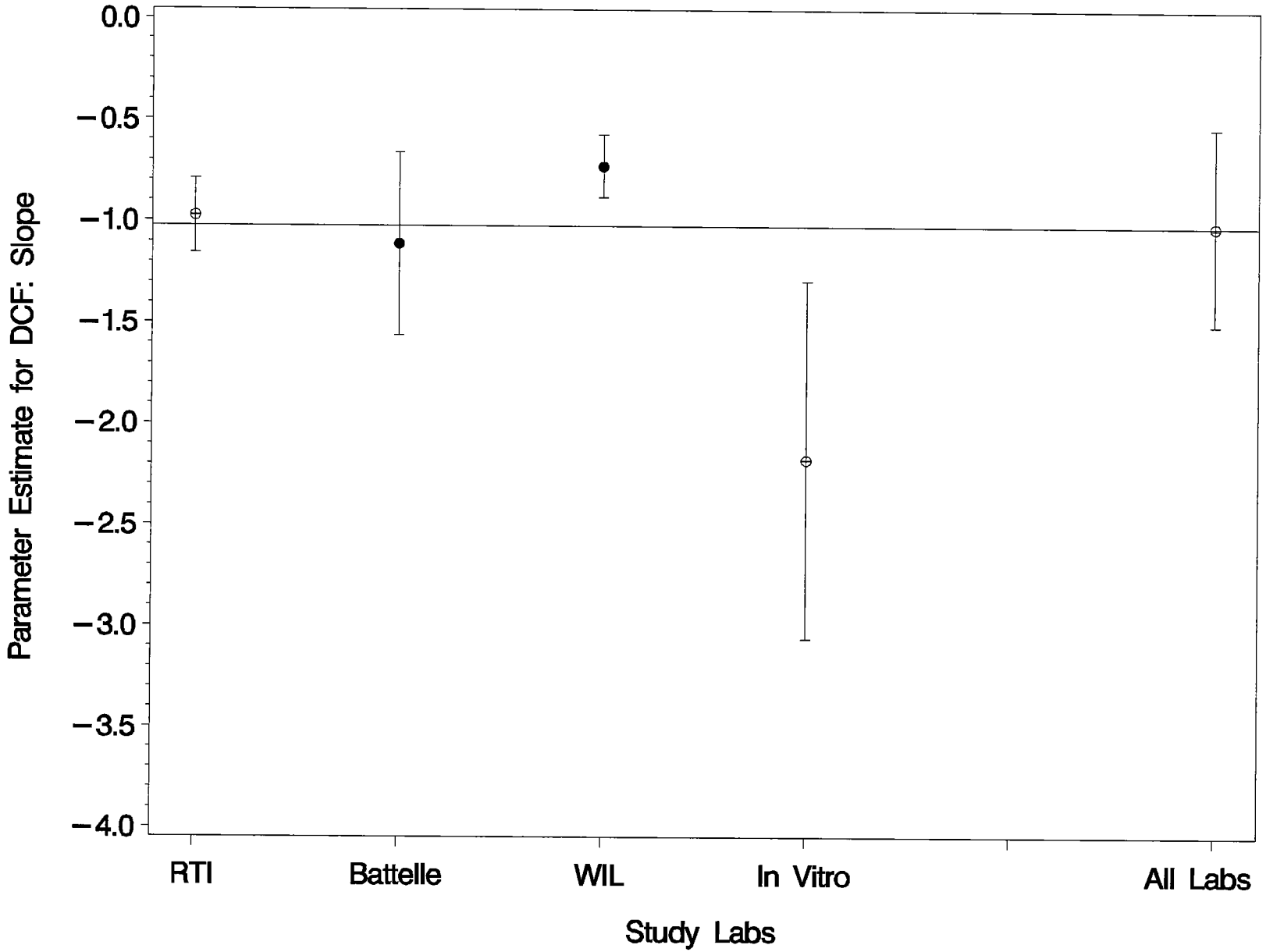


Figure 16. **Dicofol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Slope in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

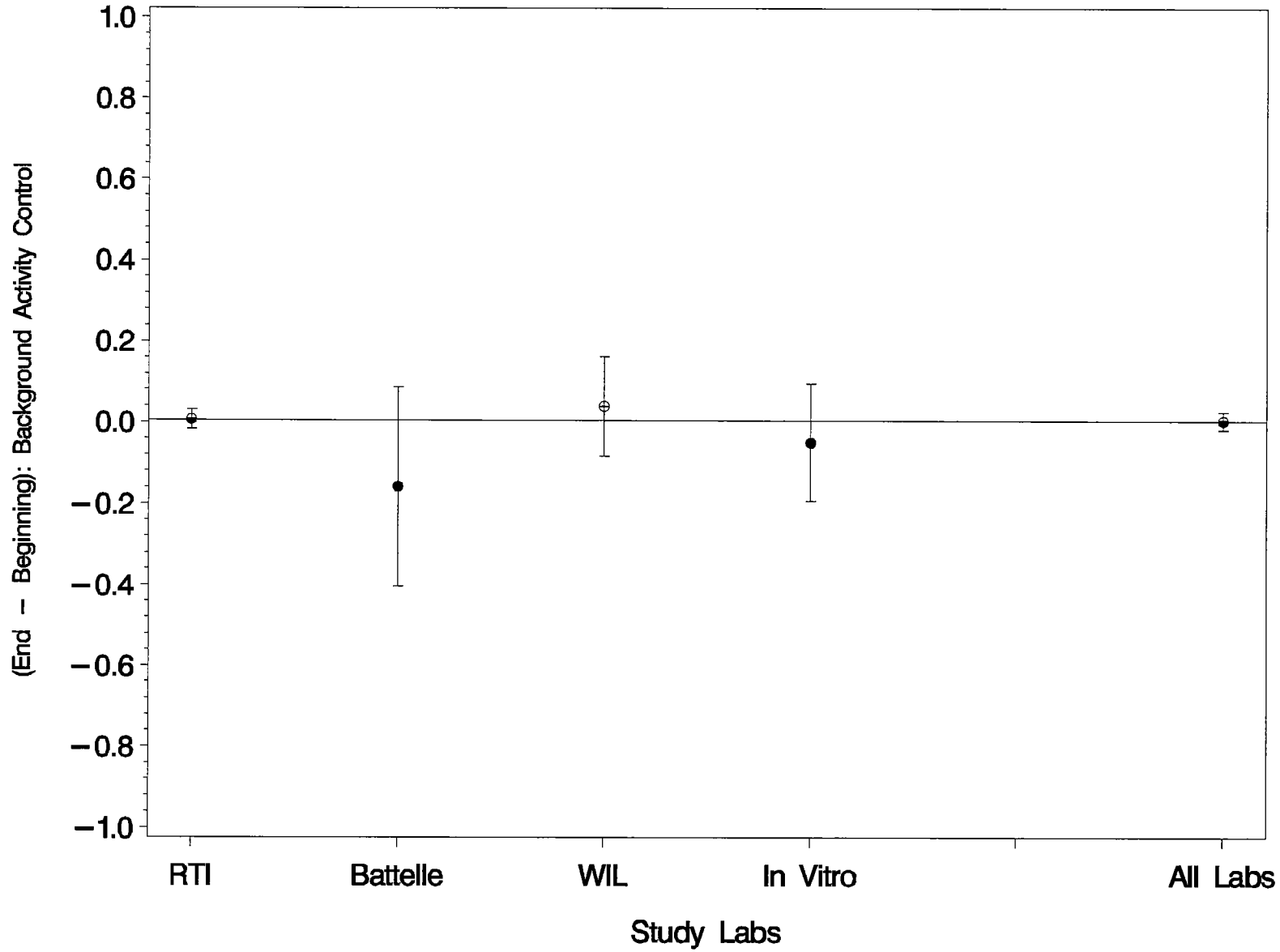


Figure 17. **Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Difference (End Minus Beginning) for Background Activity Controls in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

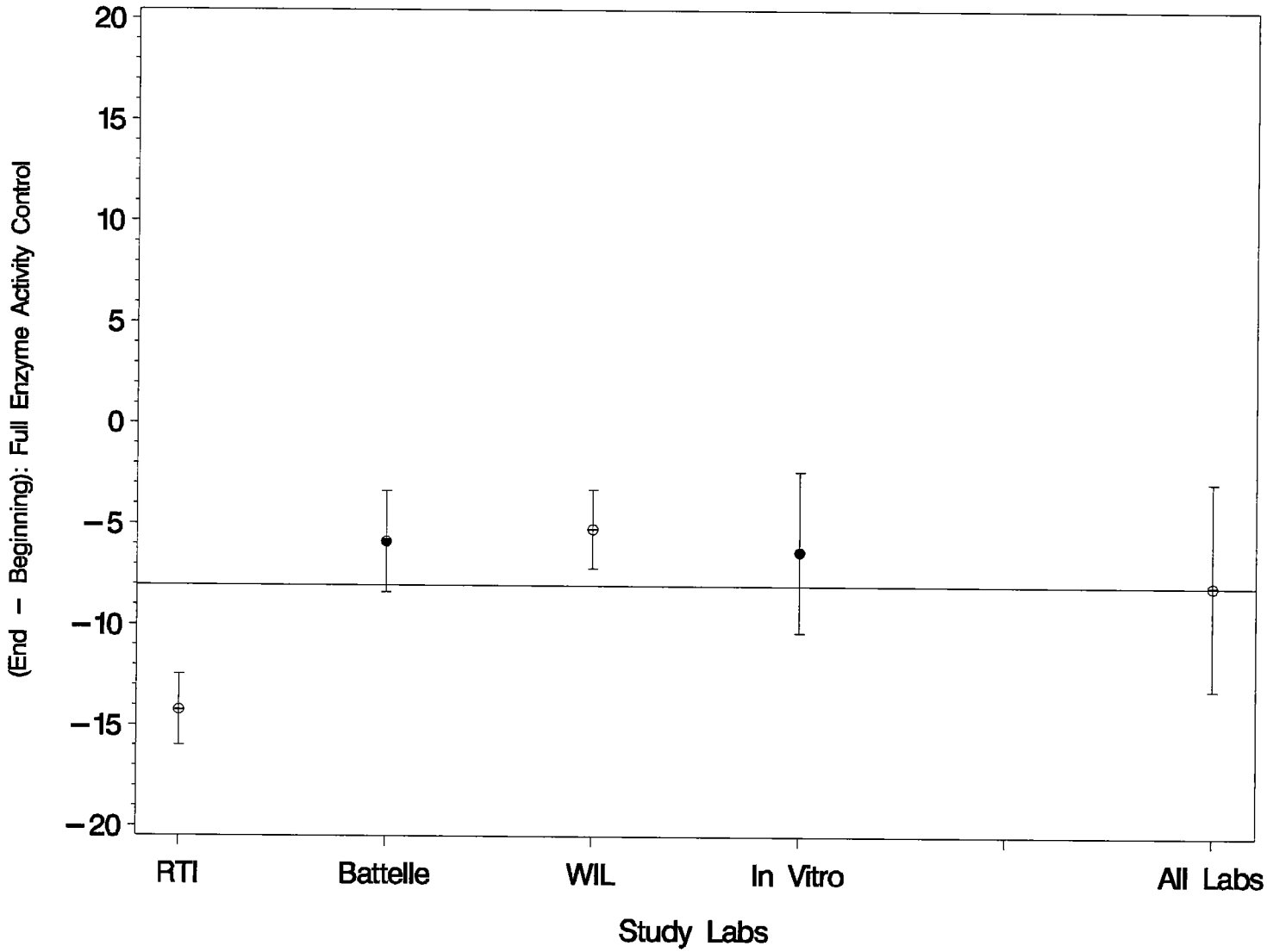


Figure 18. **Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Difference (End Minus Beginning) for Full Enzyme Activity Controls in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

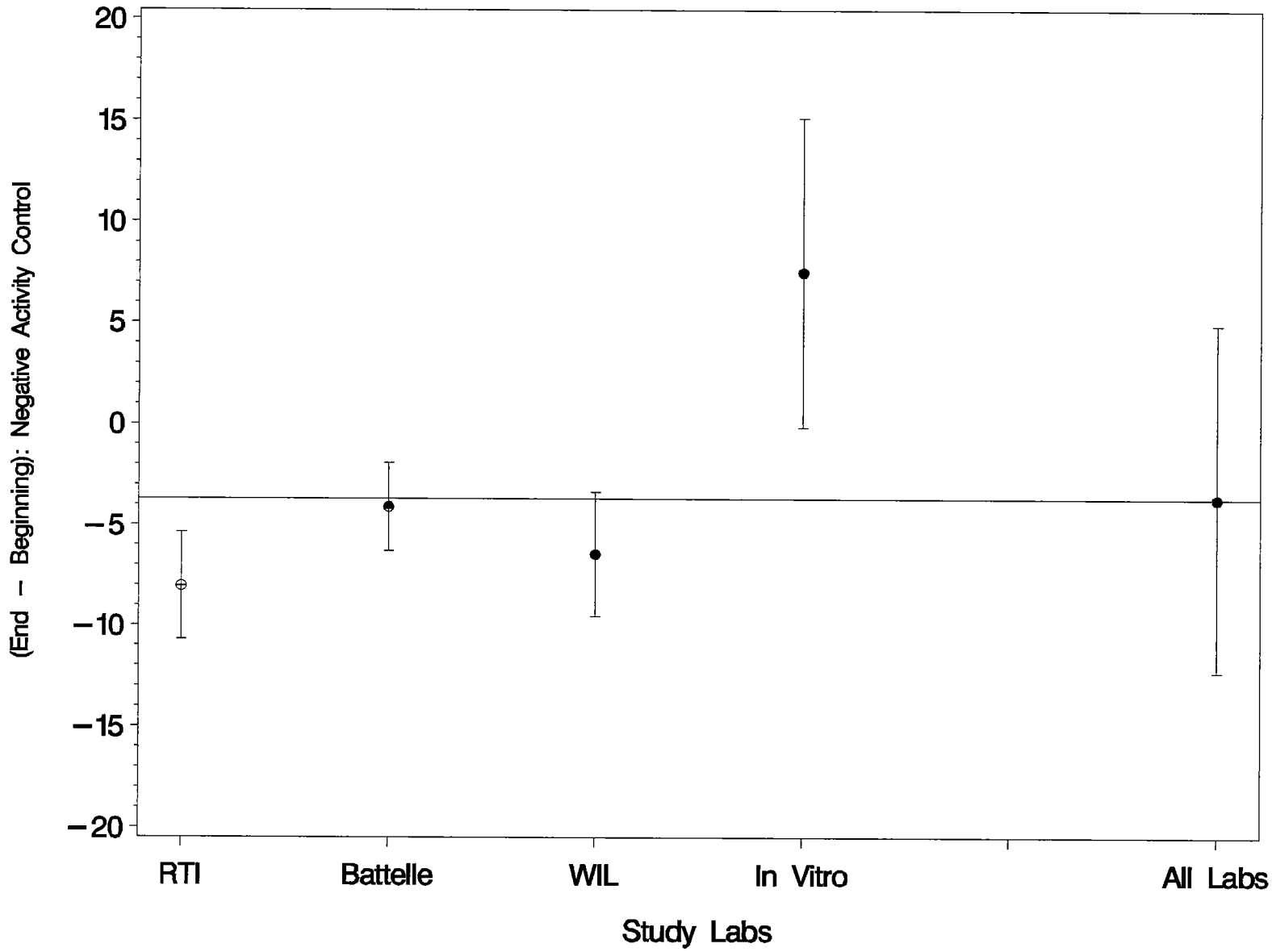


Figure 19. **Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Difference (End Minus Beginning) for Negative Controls in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

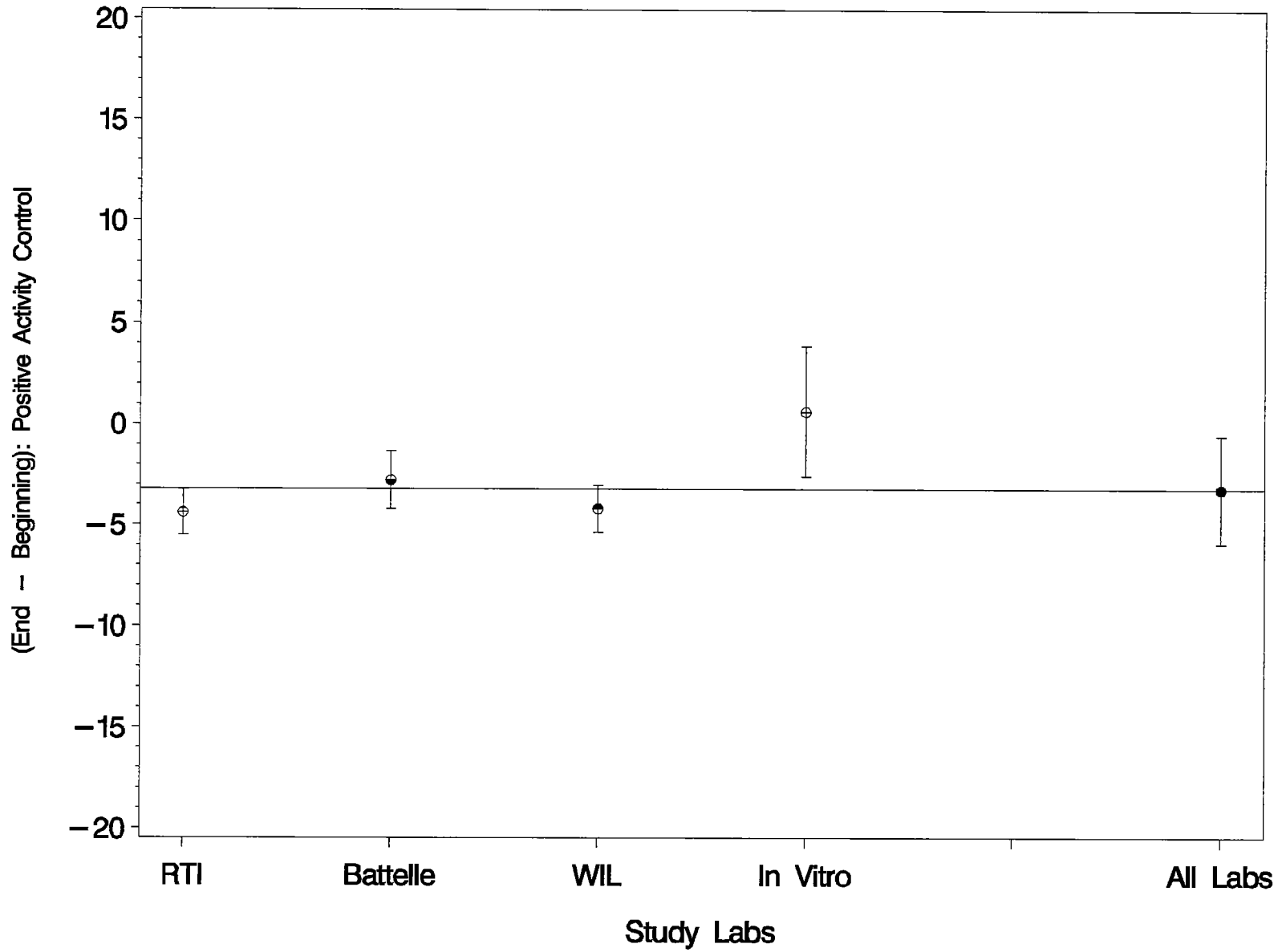


Figure 20. **Parameter Estimates and Their Associated 95 Percent Confidence Intervals for Difference (End Minus Beginning) for Positive Controls in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

Appendix A. Results of Interlaboratory Analyses for the Top and Bottom Threshold Parameters of the Concentration Response Relations.

- **Table A-1. Parameter Estimates and the 95 Percent Confidence Intervals for the Top and Bottom Threshold Parameters of the Concentration Response Relations for the Recombinant Aromatase Assay. Estimated by Chemical.**

- **Table A-2. Variance Components and Ratio of Between and Within Laboratories Variances for the Top and Bottom Threshold Parameters of the Concentration Response Relations for the Recombinant Aromatase Assay. Estimated by Chemical.**

- **Figures A-1 through A-16. Parameter Estimates and the 95 Percent Confidence Intervals for the Top and Bottom Threshold Parameters of the Concentration Response Relations for the Recombinant Aromatase Assay. Estimated by Chemical.**

Table A-1. Parameter Estimates and the 95 Percent Confidence Intervals for the Top and Bottom Threshold Parameters of the Concentration Response Relations for the Recombinant Aromatase Assay. Estimated by Chemical.

Chemical[4]	Parameter	Estimate and 95 Percent Confidence Interval					CV(percent) and 95 percent CI[3]
		RTI[1]	Battelle[1]	WIL[1]	In Vitro[1]	Overall[2]	
AG	Top	93.26(87.47,99.06)	97.65(88.69,106.61)	99.12(97.14,101.11)	110.32(99.99,120.66)	100.11(91.45,108.77)	5.98(3.51,18.81)
AG	Bottom	-0.22(-2.41,1.98)	-0.13(-1.34,1.08)	-0.14(-2.90,2.63)	-0.41(-2.57,1.75)	-0.18(-0.94,0.58)	
KCZ	Top	95.82(92.96,98.68)	101.46(83.26,119.66)	102.57(99.22,105.93)	101.69(74.38,129.01)	99.87(95.22,104.52)	3.79(2.43,8.47)
KCZ	Bottom	-0.90(-6.22,4.42)	-2.04(-6.59,2.50)	-2.48(-7.28,2.32)	4.88(0.95,8.80)	-0.27(-4.30,3.75)	
PCZ	Top	95.73(94.00,97.46)	98.34(88.49,108.19)	106.25(97.03,115.46)	96.30(53.42,139.17)	99.57(93.14,105.99)	4.78(2.90,13.08)
PCZ	Bottom	0.08(-1.40,1.56)	0.14(-0.65,0.93)	0.33(-2.35,3.01)	0.04(-1.01,1.08)	0.14(-0.40,0.67)	
NYP	Top	101.12(97.89,104.36)	97.42(78.80,116.03)	98.50(89.21,107.79)	93.49(90.80,96.19)	97.45(93.08,101.82)	3.63(2.32,8.20)
NYP	Bottom	1.22(-1.70,4.14)	0.79(-1.62,3.20)	-0.73(-4.40,2.93)	-1.58(-4.93,1.77)	-0.14(-1.30,1.02)	
FRM	Top	90.75(89.79,91.71)	99.35(97.51,101.19)	97.66(85.61,109.72)	95.49(64.51,126.48)	95.66(90.18,101.13)	4.60(2.92,10.58)
FRM	Bottom	0.89(-0.52,2.30)	0.47(-0.85,1.79)	5.73(-17.35,28.82)	10.75(-33.52,55.01)	0.74(-0.72,2.20)	
ECZ	Top	99.78(96.98,102.58)	92.95(73.77,112.13)	101.30(98.00,104.59)	95.72(73.80,117.64)	100.66(98.90,102.41)	1.29(0.78,3.51)
ECZ	Bottom	0.53(-1.01,2.07)	0.21(-0.18,0.60)	0.16(-0.47,0.79)	-0.01(-0.58,0.57)	0.16(-0.06,0.38)	
CYN	Top	91.92(89.71,94.12)	85.83(61.46,110.21)	94.19(84.30,104.08)	89.45(67.44,111.45)	92.05(89.65,94.44)	2.00(1.24,5.03)
CYN	Bottom	22.58(19.65,25.51)	21.98(13.07,30.89)	12.79(8.77,16.81)	17.40(-10.25,45.05)	18.61(12.50,24.73)	
DCF	Top	98.99(91.58,106.41)	94.07(83.44,104.70)	101.62(98.64,104.59)	89.32(81.79,96.85)	96.03(89.42,102.63)	5.32(3.30,13.31)
DCF	Bottom	-0.93(-4.60,2.73)	2.05(-1.16,5.26)	-6.00(-15.62,3.62)	41.07(12.93,69.21)	8.06(-17.72,33.83)	

1. The estimates and 95 percent confidence intervals are based on the intralaboratory analyses for the four participating laboratories. The intralaboratory analyses were performed by individual chemical.
2. The overall estimates and confidence intervals were estimated using a random effects analysis of variance, with laboratory as a random effect and with heterogeneous variances among the four laboratories. The variance for each laboratory was specified as the square of the within laboratory standard error. The degrees of freedom are given in Table A-2.
3. CV was calculated for the top parameter based on average results.
4. Concentration response relations were not fitted for dibenz[a,h]anthracene and for atrazine, since they resulted in very little aromatase inhibition (i.e. they were noninhibitors).

Table A-2. Variance Components and Ratio of Between and Within Laboratories Variances for the Top and Bottom Threshold Parameter of the Concentration Response Relations for the Recombinant Aromatase Assay. Estimated by Chemical.

Chemical[7]	Parameter	Within Laboratory Variance[1]					Among Laboratory Variance[3] and (p-value) (df=3)	Mean Variance[4,5]	Ratio and 95 percent CI[6]
		RTI	Battelle	WIL	In Vitro	Pooled Results[2]			
AG	Top	7.468/df=16.0	4.562/df=2.1	0.213/df=2.0	5.598/df=2.0	4.460/df=10.8	31.628 (0.11830)	8.970/df=3.6	7.091 (1.518, 101.998)
AG	Bottom	1.075/df=16.0	0.367/df=57.4	0.412/df=2.0	1.149/df=43.6	0.751/df=47.5	0.000 1.000)	0.144/df=47.5	0.000 (0.000, 0.000)
KCZ	Top	1.815/df=16.0	17.825/df=2.0	0.607/df=2.0	40.883/df=2.0	15.283/df=3.8	7.904 (0.15340)	3.574/df=5.9	0.517 (0.048, 7.841)
KCZ	Bottom	6.293/df=16.0	4.744/df=20.0	1.245/df=2.0	3.834/df=55.3	4.029/df=56.0	5.616 (0.18784)	2.315/df=4.6	1.394 (0.415, 19.514)
PCZ	Top	0.665/df=16.0	3.031/df=1.6	4.588/df=2.0	98.843/df=2.0	26.782/df=2.3	15.251 (0.14510)	5.659/df=4.3	0.569 (0.022, 8.994)
PCZ	Bottom	0.488/df=16.0	0.145/df=23.0	0.387/df=2.0	0.259/df=27.1	0.320/df=17.6	0.000 (1.000)	0.065/df=17.6	0.000 (0.000, 0.000)
NYP	Top	2.329/df=16.0	20.403/df=2.1	4.661/df=2.0	1.028/df=4.5	7.105/df=3.8	8.093 (0.13484)	3.134/df=5.8	1.139 (0.108, 17.249)
NYP	Bottom	1.894/df=16.0	1.418/df=38.6	0.726/df=2.0	2.592/df=21.0	1.658/df=51.1	0.000 (1.000)	0.334/df=51.1	0.000 (0.000, 0.000)
FRM	Top	0.205/df=16.0	0.370/df=3.3	7.851/df=2.0	51.509/df=2.0	14.984/df=2.6	13.528 (0.10895)	4.833/df=5.6	0.903 (0.046, 14.097)
FRM	Bottom	0.440/df=16.0	0.429/df=42.7	28.783/df=2.0	107.060/df=2.0	34.178/df=3.1	0.000 (1.000)	0.215/df=3.1	0.000 (0.000, 0.000)
ECZ	Top	1.740/df=16.0	19.643/df=2.0	0.587/df=2.0	25.150/df=2.0	11.780/df=4.3	0.000 (1.000)	0.422/df=4.3	0.000 (0.000, 0.000)
ECZ	Bottom	0.530/df=16.0	0.037/df=59.5	0.021/df=2.0	0.081/df=42.9	0.167/df=24.9	0.000 (1.000)	0.011/df=24.9	0.000 (0.000, 0.000)
CYN	Top	1.081/df=16.0	34.199/df=2.1	5.285/df=2.0	25.573/df=2.0	16.535/df=4.8	0.000 (1.000)	0.846/df=4.8	0.000 (0.000, 0.000)
CYN	Bottom	1.906/df=16.0	7.890/df=3.0	0.872/df=2.0	44.676/df=2.1	13.836/df=3.1	17.369 (0.11661)	6.157/df=5.8	1.255 (0.088, 19.312)
DCF	Top	12.237/df=16.0	5.746/df=1.9	0.477/df=2.0	3.291/df=2.1	5.438/df=14.9	21.276 (0.10490)	6.514/df=4.9	3.913 (0.941, 55.774)
DCF	Bottom	2.988/df=16.0	2.547/df=45.6	5.000/df=2.0	44.489/df=2.0	13.756/df=3.1	294.355 (0.10299)	76.787/df=3.5	21.398 (1.453, 329.632)

1. The within laboratory variance for each laboratory is the square of the standard error associated with the parameter estimate, which was reported in the intralaboratory analyses for each of the four participating laboratories.
2. Pooled average for the within laboratory variances is the unweighted average of the within laboratory variances among the four laboratories. Associated degrees of freedom were based on Satterthwaite's approximation.
3. Variance among laboratories is based on a random effects analysis of variance model with heterogeneous variances among the 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 to the pooled average within laboratory variance.
7. Concentration response relations were not fitted for dibenz[a,h]anthracene and for atrazine, since they resulted in very little aromatase inhibition (i.e. they were noninhibitors).

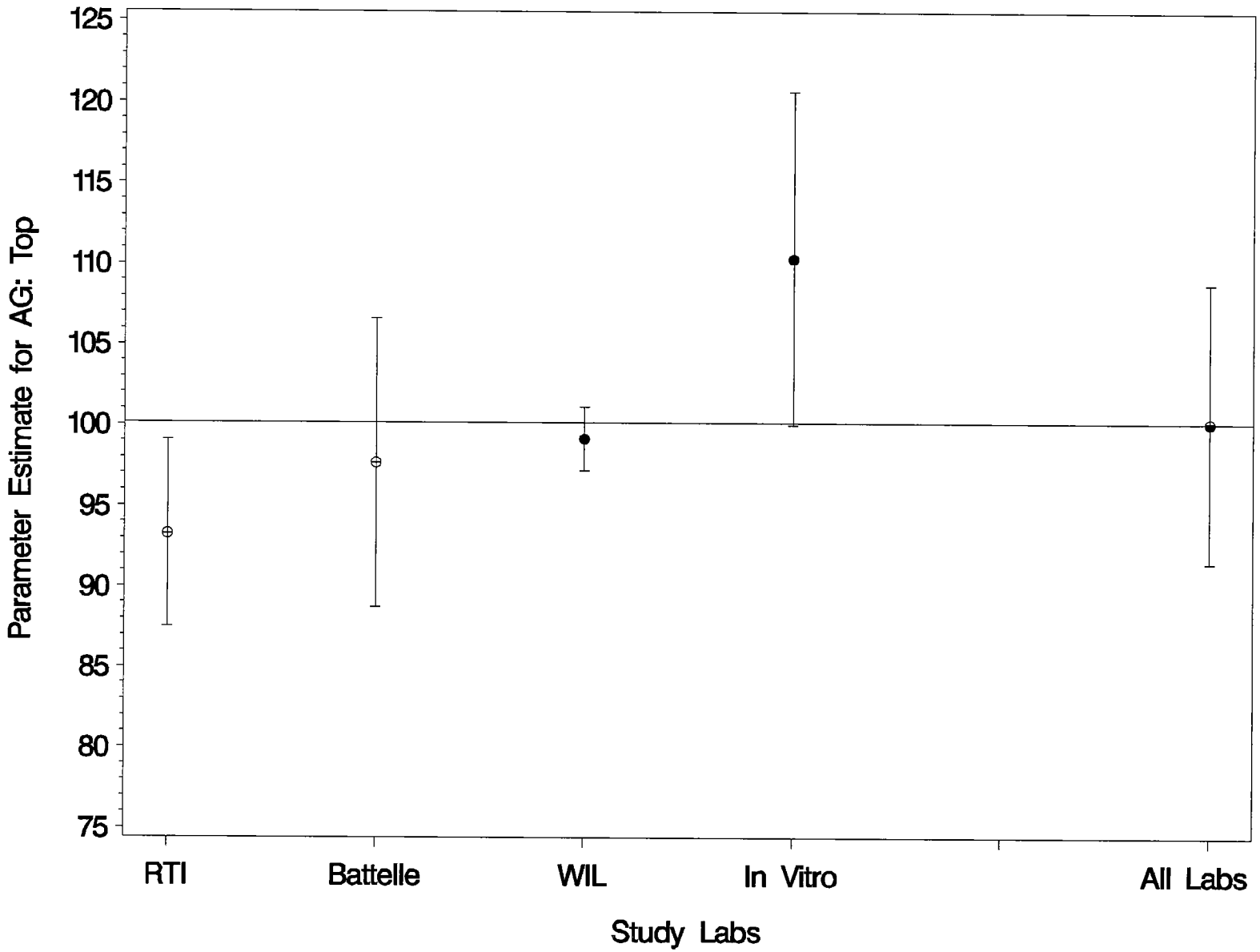


Figure A-1. **Aminoglutethimide: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Top threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

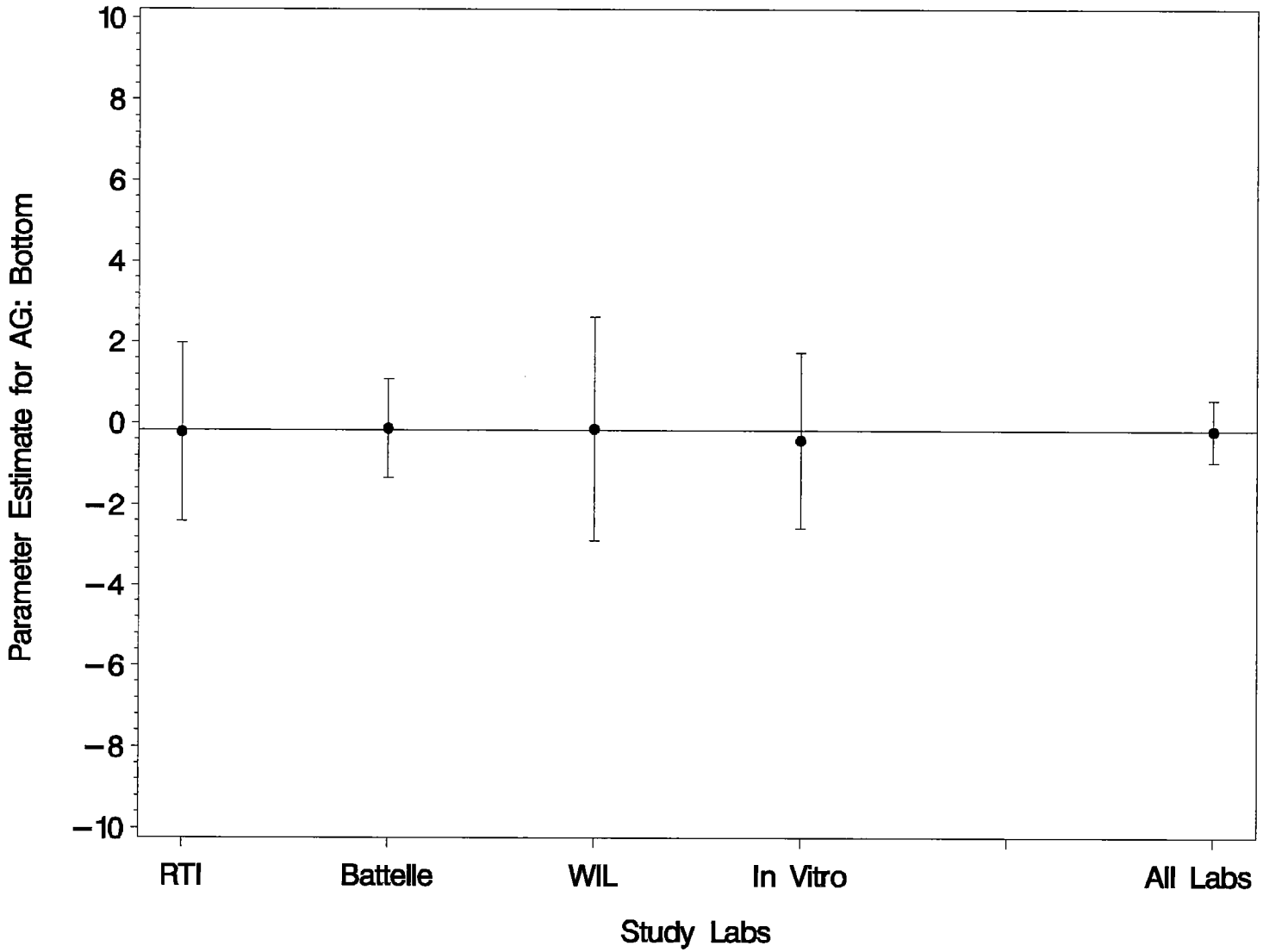


Figure A-2. **Aminoglutethimide: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Bottom Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

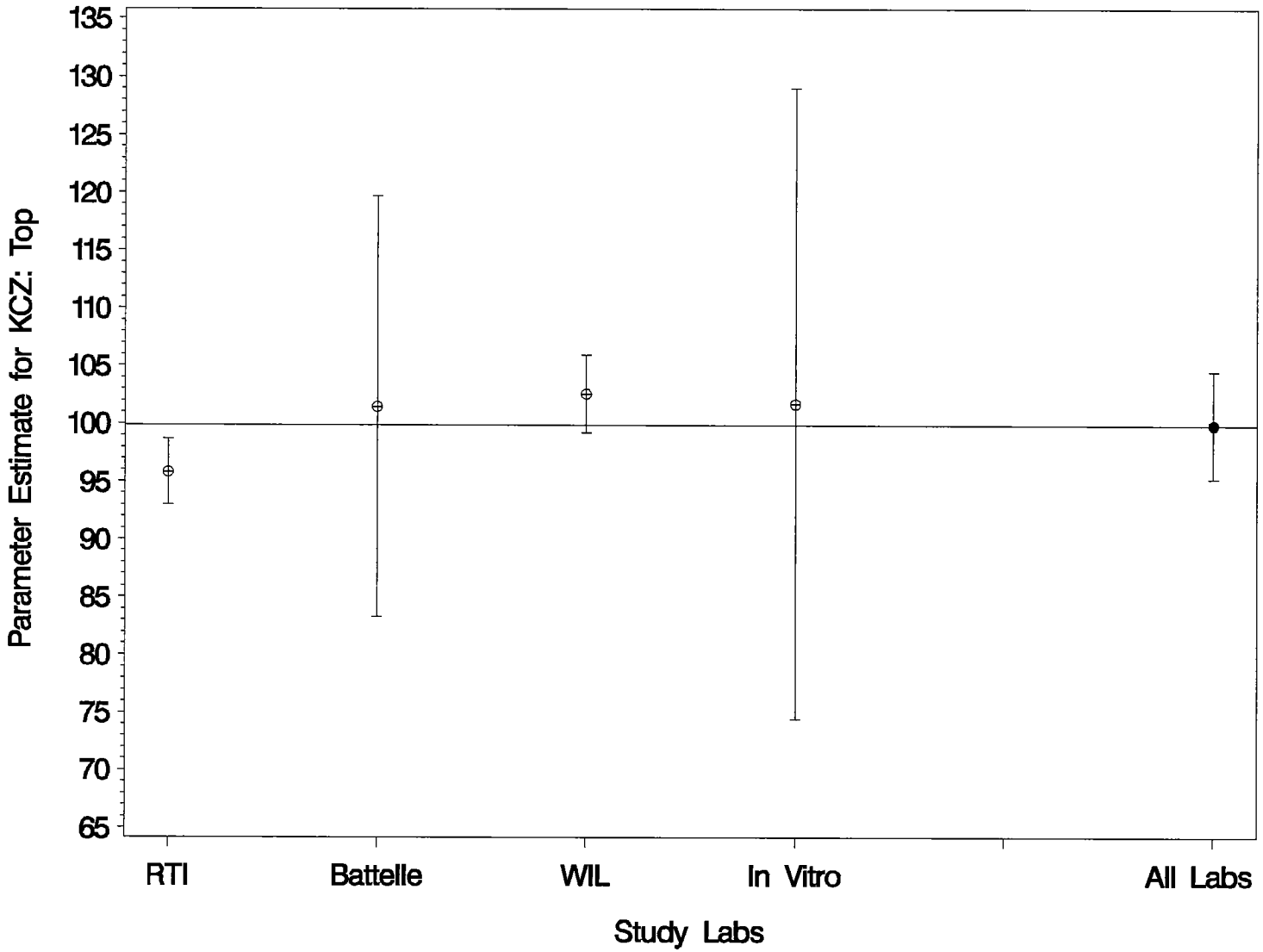


Figure A-3. **Ketoconazole: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Top Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

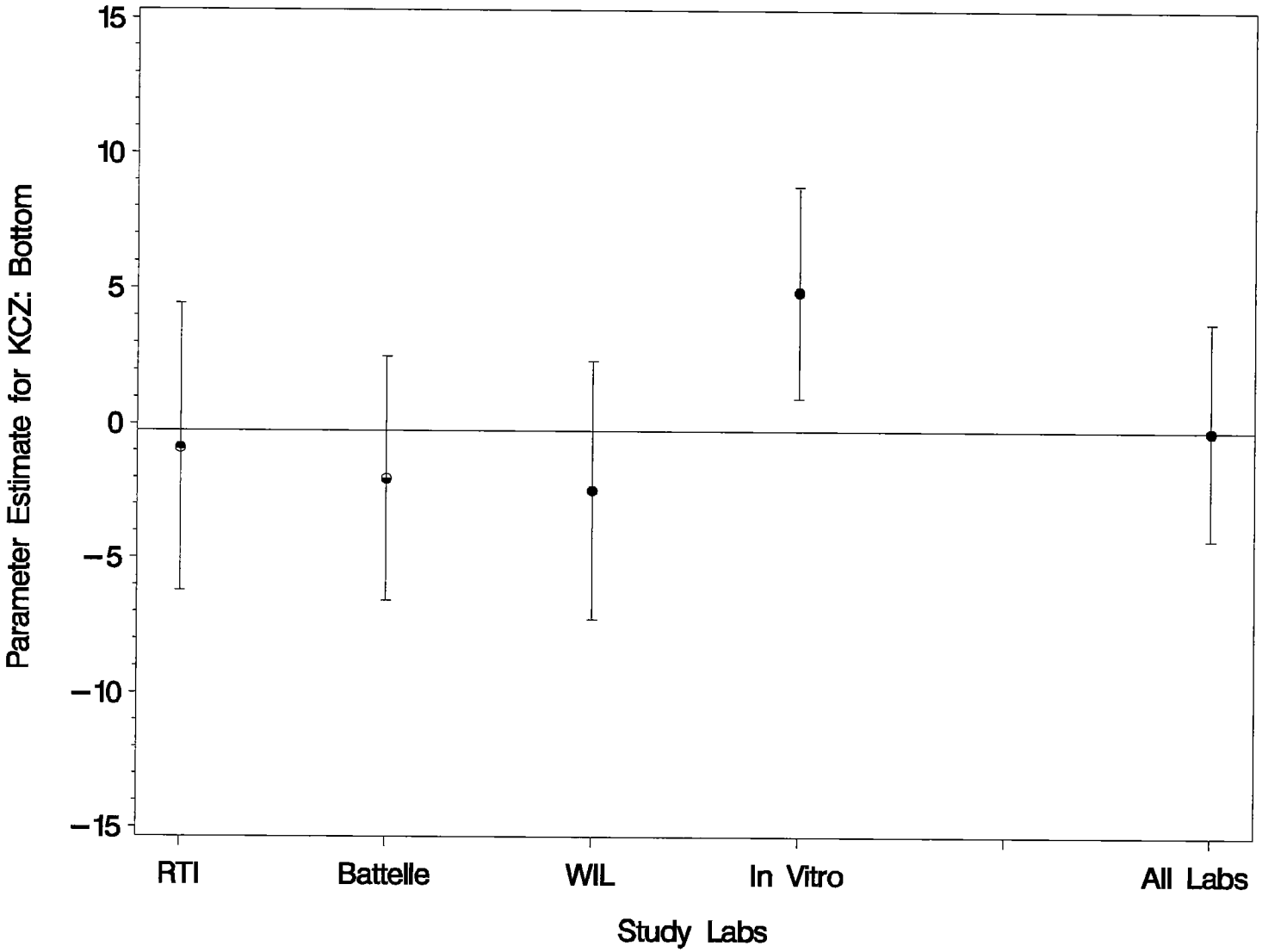


Figure A-4. **Ketoconazole: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Bottom Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

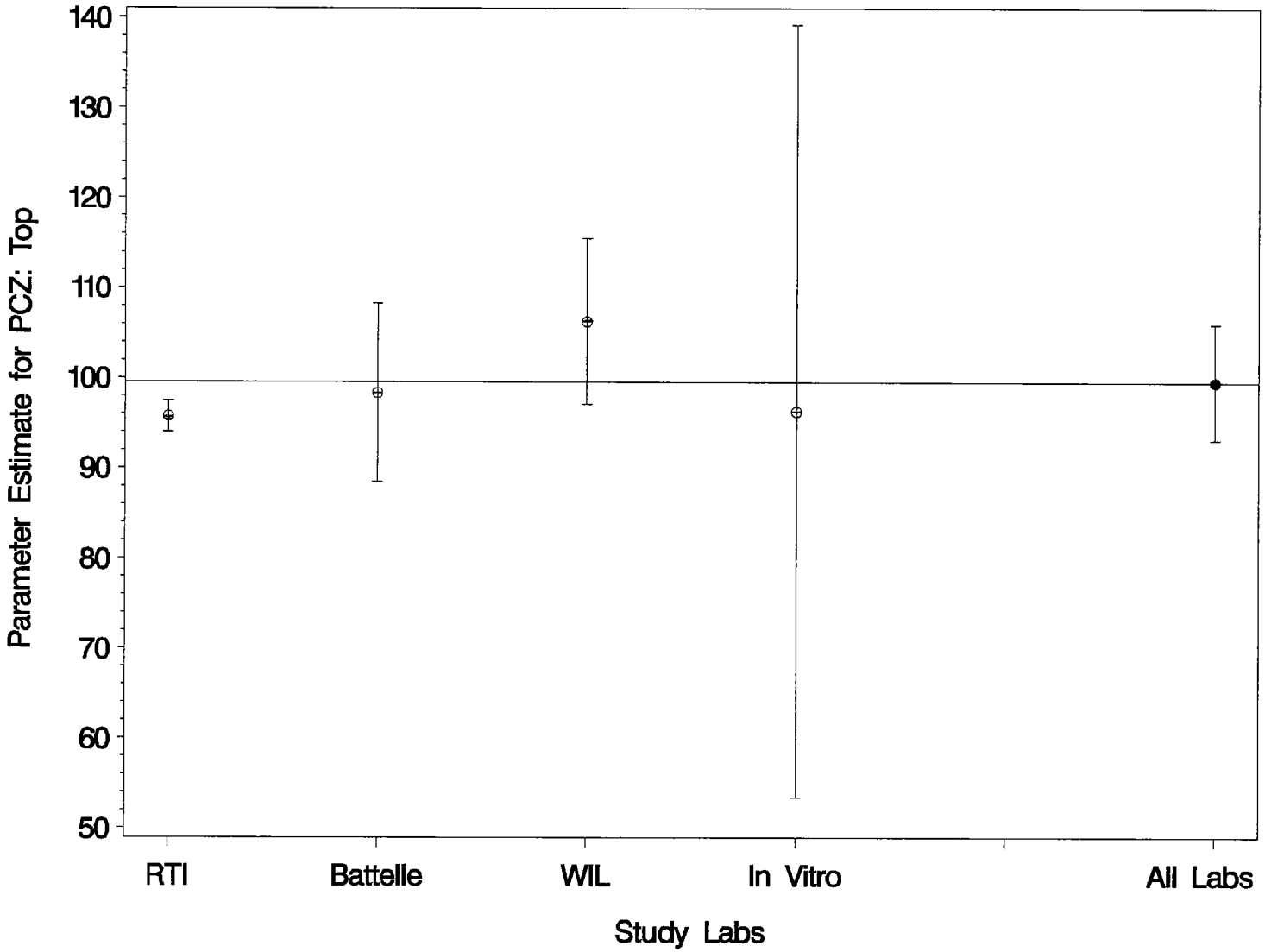


Figure A-5. **<u>Prochloraz:</u> Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Top Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

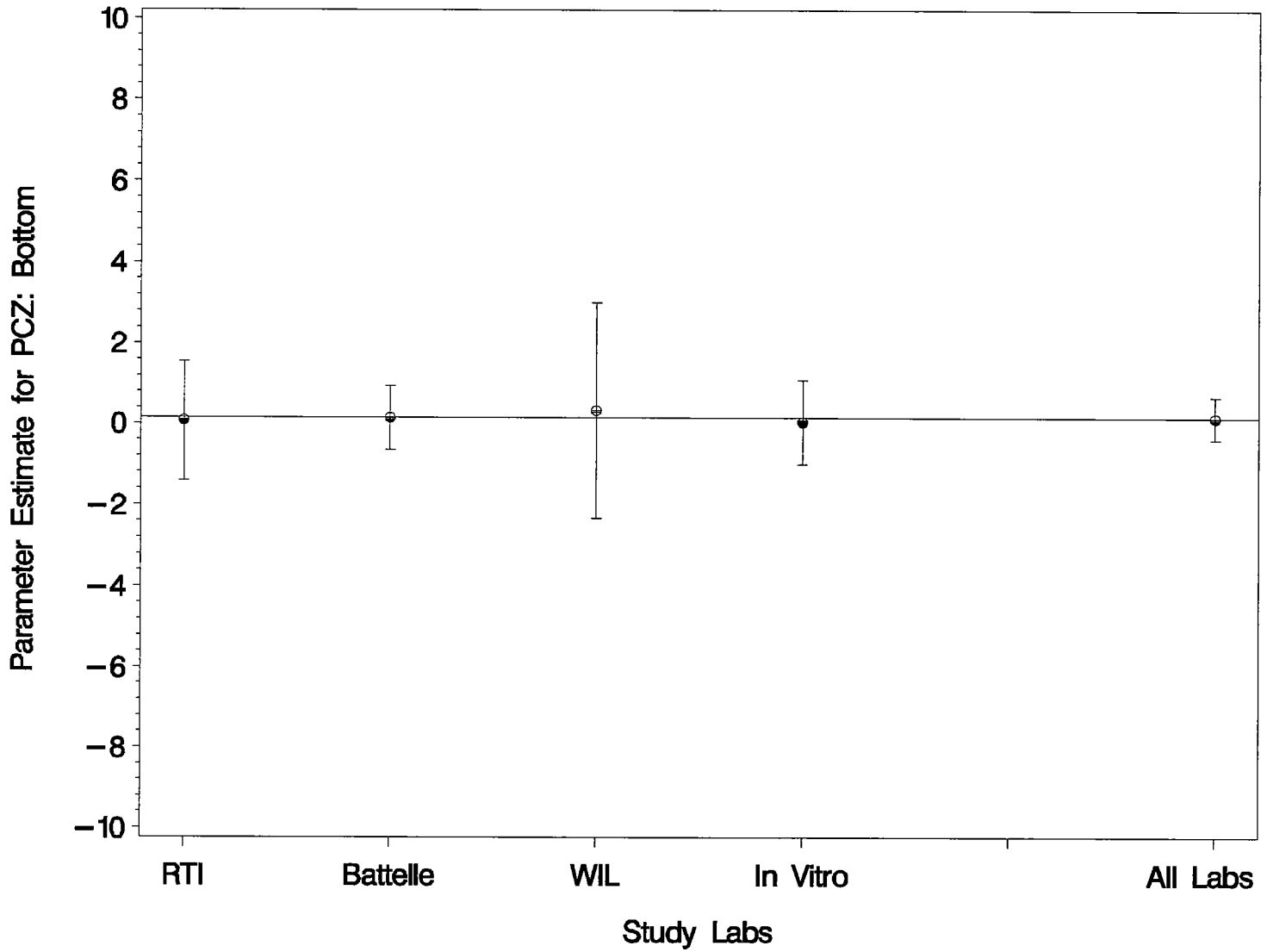


Figure A-6. **Prochloraz: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Bottom Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

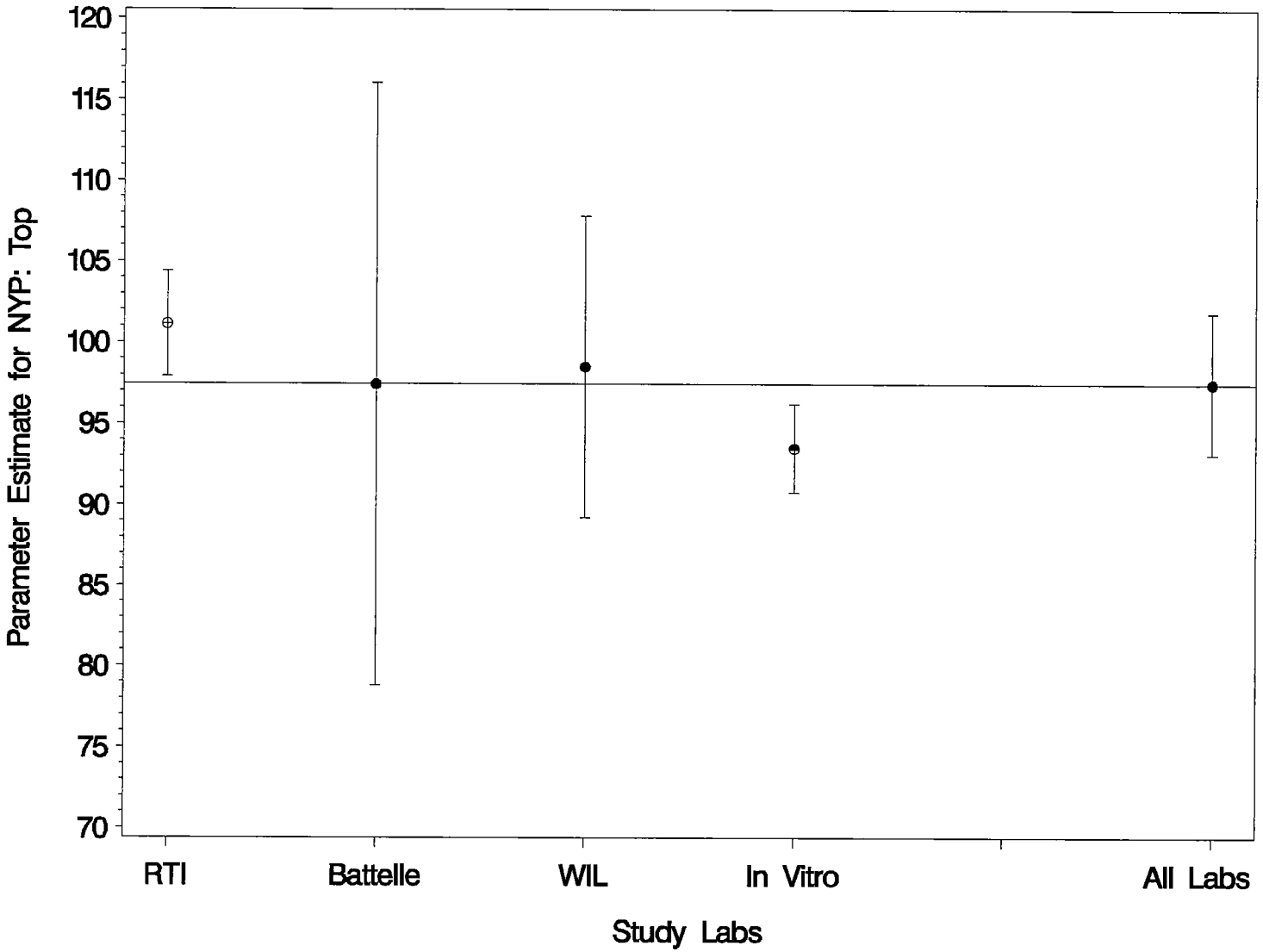


Figure A-7. **<u>4-Nonylphenol:</u> Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Top Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

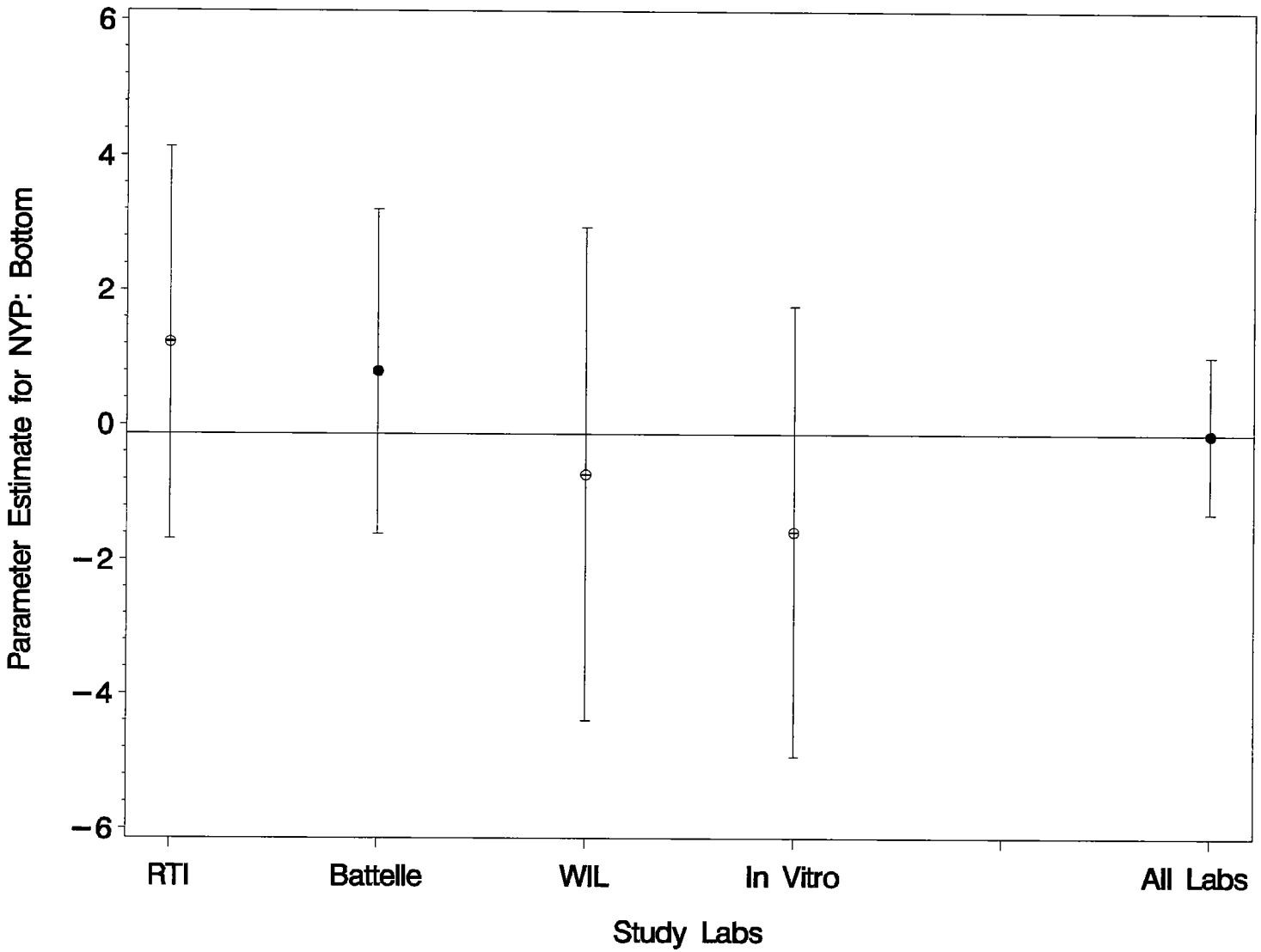


Figure A-8. **4-Nonylphenol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Bottom Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

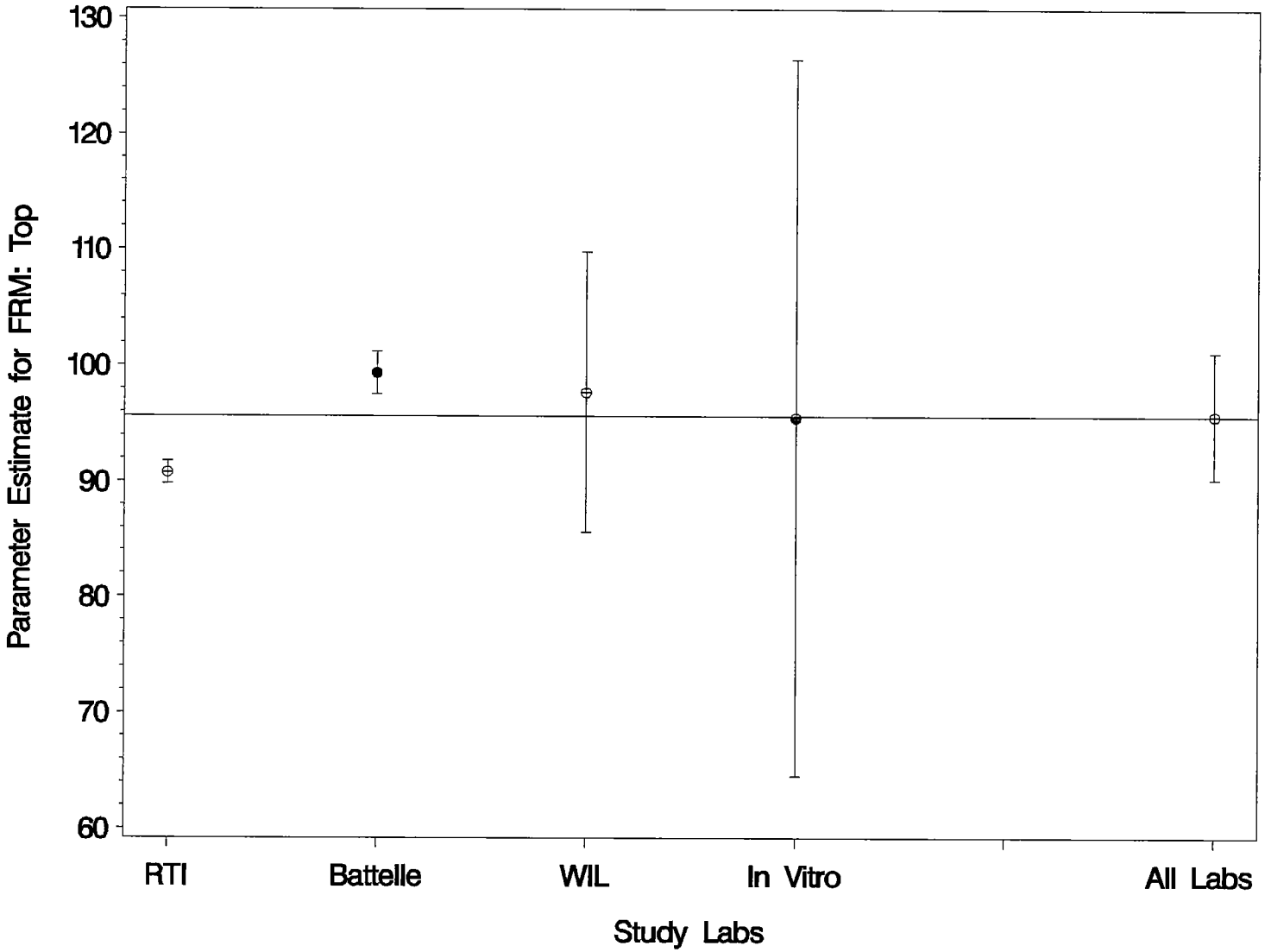


Figure A-9. **<u>Fenarimol:</u> Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Top Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

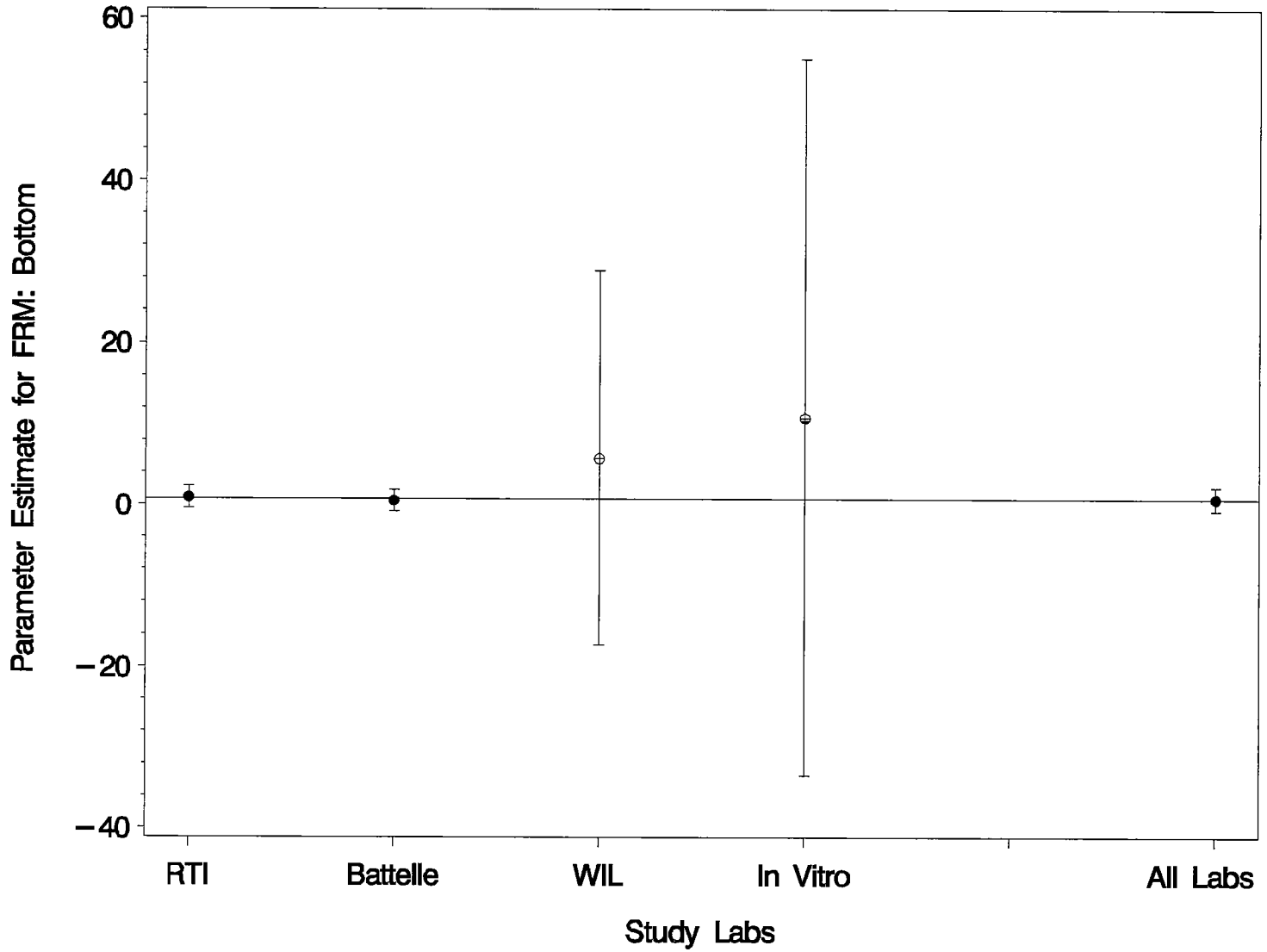


Figure A-10. **<u>Fenarimol:</u> Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Bottom Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

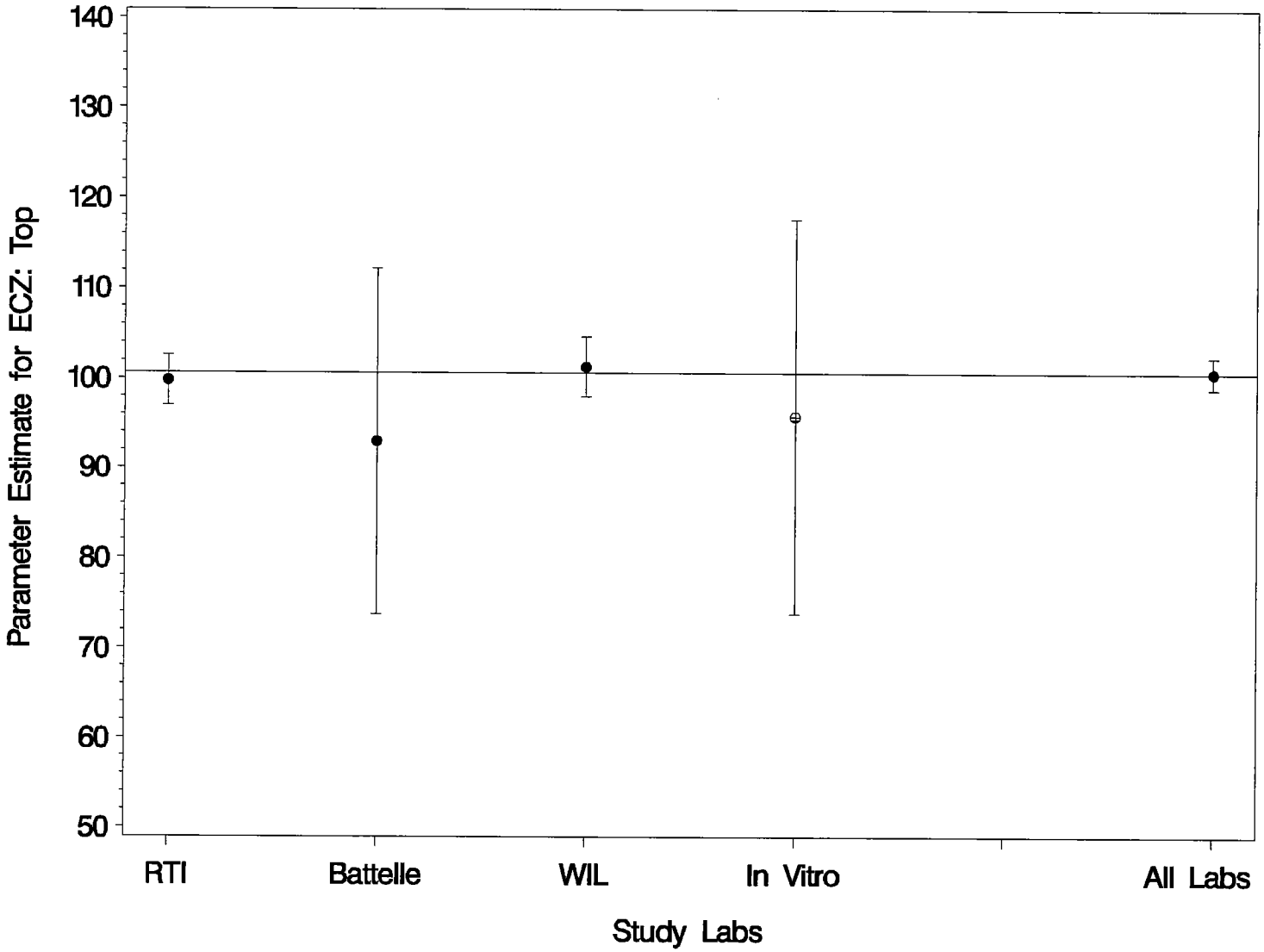


Figure A-11. **<u>Econazole:</u> Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Top Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

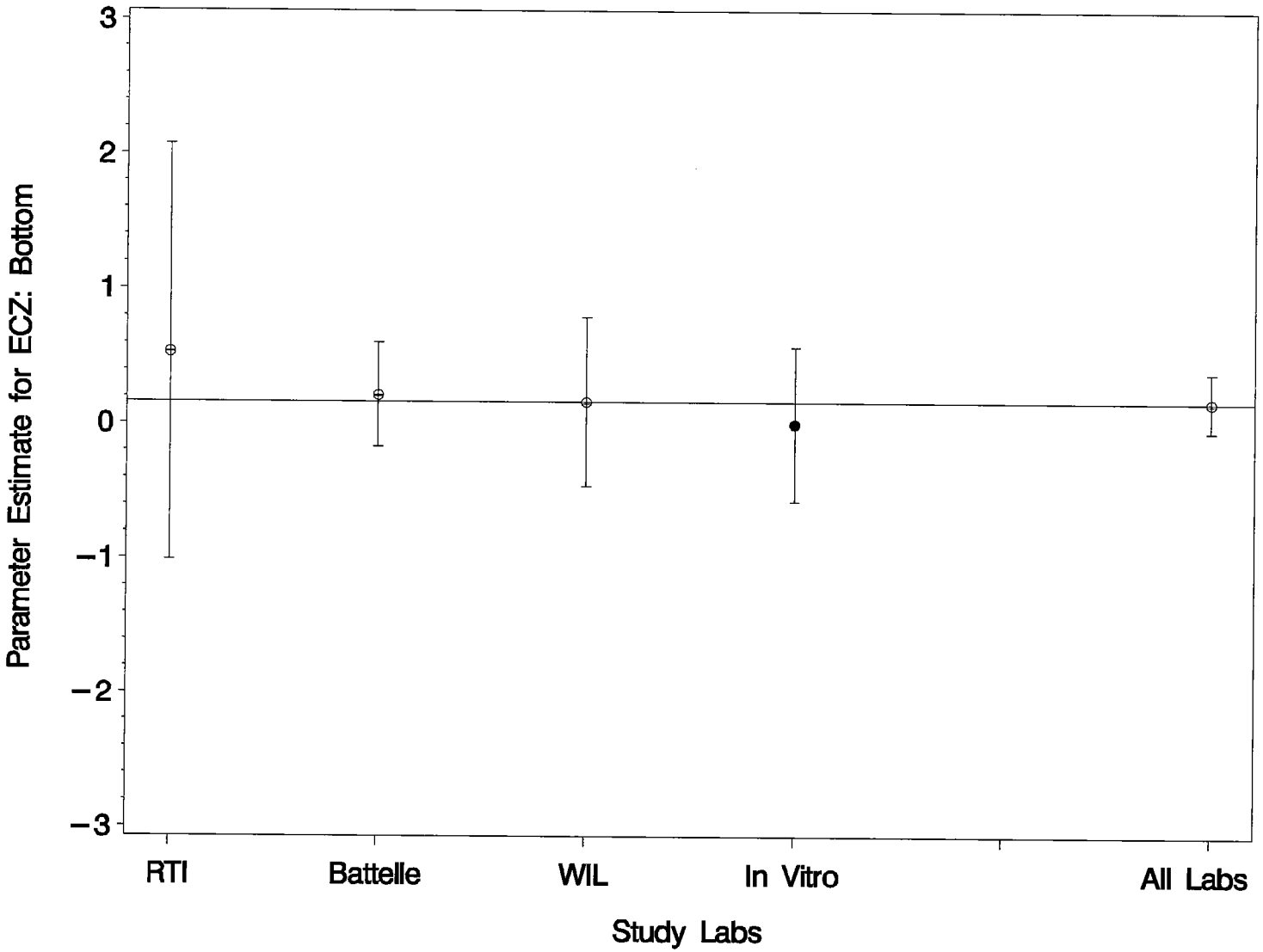


Figure A-12. **Econazole: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Bottom Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

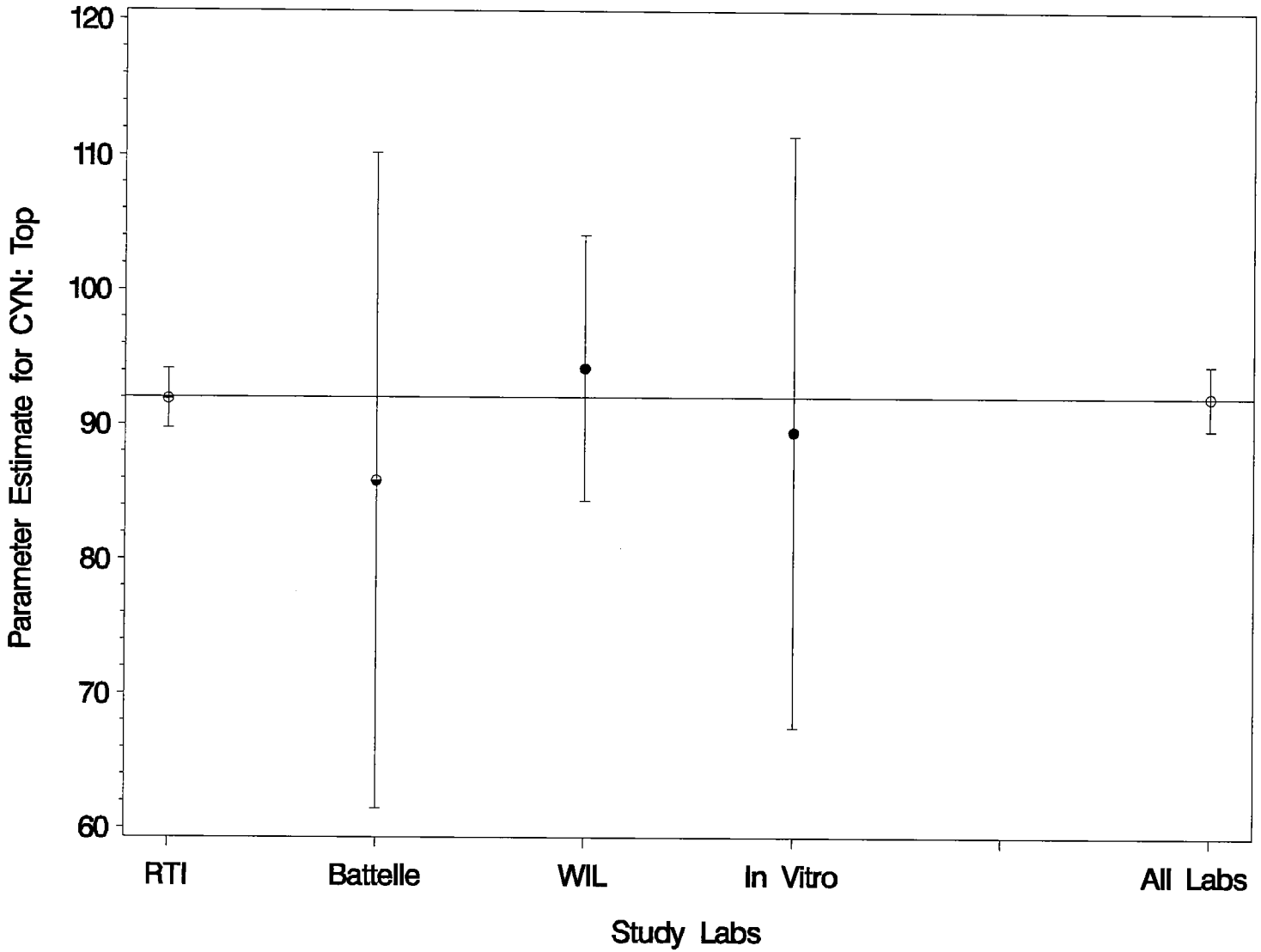


Figure A-13. **Chrysin: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Top Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

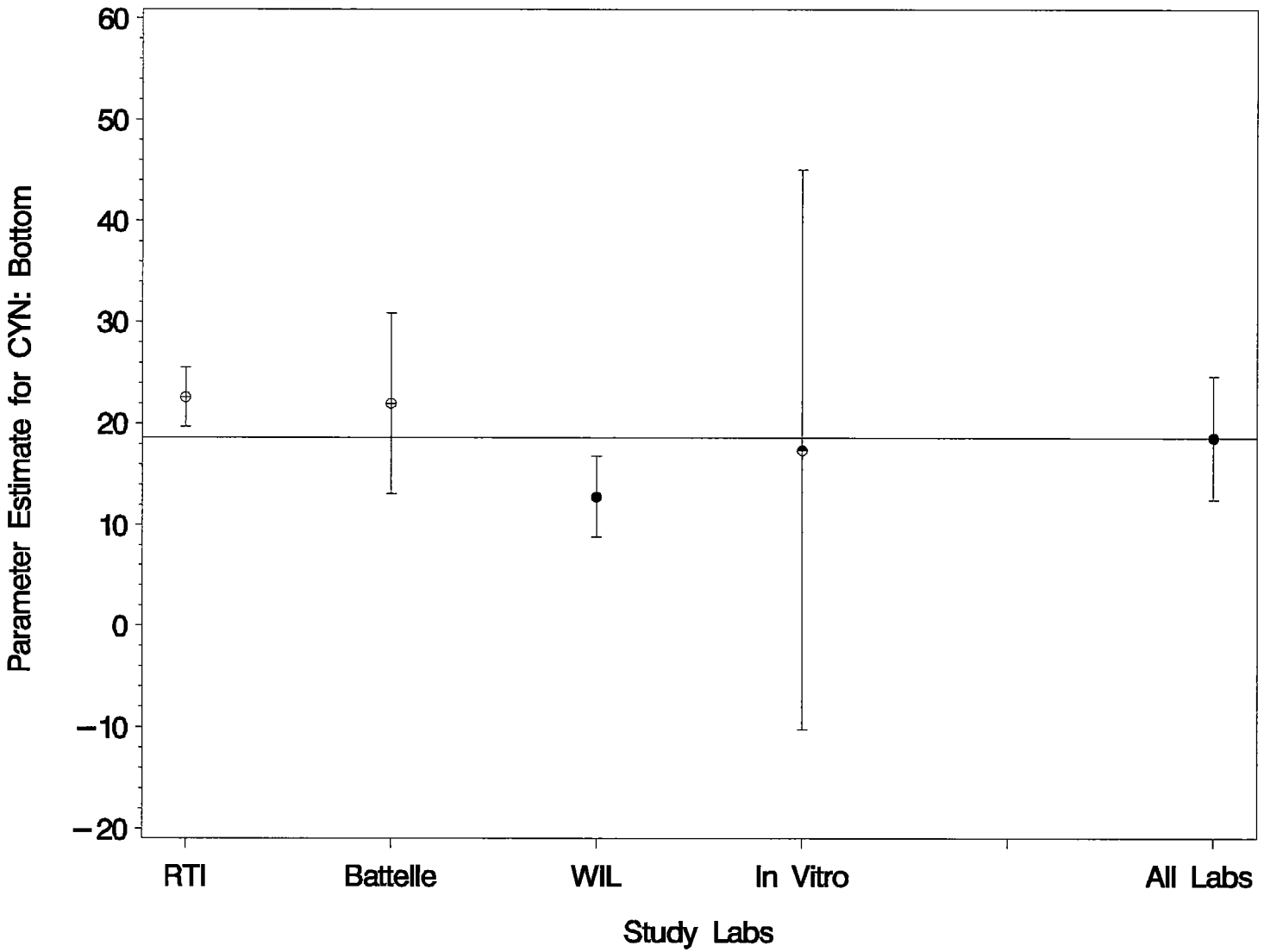


Figure A-14. **Chrysin: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Bottom Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

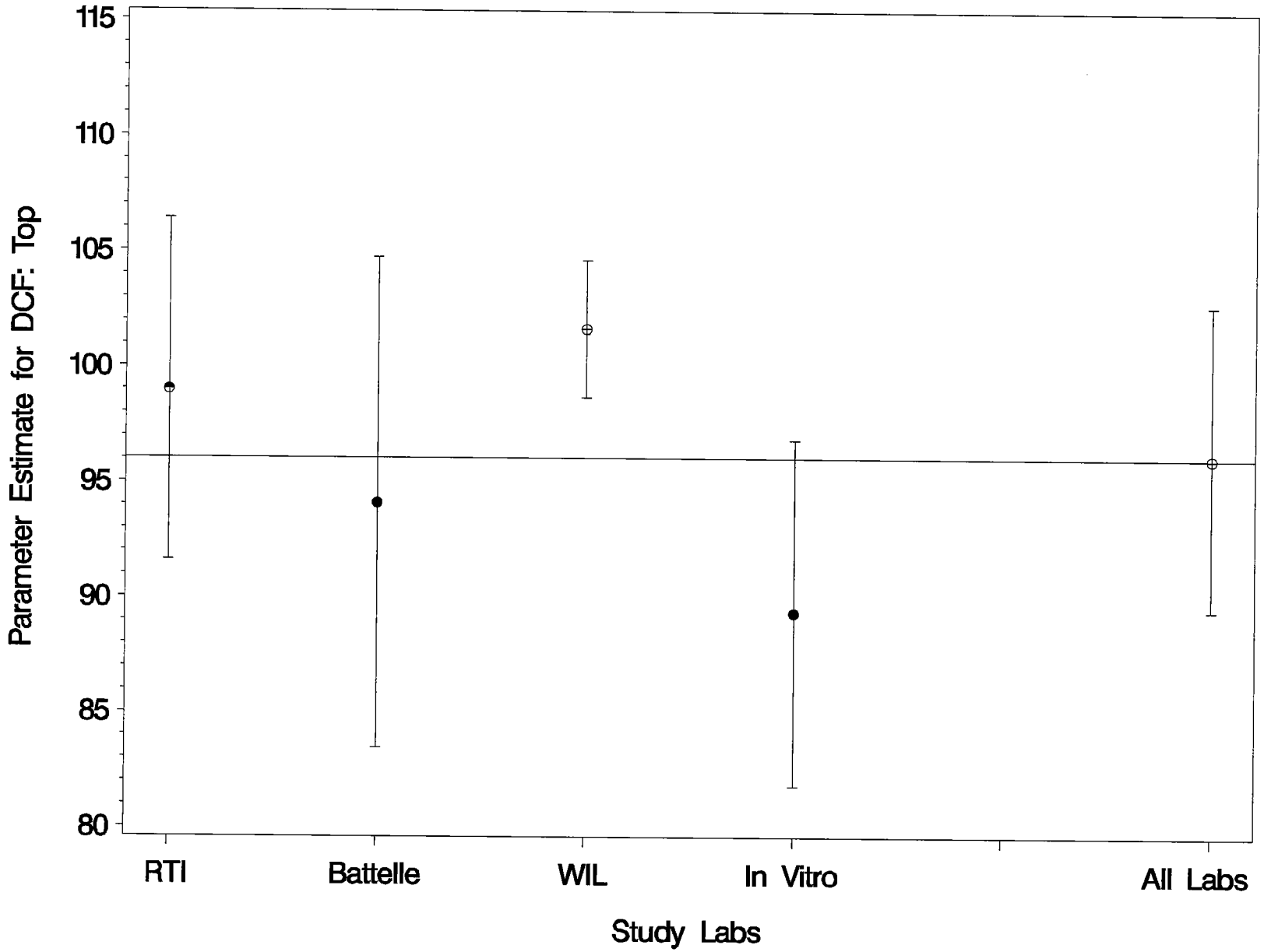


Figure A-15. **Dicofol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Top Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

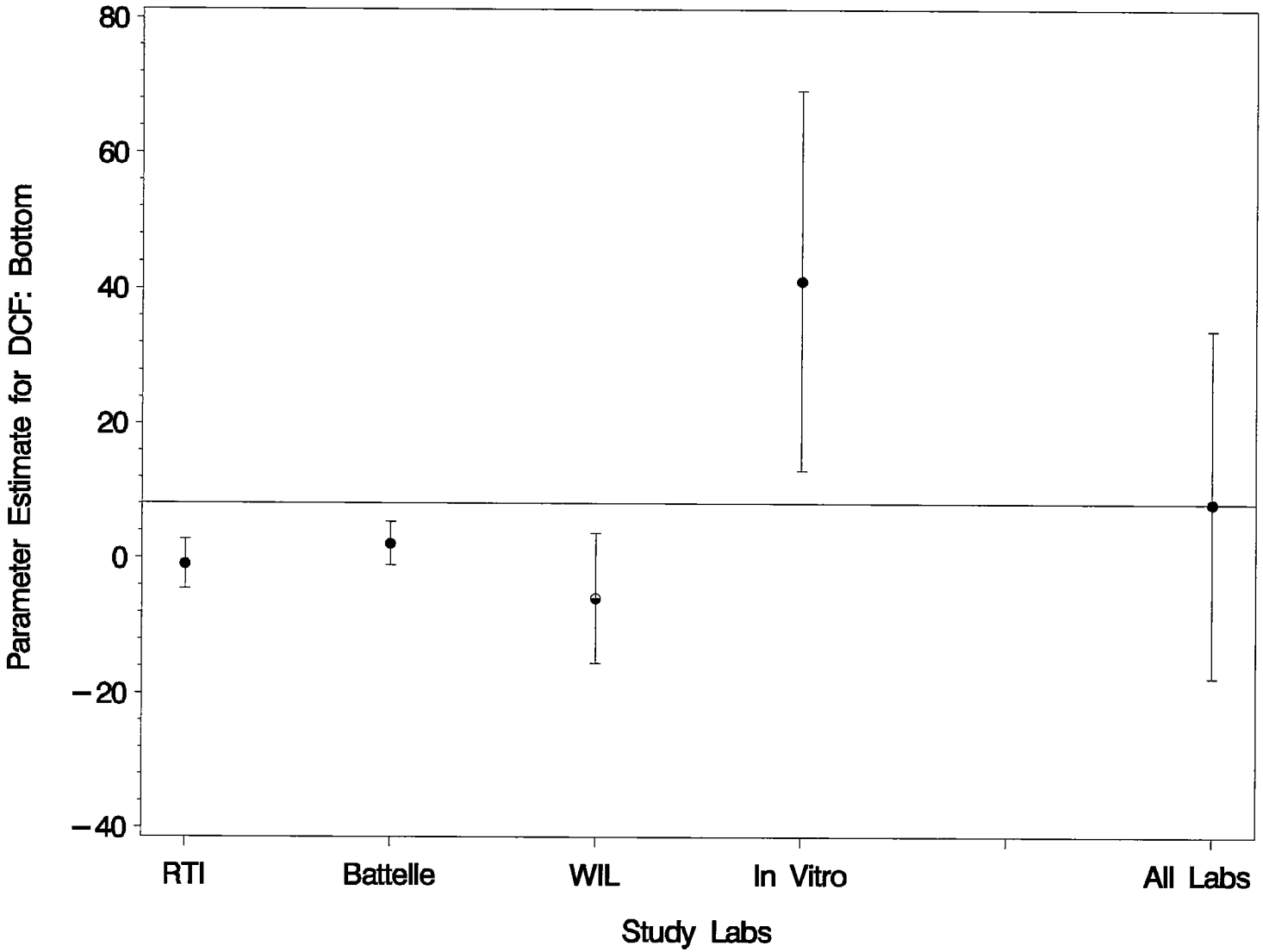


Figure A-16. **Dicofol: Parameter Estimates and Their Associated 95 Percent Confidence Intervals for the Bottom Threshold in the Recombinant Aromatase Assay. Within and Among Laboratories. The Reference Line Corresponds to the Average Across Laboratories.**

Appendix B. Results of Intralaboratory Analyses of Differences Between Beginning and End Portions for Control Data by the Four Participating Laboratories.

- **Tables B-1a and B-1b.** **Results for RTI.**
- **Tables B-2a and B-2b.** **Results for WIL.**
- **Table B-3.** **Results for Battelle.**
- **Table B-4.** **Results for In Vitro.**

Table B-1a. Least Squares Means and Associated Standard Errors for the Beginning and the End Portions. F-test Results for the Portion Effect for Each Chemical, as Reported by RTI. Calculated Results for the Differences Between the Beginning and the End Portions.

Control Parameter	Chemical	LSM (Std Error)		Degree of Freedom	F-Value (P-Value)	End-Beginning (Std Error)[1,2]
		Beginning	End			
BAC	AG	-0.007 (0.026)	0.007 (0.026)	20	0.150 (0.7017)	0.014 (0.036)
	ATZ	-0.007 (0.026)	0.006 (0.026)	20	0.130 (0.7223)	0.013 (0.036)
	CYN	-0.036 (0.026)	0.036 (0.026)	20	3.980 (0.0598)	0.072 (0.036)
	DBA	0.050 (0.026)	-0.050 (0.026)	20	7.540 (0.0125)	-0.099 (0.036)
	DCF	-0.007 (0.026)	0.006 (0.026)	20	0.130 (0.7223)	0.013 (0.036)
	ECZ	-0.036 (0.026)	0.036 (0.026)	20	3.980 (0.0598)	0.072 (0.036)
	FRM	-0.007 (0.026)	0.007 (0.026)	20	0.160 (0.6925)	0.014 (0.036)
	KCZ	-0.027 (0.026)	0.027 (0.026)	20	2.220 (0.1516)	0.054 (0.036)
	NYP	0.050 (0.026)	-0.050 (0.026)	20	7.540 (0.0125)	-0.099 (0.036)
	PCZ	-0.007 (0.026)	0.007 (0.026)	20	0.160 (0.6925)	0.014 (0.036)
FEAC	AG	104.700 (1.893)	95.300 (1.893)	20	12.330 (0.0022)	-9.400 (2.677)
	ATZ	107.310 (1.893)	92.692 (1.893)	20	29.820 (<.0001)	-14.618 (2.677)
	CYN	110.510 (1.893)	89.490 (1.893)	20	61.680 (<.0001)	-21.020 (2.676)
	DBA	103.630 (1.893)	96.366 (1.893)	20	7.380 (0.0133)	-7.264 (2.674)
	DCF	107.310 (1.893)	92.692 (1.893)	20	29.820 (<.0001)	-14.618 (2.677)
	ECZ	110.510 (1.893)	89.490 (1.893)	20	61.680 (<.0001)	-21.020 (2.676)
	FRM	109.260 (2.073)	92.280 (1.893)	20	36.610 (<.0001)	-16.980 (2.806)
	KCZ	106.540 (1.893)	93.456 (1.893)	20	23.910 (<.0001)	-13.084 (2.676)
	NYP	103.630 (1.893)	96.366 (1.893)	20	7.380 (0.0133)	-7.264 (2.674)
	PCZ	109.260 (2.073)	92.280 (1.893)	20	36.610 (<.0001)	-16.980 (2.806)
Negative	AG	95.477 (3.634)	93.961 (3.634)	20	0.140 (0.7131)	-1.516 (4.051)
	ATZ	101.800 (3.634)	96.982 (3.634)	20	1.410 (0.2492)	-4.818 (4.058)
	CYN	102.370 (3.634)	96.823 (3.634)	20	1.870 (0.1872)	-5.547 (4.057)
	DBA	105.510 (3.634)	96.993 (3.634)	20	4.400 (0.0489)	-8.517 (4.060)
	DCF	101.800 (3.634)	96.982 (3.634)	20	1.410 (0.2492)	-4.818 (4.058)
	ECZ	102.370 (3.634)	96.823 (3.634)	20	1.870 (0.1872)	-5.547 (4.057)
	FRM	110.690 (3.634)	93.313 (3.634)	20	18.280 (0.0004)	-17.377 (4.064)
	KCZ	97.207 (3.634)	90.748 (3.634)	20	2.530 (0.1276)	-6.459 (4.061)
	NYP	105.510 (3.634)	96.993 (3.634)	20	4.400 (0.0489)	-8.517 (4.060)
	PCZ	110.690 (3.634)	93.313 (3.634)	20	18.280 (0.0004)	-17.377 (4.064)
Positive	AG	59.539 (2.127)	58.309 (2.127)	20	0.520 (0.4776)	-1.230 (1.705)
	ATZ	57.381 (2.127)	56.795 (2.127)	20	0.120 (0.7338)	-0.586 (1.692)
	CYN	60.173 (2.127)	55.408 (2.127)	20	7.870 (0.0109)	-4.765 (1.698)
	DBA	55.153 (2.127)	52.112 (2.127)	20	3.210 (0.0886)	-3.041 (1.698)
	DCF	57.381 (2.127)	56.795 (2.127)	20	0.120 (0.7338)	-0.586 (1.692)
	ECZ	60.173 (2.127)	55.408 (2.127)	20	7.870 (0.0109)	-4.765 (1.698)

Control Parameter	Chemical	LSM (Std Error)		Degree of Freedom	F-Value (P-Value)	End-Beginning (Std Error)[1,2]
		Beginning	End			
	FRM	64.832 (2.127)	54.008 (2.127)	20	40.600 (<.0001)	-10.825 (1.699)
	KCZ	58.515 (2.127)	54.109 (2.127)	20	6.730 (0.0174)	-4.406 (1.698)
	NYP	55.153 (2.127)	52.112 (2.127)	20	3.210 (0.0886)	-3.041 (1.698)
	PCZ	64.832 (2.127)	54.008 (2.127)	20	40.600 (<.0001)	-10.825 (1.699)

1. For each chemical, the associated standard errors for the differences between the end and beginning were calculated as |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. 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-1b. Least Squares Means and Associated Standard Errors. Differences Between the End and the Beginning Portions (End Minus Beginning), as Reported by RTI[1].

Endpoint	Control Parameter	LS Mean[1]	Standard Error[2,3]	Degree of Freedom
End-Beginning	BAC	0.0069	0.01141	20
	FEAC	-14.2250	0.85462	20
	Negative	-8.0494	1.28355	20
	Positive	-4.4070	0.53685	20

1. The differences between the beginning and the end are averages across all chemicals.
2. 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).
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-2a. Least Squares Means for the Beginning and the End Portions, as Reported by WIL. Calculated Results for the Differences Between the Beginning and the End Portions.

Control Parameter	Chemical	LSM (SE) Beginning	LSM (SE) End	LSM (SE) Average	End-Beginning (Std Error)[1,2]
BAC	AG-KCZ	0.000 (0.093)	0.000 (0.093)	0.000 (0.066)	0.000 (0.131)
	PCZ-FRM	0.019 (0.093)	-0.019 (0.093)	0.000 (0.066)	-0.038 (0.131)
	NYP	0.009 (0.093)	-0.009 (0.093)	0.000 (0.066)	-0.018 (0.131)
	DBA	-0.001 (0.093)	0.001 (0.093)	0.000 (0.066)	0.002 (0.131)
	ECZ-CYN	-0.016 (0.093)	0.016 (0.093)	0.000 (0.066)	0.032 (0.131)
	DCF-ATZ	-0.130 (0.120)	0.130 (0.120)	0.000 (0.085)	0.260 (0.169)
FEAC	AG-KCZ	102.853 (1.216)	97.147 (1.216)	100.000 (0.860)	-5.706 (1.719)
	PCZ-FRM	104.277 (1.332)	96.436 (1.216)	100.357 (0.902)	-7.841 (1.803)
	NYP	100.428 (1.543)	99.572 (1.543)	100.000 (1.091)	-0.856 (2.182)
	DBA	102.119 (2.404)	97.881 (2.404)	100.000 (1.700)	-4.238 (3.400)
	ECZ-CYN	104.885 (1.267)	95.115 (1.267)	100.000 (0.896)	-9.770 (1.792)
	DCF-ATZ	101.527 (1.216)	98.473 (1.216)	100.000 (0.860)	-3.054 (1.719)
Negative	AG-KCZ	100.718 (2.511)	89.357 (2.511)	95.038 (2.039)	-11.361 (2.931)
	PCZ-FRM	103.538 (2.073)	97.674 (2.073)	100.606 (1.466)	-5.864 (2.931)
	NYP	101.410 (2.073)	96.484 (2.073)	98.947 (1.466)	-4.926 (2.931)
	DBA	96.000 (5.032)	95.083 (5.032)	95.541 (4.237)	-0.917 (5.429)
	ECZ-CYN	93.348 (3.664)	85.760 (3.664)	89.554 (3.358)	-7.588 (2.932)
	DCF-ATZ	100.951 (2.073)	92.805 (2.073)	96.878 (1.466)	-8.146 (2.931)
Positive	AG-KCZ	48.818 (4.260)	41.161 (4.260)	44.989 (4.180)	-7.657 (1.643)
	PCZ-FRM	70.842 (15.656)	65.279 (15.656)	68.061 (15.645)	-5.563 (1.174)
	NYP	50.814 (1.378)	49.104 (1.378)	49.959 (1.255)	-1.710 (1.138)
	DBA	50.721 (1.203)	48.991 (1.203)	49.856 (0.986)	-1.730 (1.378)
	ECZ-CYN	51.696 (1.668)	46.248 (1.668)	48.972 (1.551)	-5.448 (1.227)
	DCF-ATZ	53.424 (1.796)	50.250 (1.796)	51.837 (1.704)	-3.174 (1.135)

1. The ten chemicals were combined into subsets, resulting in a total of six combinations. For each chemical combination, the mean values and the associated standard errors at the beginning and at the end portions, as well as the averages across the two portions were reported by the laboratory. The differences between the end and the beginning were first calculated for each chemical combination. The associated standard errors for these differences were calculated as $(2(S_e^2+S_b^2)-4S_s^2)^{1/2}$, where S_e, S_b, and S_s were the standard errors associated with the end, the beginning, and the averages across the end and the beginning respectively, as provided in the intralaboratory report.
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-2b. Least Squares Means and Associated Standard Errors. Differences Between the End and the Beginning Portions (End Minus Beginning), as Reported by WIL.

Endpoint	Control Parameter	LS Mean[1]	Standard Error[2,3]	Degree of Freedom
End-Beginning	BAC	0.03967	0.05641	12
	FEAC	-5.24417	0.89276	12
	Negative	-6.46700	1.41852	12
	Positive	-4.21367	0.52884	12

1. The differences between the beginning and the end were averages across all chemicals.
2. The associated standard errors were calculated as $(1/6)(\sum s_i^2)^{1/2}$, where s_i is the standard error associated with the difference for the i^{th} chemical (Table B-2a).
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-3. Least Squares Means of Differences Between the End and the Beginning Portions (End Minus Beginning) and Associated Standard Errors, as Reported by Battelle.

Endpoint	Control Parameter	LS Mean	Standard Error[1]	Degree of Freedom
End-Beginning	BAC	-0.159	0.116	15.98
	FEAC	-5.878	1.234	35.85
	Negative	-4.115	1.049	20.39
	Positive	-2.795	0.669	12.53

1. 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-4. Least Squares Means of Differences Between the End and the Beginning Portions (End Minus Beginning) and Associated Standard Errors, as Reported By In Vitro.

Endpoint	Control Parameter	LS Mean	Standard Error[1]	Degree of Freedom
End-Beginning	BAC	-0.049	0.067	13.99
	FEAC	-6.354	1.923	23.19
	Negative	7.479	3.603	16.17
	Positive	0.619	1.519	16.94

1. The standard error calculations do not account for the constraints on the full enzyme activity and the background activity control values within replicates.

APPENDIX F

CHEMISTRY REPORTS

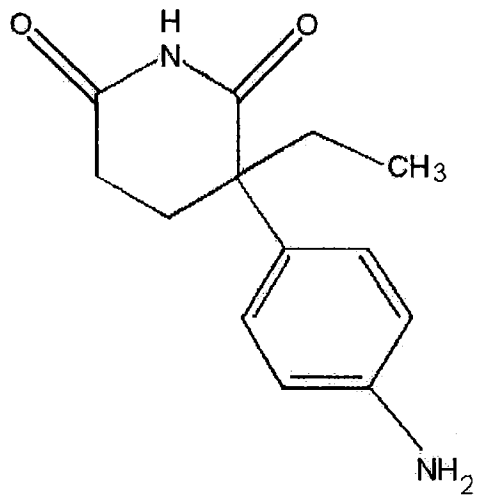
Battelle
The Business of Innovation

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, B.S.

Manager, Chemistry Technical Center

10-18-05

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 Flory 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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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

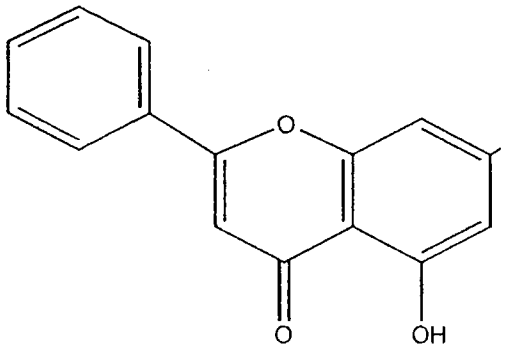
Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

Figure 1 – Certificate of Analysis, Lot No. 043K0939

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.:	Mol. Formula:
	254.24 g/mol	$C_{15}H_{10}O_4$

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:

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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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.

Battelle
The Business of Innovation

ANALYTICAL CHEMISTRY ACTIVITIES REPORT

ECONAZOLE

CAS No.: 24169-02-6

Lot No.: 123K1220 (Sigma Aldrich)

Receipt Date: 10/26/04 and 12/4/04

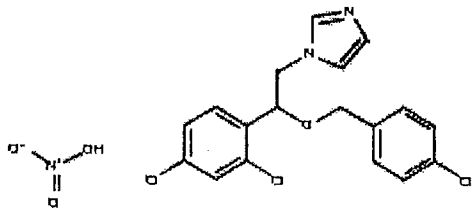
Amount Received: 10 g

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

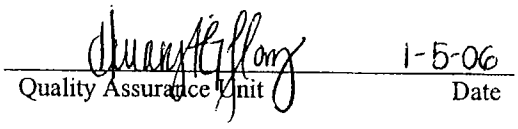
Battelle Study No. WA 4-16/17

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 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 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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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.

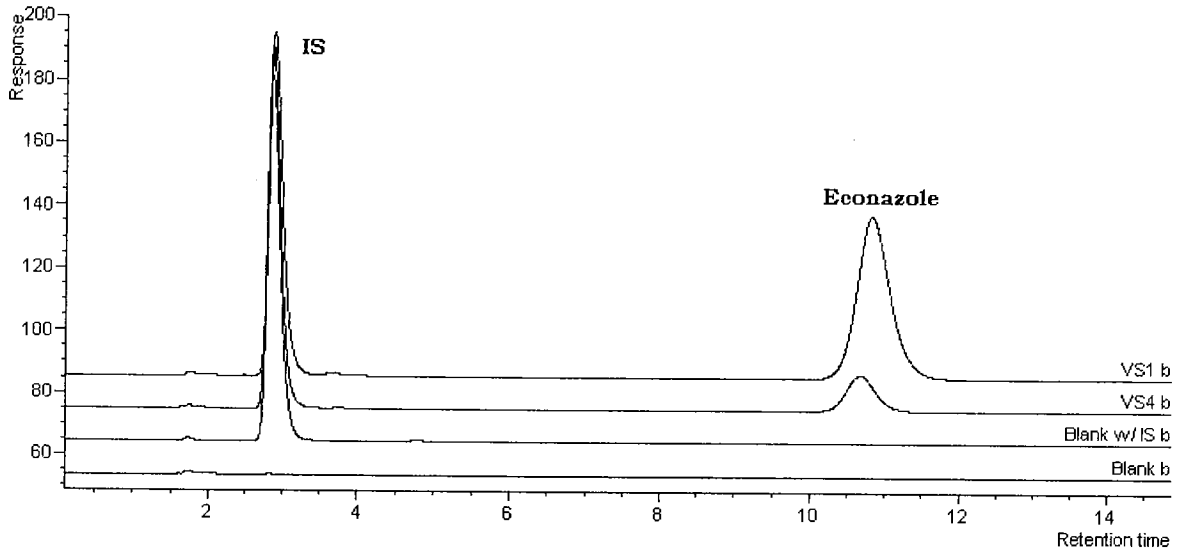


Figure 2 – Representative Overlaid Chromatograms from a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from the Validation (Shown Top to Bottom)

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

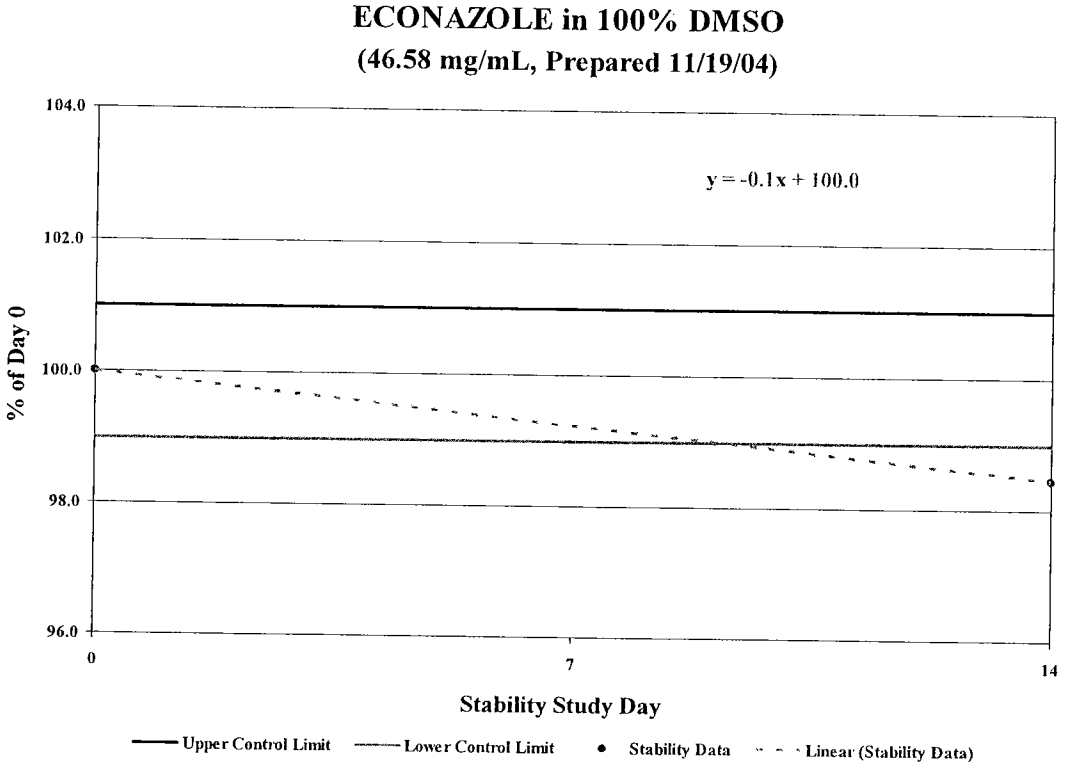
The results from the storage stability study are shown in Table 5 and presented in control chart format in Figure 3.

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.



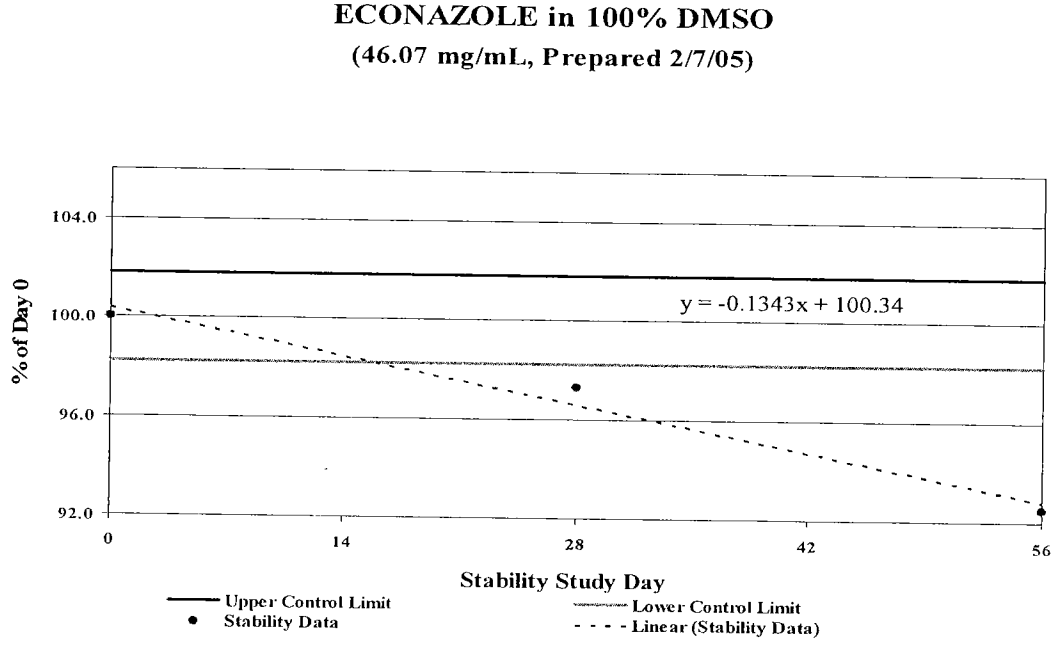


Figure 3 – Control Charts for the Storage Stability Analysis

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.

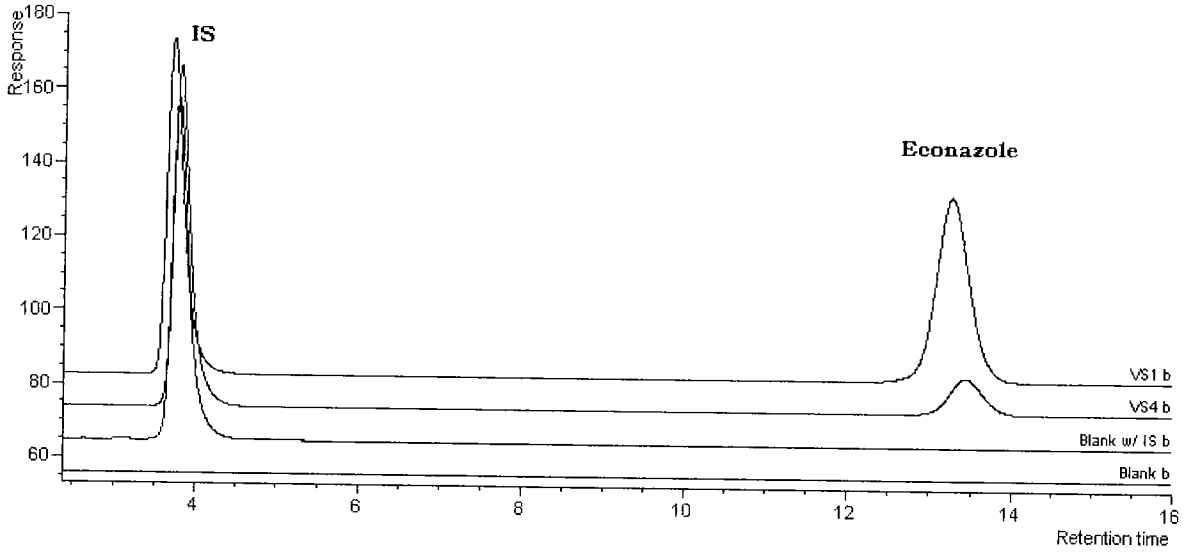


Figure 4 – Representative Overlaid Chromatograms of a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from a Formulation Analysis (Shown Top to Bottom)

The vehicle/calibration standard curve and the results of the regression analysis indicated linearity and are shown in Figure 5.

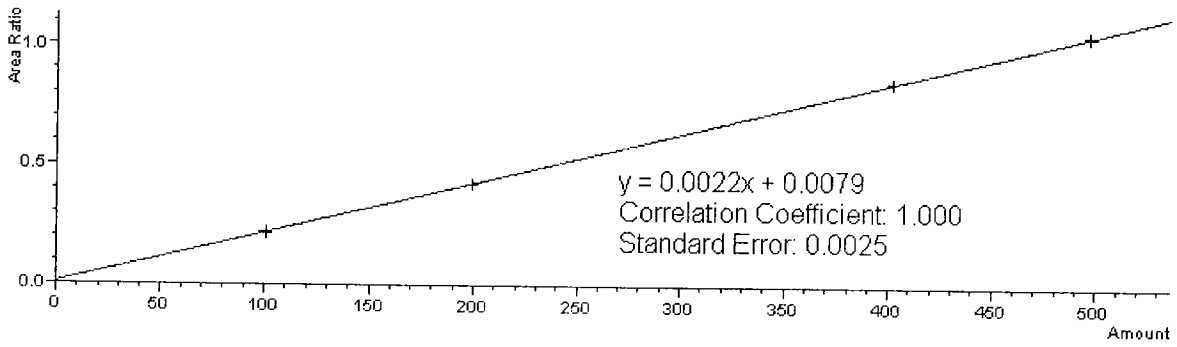


Figure 5 – Vehicle/Calibration Standard Curve and Regression Analysis Results

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.

Battelle
The Business of Innovation

ANALYTICAL CHEMISTRY ACTIVITIES REPORT

LINDANE

CAS No.: 58-89-9

Lot No.: 14419EB (Sigma Aldrich)

Receipt Date: 1/6/05

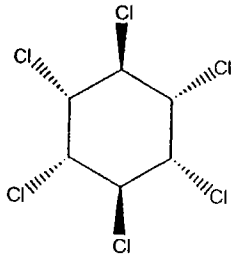
Amount Received: 10 g

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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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.

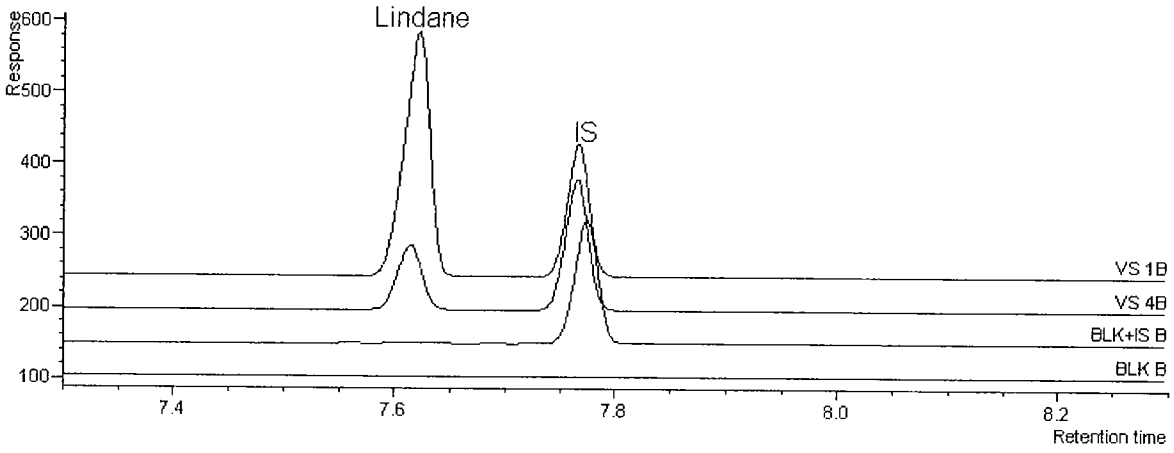


Figure 2 – Representative Overlaid Chromatograms from a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from the Validation (Shown Top to Bottom)

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.

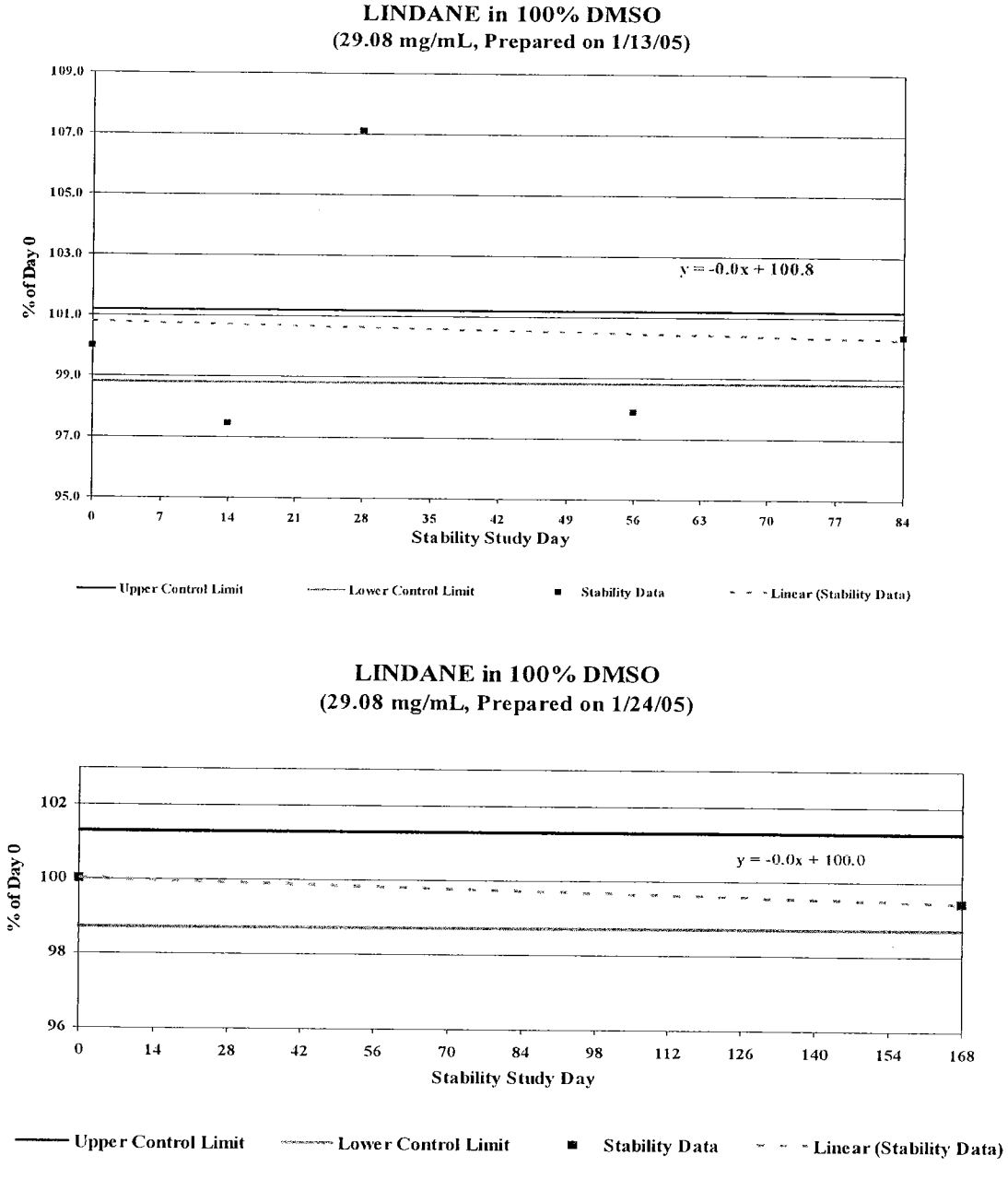


Figure 3 – Control Chart for the Storage Stability Study

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.

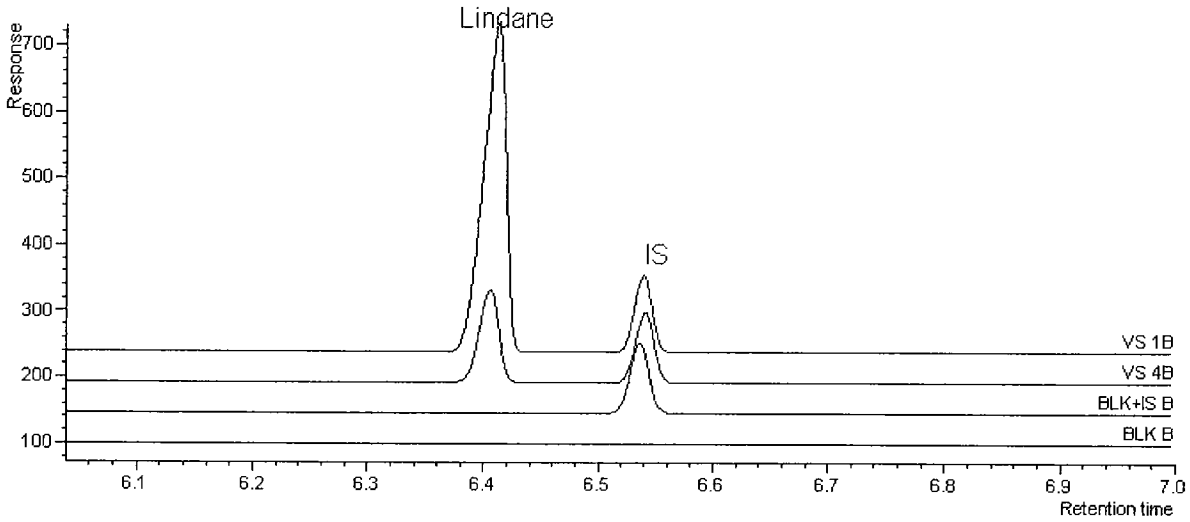


Figure 4 – Representative Overlaid Chromatograms of a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from a Formulation Analysis (Shown Top to Bottom)

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.

Battelle
The Business of Innovation

ANALYTICAL CHEMISTRY ACTIVITIES REPORT

KETOCONAZOLE

CAS No.: 65277-42-1

Receipt Date: 10/26/04

Appearance: Solid

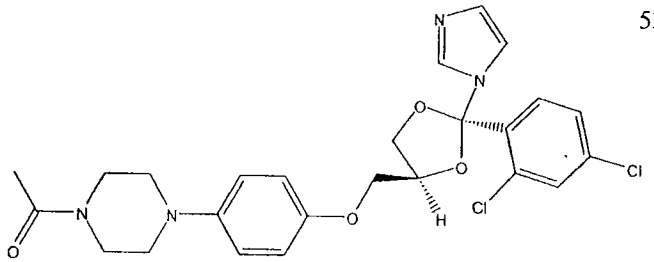
Storage Conditions (@ Battelle): Refrigerated (~5°C)

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 12/28/05

Denise A. Contos, M.S.

Approved By:

Steven W. Graves

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 — 12-27-05 Date

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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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.

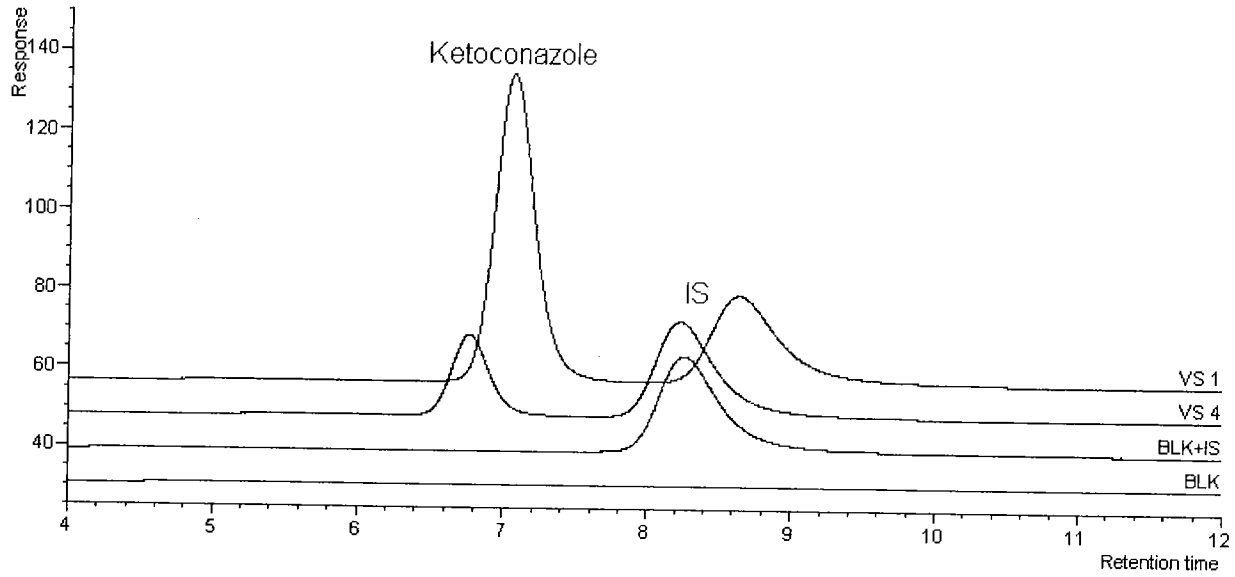


Figure 2 – Representative Overlaid Chromatograms of a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from 2/4/2005 Analysis (Shown Top to Bottom)

The regression analysis results from the standard curve for February 4, 2005 analysis indicate linearity and are shown in Figure 3.

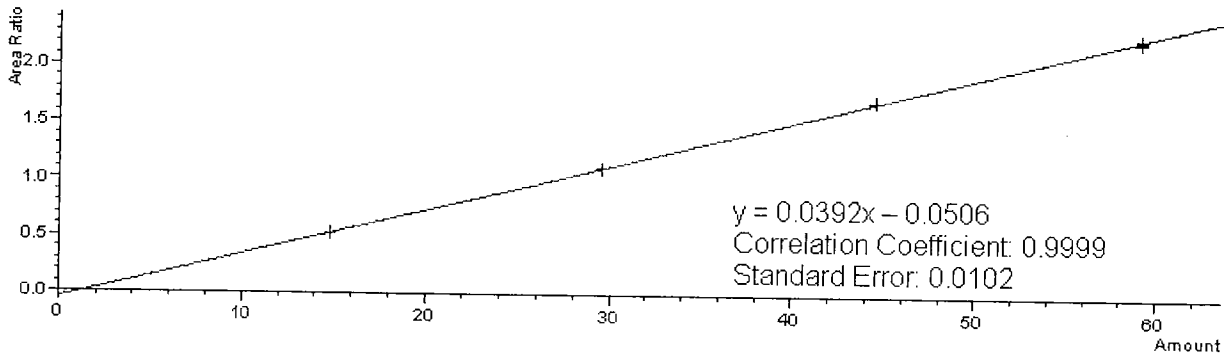


Figure 3 – Vehicle/Calibration Standard Curve for 2/4/2005 Analysis

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.

Battelle
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

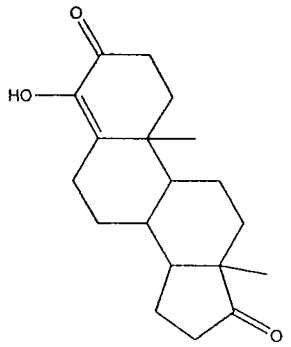
Amount Received: 3.1 g

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

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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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.

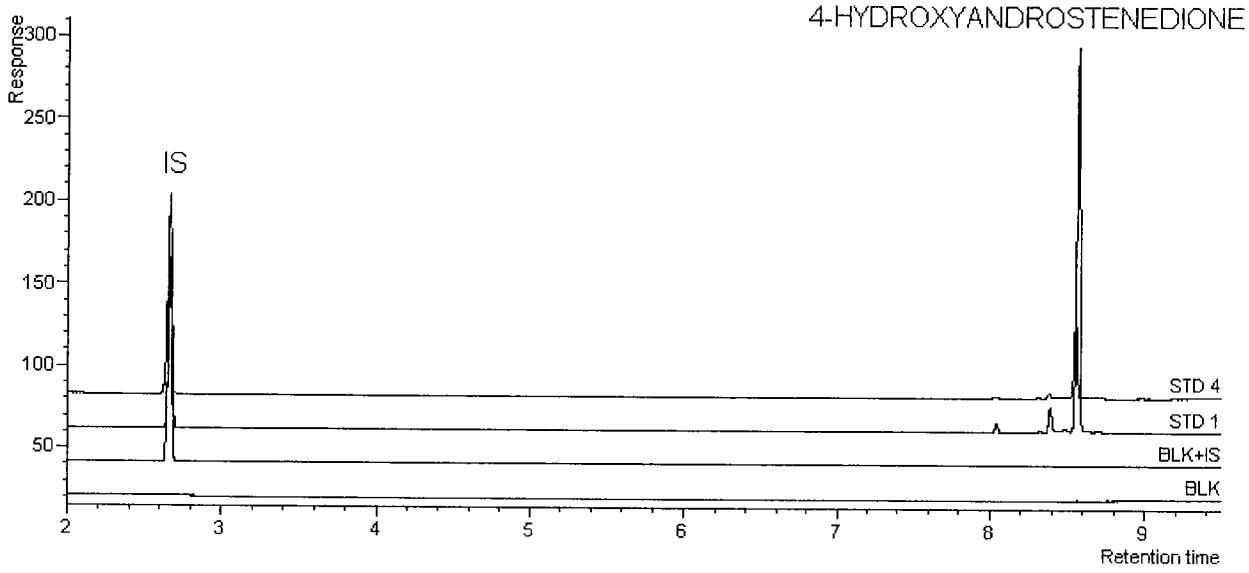


Figure 2 – Representative Overlaid Chromatograms from a Low and High Vehicle/Calibration Standard, Blank with IS, and Blank from the Validation (Shown Top to Bottom)

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

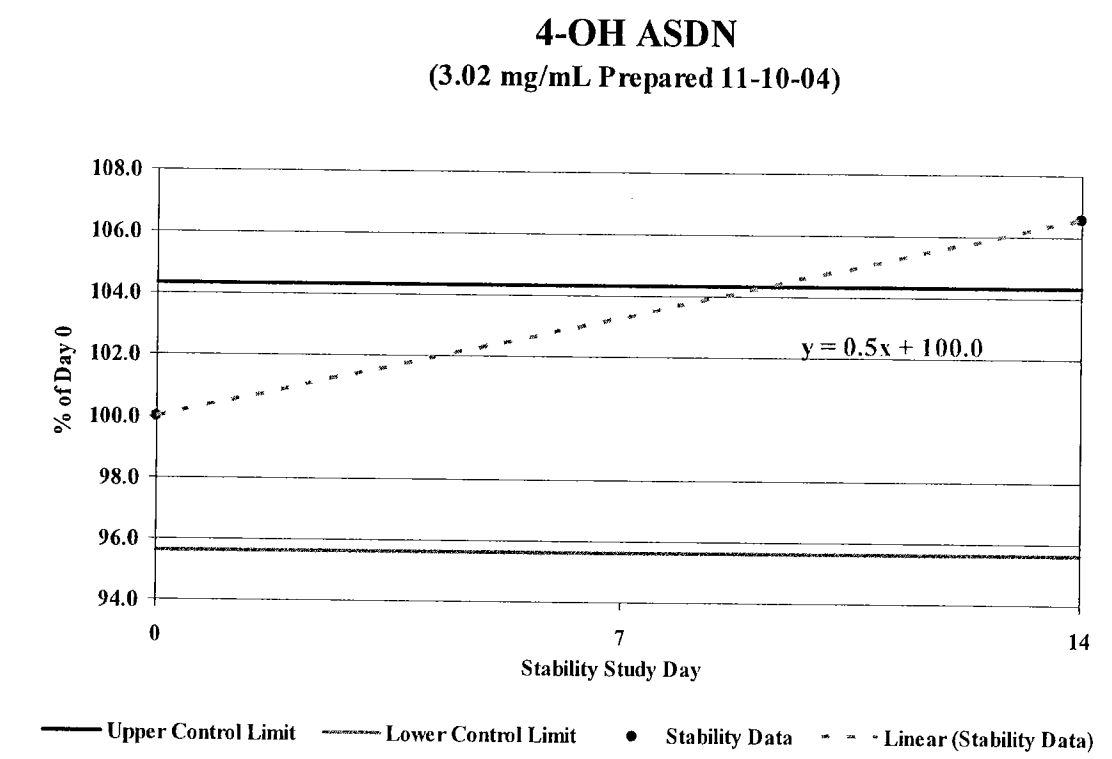
The results from the storage stability study are shown in Table 5 and presented in control chart format in Figure 3.

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.



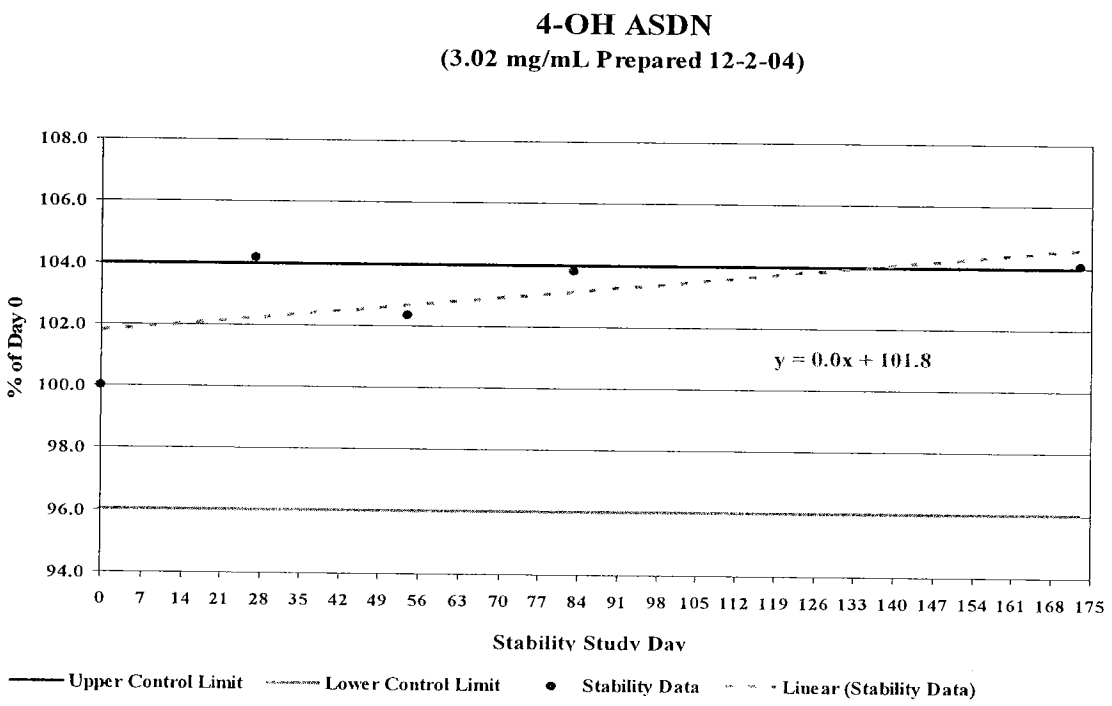


Figure 3 – Control Charts for the Storage Stability Studies

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.

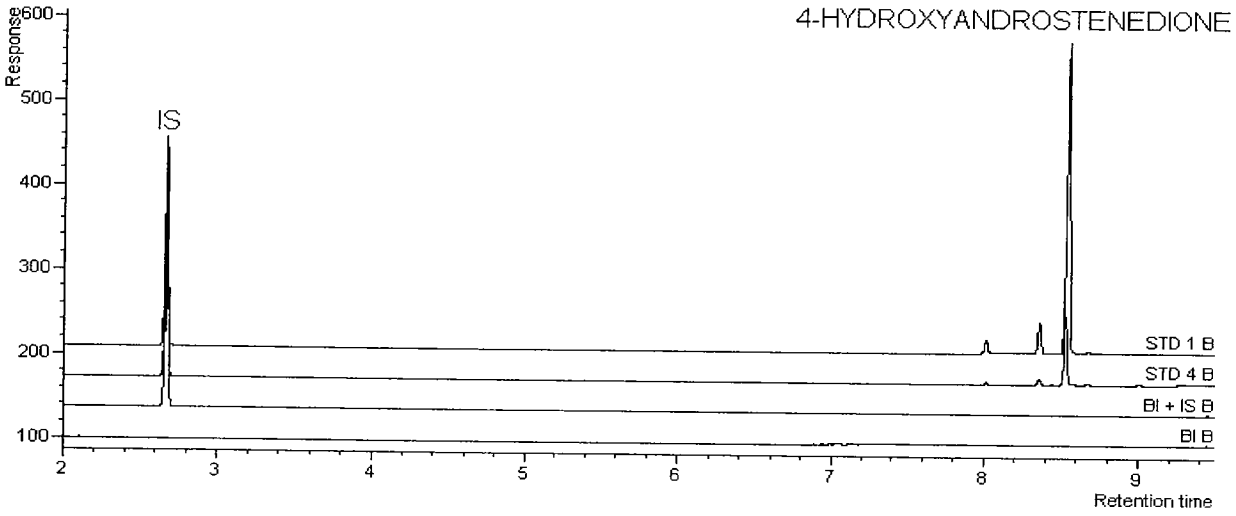


Figure 4 – Representative Overlaid Chromatograms of a High and Low Vehicle/Calibration Standard, Blank with IS, and Blank from Formulation Analysis

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 ≤ 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.

Battelle
The Business of Innovation

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
Address: 1529 West Sequim Bay Road
Sequim, WA 98382

Prepared by:

Timothy Fortman — 12-15-05
Timothy Fortman — Date
Senior Chemistry Analyst
Battelle – Marine Research Operations

Approved by:

Michael E. Cobb — 12-15-05
Michael E. Cobb — Date
EDSP Chemical Repository Study Director
Battelle – Marine Research Operations

Approved by:

Eric Crecelius — 12/15/05
Eric Crecelius — Date
Manager, EDSP Chemical Repository
Battelle – Marine Research Operations

Personnel participating in this study:

Analyst: Tim Fortman, Rebecca Wood

Chemical Repository Study Director: Michael Cobb

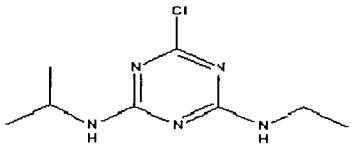
Experimental Dates:
Experimental Start 02/03/05
Experimental Termination 12/03/05

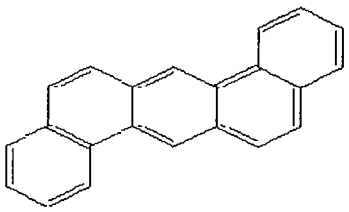
6.0 EXECUTIVE SUMMARY

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	

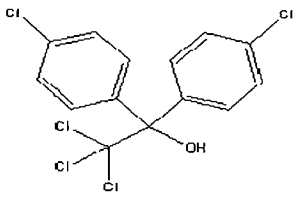
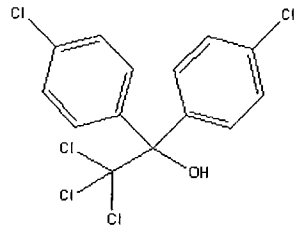
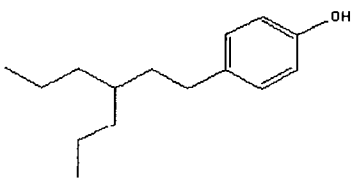
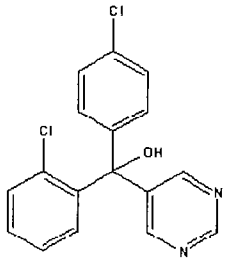
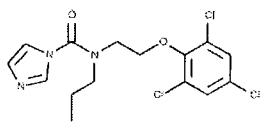
Parameter	Test Substance	Dicofol
Compound Name	Dicofol	
CAS #	115-32-2	
Central File No.	2334-1	
Molecular Weight	370.49 g/mole	
Initial Receipt Date	12/22/04	
Expiration Date	03/16/10	
Supplier	Sigma-Adrich	
Lot Number	4076X	
Vendor Purity	96.5%	
Method	EDSP.G2-025	

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

7.0 TABLE OF CONTENTS PAGE

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF FIGURES

LIST OF APPENDICES

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

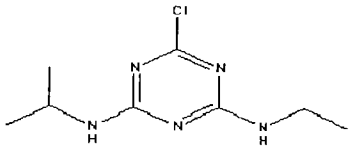
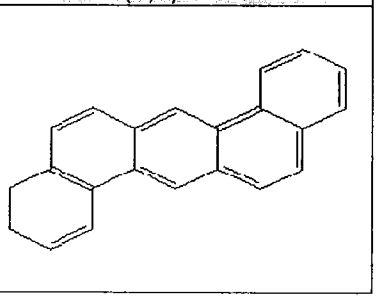
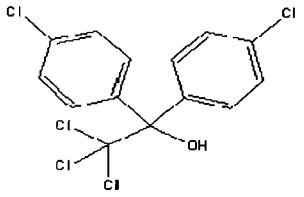
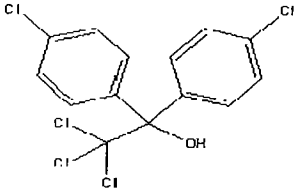
Parameter	Test Substance	Atrazine
Compound Name	Atrazine	
CAS #	1912-24-9	
Central File No.	2332-1	
Molecular Weight	215.72 g/mole	
Initial Receipt Date	12/14/04	
Expiration Date	10/07	
Supplier	Supelco/Chem Service	
Lot Number	328-137A	
Vendor Purity	98%	
Method	EDSP.G2-022	

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	

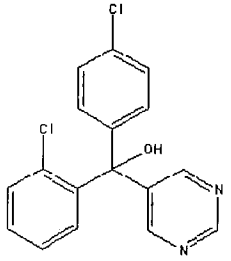
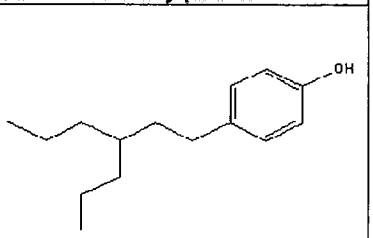
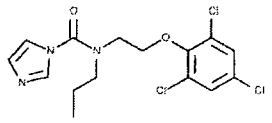
Parameter	Test Substance	Fenarimol
Compound Name	Fenarimol	
CAS #	60168-88-9	
Central File No.	2335-1	
Molecular Weight	331.21 g/mole	
Initial Receipt Date	01/10/05	
Expiration Date	07/09	
Supplier	Supelco/Chem Service	
Lot Number	325-134C	
Vendor Purity	99%	
Method	EDSP.G2-022	

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	5 MG/ML, TOLUENE; CLEAR
	YELLOW, LIGHT GREEN OR LIGHT YELLOW-GREEN	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: +49 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 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

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

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

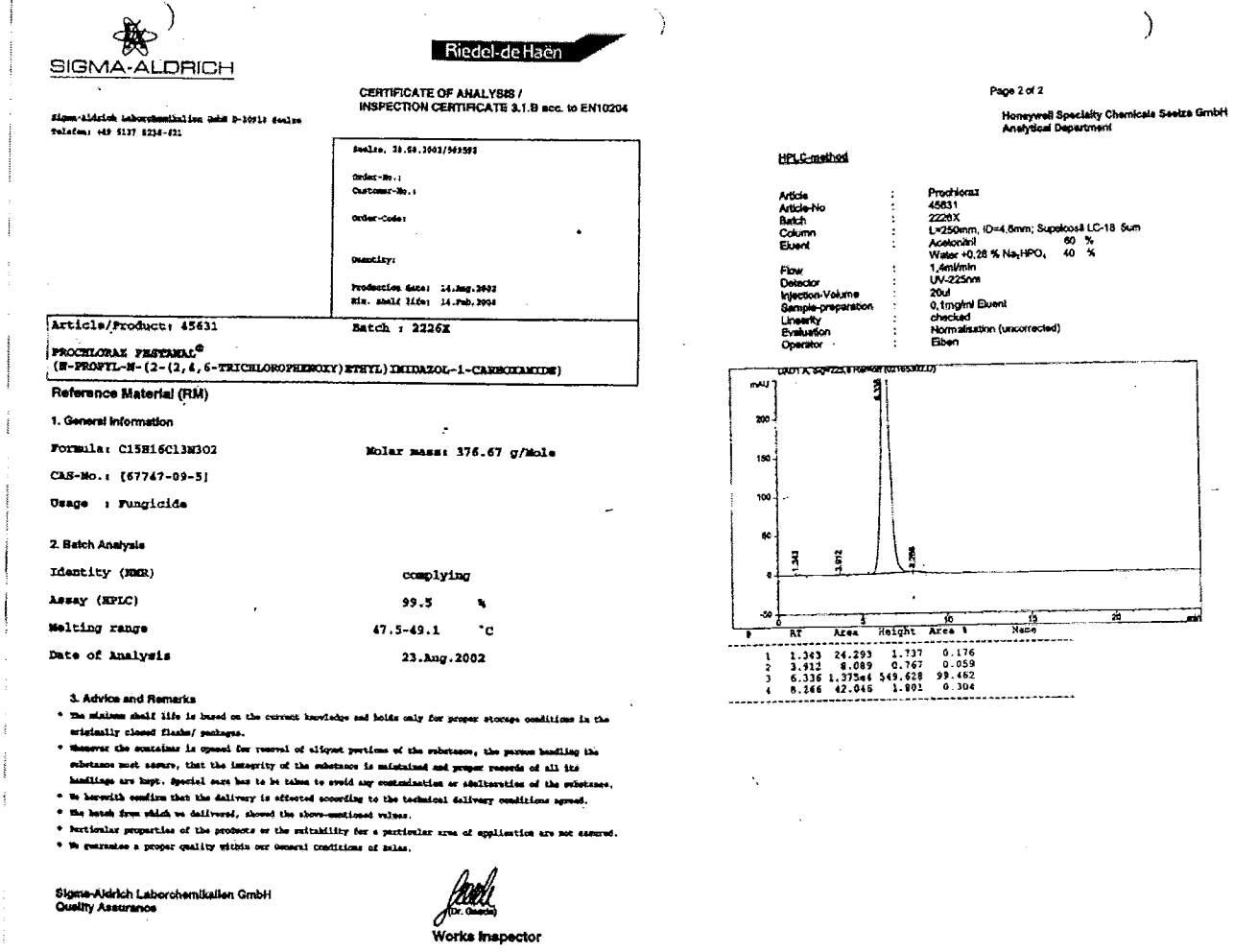
Certificate of Analysis

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

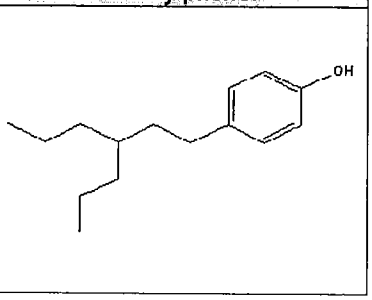
	RT	Area	Height	Area %	Name
1	1.343	24.293	1.737	0.176	
2	3.912	8.089	0.767	0.059	
3	6.336	1.375e4	549.628	99.462	
4	8.266	42.046	1.801	0.304	

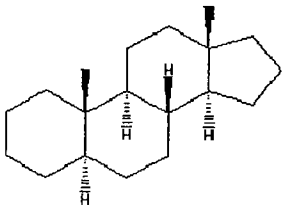
Figure 1G. Prochloraz Certificate of Analysis

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

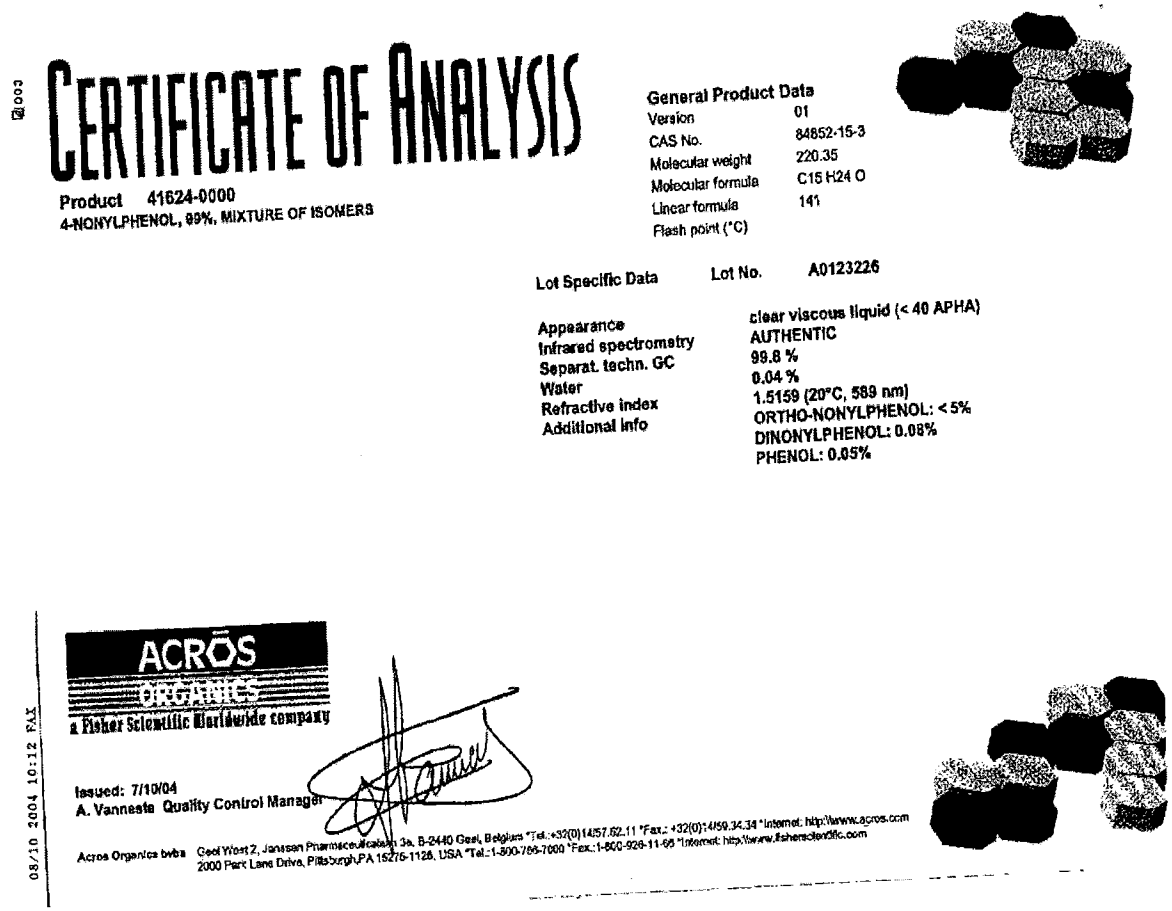
Certificate of Analysis

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	99.0%
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%

ACROS ORGANICS
a Fisher Scientific Distribute 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)14/58.34.34 *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.fishersci.com

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).

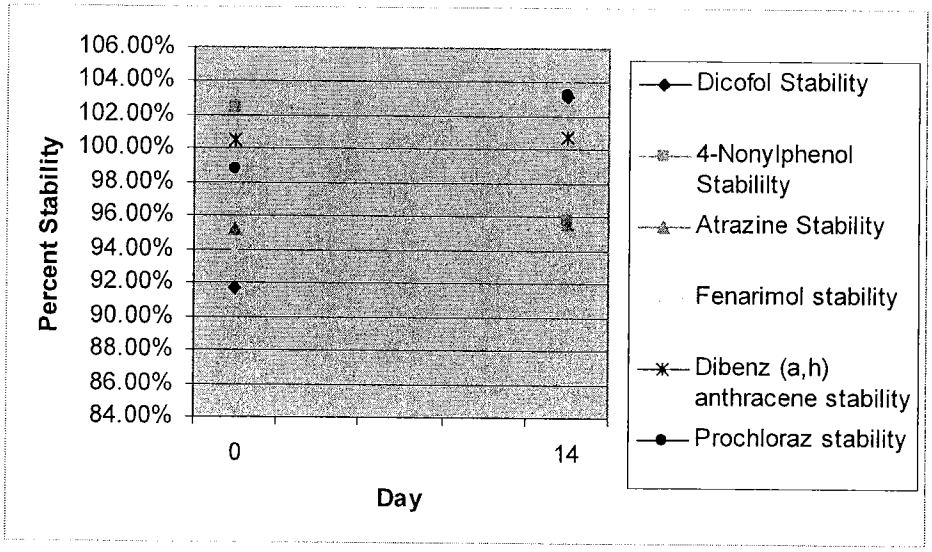


Figure 2. Two Week Stability Data

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.

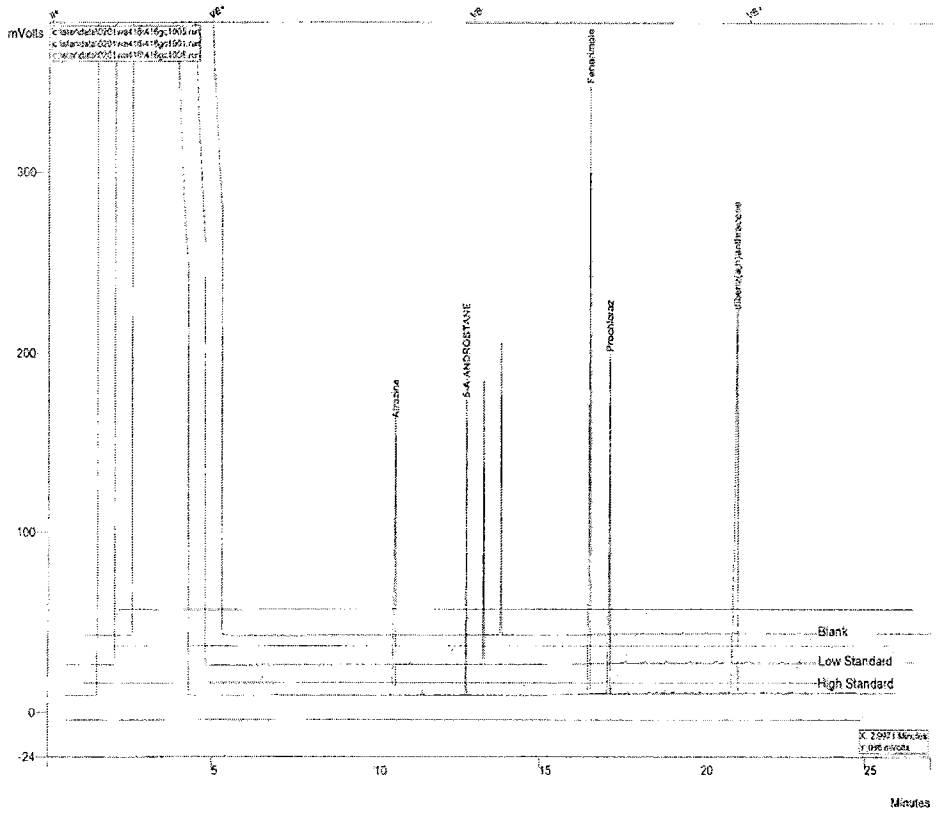


Figure 3. Calibration Standards and Blank for Atrazine, Fenarimol and Dibenz[a,h]Anthracene, (internal standard 5-α-Androstane also shown)

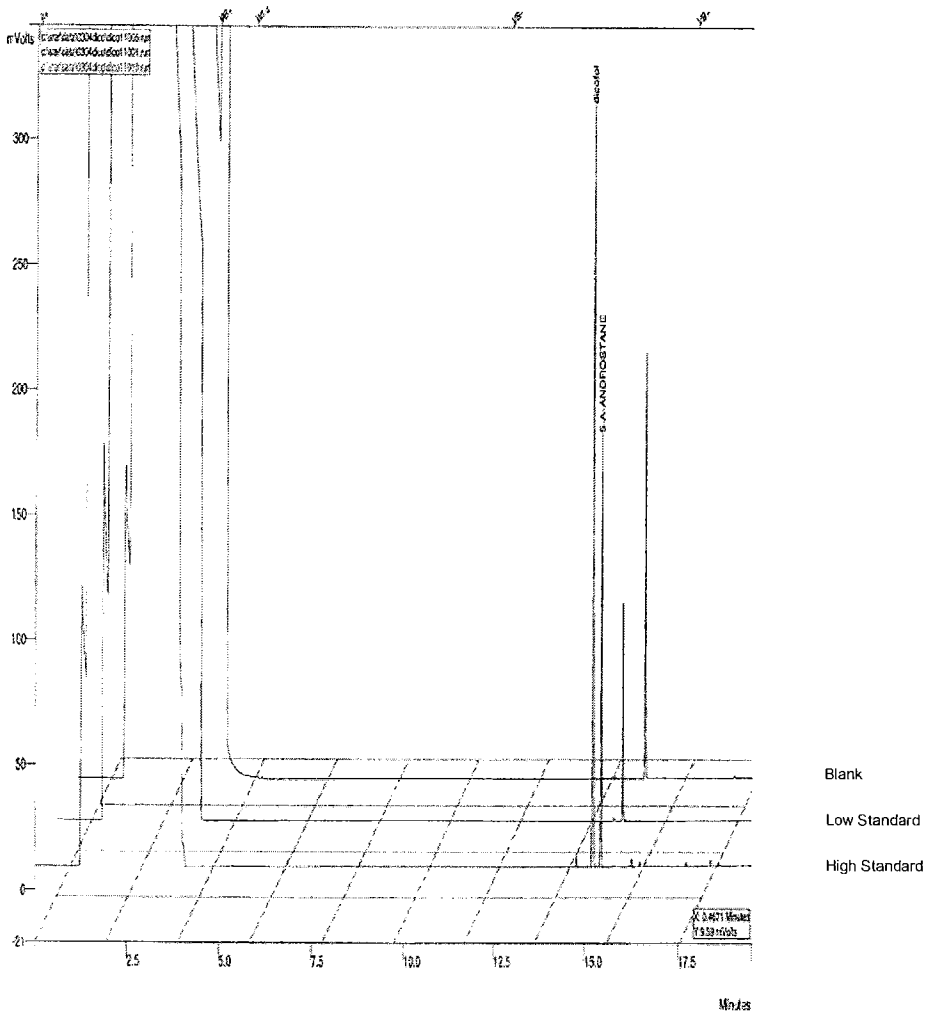


Figure 4. Chromatograms for Dicofol with internal standard 5-α-Androstane

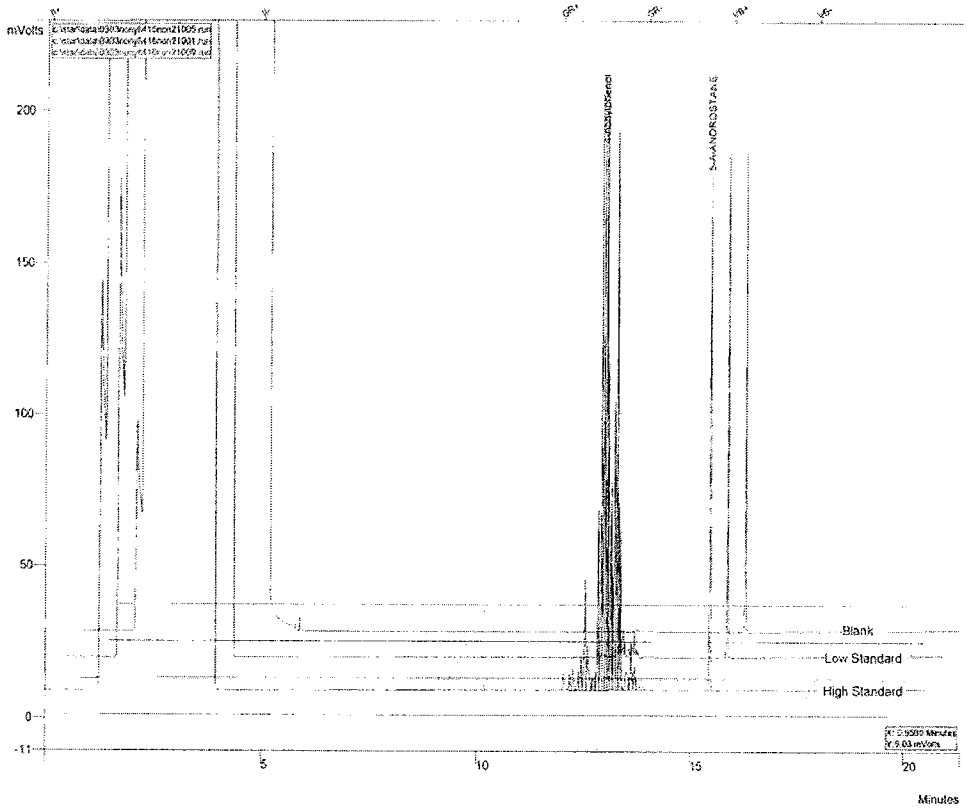


Figure 5. Chromatograms for 4-Nonylphenol, includes internal standard 5-α-Androstane

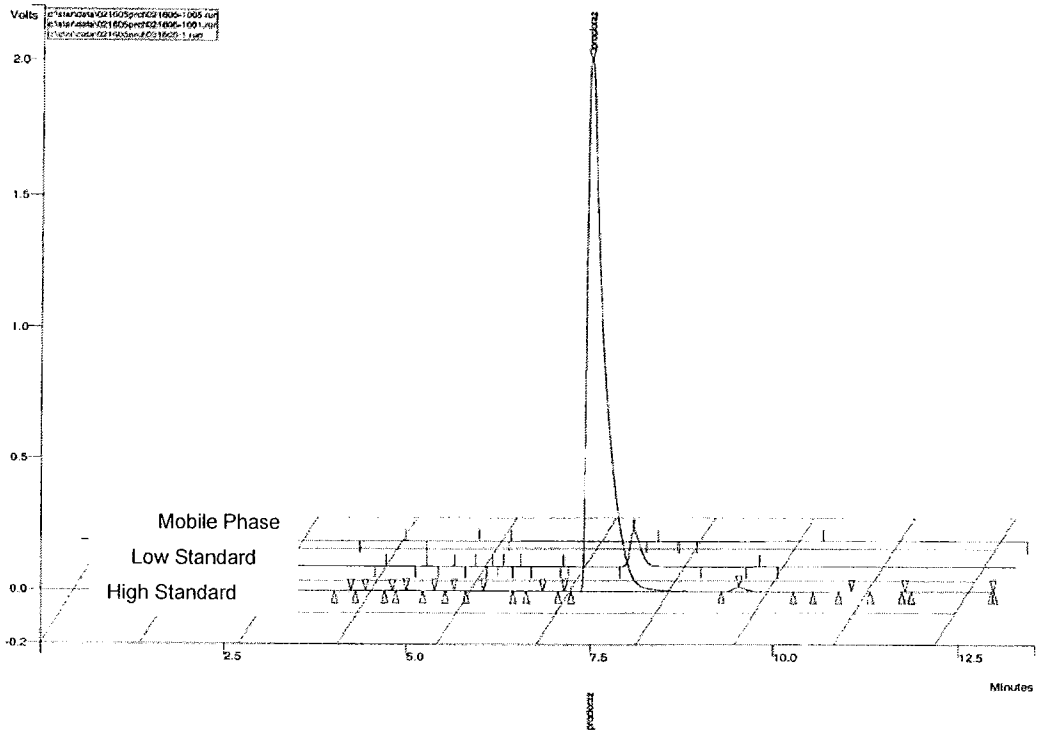


Figure 6. Chromatograms for Prochloraz

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).

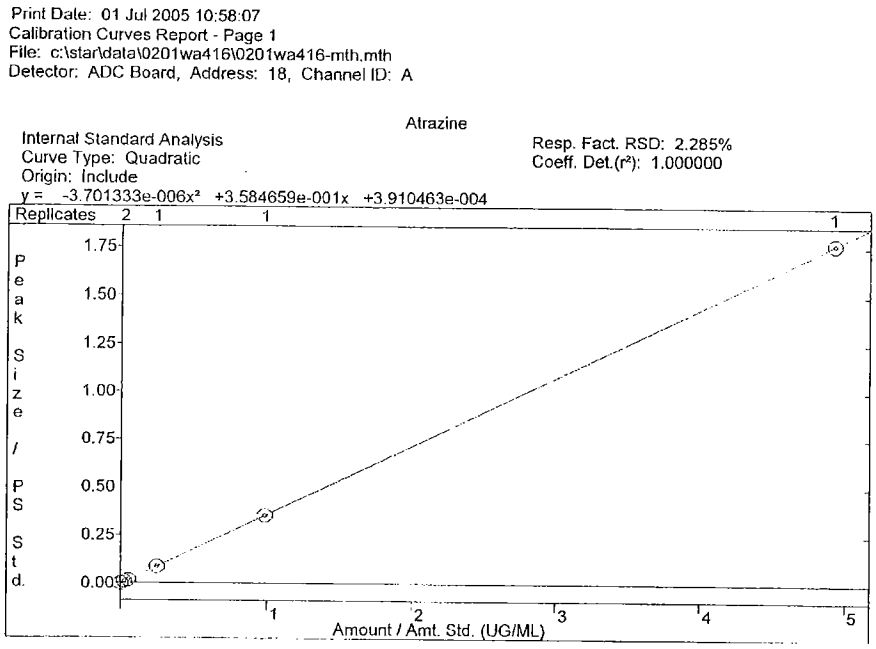


Figure 7. Atrazine Calibration Curve

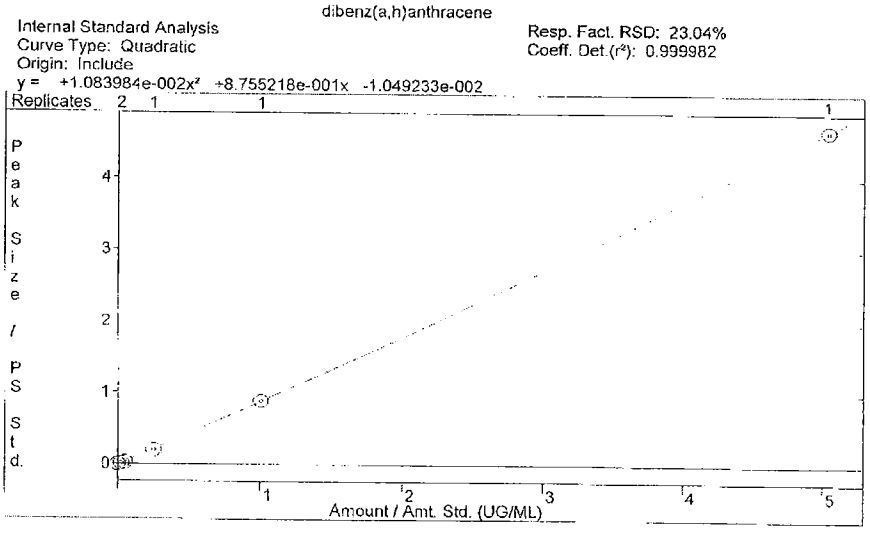


Figure 8. Dibenz[a,h]Anthracene Calibration Curve

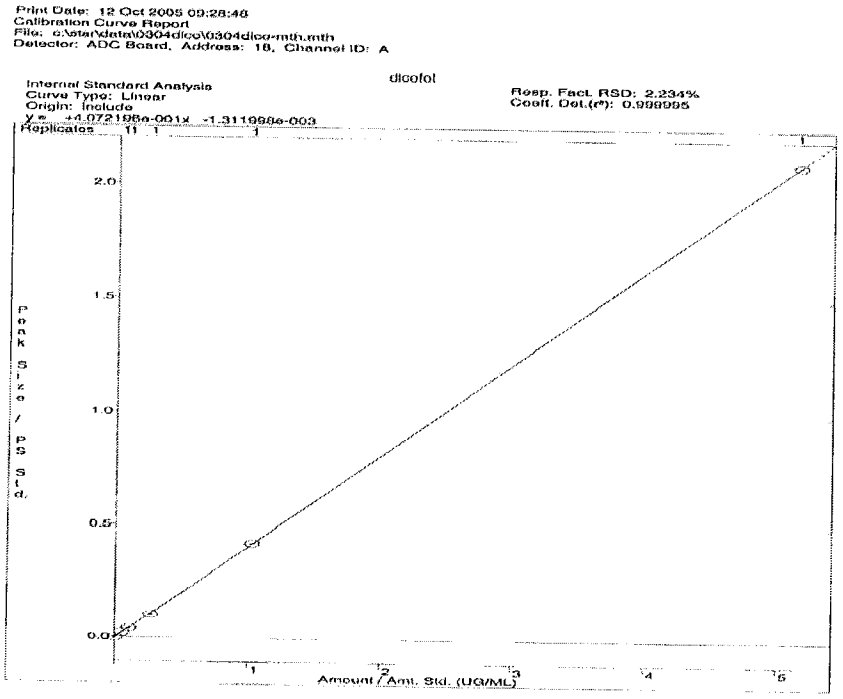


Figure 9. Dicofol Calibration Curve

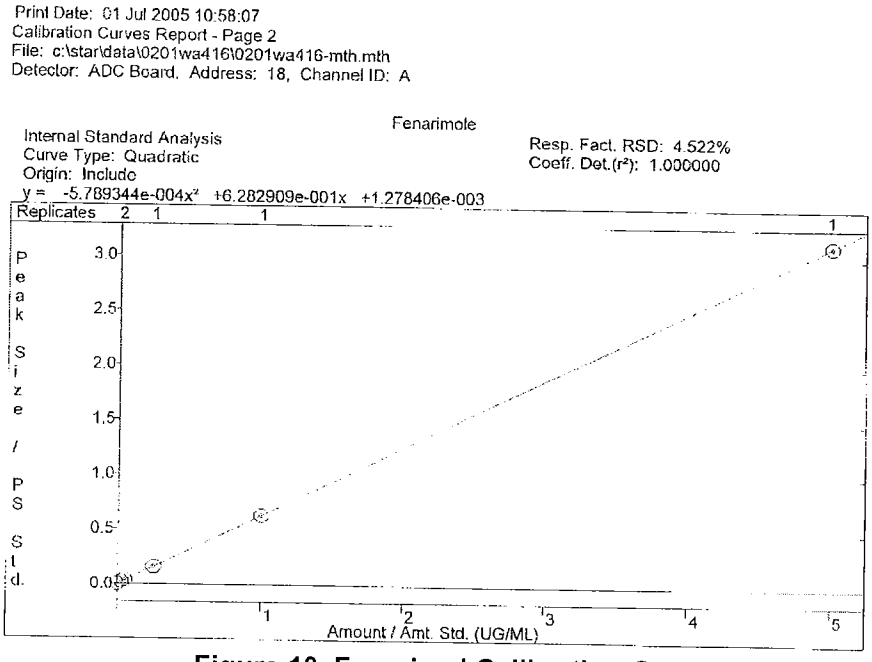


Figure 10. Fenarimol Calibration Curve

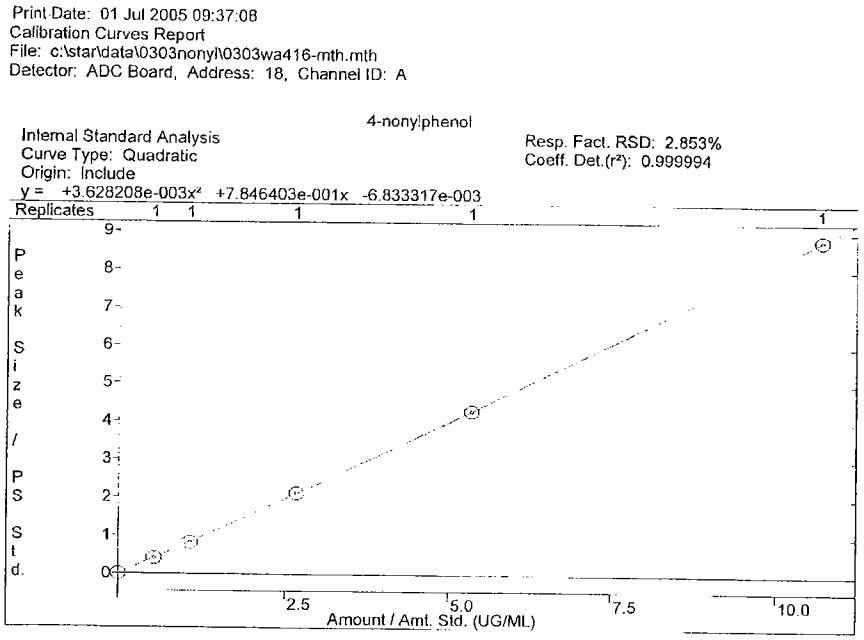


Figure 11. 4-Nonylphenol Calibration Curve

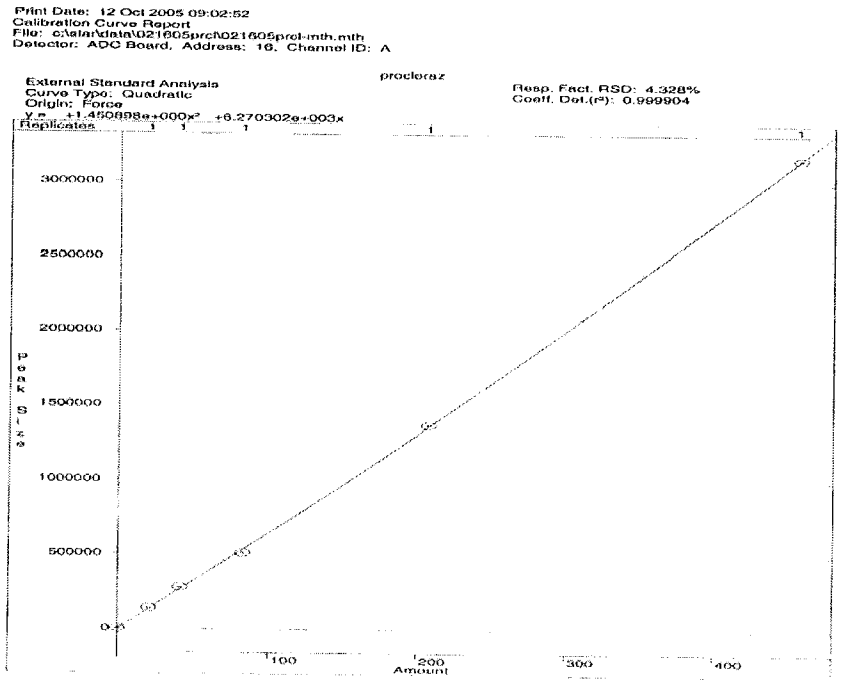


Figure 12. Prochloraz Calibration Curve

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*

Address of Testing Facility:

Battelle – Marine Research Operations
1529 West Sequim Bay Road
Sequim, Washington 98382
Ph: (360) 681-4580
FAX (360) 681-3699
Email: michael.cobb@pnl.gov

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

TABLE 1
Test Substance Abbreviations:

Chemical	Abbreviation
Atrazine	Atrz
Dibenz [A,H] Anthracene	DiBzAnt
Dicofol	Dicofol
4-Nonylphenol	4-NonF
Fenarimol	Fenrl
Prochloraz	Proclz

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 — 1/10/05 Date

Chemical Repository Manager _____ Eric Crecelius, Ph.D. — 1/13/05 Date

Sponsor Representative _____ David Houchens, Ph. D. — 1/26/05 Date

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 wrong name TW 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	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

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. Hall	Date 4/20/05
EDSP Program Management	Dirk P. Hondru	Date 3/24/05
EDSP Battelle QAM	Kimi Pelletal	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 Elwyn	Date 8/3/05
MSL Laboratory Director		Date 8/3/05
EDSP Program Management	David P. Dimmer	Date 5/9/05
EDSP Battelle QAM	Terri J Pollard	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 Jerry D Johnson Date 8-16-05

Study Director Date 8-16-05

EDSP QA Representative Mary E Lyn Date 8/19/05

MSL Laboratory Director Date 8/19/05

EDSP Program Management for David Houlden Date 8-16-05

EDSP Battelle QAM Terri L Pollock 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

1

EDSP Protocol Deviation Number EDSP.416-01-D1

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		Date 8/16/05
EDSP Program Management David Houchens, Ph.D.		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.

EDSP Protocol Deviation Number EDSP.416-01-D2

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	Jerry D Johnson	Date 10-14-05
Study Director Michael Cobb		Date 10-21-05
EDSP QA Representative Mary Lynn	Mary F Lynn	Date 10/21/05
MSL Laboratory Director Dick Ecker	Walter H Ecker FOR RLM	Date 21 OCT 05
EDSP Program Management David Houchens, Ph.D.	David P. Houchens	Date 10/18/05
EDSP Battelle QAM Terri Pollock	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

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 12/9/05
EDSP Program Management David Houchens, Ph.D.	David P. 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

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

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) Anthracene (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) Anthracene 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 2.1 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 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. 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 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 3x 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

Work Study # and Analysis _____

Date _____

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# _____

Instrument Used _____ Lot # _____

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 _____

Battelle
The Business of Innovation

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

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 5 times over the course of the stability study. 2 typo rvkb 11/7/05

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 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).

Battelle
The Business of Innovation

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	Timothy B. Fortman	2-17-05
	Signature	Date
TECHNICAL REVIEWER: Rebecca Wood	Rebecca Wood	2-17-05
	Signature	Date
CHEMICAL REPOSITORY DIRECTOR: Michael Cobb	MC	2-17-05
	Signature	Date

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
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~~.
- 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.

Battelle
The Business of Innovation

Marine Sciences Laboratory

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		03-04-05
	Signature	Date

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 1/8/05
- 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, 6 + typo

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 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%

Battelle
The Business of Innovation

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

Performing Lab: EDSP Chemical Repository
Battelle Marine Science Laboratory (MSL)
1529 West Sequim Bay Road
Sequim, WA 98382

MSL Study Number: EDSP.416-02

Work Assignment: 4-16 and 4-17

Submitted To: Dr. David P. Houchens
EDSP Program Manager
Battelle Columbus Operations
505 King Avenue
Columbus, OH, 43201-2693

Total Number of Pages: 105

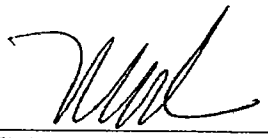
2.0 STATEMENT OF COMPLIANCE

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
Address: 1529 West Sequim Bay Road
Sequim, WA 98382

Prepared by:

12/15/05
Date

Michael E. Cobb
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

5.0 TABLE OF CONTENTS

LIST OF TABLES

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

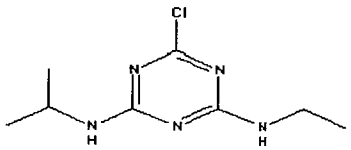
Methods of standard operation of the CR are currently addressed in MSL SOPs numbered R-001 through R-017. These procedures address chemical procurement, ordering, handling, inventory, and storage requirements; chemical receipt and chain of custody, chemical log-in and labeling, inventory, chemical storage; stock solution preparation, documentation and archiving; test solution preparation, formulation analysis, documentation and shipping; chemical disposal, and

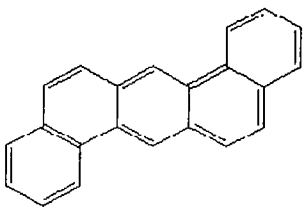
CR maintenance over time. The requirements for procurement of chemicals for use in the CR are addressed in the Quality Assurance Project Plan (QAPP) for EDSP Chemical Repository.

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	

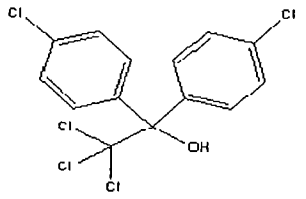
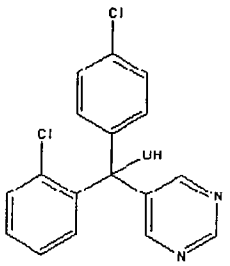
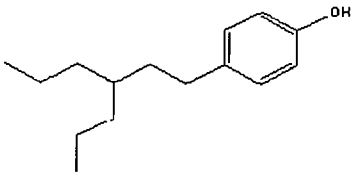
Parameter	Test Substance	Dicofol
Compound Name	Dicofol	
CAS #	115-32-2	
Central File No.	2391-1	
Molecular Weight	370.49 g/mole	
Initial Receipt Date	4/11/05	
Expiration Date	Feb. 08	
Supplier	Ultra Scientific	
Lot Number	RM00299	
Vendor Purity	96.5%	
Method	EDSP.G2-025	

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	

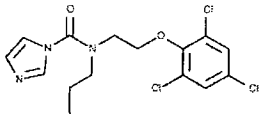
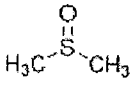
Parameter	Test Substance	Prochloraz
Compound Name	Prochloraz	
CAS #	67747-09-5	
Central File No.	2162-2	
Molecular Weight	376.67 g/mole	
Initial Receipt Date	05/20/04	
Expiration Date	02/14/08	
Supplier	Sigma/Riedel	
Lot Number	2226X	
Vendor Purity	99.5%	
Method	EDSP. H4-023	

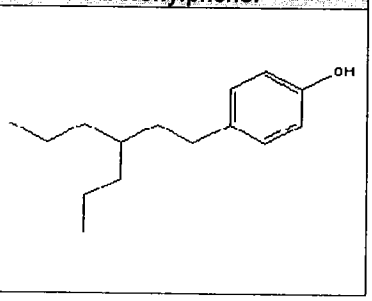
Table 2. Study Vehicle

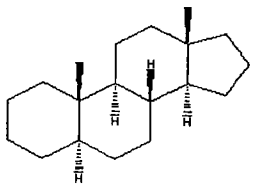
Parameter	Carrier	Dimethylsulfoxide
Compound Name	Dimethylsulfoxide	
CAS #	67-68-5	
Central File No.	Not Applicable	
Initial Receipt Date	01/20/05	
Expiration Date	January 2010	
Supplier	Sigma-Aldrich	
Lot Number	00743BD	

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

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

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

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

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.

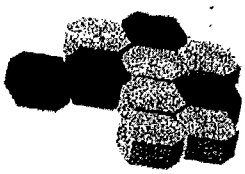
Certified By:

John Conrad

John Conrad
CSM/TC

003

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%

06/10 2004 10:12 FAX

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)14/58.34.34 *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.fishersci0lc.com

SIGMA-ALDRICH

Riedel-de Haën

Sigma-Aldrich Laborchemikalien GmbH D-30918 Seelze
Telefax: +49 5137 8210-121

CERTIFICATE OF ANALYSIS /
INSPECTION CERTIFICATE 3.1.B acc. to EN10204

Seelze, 23.08.2002/563582

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 ensure, 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

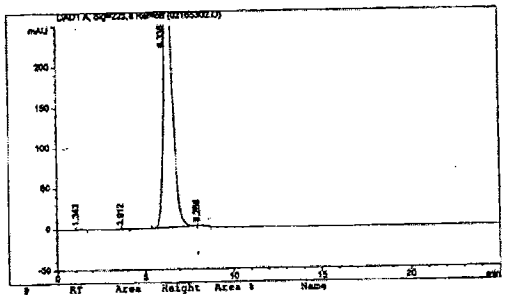
(Dr. Gaede)
Works Inspector

Page 2 of 2

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 Sum
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	:	Elben



	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	8.166	42.046	1.801	0.304	

ACROS

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
Analyst:	RK
Chemical Name:	Prochloraz
Bottle CF Number:	2162-2
CMS Barcode:	Several - See below
Supplier Name:	Sigma-Aldrich
Lot Number:	2226X
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

Cert. of Analysis:	(Y) or N
MSDS:	(Y) or N
Purity:	Min, 99.5% per mfg.
Building:	MSL-5
Room:	219
Location:	Cabinet #1, Shelf #3
CAS:	67747-09-5

Solvent

Date:	7/22/2005
Analyst:	RK
Solvent Name:	Dimethyl sulfoxide
Supplier Name:	Sigma-Aldrich
Lot Number:	00743BD
Date Received:	6/3/2005
Specific Gravity:	1.0960

Building:	MSL-5
Room:	219
Location:	Flammable Cabinet
CAS:	67-68-5
Catalog Number:	472301-2L
CMS Barcode:	253353
Expiration Date:	Jun-10

Stock Preparation

Date:	7/25/2005
Analyst:	RK
Stock Barcode:	Code 3
Expiration Date:	8/7/2005
Target Molarity (mM):	100.0
Volume Needed (mL):	50.00
Target Chemical Mass (g):	1.8834
Target Solvent Mass (g):	54.8073
Actual Molarity Achieved (mM):	100.0133

Balance Information:

Manufacturer:	Sartorius
Model:	B3100P
Serial Number:	1120122767
Calibration Interval:	12 mo.
Last Calibration:	10/18/2004
Calibration Check:	(Y) or N

(documented in Calibration 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:	✓
	Actual Solvent Mass (g):	54.8000

Chemical Dissolved 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 Room 219, MSL 5 in drawer labeled EDSP RK10-19-05

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
Analyst:	R Karle
Chemical Name:	Atrazine
Bottle CF Number:	2332-1
CMS Barcode:	253,212
Supplier Name:	Chem Service
Lot Number:	328-137A
Date Received:	12/14/2004
Expiration Date:	~~Oct-05~~ 10/07
Formula Weight:	215.72

Cert. of Analysis:	(Y) or N
MSDS:	(Y) or N
Purity:	Min, 98% per mfg.
Building:	MSL-5
Room:	219
Location:	Cabinet #1, Shelf #1
CAS:	1912-24-9

RK 12-1-05 COMPUTER ENTRY ERROR

Solvent

Date:	8/22/2005
Analyst:	RKarle
Solvent Name:	Dimethyl sulfoxide
Supplier Name:	Sigma-Aldrich
Lot Number:	00743BD
Date Received:	6/3/2005
Specific Gravity:	1.0960

Building:	MSL-5
Room:	219
Location:	Flammable Cabinet
CAS:	67-68-5
Catalog Number:	472301-2L
CMS Barcode:	253353
Expiration Date:	Jun-10

Stock Preparation

Date:	8/22/2005
Analyst:	RKarle
Stock Barcode:	Code 10
Expiration Date:	9/5/2005
Target Molarity (mM):	100.0
Volume Needed (mL):	50.00
Target Chemical Mass (g):	1.0786
Target Solvent Mass (g):	54.8000
Actual Molarity Achieved (mM):	100.0000

Balance Information:

Manufacturer:	Sartorius
Model:	B3100P
Serial Number:	1120122767
Calibration Interval:	12 mo.
Last Calibration:	10/18/2004

Calibration Check: (Y) or N (documented in Calibration Notebook; room MSL-5, 219)

Step 1:	Tare Stock Bottle:	✓
	Add Chemical:	✓
	Actual Chemical Mass (g):	1.07860
Step 2:	Tare Stock Bottle again:	✓
	Add Solvent:	✓
	Actual Solvent Mass (g):	54.8000

Sonicated 10-15 minutes and chemical is still visable. Swirling, cloud

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 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			Balance Information:	
Date:	9/13/2005		Manufacturer:	Sartorius
Analyst:	RK		Model:	B3100P
Stock Barcode:	Code 5		Serial Number:	1120122767
Expiration Date:	9/28/2005		Calibration Interval:	12 mo.
Target Molarity (mM):	10.0		Last Calibration:	10/18/2004
Volume Needed (mL):	50.00		Calibration Check:	(Y) or N
Target Chemical Mass (g):	0.1392		(documented in Calibration Notebook; room MSL-5, 219)	
Target Solvent Mass (g):	54.8098			
Actual Molarity Achieved (mM):	10.0000			
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
Analyst:	RKarls
Chemical Name:	Dicofol
Bottle CF Number:	2391-1
CMS Barcode:	253313
Supplier Name:	Ultra
Lot Number:	RM00299
Date Received:	4/11/2005
Expiration Date:	Feb-08
Formula Weight:	370.49

Cert. of Analysis:	(Y) or N
MSDS:	(Y) or N
Purity:	Min, 96.5% per mfg.
Building:	MSL-5
Room:	219
Location:	Cabinet #1, Shelf #1
CAS:	115-32-2

Solvent

Date:	8/22/2005
Analyst:	RKarls
Solvent Name:	Dimethyl sulfoxide
Supplier Name:	Sigma-Aldrich
Lot Number:	00743BD
Date Received:	6/3/2005
Specific Gravity:	1.0960

Building:	MSL-5
Room:	219
Location:	Flammable Cabinet
CAS:	67-68-5
Catalog Number:	472301-2L
CMS Barcode:	253353
Expiration Date:	Jun-10

Stock Preparation

Date:	8/22/2005
Analyst:	RKarls
Stock Barcode:	Code 9
Expiration Date:	9/5/2005
Target Molarity (mM):	100.0
Volume Needed (mL):	50.00
Target Chemical Mass (g):	1.8525
Target Solvent Mass (g):	54.8015
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

Balance Information:

Manufacturer:	Sartorius
Model:	B3100P
Serial Number:	1120122767
Calibration Interval:	12 mo.
Last Calibration:	10/18/2004
Calibration Check:	(Y) or N
	(documented in Calibration Notebook; room MSL-5, 219)
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 (stock) 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:	LB326-134C	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:	8/3/2005	CMS Barcode:	253353
Specific Gravity:	1.0960	Expiration Date:	~~Jun-05~~ 06/10

RK 12-1-05 COMPUTER ENTRY ERROR

Stock Preparation				
Date:	7/25/2005	Balance Information:		
Analyst:	RK	Manufacturer:	Sartorius	
Stock Barcode:	Code 6	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):	1.6561	Calibration Check:	(Y) or N	
Target Solvent Mass (g):	54.8116	(documented in Calibration 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 Dissolved 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 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:	~~A012322601~~ ~~A019271201~~ RK 12-1-05 RE; A0123226 RK 12-1-05 cc	CAS:	84852-15-3
Date Received:	2/5/2001		
Expiration Date:	Feb-08		
Formula Weight:	220.35		

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 4	Model:	B3100P
Expiration Date:	9/28/2005	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):	1.1018	Calibration Check:	(Y) or N
Target Solvent Mass (g):	54.8025	(documented in Calibration 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:	✓
	Add Solvent:	✓
	Actual Solvent Mass (g):	54.8000

Chemical Dissolved 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 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. Mathews 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

7/27/05 1500

M-McCARTHY BATTELLE

Printed Name Company

Received By:

Pete Rosmi 7-28-05 1130 hrs

Signature Date Time

PETE RESNIS WIL RESEARCH

Printed Name Company

Certified Copy

Init: NLL **Date:** 07.28.05

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

M. McCarthy 7/27/05 1500

Printed Name Company

BATTELLE

Received By:

Signature Date Time

28 Jul 05 1530

Tony Town

Printed Name Company

Invitro

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:

Shaun Norton 7-28-05 1:00pm
Signature Date Time

Shaun 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. M. Martin 8/24/05 1500
Signature Date Time

M. M. MARTIN BATTELLE
Printed Name Company

Received By:

Pete Resnis 8-25-05 1140
Signature Date Time

Pete Resnis WIL RESEARCH
Printed Name Company

RECEIVED
AUG 25 2005

CERTIFIED COPY

DATE 8-25-05 INITIALS AC

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 N. Cobb

Signature Date 16/08/05 Time

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 8-30-05 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:

9/14/05 1510
Signature Date Time

MCCARTHY BATTELLE
Printed Name Company

Received By:

Don Hubbard 9-15-05 2:30 p.m.
Signature Date Time

DON HUBBARD RTI INTERNATIONAL
Printed Name 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: [signature] Date: 9/14/05 Time: 1510

Printed Name: MCCARTHY Company: BATTELLE

Received By:

Signature: [signature] Date: 9-15-05 Time: 1255

Printed Name: PETE RESNIS Company: WIL RESEARCH LABS

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:

TONY L 9/15/05 900
Signature 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: MURPHY Company: BATTELLE

Received By:

Signature: [signature] Date: 9/15/05 Time: 12:45PM

Printed Name: Leslee Singh 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

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. 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.

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. 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
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 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:

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. 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.

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. 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:

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 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.

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. 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

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

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.

Q#	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 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

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 11/6/05

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 fixed 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 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. and 6 Typo fixed 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 3x 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 # _____

Purpose of Standard _____ Storage Location _____

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
Lot #
CF#
Sampling Interval ____ days
Sampling Date
Sampler
Analysis Date
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

Battelle

The Business of Innovation

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

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.

Multi-peaks 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 HPC 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).

Battelle
The Business of Innovation

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	Timothy B. Fortman	2-17-05
	Signature	Date
TECHNICAL REVIEWER: Rebecca Wood	Rebecca Wood	2-17-05
	Signature	Date
CHEMICAL REPOSITORY DIRECTOR: Michael Cobb		2-17-05
	Signature	Date

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.G2.024-00-D2
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.

Battelle
The Business of Innovation

Marine Sciences Laboratory

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	Timothy Fortman	3-4-05
	Signature	Date
TECHNICAL REVIEWER: Rebecca Wood	Rebecca Wood	3-4-05
	Signature	Date
CHEMICAL REPOSITORY DIRECTOR: Michael Cobb		03-04-05
	Signature	Date

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 4/8/05
- 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.

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 4-17
Recombinant Aromatase Validation Study

Task 4. Conduct Multiple Chemical Studies with Recombinant Microsomes

for

EPA CONTRACT NUMBER 68-W-01-023

June 7, 2005

SIGNATURE PAGE

Quality Assurance Project Plan for WA 4-17, Task 4
Recombinant Aromatase Validation Study
EPA CONTRACT NUMBER 68-W-01-023

Concurrences and Approvals

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

Terri L Pollock 6-29-05
Signature Date

David P. Houchens, Ph.D.
EDSP Program Manager
Battelle
Columbus, OH

David P. Houchens 6/29/05
Signature Date

Jerry D. Johnson, Ph.D., DABT
EDSP Work Assignment Leader
Battelle
Columbus, OH

David P. Houchens for 6/29/05
Signature Date

Gary Timm, M.S., M.A.
EPA Work Assignment Manager
U.S. EPA
Washington, DC

Linda J. Phillips for Gary Timm 6/29/05
Signature Date

J. Thomas McClintock, Ph.D.
EPA Quality Assurance Manager
U.S. EPA
Washington, DC

Thomas M McClintock 6/30/05
Signature Date

Linda J. Phillips, Ph.D.
EPA Project Officer
U.S. EPA
Washington, DC

Linda J. Phillips 6/29/05
Signature Date

2.0 TABLE OF CONTENTS

List of Figures

List of Tables

List of Appendices

3.0 DISTRIBUTION LIST

Debra A. Drissel, B.S.
Quality Assurance Unit Manager
RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194
919-541-6587

James M. Mathews, Ph.D., DABT
Study Director
RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194
919-541-7461

Terri Pollock, B.A.
Quality Assurance Program Manager
Battelle
505 King Ave
Columbus, OH 43201
614-424-5883

David P. Houchens, PhD
Program Manager
Battelle
505 King Avenue
Columbus, OH 43201
614-424-3564

Jerry D. Johnson, Ph.D., DABT
Work Assignment Leader
Battelle
505 King Avenue
Columbus, OH 43201
614-424-4499

J. Thomas McClintock, Ph.D.
Quality Assurance Manager
U.S. Environmental Protection Agency
Room 4121-A
1201 Constitution Ave., NW
Washington, DC 20004
202-564-8488

Gary Timm, M.S., M.A.
EPA Work Assignment Manager
U.S. Environmental Protection Agency
EPA East Building
Room 4106-L, Mail Code 7201M
1201 Constitution Ave., NW
Washington, DC 20004
202-564-8474

Linda J. Phillips, Ph.D.
EPA Project Officer
U.S. Environmental Protection Agency
EPA East Building
Room 4106-G, Mail Code 7203M
1201 Constitution Ave., NW
Washington, DC 20004
202-564-1264

Christopher J. Bowman, Ph.D.
Staff Toxicologist, Developmental and
Reproductive Toxicology
WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-9281
(419) 289-8700

Neil S. Jensen, Ph.D.
Director, Technology Development
1450 South Rolling Road
In Vitro Technologies, Inc.
Baltimore, MD 21227
(410) 455-1242

Bozena D. Lusiak, Ph.D.
Principal Research Scientist
PK/TK and ADMET
Battelle
505 King Avenue
Columbus, OH 43201
(614) 424-6310

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 recombinant microsomes, demonstrate the utility of the microsomal assay to detect known aromatase inhibitors, and compare the performance of the recombinant assay system and the placental 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 objectives of this work assignment are to use the now optimized assay: (1) to obtain intra- and interlaboratory assay variability estimates by conducting positive control experiments at multiple laboratories, and (2) 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 Tasks 3 and 4 of the work assignment.

A summary of the work assignment organization is shown in Figure 4-1.

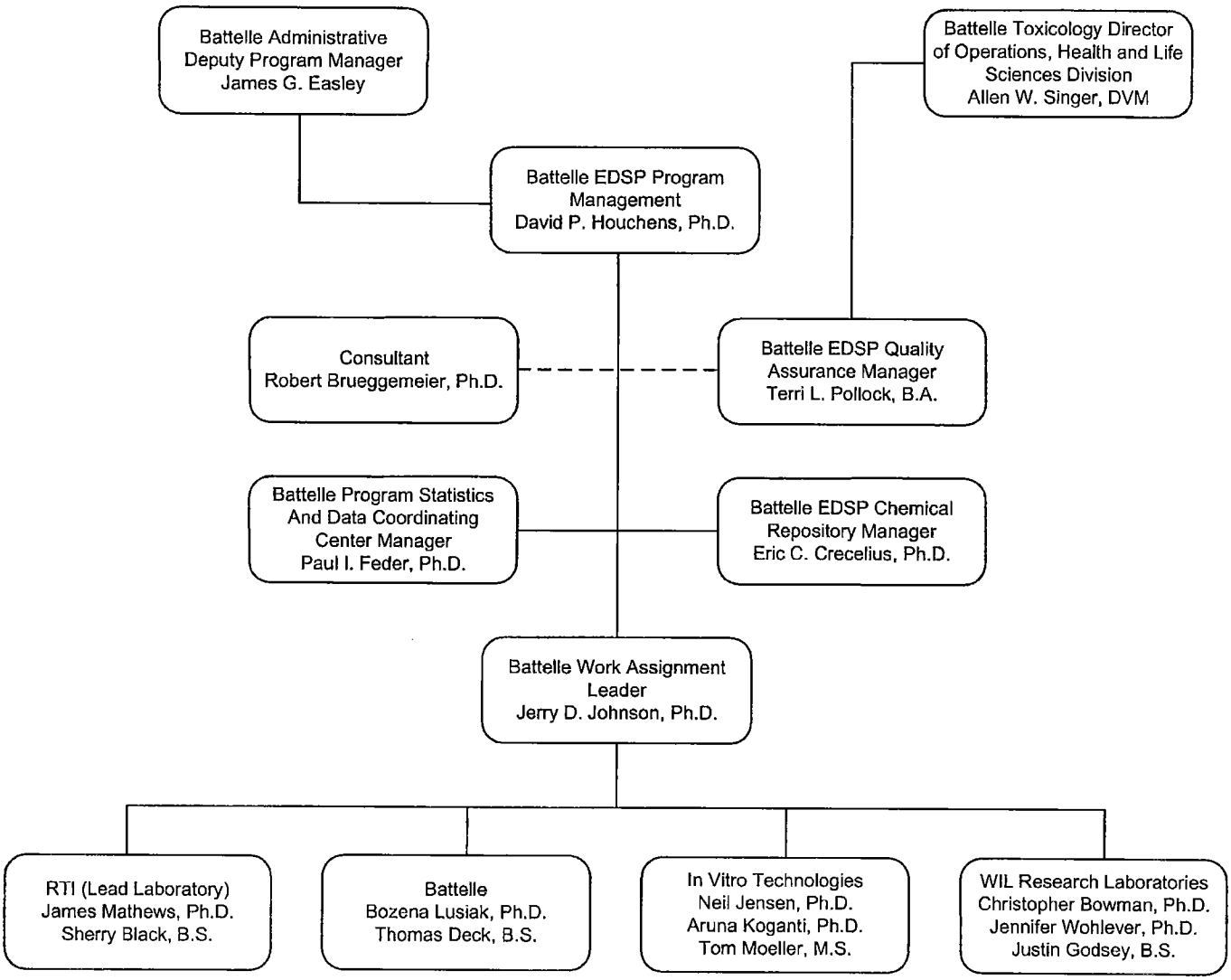


Figure 4-1. WA 4-17 Project Organization Overview

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 recombinant aromatase assay (WA 2-24) were conducted to optimize the microsomal aromatase assay protocol for recombinant microsomes, demonstrate the utility of the microsomal assay to detect known aromatase inhibitors, and compare the performance of a recombinant assay system and the human placental 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).

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 recombinant aromatase assay. A companion work assignment (WA 4-16) has been issued for the conduct of the human placental aromatase assay.

The work assignment is comprised of 6 tasks of which two tasks involve experimentation. The work in Tasks 3 and 4 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	Conduct Positive Control Studies in the Participating Laboratories	3 Participating Laboratories
4	Conduct Multiple Chemical Studies with Microsomes Supplied by RTI (RTI/Participating Laboratories)	Lead Laboratory + 3 Participating Laboratories
5	Prepare Study Reports (RTI/Participating Laboratories)	Not an experimental task
6	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 $P450_{arom}$ 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 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 recombinant microsomal aromatase assay was recommended as the *in vitro* aromatase screening assay to be included in the Tier 1 Screening Battery. This assay will detect environmental toxicants that possess the ability to inhibit aromatase activity. Prevalidation studies on recombinant aromatase (WA 2-24) were conducted to optimize the microsomal aromatase assay protocol, demonstrate the utility of the microsomal assay to detect known aromatase inhibitors, and compare the performance of a recombinant assay system and the placental microsomal assays. 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 recombinant microsome aromatase assay.

6.0 PROJECT/TASK DESCRIPTION

Only Task 4 is under the control of this QAPP. However, this QAPP also describes the other experimental task 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 4 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 3: Conduct of the 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 recombinant 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 were also 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.

Task 4: Conduct Studies on Reference Chemicals

Each participating laboratory will conduct the studies in this task with recombinant microsomes supplied by RTI.

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

The endpoints for WA 4-17 include the aromatase activity measured in the control and inhibitor samples, the inter- and intralaboratory variance, and the IC_{50} and slope values for each inhibitor tested.

7.1 Data Quality Indicators

7.1.1 Precision

The mean positive control activity for each assay/laboratory should be within the overall mean ± 15% 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 positive control activity between and within laboratories should be statistically equivalent at the $p > 0.1$ level. Any modifications to this criterion would be discussed with the sponsor and added to the QAPP by amendment.

IC_{50} and slope values calculated for each inhibitor should be statistically equivalent at the $p>0.1$ level both between and within laboratories. If data from an assay are statistical outliers, the assay may be repeated.

7.1.2 Bias

The positive and background activity samples that are run with each assay are used to control for bias. If the control samples for any assay do not meet the precision criteria described above, the assay may be rerun.

7.1.3 Accuracy

Accuracy of the liquid scintillation spectrometry (LSS) data (from which is derived the aromatase activity) is 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 would not be used. Samples may 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.

Staff from the participating laboratories have been trained on the performance of the aromatase assay at RTI International as part of Task 3 of WA 4-16. 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 Control and Reference Chemical Solutions

The control and reference chemical stock solutions will be transferred to the Laboratories' Material Handling Facility with a study specific transfer of material form. All reference chemicals will be coded by Battelle. 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 4

EPA Contract No.: 68-W-01-023
EPA Work Assignment No.: 4-17
Task Number: 4
RTI Study Code:

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

SPONSOR: Battelle
505 King Ave.
Columbus, OH 43201

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

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

AMENDMENTS:

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

Approved By:

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

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

Linda J. Phillips, Ph.D. — Date
EPA Project Officer

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

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

Reviewed By:

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

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

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

Table of Contents

List of Tables

1.0 OBJECTIVES

TASK 4: CONDUCT MULTIPLE CHEMICAL STUDIES WITH RECOMBINANT MICROSOMES

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

1.1 Justification for Test System

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

1.2 Test Method

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

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

2.0 MATERIALS RECEIPT AND/OR PREPARATION

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

2.1 Substrate

2.1.1 Substrate Name/Supplier

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

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

2.1.2 Preparation of Substrate Solution for use in Aromatase Assay

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

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

2.2 *Reference Chemicals*

The reference chemicals for Task 4, their properties and rationale for selection are listed in Table 1. Each chemical will initially be tested over the concentration range 10^{-3} to 10^{-10} M (final concentration) but the range may be adjusted as described in Section 5.

Table 1. Reference Chemicals for Aromatase Assay Validation

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

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

2.2.1 Reference Chemical Formulation and Analysis

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

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

2.3 Control Substances

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

Table 2. Control Substances

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

2.3.1 Control Substance Formulation and Analysis

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

2.4 Microsomes

Recombinant microsomes will be supplied to each laboratory by RTI. The microsomes must be stored at -70 to -80 °C. The approximate protein content of the microsomes will be provided by the supplier.

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

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

On the day of use, microsomes will be thawed quickly in a 37 ± 1 °C water bath and then will be immediately transferred to an ice bath. The microsomes will be rehomogenized using a Potter-Elvejhem homogenizer (about 5-10 passes) or by vortexing about 5 seconds prior to use. The microsomes will be diluted in buffer (serial dilutions may be necessary) to an approximate protein concentration of 0.008 mg/mL. The addition of 1 mL of that microsome dilution will result in a final approximate protein concentration of 0.004 mg/mL in the assay tubes. All microsome samples must be kept on ice until they are placed in the water bath just prior to their addition to the aromatase assay. Microsomes are not to be left on ice for longer

than approximately 1 h before proceeding with the assay. Appropriate documentation of time from thaw to use must be maintained.

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

2.5 Other Assay Components

2.5.1 Buffer

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

2.5.2 Propylene Glycol

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

2.5.3 NADPH

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

3.0 PROTEIN ASSAY

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

polystyrene cuvettes and the absorbance (@ 750 nm) will be measured using a spectrophotometer. The protein concentration of the microsomal sample will be determined by interpolation of the absorbance value using the curve developed from the protein standards.

4.0 AROMATASE ASSAY METHOD

The assays will be performed in 13 x 100 mm test tubes maintained at 37 ± 1 °C in a shaking water bath. Each test tube will be uniquely identified by applying a label or writing directly on the test tube. Propylene glycol (100 µL), [^{3}H]ASDN, NADPH, and buffer (0.1 M sodium phosphate buffer, pH 7.4) will be combined in the test tubes (total volume 1 mL). The final concentrations for the assay components are presented in Table 3. The tubes and the microsomal suspension will be placed at 37 ± 1°C in the water bath for 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)	Recombinant
Microsomal Protein (mg/mL)[a]	0.004
NADPH (mM)[a]	0.3
[^{3}H]ASDN (nM)[a]	100
Incubation Time (min)	15

[a] Final concentrations

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

5.0 DETERMINATION OF THE RESPONSE OF AROMATASE ACTIVITY TO REFERENCE CHEMICALS

Ten reference chemicals will be tested. The reference chemicals must be coded prior to distribution to the assaying technicians in order that the replicates are conducted blind for reference chemical identity. Each reference chemical will be tested at eight concentrations and there will be three (triplicate) repetitions for each concentration of a given replicate. All three replicates for a given reference chemical must be conducted by the same technician. However, the same technician is not required to perform the three replicates for all ten reference chemicals. Multiple reference chemicals may be conducted by a single technician in a given day. Each replicate for a given reference chemical must be conducted entirely independently of the other replicates for that reference chemical. Thus, it is recommended that if multiple replicates are conducted on a given day by a single technician, those replicates should use different reference chemicals. A single replicate study of a given reference chemical is described in Table 4.

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

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

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

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

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

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

Table 4. Reference Chemical Study Design

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

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

6.0 DATA ANALYSIS

6.1 Aromatase Activity and Percent of Control Calculations

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

6.2 Statistical Analyses

6.2.1 Concentration Response Fits for the Reference Chemicals

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

For each replicate two repeat tubes of the full enzyme activity controls, the background activity controls and the positive and negative controls will be run prior to the 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 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 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 TWP 5-30-06	Date	
Program Management	Deb P. Barnes	Date	5/30/06
EDSP Battelle QAM	Terri J Pollock	Date	5-30-06